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Kenneth V. Honn
Darryl C. Zeldin *Editors*

The Role of Bioactive Lipids in Cancer, Inflammation and Related Diseases

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Editors

The Role of Bioactive Lipids in Cancer, Inflammation and Related Diseases

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About the Editors

Kenneth V. Honn, Ph.D., Distinguished Professor and Director, Bioactive Lipids Research Program, Wayne State University Kenneth Honn, Ph.D., is a distinguished professor and an adjunct professor in the Wayne State University School of Medicine Departments of Pathology and Oncology and Department of Chemistry, respectively. He is director of the Bioactive Lipids Research Program and serves as a member of the Cancer Biology Graduate Program and of the Barbara Ann Karmanos Cancer Institute. He is the founding member and president of the Eicosanoid Research Foundation and chairman of the International Conference on Bioactive Lipids in Cancer, Inflammation and Related Diseases, a biennial international conference he initiated in 1989. He received his Ph.D. in Endocrinology from Wayne State University in 1977. With more than 30 years of experience in the fields of cancer, inflammation, and bioactive lipids, his laboratory focuses on bioactive lipids and integrin receptors and the role they play in various aspects of tumor progression, namely, cell growth and apoptosis, angiogenesis, and tumor cell matrix interactions. His lab concentrates on lipoxygenases, in particular 12-lipoxygenase and its metabolic product 12(*S*)-HETE. In addition to his research on bioactive lipids in tumor progression, he has collaborated with scientists at the Perinatology Research Branch of the National Institutes of Health for the past 4 years, studying the role of lipids in human parturition, in particular their role in preterm labor and term labor. Research efforts in his laboratory have directly led to six

clinical trials, and he holds 17 US patents, 7 of which are based on the generation of novel chemotherapeutic/radiation sensitizing compounds. He is the author of more than 300 published works. He has had continuous external funding with more than 50 grants totaling in excess of $25 million. He also has and continues to serve on numerous study sections, reviewing grants for the National Cancer Institute and the Department of Defense, and provides consultation to pharmaceutical companies. In addition, he is a member of the editorial boards of 12 scientific journals and is coeditor in chief of *Cancer and Metastasis Reviews*.

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Darryl C. Zeldin, M.D., is the scientific director at the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH). He is an internationally recognized expert on eicosanoids (lipid mediators) and their role in regulating respiratory and cardiovascular function. He received his medical degree from Indiana University in 1986 and completed his Internal Medicine Residency at Duke University Medical Center in 1989 and a Fellowship in Pulmonary/Critical Care Medicine at Vanderbilt University in 1993. He was recruited to the NIH in 1994 and promoted to senior investigator with tenure in 2001. He served as the NIEHS clinical director from 2007 to 2011 prior to becoming scientific director in 2011. He is board certified in Internal Medicine and Pulmonary Medicine, is a fellow in the American College of Chest Physicians and in the American Heart Association, and is an elected member of the American Society for Clinical Investigation and Association of American Physicians. He has coauthored over 340 primary peer-reviewed manuscripts, and his work has been cited over 25,000 times (h-index 86). He has mentored over 75 postbaccalaureate fellows, graduate students, medical students, postdoctoral research fellows,

and clinical research fellows. As the NIEHS scientific director, he is responsible for one of the largest intramural research programs at the NIH with over 1000 scientists in 10 departments and 15 core facilities and an annual budget of over $130M. His own research has also been featured on NPR, Good Morning America, *USA Today*, US News & World Report, PBS, and on other internationally recognized media venues.

1 Introduction

Kenneth V. Honn and Darryl C. Zeldin

In addition to this introduction, this book contains 18 outstanding chapters based on comprehensive and detailed reviews of timely topics presented at the 15th International Conference on Bioactive Lipids in Cancer, Inflammation and Related Diseases held in Puerto Vallarta, Mexico on October 22–25, 2017.

Held biennially since 1989, this ongoing conference series provides a scientific program that is comprehensive and structured to highlight research at the cutting edge of science on the role of lipid mediators in various physiological and pathological processes in a wide range of therapeutic areas. Over 315 basic researchers and clinicians representing 32 countries attended this most recent meeting. Dr. Jeffrey M. Drazen, Editor-in-Chief of the *New England Journal of Medicine*, received the Exceptional Contributions to Human Physiology and Translational Medicine Award and presented an opening lecture on "Data Sharing in Clinical Trials." Dr. Charles N. Serhan received the Lifetime Achievement Award and presented a lecture on "Decoding New Lipid Mediators and Mechanisms of Resolution of Inflammation, Infections and Tissue Regeneration." Drs. Bruce D. Hammock, Jorge H. Capdevila and Darryl C. Zeldin shared the Outstanding Achievement Award for their work on the P450 epoxygenase pathway of arachidonic acid metabolism and delivered lectures on the role of fatty acids in neuropathic pain, cardiovascular disease and lung inflammation. There were three Eicosanoid Research Foundation Young Investigator awards presented to Drs. Karin Larsson (cancer), Jesmond Dalli (inflammation) and Scott Hansen (structure-function). Dr. Shu Xu from the University of Toledo was presented with the Santosh Nigam Memorial Outstanding Young Scientist award for his presentation on "The Structure of the Catalytic Domain of 12-Lipoxygenase." There were 50 lectures organized into 3 Plenary Sessions, 14 scientific sessions and 140 poster presentations.

The specialized pro-resolving lipid mediators or SPMs (e.g., resolvins, lipoxins, maresins) have received wide-spread attention as to their role in inflammatory processes. Reviews presented in this book detail recent advances in understanding the role of SPMs in inflammatory conditions of the eye, brain and cardiovascular system. Dr. Elizabeth Berger (Chap. 2) summarized recent literature on infectious keratitis (bacterial, viral, fungal) and endophthalmitis with an emphasis on

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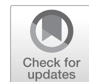
the host response and the role of SPMs in resolution of the eye inflammation. Drs. Darlene Dartt, Robin R. Hodges, and Charles N. Serhan (Chap. 3) provided a comprehensive summary of the role of resolvin D1 (RvD1), its cognate receptor (ALX/FPR2 or GPR32) and downstream signaling pathways (PLC, PLD, PLA2, EGFR, and ERK) in preserving ocular surface homeostasis and regulating conjunctival goblet cell mucin secretion. Dr. Niccolo Terrando (Chap. 4) reported on the role of SPMs in the brain with an emphasis on postoperative neuroinflammation and cognitive function. Dr. Rafael Bojalil (Chap. 5) reviewed the role of pro-resolving mediators in cardiovascular diseases, notably in regulating inflammation. Dr. Ganesh Halade (Chap. 6) summarized recent literature on the role of SPMs in resolution of cardiac inflammation, tissue repair and remodeling in heart failure models. Dr. Jesmond Dalli (Chap. 7) reported on the biological actions of resolvins in the cardiovascular system and their role in mediating the actions of statins.

The book contains two chapters that overview recent advances in cyclooxygenase-derived eicosanoids in the brain. Dr. Philip Kingsley (Chap. 8) reported on the identification and role of prostaglandin glycerol esters (PG-Gs) in the brain, detailing their production, metabolism and bioactivity. Dr. Karin Larsson (Chap. 9) summarized recent data on the role of prostaglandin E2 (PGE2) in neuroblastoma, in particular its actions in regulating angiogenesis, immunosuppression in the tumor microenvironment, and tumor growth. There are three chapters that provide an overview on the biological relevance of cytochrome P450 (CYP) and lipoxygenase-derived eicosanoids. Dr. Pei-an Shih (Chap. 10) reported that CYP-derived eicosanoids are prognostic biomarkers for certain neuropsychiatric disorders such as depression. Dr. Guodong Zhang and coworkers (Chap. 11) discussed recent studies on the roles of CYP-derived eicosanoids and soluble epoxide hydrolase in the pathogenesis of colonic inflammation and colorectal cancer. Dr. Klaus Van Leyen and co-workers (Chap. 12) summarized the biological relevance of lipoxygenase-derived eicosanoids in ischemic stroke and neuronal cell death in the brain.

Sphingolipids are a unique class of bioactive lipids with significant chemical diversity. Recent advances in the field of sphingolipids and their role in the eye, brain, intestine and immune system are discussed in this book. Dr. Yasuyuki Kihara (Chap. 13) provided a review of eicosanoid and other bioactive lipid signaling involvement in the neuroinflammatory mechanisms underlying multiple sclerosis. Drs. Koushik Mondal and Nawajes Mandal (Chap. 14) reported on the role of bioactive sphingolipids including ceramide and sphingosine in ocular inflammation and eye diseases such as uveitis, diabetic retinopathy and glaucoma. Drs. Sabrin Albeituni and Johnny Stiban (Chap. 15) reported on the role of ceramides and other sphingolipids in immune cell function and inflammation in cancer, multiple sclerosis and inflammatory bowel disease.

The endocannabinoids and their receptors affect both central nervous system and peripheral processes. There are two chapters on recent advances in endocannabinoids in the brain. Dr. Heather Bradshaw and colleagues (Chap. 16) reported on the role of endogenous cannabinoids and their receptors in the pathogenesis of traumatic brain injury. Dr. Aditi Das (Chap. 17) summarized recent information on the anti-inflammatory and vasoactive role of omega-3 endocannabinoid epoxide metabolites in the brain. In addition, there is a chapter by Dr. Andres Trostchansky (Chap. 18) on the relationship of lipid metabolism and lipid derived metabolites with the onset and progression of amylotrophic latereral sclerosis in humans. This book is capped off with a concise, scholarly review by Drs. Carlo Barnaba and Ilce G. Medina-Meza on the role of flavonoids in the reduction of inflammation and lipid oxidation in the pathogenesis of cardiovascular disease (Chap. 19).



2 Understanding the Role of Pro-resolving Lipid Mediators in Infectious Keratitis

Elizabeth A. Berger

2.1 Infectious Keratitis

Keratitis is a sight-threatening inflammatory condition of the cornea that can be caused by both infectious and non-infectious agents. Physical or chemical trauma are typically related to non-infectious keratitis, which may then become secondarily infected or remain non-infected. Etiology of infectious keratitis is most often associated with bacteria; but viruses, fungi, and parasites are common causative pathogens as well. As a global concern, common risk factors include: systemic immunosuppression (secondary to malnutrition, alcoholism, diabetes, steroid use), previous corneal surgery (refractive corneal surgery, penetrating keratoplasty), extended wear contact lens use, pre-existing ocular surface diseases (dry eye, epithelial defect) and ocular trauma (agriculture- or farm-related) [1–8]. Annual rates of incidence include nearly one million clinical visits due to keratitis in the United States, while it has been reported that roughly two million people develop corneal ulcers in India. Clinically, patients may show signs of eye pain (ranging from mild to severe), blurred vision, photophobia, chemosis and redness. Pathogenesis is generally characterized by rapid progression, focal white infiltrates with underlying stromal inflammation, corneal thinning, stromal edema, mucopurulent discharge and hypopyon, which can lead to corneal scarring, endophthalmitis, and perforation. In fact, corneal opacity is not only a complication of keratitis, but among the leading causes of legal blindness worldwide. Despite that empirical treatment effectively controls most of the pathogens implicated in infectious keratitis, improved clinical outcomes are not guaranteed. Further, if treatment is not initiated in a timely manner, good visual outcome is reduced to approximately 50% of keratitis patients [9]. Moreover, resultant structural alterations, loss of tissue and an unresolved host response remain unaddressed through current clinical management of this condition.

2.2 Host Response to Infectious Keratitis

A large body of work has been carried out therefore elucidating the keratitis-induced host innate immune response. During infection, corneal ulceration progresses due to inflammatory mediators released by the pathogen, as well as from corneal epithelial and stromal cells and infiltrated inflammatory cells [10]. Though infiltrating leukocytes are a requisite feature of an effective

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inflammatory response, persistence and chronic activation of macrophages, neutrophils/PMN and T cells contribute to disease pathogenesis through sustained production of inflammatory mediators, proteolytic enzymes and resultant tissue necrosis. Similarly, cytokines and chemokines such as IL-1α/β, TNF-α, IFN-γ and MIP-2 promote antimicrobial effector mechanisms, activate phagocytic killing and recruit additional inflammatory cells. However, the continued production of such pro-inflammatory cytokines/chemokines contributes to the destruction of the corneal microenvironment. In addition to an upregulation of cytokines, lipid mediators are also released, including prostaglandins, thromboxanes and platelet-activating factor. It is well-known that these arachidonic acid-derived mediators play key roles in the initiation of inflammation, including the recruitment of leukocytes (particularly neutrophils). Although necessary, it is imperative that this biological cascade is both inhibited and dissolved. These initial pro-inflammatory pathways are balanced by proresolving lipid signals known as specialized pro-resolving lipid mediators (SPMs). In fact, it has been shown that prostaglandins (PGE_2 and PGD_2) initiate lipid mediator class switching [11]. Biosynthesized during the active phase of resolution, SPMs play a pivotal role as endogenous agonists stimulating the resolution of inflammation – a key feature of a healthy, productive inflammatory response [12]. Proresolving actions include limiting PMN infiltration, regulating the cytokine profile [13, 14], reducing pain [15], and efferocytosis [16, 17]. SPMs best associated with the eye are derived from two major polyunsaturated fatty acids; arachidonic acid (AA) and docosahexaenoic acid (DHA) and are targeted by lipoxygenase (LOX) enzymes, which comprise the main pathways for SPM biosynthesis (Fig. 2.1). Studies have demonstrated LOX activity in the cornea with 15-LOX appearing to be largely protective as a key enzyme for the generation of SPMs [12, 18–20] . Alox15 is the most abundant lipoxygenase in the healthy murine cornea [21–23]. *Gronert* et al. have established the presence of functional 5-LOX and 12/15-LOX in mouse corneal epithelium and corneas [19]. Furthermore, both healthy and inflamed corneas express 12/15-LOX as detected from epithelial cells and infiltrated neutrophils [19]. AA-derived lipoxin A4 (LXA4) can

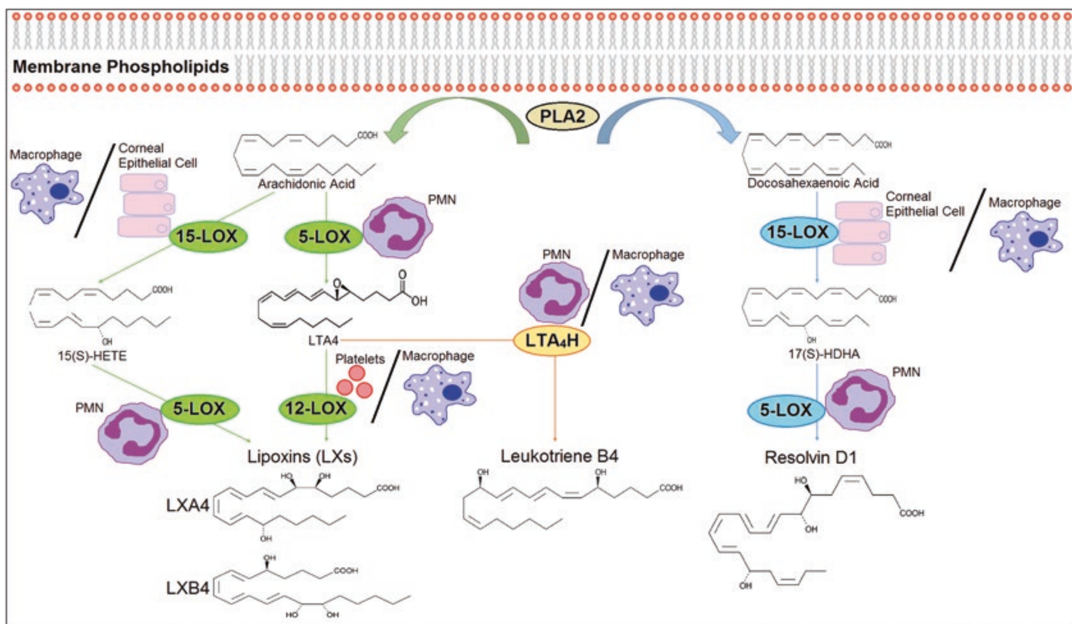

Fig. 2.1 Schematic of major SPM pathways involved in the cornea

be transcellularly biosynthesized during leukocyte interactions with mucosal cells, such as PMN interactions with epithelial cells [24]. Studies have revealed a protective role for LXA4, whereby it has been shown to accelerate corneal re-epithelization and limit neovascularization using a thermal injury model [25]. In addition to AA-derived lipoxins, DHA is targeted by phospholipase A2 and subsequently LOX and COX-2 produce di- and trihydroxylated DHA derivatives including D series resolvins, protectins and maresins [26]. These SPMs are particularly relevant to the eye as studies have shown that RvD1 reduces the inflammatory response during endotoxin-induced uveitis [25], allergic eye disease [27], and dry eye [28].

2.3 Current Treatments for Infectious Keratitis

This collective work illuminates host factors that exacerbate the progression of keratitis, indicating that the pathogenicity of disease is a combination of microbe-induced damage and an unresolved host response – the latter of which may be due to ocular immune privilege. Regardless, a number of adjunctive treatments have been considered, including cross-linking, anti-collagenases, anti-inflammatories, and corticosteroids. Collagen cross-linking was found to improve corneal re-epithelization and resistance to enzymatic degradation, reduce corneal melting, and exert bactericidal effects [29–31]. However, complications include endothelial cell loss and reactivation of HSV infections, thus warranting further investigation. Non-steroidal anti-inflammatory treatments have been investigated in an attempt to prevent excessive inflammation; but shown to exacerbate the disease response in the absence of concomitant antibiotic therapy or exhibit toxicity issues complicated by corneal melting [32–34]. Indecision continues to exist regarding the benefits and risks associated with use of corticosteroids. The Steroids for Corneal Ulcers Trial (SCUT) provides evidence for improved visual acuity in bacterial keratitis patients with no reported increase in adverse events. Yet, other studies have indicated delayed epithelial healing and worsened disease outcome [35–37]. Additionally, a comprehensive review evaluating the effects of corticosteroids from 1950 to 2000 concluded that the role of corticosteroids in the adjunctive treatment of infectious keratitis remains unsubstantiated [38]. Further, a review by *McGhee* et al. states that corticosteroid-induced ocular morbidity includes: ocular hypertension, glaucoma, cataract formation, tear film instability, and crystalline keratopathy [39]. To this end, this review will highlight the compelling evidence for the development of pro-resolving molecules as an adjuvant therapy for the treatment of infectious keratitis – with focus on bacterial, viral and fungal infections.

2.4 Bacterial Keratitis

Bacteria remain the most common cause of infectious keratitis with studies indicating that bacterial keratitis comprises approximately 90% of infectious keratitis cases. Epidemiological studies have revealed that between 54–94% of all corneal infections are due to bacteria. *Pseudomonas aeruginosa* and *Staphylococcus aureus* have been reported as the more prevalent bacteria associated with infectious keratitis [40–42]. *Staphylococcus aureus* as the most common ocular isolate [43]; while *Pseudomonas aeruginosa* is the most frequently isolated of Gram negative ocular infections [22]. Moreover, *Pseudomonas aeruginosa* ulcers have been shown to result in a more progressive disease with large infiltrate and scarring [43, 56] and not benefit from the addition of corticosteroids [36]. Current treatment of microbial keratitis consists primarily of fourth generation ophthalmic fluoroquinolones (moxifloxcin and gatifloxacin), which are effective against both Gram-positive and Gram-negative bacteria. This class of antibiotics also exhibits better mutant prevention characteristics and penetrates well into ocular tissues. However, antibiotic-resistance is of major concern; in the U.S., roughly 80% of methicillin-resistant *S. aureus* ocular isolates have been reported as resistant to

fluoroquinolones [44–46]. Multi-drug resistance rates for *Pseudomonas* are on the rise, as well [47, 48].

Work using a mouse model of *Pseudomonas*-induced keratitis has revealed the importance of SPM circuits in the resolution of inflammation [23]. Lipid mediator profiles were characterized in susceptible (corneal thinning, perforation) and resistant (resolution) phenotypes and indicated that activation of SPM pathways are requisite for a balanced inflammatory response characterized by the resolution of disease. Furthermore, this study indicated a critical role for 15-LOX in influencing inflammatory cell function. Without 15-LOX enzymatic activity, essential innate inflammatory functions (phagocytosis, efferocytosis) were impaired, leading to an exacerbated disease response. This supports previous work that showed 15-LOX$^{-/-}$ mice resulted in impaired wound healing and amplified inflammatory angiogenesis [19, 49, 50]. In addition, potential experimental treatments that have been shown to improve disease response to *Pseudomonas*-induced keratitis appear to have a common theme – the activation of SPM circuitry. Vasoactive intestinal peptide or VIP has been demonstrated to exert its immunoregulatory effects, in part, through upregulation of lipoxygenase enzymes in corneas of *Pseudomonas*-infected mice [51, 52]. Additional work in the mouse model has revealed that VIP increases RvD1 and modulates the ALX/FPR2 receptor axis toward inflammation resolution [52]. Ligands for this receptor include both LXA4 and RvD1 [53]; the latter of which has been shown to reduce corneal neovascularization [54]. Evidence also indicates that thymosin beta-4 (Tβ4), another therapeutic molecule, may carry out its profound effects through activation of SPM circuits [55]. Adjunct Tβ4 treatment with antibiotic resulted in increased 12- and 12/15-LOX mRNA levels, while COX-2 and 5-LOX were decreased after infection [55]. This is further supported by increased RvD1 and its receptor, ALX/FPR2. In contrast, pro-inflammatory lipid mediator pathways were affected, as evidenced by lower levels of LTB4 and its receptor, BLT1. This work underscores the importance of SPM circuit activation in the resolution of inflammation following corneal infection. Further, it highlights the significant potential of combination treatments – where antibiotics alone cannot activate the resolution arm of inflammation; yet without the antibiotic, Tβ4 (and other activators of SPM pathways) may not be able to overcome the bacterial burden and subsequently move toward active resolution. Work carried out by the Pearlman group investigated the role of RvE1 in the corneal inflammatory response induced by LPS and antibiotic-killed *Pseudomonas aeruginosa* and *Staphylococcus aureus* [56]. Cytokine production was significantly reduced in human corneal epithelial cells and neutrophils, as well as mouse cornea and macrophages. In addition, a decrease in neutrophil infiltrate and reduced corneal haze was observed after RvE1 treatment following bacteria- and LPS-induced corneal inflammation.

2.5 Viral Keratitis

Herpes simplex virus (HSV) keratitis affects an estimated 500,000 people in the US and an estimated 1.5 million globally per annum [57, 58]. Though it begins as a subclinical infection of the cornea, HSV establishes latency within the trigeminal ganglia and when reactivated, it causes recurrent infections that result in decreased corneal sensation, neurotrophic keratitis, stromal opacity, scarring, and blindness. In fact, HSV keratitis is the predominant cause of unilateral infectious corneal blindness in most developed nations [59]. Though rare, bilateral infection does occur in approximately 1.3–12% of patients [60]. Primary HSV infections are typically overlooked in the clinic as adenoviral conjunctivitis. Epithelial infection is typically caused by actively replicating virus and presents itself as the classic herpetic corneal lesion – a dendritic ulcer – with minimal stromal inflammation [61]. Once resolved, a dendritic scar or ghost dendrite may persist in the superficial stroma. A more progressive geographic ulcer (also caused by live replicating virus) can occur in immunocompromised patients and

results in a larger epithelial defect. Unfortunately, insufficient corneal wound healing secondary to antiviral toxicity, loss of innervation, and chronic stromal inflammation can result in trophic ulcers. The greatest morbidity is associated with recurrent stromal keratitis and HSV endothelitis due to excessive corneal scarring and neovascularization commonly in response to non-replicating viral particles. Herpes stromal keratitis or HSK can range from focal, multifocal, or diffuse stromal opacities. Disciform keratitis results from endothelial dysfunction in response to viral antigens. Necrotizing keratitis is most commonly observed in patients with multiple recurrent HSV infections and can result in corneal melting and perforation. Less common viral pathogens include varicella-zoster virus (VZV) and cytomegalovirus (CMV). Though treatment varies based on the severity of disease, trifluridine is the most commonly prescribed topical antiviral medication for HSV keratitis in the US [62]. However, due to low bioavailability and ocular surface toxicity, its use is more limited. While in the rest of the world, acyclovir is the preferred treatment as it is equally effective and has lower toxicity issues. Topical corticosteroids, under antiviral cover, are used for the treatment of HSK. Ganciclovir has been found to be just as effective as acyclovir with less toxicity, less likely to promote resistance, and effective against HSV, VZV and CMV. Despite prompt treatment however, scarring is a major complication associated with viral keratitis. Furthermore, since all of the available antivirals for HSV keratitis are nucleoside analogues that inhibit viral replication, there remains the toxicity issue due to host DNA synthesis interference.

Similar to bacterial keratitis, the therapeutic potential of SPMs regarding viral keratitis has been investigated. Work by *Rajasagi* et al. revealed that topical application of aspirin-triggered (AT)-RvD1 reduced lesion severity, corneal neovascularization, pro-inflammatory mediators, and infiltration of effector CD4+ T cells and neutrophils using a mouse model of HSK [63]. Further, they found that AT-RvD1 was equally efficacious regardless of whether treatment was started before or after the induction of infection. The effect on infiltrating inflammatory leukocytes is particularly relevant given that HSK-induced lesions are primarily driven by neutrophils and CD4+ T cells [63]. Work in a corneal transplant model suggests that the observed effect on T cells may be due to an inhibition of dendritic cell maturation and activation [64]. While *in vitro* studies revealed that AT-RvD1 decreased IFN-γ and IL-12 – the latter of which is a requisite signal for Th1 polarization [63]. Another study by the Rouse group found that RvE1 similarly improved HSV-induced keratitis through reduced neovascularization, reduced corneal infiltrate of neutrophils and T cells (Th1/Th17), downregulation of pro-inflammatory cytokines and chemokines, along with less severe lesions [65]. Neuroprotectin D1(NPD1), another DHA-derived pro-resolving mediator [66], was shown to modulate corneal inflammation through similar mechanisms following HSV-induced stromal keratitis in the mouse, as well [67]. These results are in line with a number of studies that have reported NPD1 pro-resolving bioactivities, including reduced T cell migration [68] and neutrophil influx [69], enhanced efferocytosis of apoptotic neutrophils [13], and decreased production of proinflammatory mediators (cytokines, chemokines, ROS) [70, 71]. Taken together, this body of work indicates promise for DHA-derived pro-resolving mediators in the treatment of viral keratitis.

2.6 Fungal Keratitis

Fungal keratitis tends to be more prevalent in tropical and subtropical climates, where it has been reported that up to 50% of infectious ulcers are caused by fungi – predominately *Fusarium* and *Aspergillus* [72–74]. However, fungal keratitis can occur in more temperate regions as demonstrated by the *Fusarium* outbreak related to contact lens solution contamination [75–77]. The prognosis of fungal keratitis is worse than that of bacterial keratitis. Surprisingly, no new treatments have been introduced since natamycin in the 1960s [36]. This topical polyene remains the

first choice of treatment; though amphotericin B is preferred for *Aspergillus* and *Candida* keratitis. Limitations exist for both however; natamycin exhibits poor penetration into the corneal stroma and topical amphotericin B has toxicity issues [78]. Although voriconazole, a newer-generation triazole, demonstrates excellent ocular penetration [79] and high susceptibility among common fungal isolates [80], natamycin remains superior to voriconazole in the topical treatment of fungal keratitis [36, 81]. However, there are no reports examining the effects of SPMs in the treatment of this disease either as a monotherapy or as an adjunct to available antifungals. *In vitro* antifungal susceptibility testing and *in vivo* fungal keratitis models could provide valuable insight into the potential use of SPMs for fungal keratitis given the diversity of fungal aetiology and emergence of drug resistance.

2.7 Endophthalmitis

In rare cases, infectious keratitis can extend from the cornea into the vitreous and/or aqueous humor and is termed endophthalmitis. Though uncommon, it is among the most devastating eye infections that bears the worst possible outcome – irreversible blindness that can occur within even hours of symptom onset [82]. Incidence of disease ranges from 0.03–1.3% with cataract surgery to as high as 30–40% following open globe injuries [83]. The most prevalent pathogens related to endogenous endophthalmitis are bacteria (*Staphylococcus* sp., *Streptococcus* sp.) or fungi (*Candida*, *Aspergillus*). With more than 50% of keratitis-related endophthalmitis cases due to mold, *Fusarium* and *Aspergillus* are the most common etiologies [84]. Moreover, fungal endophthalmitis is particularly difficult to treat due to delayed diagnosis and poor ocular penetration of antifungals compounded by toxicity issues. Despite this, treatment typically consists of intravitreal antibiotics. In addition, vitrectomy is often carried out due to the rapidity and severity of disease progression.

Initial studies in a *S. aureus*-induced model of endophthalmitis show promise for pro-resolving mediators in the treatment of this disease. Intravitreal injections of RvD1 was shown to attenuate the progression of disease while preserving retinal function [85]. This was indicated by reduced levels of proinflammatory mediators, reduced bacterial burden, fewer infiltrated PMN, and induction of antimicrobial molecules. Further, it was suggested that this effect may involve TLR signaling pathways as TLR2 deficient mice were not protected by RvD1 treatment. Similar to fungal keratitis though, therapeutic potential of pro-resolving mediators have yet to be examined in fungal endopthalmitis.

2.8 Conclusion

Although currently available antibiotics, antivirals, and antifungals are efficacious in successfully controlling most of the pathogens implicated in infectious keratitis, clinical outcomes remain poor. A common limitation among these therapies is evident – a sustained host response that compromises corneal integrity. The cornea demonstrates a deficiency in switching from active inflammation to active resolution – perhaps due to ocular immune privilege – resulting in structural alterations and loss of tissue. There exists a clear need for treatments that modify the immune response toward resolution and activate tissue restoration. In fact, a review on the clinical management of infectious keratitis suggested that the development of future adjuvant therapies with the greatest potential are likely to be multidimensional that are aimed at modifying the immune response to infection [36]. The current review provides evidentiary support for using pro-resolving molecules to treat infectious keratitis.

SPMs are being targeted as a beneficial monotherapy and adjuvant for infectious keratitis. The concept of combining SPMs with other drugs has been demonstrated to be highly effective in resolving inflammation [86]. This class of pro-resolving lipid mediators holds promise in the treatment of drug-resistant infections while avoiding ocular surface toxicity issues that currently impede proper treatment of infectious keratitis. As reviewed, SPMs function to dis-

solve inflammation through the regulation of leukocyte infiltration, activation and function. Further, chemical mediators (cytokines, chemokine's, ROS) that would otherwise contribute to chronic inflammation are influenced by SPMs – all of which enables the host response to return to homeostasis and is critical to restoration of corneal function and visual acuity.

One aspect of SPM-induced corneal restoration that has yet to be properly examined in the infectious keratitis model is corneal nerve regeneration. As the most innervated tissue in the body, the cornea contains predominately sensory nerves along with sympathetic and parasympathetic. Extensive work has been carried out investigating the role of corneal nerves in maintaining a healthy ocular surface, during wound healing and following infection [22, 87–90]. Clinically, damaged corneal nerves lead to decreased sensitivity, blink reflexes, tear secretion, dry eye, and neurotropic keratitis. The Bazan group has revealed a combination of DHA and pigment epithelial-derived factor (PEDF) increases NPD1 levels and corneal nerve regeneration using a lamellar keratectomy injury model in the rabbit [91–93]. Further, they found that epithelial wound healing was accelerated after nerve damage, as well. Though these results indicate that pro-resolving mediators will also influence corneal nerves following infection, a more comprehensive assessment is required. Overall, it is anticipated that SPMs will provide a promising avenue of therapy that contributes to the significant advancement in the treatment of infectious keratitis.

References

1. Rattanatam T, Heng WJ, Rapuano CJ, Laibson PR, Cohen EJ (2001) Trends in contact lens-related corneal ulcers. Cornea 20:290–294
2. Stapleton F, Naduvilath T, Keay L, Radford C, Dart J, Edwards K, Carnt N, Minassian D, Holden B (2017) Risk factors and causative organisms in microbial keratitis in daily disposable contact lens wear. PLoS One 12:e0181343
3. Green M, Apel A, Stapleton F (2008) Risk factors and causative organisms in microbial keratitis. Cornea 27:22–27
4. Keay L, Stapleton F, Schein O (2007) Epidemiology of contact lens-related inflammation and microbial keratitis: a 20-year perspective. Eye Contact Lens 33:346–353. discussion 362–343
5. Thylefors B (1992) Epidemiological patterns of ocular trauma. Aust N Z J Ophthalmol 20:95–98
6. Sheng XL, Li HP, Liu QX, Rong WN, Du WZ, Ma L, Yan GH, Ma RQ, Zhang JL, Xu HF, Zou WQ, Bi XJ (2014) Prevalence and associated factors of corneal blindness in Ningxia in Northwest China. Int J Ophthalmol 7:557–562
7. Saha S, Banerjee D, Khetan A, Sengupta J (2009) Epidemiological profile of fungal keratitis in urban population of West Bengal, India. Oman J Ophthalmol 2:114–118
8. Nirmalan PK, Katz J, Tielsch JM, Robin AL, Thulasiraj RD, Krishnadas R, Ramakrishnan R (2004) Ocular trauma in a rural south Indian population: the Aravind comprehensive eye survey. Ophthalmology 111:1778–1781
9. Jones DB (1981) Decision-making in the management of microbial keratitis. Ophthalmology 88:814–820
10. Hazlett LD (2004) Corneal response to *Pseudomonas aeruginosa* infection. Prog Retin Eye Res 23:1–30
11. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN (2001) Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol 2:612
12. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192:1197–1204
13. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196:1025–1037
14. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN (2003) Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem 278:14677–14687
15. Xu ZZ, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji RR (2010) Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 16:592–597. 591p following 597
16. Dalli J, Serhan CN (2012) Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood 120:e60–e72
17. Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447:869–874
18. Wei J, Gronert K (2017) The role of pro-resolving lipid mediators in ocular diseases. Mol Asp Med 58:37–43

19. Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M (2005) A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. J Biol Chem 280:15267–15278
20. Liclican EL, Gronert K (2010) Molecular circuits of resolution in the eye. Scientific World J 10:1029–1047
21. Gronert K (2005) Lipoxins in the eye and their role in wound healing. Prostaglandins Leukot Essent Fatty Acids 73:221–229
22. Kenchegowda S, Bazan HE (2010) Significance of lipid mediators in corneal injury and repair. J Lipid Res 51:879–891
23. Carion TW, Greenwood M, Ebrahim AS, Jerome A, Suvas S, Gronert K, Berger EA (2018) Immunoregulatory role of 15-lipoxygenase in the pathogenesis of bacterial keratitis. FASEB J 32:5026–5038
24. Fredman G, Oh SF, Ayilavarapu S, Hasturk H, Serhan CN, Van Dyke TE (2011) Impaired phagocytosis in localized aggressive periodontitis: rescue by Resolvin E1. PLoS One 6:e24422
25. Settimio R, Clara DF, Franca F, Francesca S, Michele D (2012) Resolvin D1 reduces the immunoinflammatory response of the rat eye following uveitis. Mediat Inflamm 2012:318621
26. Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101
27. Saban DR, Hodges RR, Mathew R, Reyes NJ, Yu C, Kaye R, Swift W, Botten N, Serhan CN, Dartt DA (2019) Resolvin D1 treatment on goblet cell mucin and immune responses in the chronic allergic eye disease (AED) model. Mucosal Immunol 12:145–153
28. Dartt DA, Hodges RR, Li D, Shatos MA, Lashkari K, Serhan CN (2011) Conjunctival goblet cell secretion stimulated by leukotrienes is reduced by resolvins D1 and E1 to promote resolution of inflammation. J Immunol 186:4455–4466
29. Makdoumi K, Mortensen J, Crafoord S (2010) Infectious keratitis treated with corneal crosslinking. Cornea 29:1353–1358
30. Papaioannou L, Miligkos M, Papathanassiou M (2016) Corneal collagen cross-linking for infectious keratitis: a systematic review and meta-analysis. Cornea 35:62–71
31. Alio JL, Abbouda A, Valle DD, Del Castillo JM, Fernandez JA (2013) Corneal cross linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. J Ophthalmic Inflamm Infect 3:47
32. Gritz DC, Lee TY, Kwitko S, McDonnell PJ (1990) Topical anti-inflammatory agents in an animal model of microbial keratitis. Arch Ophthalmol 108:1001–1005
33. Flach AJ (2001) Corneal melts associated with topically applied nonsteroidal anti-inflammatory drugs. Trans Am Ophthalmol Soc 99:205–210. discussion 210–202
34. Guidera AC, Luchs JI, Udell IJ (2001) Keratitis, ulceration, and perforation associated with topical nonsteroidal anti-inflammatory drugs. Ophthalmology 108:936–944
35. Gritz DC, Kwitko S, Trousdale MD, Gonzalez VH, McDonnell PJ (1992) Recurrence of microbial keratitis concomitant with antiinflammatory treatment in an animal model. Cornea 11:404–408
36. Austin A, Lietman T, Rose-Nussbaumer J (2017) Update on the management of infectious keratitis. Ophthalmology 124:1678–1689
37. Cohen EJ (2009) The case against the use of steroids in the treatment of bacterial keratitis. Arch Ophthalmol 127:103–104
38. Wilhelmus KR (2002) Indecision about corticosteroids for bacterial keratitis: an evidence-based update. Ophthalmology 109:835–842. quiz 843
39. McGhee CN, Dean S, Danesh-Meyer H (2002) Locally administered ocular corticosteroids: benefits and risks. Drug Saf 25:33–55
40. Orlans H, Hornby S, Bowler I (2011) In vitro antibiotic susceptibility patterns of bacterial keratitis isolates in Oxford, UK: a 10-year review. Eye 25:489
41. Lichtinger A, Yeung SN, Kim P, Amiran MD, Iovieno A, Elbaz U, Ku JY, Wolff R, Rootman DS, Slomovic AR (2012) Shifting trends in bacterial keratitis in Toronto: an 11-year review. Ophthalmology 119:1785–1790
42. Chang VS, Dhaliwal DK, Raju L, Kowalski RP (2015) Antibiotic resistance in the treatment of *Staphylococcus aureus* keratitis: a 20-year review. Cornea 34:698–703
43. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M (2017) Bacterial profile of ocular infections: a systematic review. BMC Ophthalmol 17:212
44. Asbell PA, Colby KA, Deng S, McDonnell P, Meisler DM, Raizman MB, Sheppard JD Jr, Sahm DF (2008) Ocular TRUST: nationwide antimicrobial susceptibility patterns in ocular isolates. Am J Ophthalmol 145:951–958.e951
45. Haas W, Pillar CM, Torres M, Morris TW, Sahm DF (2011) Monitoring antibiotic resistance in ocular microorganisms: results from the ARMOR 2009 surveillance study. Am J Ophthalmol 152:567–574
46. Alster Y, Herlin L, Lazar M, Loewenstein A (2000) Intraocular penetration of vancomycin eye drops after application to the medial canthus with closed lids. Br J Ophthalmol 84:300–302
47. Livermore DM (2002) Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin Infect Dis 34:634–640
48. Su H-C, Ramkissoon K, Doolittle J, Clark M, Khatun J, Secrest A, Wolfgang MC, Giddings MC (2010) The development of ciprofloxacin resistance in Pseudomonas aeruginosa involves multiple response stages and multiple proteins. Antimicrob Agents Chemother 54:4626–4635
49. Biteman B, Hassan IR, Walker E, Leedom AJ, Dunn M, Seta F, Laniado-Schwartzman M, Gronert K (2007) Interdependence of lipoxin A4 and heme-

oxygenase in counter-regulating inflammation during corneal wound healing. FASEB J 21:2257–2266
50. Leedom AJ, Sullivan AB, Dong B, Lau D, Gronert K (2010) Endogenous LXA4 circuits are determinants of pathological angiogenesis in response to chronic injury. Am J Pathol 176:74–84
51. Carion TW, McWhirter CR, Grewal DK, Berger EA (2015) Efficacy of VIP as treatment for bacteria-induced keratitis against multiple Pseudomonas aeruginosa strains. Invest Ophthalmol Vis Sci 56:6932–6940
52. Carion TW, Kracht D, Strand E, David E, McWhirter C, Ebrahim AS, Berger EA (2018) VIP modulates the ALX/FPR2 receptor axis toward inflammation resolution in a mouse model of bacterial keratitis. Prostaglandins Other Lipid Mediat 140:18–25
53. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN (2012) Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs. Am J Pathol 180:2018–2027
54. Jin Y, Arita M, Zhang Q, Saban DR, Chauhan SK, Chiang N, Serhan CN, Dana R (2009) Anti-angiogenesis effect of the novel anti-inflammatory and pro-resolving lipid mediators. Invest Ophthalmol Vis Sci 50:4743–4752
55. Carion TW, Ebrahim AS, Kracht D, Agrawal A, Strand E, Kaddurah O, McWhirter CR, Sosne G, Berger EA (2018) Thymosin beta-4 and ciprofloxacin adjunctive therapy improves Pseudomonas aeruginosa-induced keratitis. Cell 7
56. Lee JE, Sun Y, Gjorstrup P, Pearlman E (2015) Inhibition of corneal inflammation by the resolvin E1. Invest Ophthalmol Vis Sci 56:2728–2736
57. Farooq AV, Shukla D (2012) Herpes simplex epithelial and stromal keratitis: an epidemiologic update. Surv Ophthalmol 57:448–462
58. Lairson DR, Begley CE, Reynolds TF, Wilhelmus KR (2003) Prevention of herpes simplex virus eye disease: a cost-effectiveness analysis. Arch Ophthalmol 121:108–112
59. Liesegang TJ, Melton L 3rd, Daly PJ, Ilstrup DM (1989) Epidemiology of ocular herpes simplex: incidence in Rochester, minn, 1950 through 1982. Arch Ophthalmol 107:1155–1159
60. Souza PM, Holland EJ, Huang AJ (2003) Bilateral herpetic keratoconjunctivitis. Ophthalmology 110:493–496
61. Tsatsos M, MacGregor C, Athanasiadis I, Moschos MM, Jameel S, Hossain P, Anderson D (2017) Herpes simplex virus keratitis: an update of the pathogenesis and current treatment with oral and topical antiviral agents – comment. Clin Exp Ophthalmol 45:932
62. Morfin F, Thouvenot D (2003) Herpes simplex virus resistance to antiviral drugs. J Clin Virol 26:29–37
63. Rajasagi NK, Bhela S, Varanasi SK, Rouse BT (2017) Frontline science: aspirin-triggered resolvin D1 controls herpes simplex virus-induced corneal immunopathology. J Leukoc Biol 102:1159–1171
64. Hua J, Jin Y, Chen Y, Inomata T, Lee H, Chauhan SK, Petasis NA, Serhan CN, Dana R (2014) The resolvin D1 analogue controls maturation of dendritic cells and suppresses alloimmunity in corneal transplantation. Invest Ophthalmol Vis Sci 55:5944–5951
65. Rajasagi NK, Reddy PBJ, Suryawanshi A, Mulik S, Gjorstrup P, Rouse BT (2011) Controlling herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1. J Immunol 186:1735–1746
66. Serhan CN, Arita M, Hong S, Gotlinger K (2004) Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. Lipids 39:1125–1132
67. Rajasagi NK, Reddy PB, Mulik S, Gjorstrup P, Rouse BT (2013) Neuroprotectin D1 reduces the severity of herpes simplex virus-induced corneal immunopathology. Invest Ophthalmol Vis Sci 54:6269–6279
68. Ariel A, Li PL, Wang W, Tang WX, Fredman G, Hong S, Gotlinger KH, Serhan CN (2005) The docosatriene protectin D1 is produced by TH2 skewing and promotes human T cell apoptosis via lipid raft clustering. J Biol Chem 280:43079–43086
69. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA (2006) Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol 176:1848–1859
70. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN (2005) Molecular circuits of resolution: formation and actions of resolvins and protectins. J Immunol 174:4345–4355
71. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG (2004) Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci U S A 101:8491–8496
72. Srinivasan M, Gonzales CA, George C, Cevallos V, Mascarenhas JM, Asokan B, Wilkins J, Smolin G, Whitcher JP (1997) Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, South India. Br J Ophthalmol 81:965–971
73. Gopinathan U, Garg P, Fernandes M, Sharma S, Athmanathan S, Rao GN (2002) The epidemiological features and laboratory results of fungal keratitis: a 10-year review at a referral eye care center in South India. Cornea 21:555–559
74. Deorukhkar S, Katiyar R, Saini S (2012) Epidemiological features and laboratory results of bacterial and fungal keratitis: a five-year study at a rural tertiary-care hospital in western Maharashtra, India. Singap Med J 53:264–267
75. Chang DC, Grant GB, O'Donnell K, Wannemuehler KA, Noble-Wang J, Rao CY, Jacobson LM, Crowell CS, Sneed RS, Lewis FM (2006) Multistate outbreak of Fusarium keratitis associated with use of a contact lens solution. JAMA 296:953–963
76. Bernal MD, Acharya NR, Lietman TM, Strauss EC, McLeod SD, Hwang DG (2006) Outbreak of

Fusarium keratitis in soft contact lens wearers in San Francisco. Arch Ophthalmol 124:1051–1053
77. Gower EW, Keay LJ, Oechsler RA, Iovieno A, Alfonso EC, Jones DB, Colby K, Tuli SS, Patel SR, Lee SM, Irvine J, Stulting RD, Mauger TF, Schein OD (2010) Trends in fungal keratitis in the United States, 2001–2007. Ophthalmology 117:2263–2267
78. O'Day DM, Head WS, Robinson RD, Clanton JA (1986) Corneal penetration of topical amphotericin B and natamycin. Curr Eye Res 5:877–882
79. Hariprasad SM, Mieler WF, Lin TK, Sponsel WE, Graybill JR (2008) Voriconazole in the treatment of fungal eye infections: a review of current literature. Br J Ophthalmol 92:871–878
80. Walsh TJ, Pappas P, Winston DJ, Lazarus HM, Petersen F, Raffalli J, Yanovich S, Stiff P, Greenberg R, Donowitz G, Schuster M, Reboli A, Wingard J, Arndt C, Reinhardt J, Hadley S, Finberg R, Laverdière M, Perfect J, Garber G, Fioritoni G, Anaissie E, Lee J (2002) Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 346:225–234
81. Walsh TJ, Pappas P, Winston DJ, Lazarus HM, Petersen F, Raffalli J, Yanovich S, Stiff P, Greenberg R, Donowitz G, Schuster M, Reboli A, Wingard J, Arndt C, Reinhardt J, Hadley S, Finberg R, Laverdiere M, Perfect J, Garber G, Fioritoni G, Anaissie E, Lee J, National Institute of, A., and Infectious Diseases Mycoses Study, G (2002) Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 346:225–234
82. Durand ML (2017) Bacterial and fungal endophthalmitis. Clin Microbiol Rev 30:597–613
83. Guest JM, Singh PK, Revankar SG, Chandrasekar PH, Kumar A (2018) Isavuconazole for treatment of experimental fungal endophthalmitis caused by Aspergillus fumigatus. Antimicrob Agents Chemother 62:e01537–e01518
84. Shen YC, Wang CY, Tsai HY, Lee HN (2010) Intracameral voriconazole injection in the treatment of fungal endophthalmitis resulting from keratitis. Am J Ophthalmol 149:916–921
85. Singh PK, Kumar A, Kumar A (2014) Resolvin D1 (RvD1) promotes the resolution of inflammation and protects mice from *S. aureus* endophthalmitis via toll-like receptor 2 signaling. Invest Ophthalmol Vis Sci 55:3575–3575
86. Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Serhan CN (2012) Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature 484:524–528
87. Eguchi H, Hiura A, Nakagawa H, Kusaka S, Shimomura Y (2017) Corneal nerve fiber structure, its role in corneal function, and its changes in corneal diseases. Biomed Res Int 2017:3242649
88. Muller RT, Abedi F, Cruzat A, Witkin D, Baniasadi N, Cavalcanti BM, Jamali A, Chodosh J, Dana R, Pavan-Langston D, Hamrah P (2015) Degeneration and regeneration of subbasal corneal nerves after infectious keratitis: a longitudinal in vivo confocal microscopy study. Ophthalmology 122:2200–2209
89. Cruzat A, Qazi Y, Hamrah P (2017) In vivo confocal microscopy of corneal nerves in health and disease. Ocul Surf 15:15–47
90. Mastropasqua L, Massaro-Giordano G, Nubile M, Sacchetti M (2017) Understanding the pathogenesis of neurotrophic keratitis: the role of corneal nerves. J Cell Physiol 232:717–724
91. Cortina MS, He J, Li N, Bazan NG, Bazan HE (2010) Neuroprotectin D1 synthesis and corneal nerve regeneration after experimental surgery and treatment with PEDF plus DHA. Invest Ophthalmol Vis Sci 51:804–810
92. Pham TL, He J, Kakazu AH, Jun B, Bazan NG, Bazan HEP (2017) Defining a mechanistic link between pigment epithelium–derived factor, docosahexaenoic acid, and corneal nerve regeneration. J Biol Chem 292:18486–18499
93. Kenchegowda S, He J, Bazan HE (2013) Involvement of pigment epithelium-derived factor, docosahexaenoic acid and neuroprotectin D1 in corneal inflammation and nerve integrity after refractive surgery. Prostaglandins Leukot Essent Fatty Acids 88:27–31

3 Immunoresolvent Resolvin D1 Maintains the Health of the Ocular Surface

Darlene A. Dartt, Robin R. Hodges, and Charles N. Serhan

Abstract

The present review focuses on the role of one of the D-series resolvins (Rv) RvD1 in the regulation of conjunctival goblet cell secretion and its role in ocular surface health. RvD1 is the most thoroughly studied of the specialized proresolution mediators in the goblet cells. The anterior surface of the eye consists of the cornea (the transparent central area) and the conjunctiva (opaque tissue that surrounds the cornea and lines the eyelids). The secretory mucin MUC5AC produced by the conjunctival goblet cells is protective of the ocular surface and especially helps to maintain clear vision through the cornea. In health, a complex neural reflex stimulates goblet cell secretion to maintain an optimum amount of mucin in the tear film. The specialized pro-resolution mediator, D-series resolvin (RvD1) is present in human tears and induces goblet cell mucin secretion. RvD1 interacts with its receptors ALX/FPR2 and GPR32, activates phospholipases C, D, and A_2, as well as the EGFR. This stimulation increases the intracellular $[Ca^{2+}]$ and activates extracellular regulated kinase (ERK) 1/2 to cause mucin secretion into the tear film. This mucin secretion protects the ocular surface from the challenges in the external milieu thus maintaining a healthy interface between the eye and the environment. RvD1 forms a second important mechanism along with activation of a neural reflex pathway to regulate goblet cell mucin secretion and protect the ocular surface in health.

Keywords

Conjunctiva · Goblet cells · Allergic eye disease · Dry eye disease · Ocular surface · Mucins · Tear film · Signaling pathways · Resolvin D1 · Inflammation · Secretion

3.1 Tear Film, Cornea, and Conjunctiva

3.1.1 Introduction

The present review focuses on the role of one of the D-series resolvins (Rv) RvD1 in the regulation

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of conjunctival goblet cell secretion. RvD1 is the most thoroughly studied of the specialized pro-resolution mediators in the goblet cells. This review is also focused on the role of RvD1 in maintenance of ocular surface health, but not its role in disease.

3.1.2 Function of Tear Film and Ocular Surface

The eye is a unique, specialized organ whose function is to provide a clear optical path for image presentation on the retina and its analysis by the brain (Fig. 3.1). The first optical surface in the eye is the tear film that overspreads the cornea, a transparent avascular tissue. Together the tear film and cornea provide most of the refractive power of the eye [1]. The tear film and the cornea are among the first components of the anterior eye aided by the conjunctiva to interact with the external environment. The conjunctiva is a vascular, optically dense tissue that surrounds the cornea and lines the eyelids. Together the tear film and ocular surface tissues (cornea and conjunctiva) form the outermost protective layer that functions to maintain a transparent, healthy and impermeable cornea [1]. These components each have unique properties that contribute to the protection of the eye from mechanical, thermal, and chemical injury; desiccation; allergens and pollutants; and pathogens from the external environment.

At the basal aspect, the cornea consists of a single layered endothelium, overlaid by stroma containing keratocytes, and on the apical side a multi-layered epithelium. One of the major protective properties of the cornea is that it is a very tight epithelium with limited permeability [1]. It is difficult to permeate the corneal epithelium because of the extensive tight junctions and other types of junctions between the topmost layers of cells. For example, bacteria cannot penetrate the corneal epithelium unless it is damaged [2]. Furthermore, the cornea is an immune privileged site and maintains an immunosuppressive and anti-inflammatory environment preventing immune cells from migrating into the cornea or

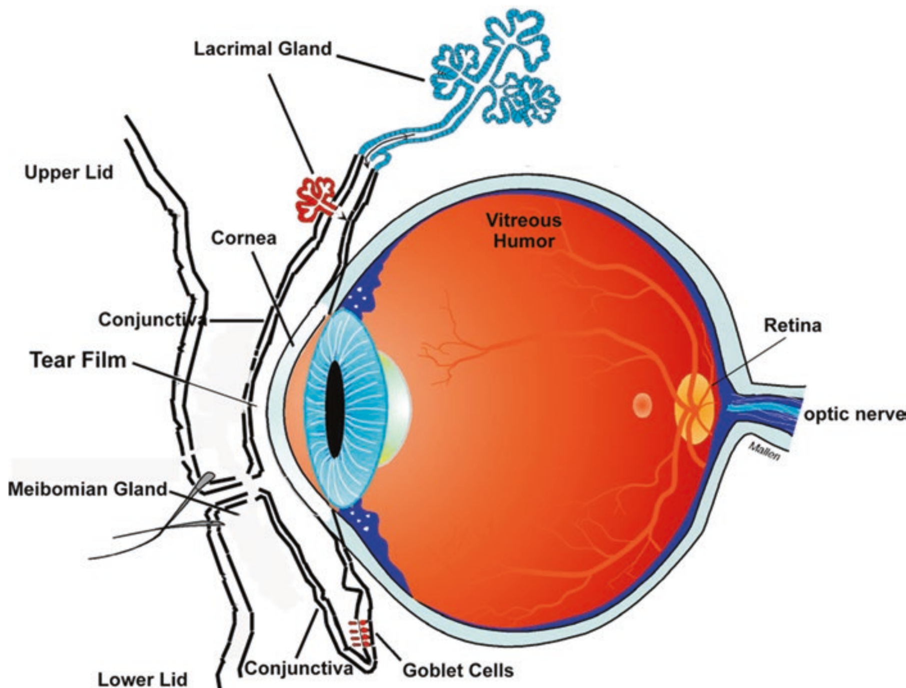

Fig. 3.1 Scehmatic diagram of the eye and ocular surface

aqueous humor in response to an immune or inflammatory challenge [3]. When injured, the cornea normally heals rapidly with minimal scarring and no blood vessel ingrowth. The number of mechanisms that the cornea can use to respond to the external environment is limited and often constrained, as this tissue needs to preserve transparency and remain avascular to ensure clear vision.

The conjunctiva, the focus of this review, surrounds the cornea and lines the lids. One of its major functions is to secrete electrolytes, water, and multiple types of mucins that together form the glycocalyx and the inner layer of the tear film [4]. The conjunctiva is a stratified squamous epithelium that overlies a loose, disorganized stroma containing plentiful blood vessels and nerves. This epithelium consists of two major cell types, stratified squamous cells and goblet cells (Fig. 3.2) [4]. Stratified squamous cells are multilayered, have complex interdigitated lateral membranes, and contain a plethora of small clear vesicles. The second cell type is the goblet cells [5]. These cells occur singly or in clusters and span the entire epithelium in rodents, but only occupy the mid portion of the epithelium reaching to the surface in humans [5]. The goblet cells are polarized with a basal nucleus, substantial Golgi apparatus, and a plethora of secretory granules that occupy most of the cell volume and reach to the apical membrane and the tear film. The stratified squamous cells and the goblet cells both produce the mucous layer of the tear film but release different types of mucins. Both cells secrete electrolytes and water.

Secretion of mucins is one of the major protective mechanisms of the conjunctiva. The mucins produce an extensive glycocalyx and the soluble mucous layer that overspreads both the cornea and conjunctiva. Stratified squamous cells produce the membrane spanning mucins MUC1, MUC4, MUC16, and MUC20 (for a review see [6]). These mucins help form the glycocalyx, a thick coat of carbohydrates that emerges from the apical membranes of epithelial cells and protects the corneal and conjunctival surface. The glycocalyx interfaces with the tear film. The glycocalyx is critical to maintaining the barrier function of the cornea, preventing access of microbes to the plasma membrane, and preventing apical adhesion [7]. In contrast, the goblet cells secrete the large soluble, gel-forming mucin MUC5AC [8]. This mucin is the major component of the inner mucous layer of the tear film and is essential for ocular surface health. MUC5AC upon secretion by the goblet cells overspreads the entire ocular surface including the cornea. It protects the underlying epithelia as a mucus gel by preventing attachment of bacteria and keeping them suspended in the gel. The water and electrolytes in the gel keep the ocular surface hydrated. The movement of the mucus gel across the ocular

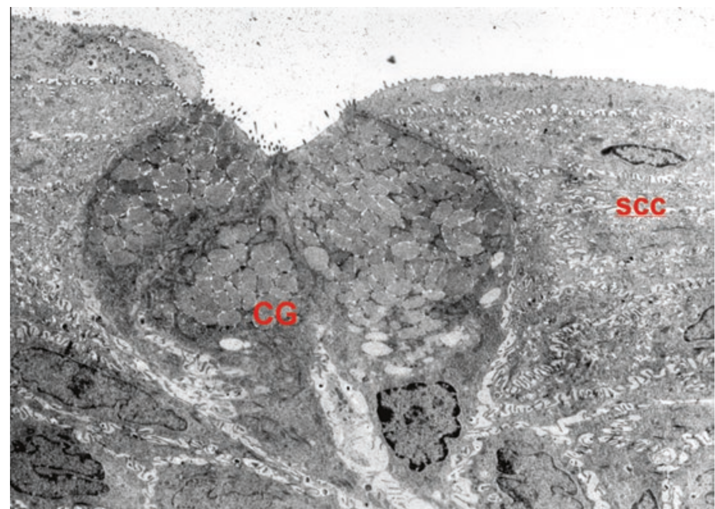

Fig. 3.2 An electron micrograph of rat conjunctival goblet cells (CG). Numerous secretory vesicles can be seen in the apical portion of the cells, while nuclei can be seen in the basal portion. *SCC* stratified squamous cells. Magnification ×6000. Reprinted from Dartt [4]

surface into the lacrimal drainage ducts removes particles, pollutants, and other environmental components from the ocular surface. Goblet cells and their secretion of MUC5AC are regulated by nerves. Afferent sensory nerves and efferent parasympathetic and sympathetic nerves extend into the conjunctival epithelium interdigitating between the stratified squamous cells. Efferent parasympathetic and sympathetic nerves surround the goblet cells. The localization of the nerves in the epithelium provides the basis for these epithelial cells to respond quickly to changes in the external environment by secretion of MUC5AC into the tears.

The conjunctiva has multiple protective mechanisms in addition to the secretion of MUC5AC. As the conjunctival epithelium is opaque, well vascularized, and very permeable, unlike the cornea, it is not constrained in its responses to the environment. The conjunctiva in fact can respond very robustly. The conjunctiva contains conjunctival associated lymphoid tissue that is part of the mucosal immune system [9]. In addition the conjunctival stroma contains numerous different cells for innate defense (macrophages, neutrophils, and mast cells) and for immune protection (lymphocytes, plasma cells, and dendritic cells). In addition for the innate defense system the conjunctiva has most of the Toll-like receptors [10], multiple NOD-like receptors, and a constitutively assembled NLRP3 inflammasome poised for activation [11, 12]. The conjunctiva also has goblet cell associated passages (GAPs) that are openings between goblet cells that allow the stroma to sample antigens and other immune activating material in the external environment [13]. These GAPs are under parasympathetic muscarinic control.

3.1.3 Role Goblet Cells in Ocular Surface Health

As goblet cells and their secretion of MUC5AC are critical for ocular surface health, both a decrease and an increase in goblet cell mucin secretion leads to ocular surface disease. Loss of goblet cells from the conjunctival epithelium and depletion of MUC5AC in the tear film lead to serious damaging, painful ocular surface diseases such as dry eye and vitamin A deficiency [14]. Increase in goblet cell mucin in the tear film is also characteristic of specific diseases, such as ocular allergy, that upset ocular surface homeostasis and can be damaging to the ocular surface. The finding that both a decrease and an increase in goblet cell mucin secretion leads to disease suggests that goblet cell mucin secretion must be tightly regulated to maintain an optimal amount of mucin in the tear film [6, 8, 15].

In health neural regulation of goblet cell mucin secretion provides this regulation [16, 17]. Using a complex neural reflex, goblet cell mucin secretion can be exquisitely regulated to respond to changes in the external environment to secrete mucins as needed to protect the occur surface [18]. Corneal and conjunctival afferent sensory nerves are activated by changes in temperature, acid or bases, or mechanical stimuli (including trauma and particulates that occur in pollution), for examples. The stimulated afferent nerves then activate the trigeminal ganglion that by a complex neural reflex within the brain activates efferent parasympathetic nerves In the conjunctiva parasympathetic nerve endings surround the goblet cells and stimulate them to secrete mucins into the tear film [17]. The goblet cells also likely secrete electrolytes and water, but the evidence for this is indirect [19, 20].

Parasympathetic nerves are the major stimulus of goblet cell mucin secretion in health [16, 21, 22] (Fig. 3.3). There is no published evidence for the role of sympathetic nerves in this secretion. Parasympathetic nerves release the neurotransmitters acetylcholine that activate muscarinic receptors (MAchR) type 1, 2, and 3 and vasoactive intestinal peptide (VIP) that uses the VPAC1 and 2 receptors [21]. Both acetylcholine (carbachol is used experimentally) and VIP stimulate secretion by increasing intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) and activating extracellular regulated kinase (ERK)1/2 also known as p44/p42 mitogen activated protein kinase (MAPK) [13, 23]. In addition VIP activates adenylyl cyclase to increase the cellular cAMP level (For a review see [24]). Acetylcholine (carbachol), but not VIP,

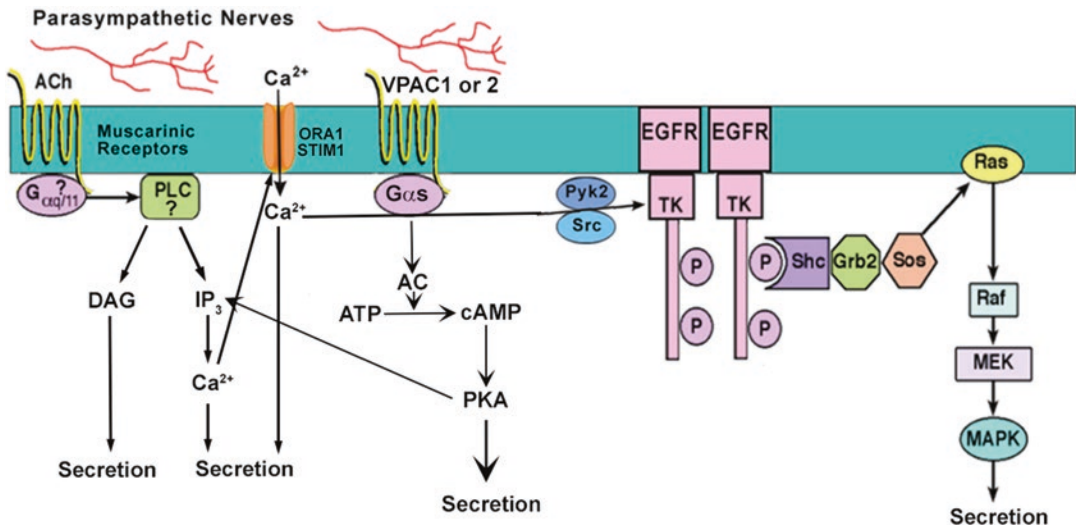

Fig. 3.3 Schematic representation of cholinergic pathway leading to goblet cell mucin secretion. Muscarinic receptors activate phospholipase C (PLC) to generate the production of inositol trisphosphate (IP_3), which releases intracellular Ca^{2+} and diacylglycerol (DAG), which activates protein kinase C (PKC). The EGF receptor (EGFR) is transactivated leading to the activation of Ras, Raf, mitogen activated kinase kinase (MEK), and ERK 1/2. Vasoactive intestinal peptide (VIP) binds to its receptors VPAC1 or 2 to activate adenylyl cyclase (AC) to generate cAMP from ATP. cAMP then activates protein kinase A (PKA) to stimulate secretion. Reprinted from Hodges et al. Encyclopedia of the Eye, Dartt D, Dana R, Besharse J eds. Elsevier, 2010

also works by activating a matrix metalloproteinase to cause ectodomain shedding of EGF to stimulate the EGFR that in turn increases $[Ca^{2+}]_i$ and activates ERK1/2 to stimulate goblet cell secretion [22].

Thus under normal conditions, the parasympathetic neurotransmitters use a common intracellular signaling pathway, an increase in $[Ca^{2+}]_i$ and activation of ERK1/2 to stimulate mucin secretion by inducing exocytosis and release of all the secretory granules within a given goblet cell [25]. The molecular mechanism by which the exocytosis occurs is unstudied in the goblet cell. This pathway is tightly regulated in response to neural activation of the cornea and conjunctiva to maintain an optimal mucin layer that is critical for a healthy ocular surface.

As there is far less research on the conjunctiva than the cornea, the mechanisms used by the conjunctiva to respond to the environment are only beginning to be described. The role of the specialized pro-resolving mediator (SPM) resolvin D1 (RvD1) is one of these mechanisms and is the topic of the present review.

3.2 Specialized Pro-resolving Mediators

The SPMs comprise four families, lipoxins, resolvins, protectins, and maresins. The SPMs function in the resolution phase of acute inflammatory diseases and each family possess unique bioactions to resolve inflammation [26]. Each family of mediators has very potent actions as well as being structurally distinct, displaying stereospecific actions, and utilizing different biosynthetic pathways. Lipoxins are biosynthesized from the omega 6 fatty acid arachidonic acid after class switching from production of pro-inflammatory mediators. The omega 3 fatty acid docosahexaenoic acid (DHA) is precursor for the biosynthesis the D-series resolvins, the protectins, and the maresins. The omega 3 fatty acid, eicosapentaenoic acid (EPA) is precursor for the biosynthesis of the potent E-series resolvins. Each member of the RvD (RvD1-6) and RvE (RvE1 and 2) families possesses unique structures and has distinct functions in the treatment of disease in animal models and in cells.

The enzymes responsible for this biosynthesis are the lipoxygenases (LOX) 5-LOX and 12/15 LOX. The location and specificity of the LOXs are cell and tissue specific. This determines the type and amount of SPM produced. In general 15-LOX and 5-LOX are needed to produce lipoxins from arachidonic acid. 5-LOX is required to biosynthesize E-series resolvins. 15-LOX and 5-LOX biosynthesize D-series resolvins and protectin (Fig. 3.4). 12-LOX biosynthesizes maresins. There is little published information on LOX enzymes in the conjunctiva and especially the goblet cells. Several articles from the 1980s found that the normal, uninflamed conjunctiva from various species of animals has the capacity to synthesize both cyclooxygenases and lipoxygenases [27, 28]. The cycloxygenase was higher than the lipoxygenase activity. The major lipoxygenases produced by the conjunctiva were 12-HETE, 5-HETE, and 5,12-diHETE suggesting production of hepoxilin and LTA_4. Subsequently conjunctiva and eyelids were demonstrated to possess EPA lipoxygenase products of the 5-series suggesting the capability of producing E-series resolvins [29].

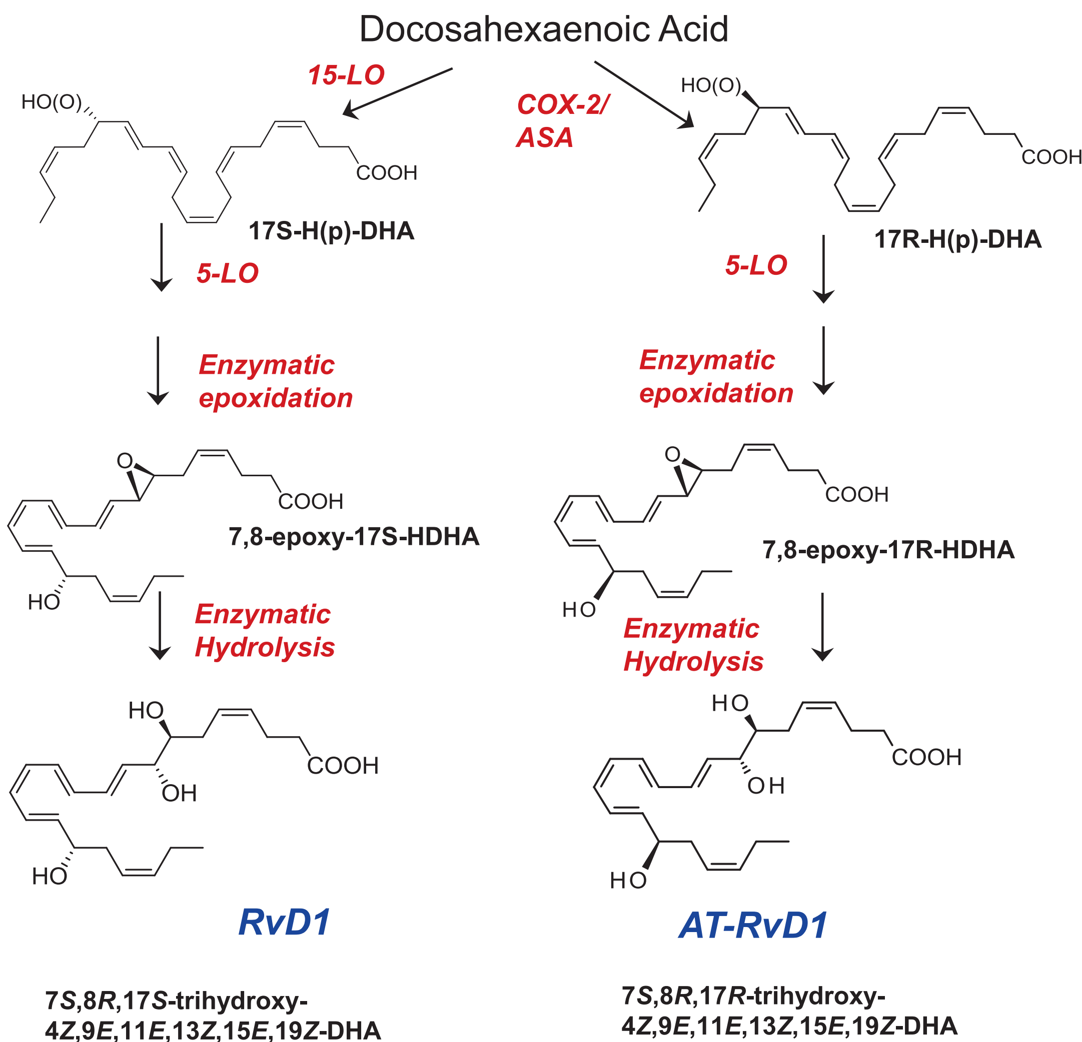

Fig. 3.4 Synthetic Pathways of the D-series specialized pro-resolving mediators. Each is derived from DHA. 5-LO: 5 lipoxygenase Modified from Sun Y, Oh SF et al. J Biol Chem 282:9323–9234, 2007

There is substantial work on the LOX enzymes in the cornea and the draining lymph nodes of the eye that contribute to ocular surface disease especially [30–32], 5- and 15-LOX are the rate limiting enzymes responsible for the generating SPMs [33]. The corneal epithelium has high expression of 15-LOX and functions as part of a LXA_4-ALX/FPR2 circuit that controls wound healing and immune response in the cornea. In particular there is a population of tissue specific polymorphonuclear leukocytes (PMNs) that contain especially high amounts of 15-LOX and that in a sex-specific manner play a role in exacerbating autoimmune dry eye disease in females. These PMNs were detected in the lacrimal gland, draining lymph nodes, and limbus of the cornea. As the cornea and its limbus is adjacent to the conjunctiva, it is possible that conjunctival epithelial cells also have elevated levels of 15-LOX. Furthermore, conjunctival goblet cells are a target of disease in autoimmune dry eye. Study of ocular surface disease has shown the presence of high levels of the biosynthetic enzymes for the SPMs and the circuits for their regulation. These enzymes could also function in health especially as during eye closure in sleep, PMNs migrate into the tear film and could interact with the conjunctival epithelial cells to set up a transient, low level activation of a LXA_4-ALX/FPR2 like circuit or other circuits potentially in the conjunctiva. The same enzymes produce RvD1 and could be critical for regulating RvD1 biosynthesis in conjunctival goblet cells.

In addition to the production of SPMs in disease, SPMs are also endogenously produced in human tissues rich in omega 3 fatty acids in the absence of disease [34]. Thus SPMs are produced in human milk [35, 36], blood [37, 38], brain [39], and retina [40]. SPMs are major players in the regulation of ocular surface health as SPMs are detected in the tear film and stimulate conjunctival goblet cell mucin secretion under physiologic conditions. They function to maintain an optimum mucin layer of the tear film in response to the normal changes in the cornea and conjunctiva and to the extracellular environment. Multiple SPM family members are present within emotional human tears and stimulate goblet cell secretion to maintain the healthy tear film [41] . Herein we will review the evidence for the role of the D-series resolvin RvD1 in conjunctival health.

3.3 RvD1 Is Present in Tears from Healthy Individuals

A number of eicosanoids as well as SPMs are found in human tears. Human emotional tears were collected from six male and six female subjects and the lipid profile analyzed using an LC-MS-MS based metabololipidomics along with deuterium-labeled SPM as internal standards for quantitation [41]. We documented the presence of pro-inflammatory prostaglandins and the leukotriene B_4. For the SPMs, the D-series resolvins (RvD1, RvD2, RvD5), protectin D1, and lipoxin $A_{4,}$ but neither the maresins nor E-series resolvins, were identified in these samples from healthy human subjects. The SPM biosynthesis pathway markers 17-HDHA, 14-HDHA, and 18-HEPE were also identified. These compounds could be bioactive themselves [42] or could suggest that both D- and E-series resolvins may be present in higher amounts locally than measured in the present study. The presence of pathway markers would also be consistent with the further metabolism of these bioactive resolvins to their oxo- and dehydro-resolvin products that were not profiled in the present study and are usually less or devoid of bioactivity. Thus, RvD1 was present in human tears and available to regulate the function of the conjunctival goblet cells. There are two other studies on SPMs in tears that are in agreement with that of English et al. [41]. Walter et al. [43] found DHA, the ω 3 fatty acid from which RvD1 is biosynthesized, in tears. RvD1 was not measured directly in this study. Masoudi S et al. [44] found RvD1 in tears, but this was the only SPM analyzed.

Surprisingly the lipid profile of male and female tears differed substantially [41]. Use of a principal component analysis demonstrated a gender difference in the SPMs in tears (Fig. 3.5). The loading plot calculated from LC-MS-MS identified RvD1, RvD2, RvD5 and protectin D1 in male tears. In contrast LXA_4 and aspirin-triggered LXA_4 were detected in female tears. When the ratio of SPMs,

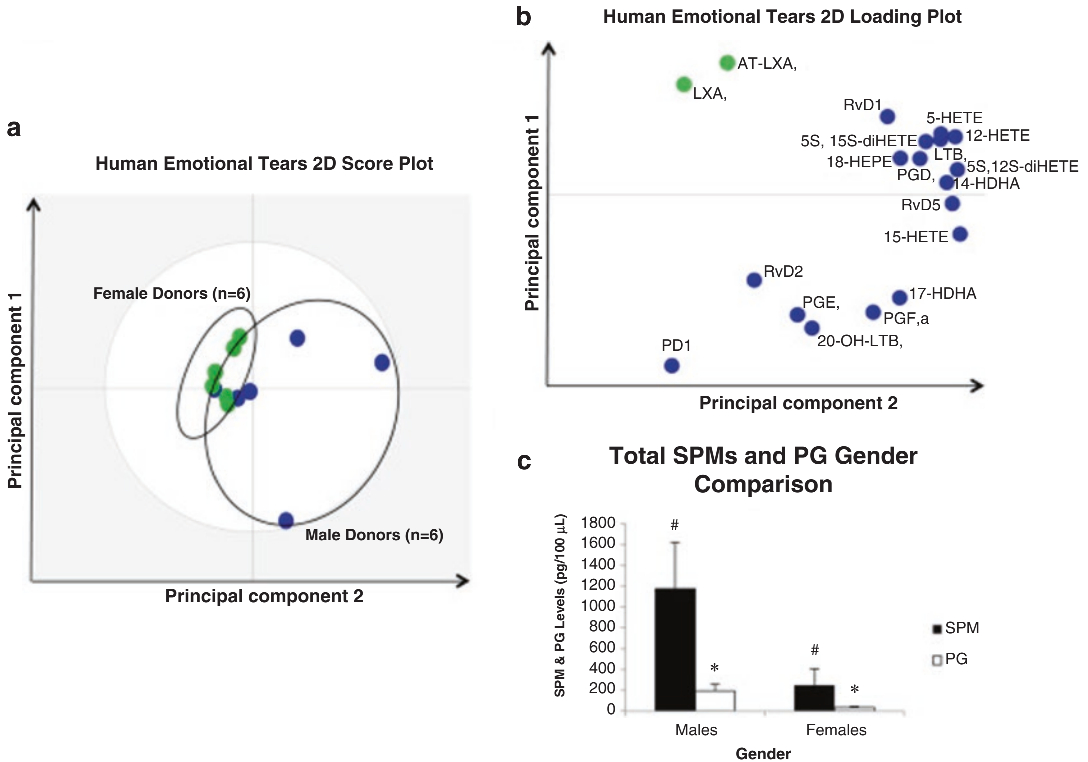

Fig. 3.5 PCA and quantitative ratio by gender for LM-SPMs identified in human emotional tears. (**a**) 2-dimensional score plot of human emotional tear donors; blue circles (n = 6) are representative of males, while green circles (n = 6) are representative of females. Gray ellipse denotes 95% confidence interval. (**b**) 2-dimensional loading plot of LM-SPMs identified in human emotional tears; blue circles are those mediators associated with male donors & green circles are associated with female donors. (**c**) Bar graph depicting the ratio of total SPMs including RvD1, RvD2, RvD5, PD1, LXA_4, AT-LXA_4, 17-HDHA, and 18-HEPE compared to PGE_2 and $PGF_{2\alpha}$ (pg/100 µl), in males compared females (n = 6 for each gender; $^{\#}P < 0.05$ for male donors vs. female donors; $*P < 0.05$, females vs. males). Reprinted from English et al. [41]

17-HDHA, and 18-HEPE versus the pro-inflammatory compounds PGE2 and PGF2a was compared between males and females, the ratio of SPMs to prostaglandins was much higher in males.

3.4 Exogenous RvD1 Stimulates Conjunctival Goblet Cell Mucin Secretion Using Multiple Intracellular Signaling Pathways

3.4.1 RvD1 Stimulates Goblet Cell Mucin Secretion

As the major function of goblet cells is to secrete mucins, the action of RvD1 on mucin secretion was our focus. RvD1 stimulated human and rat conjunctival goblet cell high molecular weight glycoconjugate secretion (that includes mucin) [45, 46]. Secretion peaked at 1 h and then declined over time for the next 3 h. RvD1 stimulated secretion by increasing the $[Ca^{2+}]_i$ and activating ERK1/2 [45], similarly to the effect of cholinergic agonists and VIP [21] (Fig. 3.6). RvD1 was effective in the range of 10^{-10}–10^{-8} M, a much lower concentration range than for carbachol (10^{-6}–10^{-4} M), but similar to VIP (10^{-11}–10^{-7} M) [21, 23]. The RvD1 stimulation of secretion was blocked by chelating the intracellular Ca^{2+} with BAPTA and by inhibiting ERK1/2 activation with U0126 [47] substantiating the role of these signaling molecules in RvD1 stimulation of goblet cell secretion.

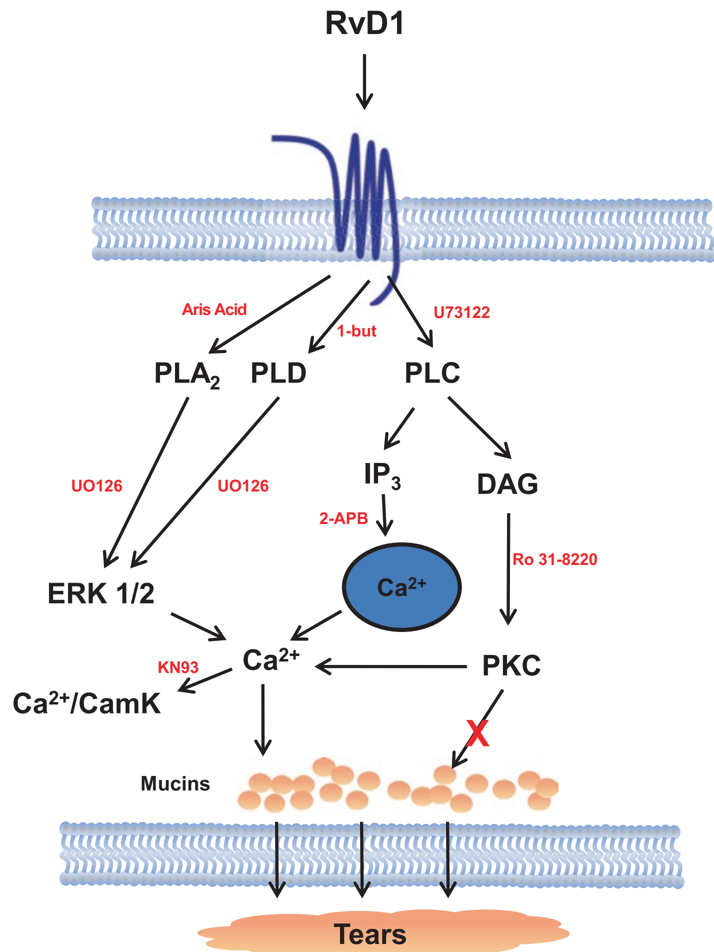

Fig. 3.6 Schematic diagram of signaling pathways of RvD1 in conjunctival goblet cells. RvD1 binds to the ALX/FPR2 receptor and activates the signaling pathways of PLA_2, PLC, and PLD. PLA_2 and PLD are believed to activate ERK 1/2 to increase $[Ca^{2+}]_i$ and Ca^{2+}/CamK. PLC increases IP_3 and DAG. IP_3 releases Ca^{2+} while DAG activates PKC. Activation of these pathways leads to mucin secretion. PLA_2-phospholipase A_2; PLD- phospholipase D; PLC- phospholipase C; ERK 1/2- extracellular regulated kinase 1/2; IP_3- inositol trisphosphate; DAG-diacylglycerol; Ca^{2+}/CamK- calcium/calmodulin dependent kinase; PKC- protein kinase C. Inhibitors to pathways are shown in red. Reprinted from Lippestad et al. [47]

To induce goblet cell function the appropriate receptors must be present on conjunctival goblet cells. RvD1 uses the ALX/FPR2 receptor in rat and the GPR32/DRV1 receptor in human goblet cells. PCR, western blot and immunofluorescence microscopy demonstrated that the ALX/FPR2 receptor was present on rat conjunctival goblet cells in vivo and in culture [48, 49]. There is as yet no published report of GPR32/DRV1 on human goblet cells. There is also functional evidence for the presence of ALX/FPR2 receptor in rat conjunctival goblet cells. To address whether RvD1 uses the ALX/FPR2 receptor in the rat, both the ALX/FPR2 inhibitor N-Boc-Phe-Leu-Phe-Leu-Phe (BOC-2) and siRNA for ALX/FPR2 blocked RvD1 stimulated increase in $[Ca^{2+}]_i$ [45, 47]. Functional evidence also demonstrates that RvD1 uses the GPR32/DRV1 receptor in human goblet cells. In human cells inhibition of ALX/FPR2 with its inhibitor BOC-2 does not block RvD1 stimulated increase in $[Ca^{2+}]_i$ [49]. Furthermore, in desensitization experiments in human cells in which LXA_4 and RvD1 were used sequentially, RvD1 does not desensitize LXA_4 response [49]. This suggests use of separate receptors in human goblet cells. Thus RvD1 activates ALX/FPR2 in rat goblet cells and GPR32/DRV1 in human goblet cells. Further experiments using GPR32 siRNA in human goblet cells is warranted.

3.4.2 Cellular Signaling Pathways Activated by RvD1

3.4.2.1 Phospholipase C Pathway

RvD1 activates a several specific intracellular signaling pathways to stimulate mucin secretion from rat goblet cells. One pathway is activation of phospholipase (PL) C that produces water soluble 1,4,5-inositol trisphosphate (IP3) and lipid soluble (membrane-bound) diacylglycerol (DAG) (Fig. 3.6). IP3 binds to its receptors (IP3R) on the endoplasmic reticulum that releases Ca^{2+} from this intracellular store to increase the cytosolic [Ca^{2+}] that stimulates exocytosis and mucin secretion. The DAG produced activates protein kinase (PK)C that phosphorylates as yet unidentified substrates to stimulate secretion. We used multiple techniques and inhibitors to determine if a PLC-dependent pathway plays a role in RvD1 stimulated goblet cell secretion [47]. First the active PLC inhibitor U73122 blocked RvD1 stimulated increase in $[Ca^{2+}]_i$ and secretion as well as the increase caused by the positive control the cholinergic agonist carbachol. As expected, the inactive inhibitor U73343 did not block either the RvD1 or cholinergic agonist stimulation of secretion nor the cholinergic agonist induced increase in $[Ca^{2+}]_i$. Unfortunately the inactive inhibitor blocked the RvD1 induced increase in $[Ca^{2+}]_i$ but was not as effective as the active analog. As the inactive inhibitor did not block three out of four responses, we concluded that RvD1 activates PLC in conjunctival goblet cells. To investigate IP3 interaction with its receptor to release Ca^{2+} from intracellular stores, an inhibitor of the IP3 receptor 2-aminoethyl diphenylborate (2-APB) was used. 2-APB blocked both the RvD1 stimulated increase in $[Ca^{2+}]_i$ and secretion. A second method of determining the role of intracellular Ca^{2+} stores is the use of thapsigargin that blocks the re-uptake of Ca^{2+} into the stores, thereby depleting them of Ca^{2+}. If RvD1 used intracellular Ca^{2+} stores, the addition of thapsigargin before RvD1 should prevent the increase in $[Ca^{2+}]_i$ by RvD1. In goblet cells this did not occur [45]. However, that depletion of extracellular Ca^{2+} blocked the RvD1 stimulated elevation in $[Ca^{2+}]_i$ supported the activation of Ca^{2+} influx by RvD1 [45].

The DAG arm of the PLC pathway was investigated by determining the role of PKC using the PKC inhibitor Ro 31-8220. The PKC inhibitor blocked the RvD1-stimulated increase in $[Ca^{2+}]_i$, but not in secretion. Activation of PKC may be important for the increase in $[Ca^{2+}]_i$, but not for secretion. There are multiple isoforms of PKC that are differentially activated by Ca^{2+} and diacylglycerol and can have opposing effects on a given process [50]. Thus investigation of the types of PKC isoforms present in goblet cells and inhibition of single isoforms would demonstrate more accurately whether PKC isoforms are involved in goblet cell secretion.

We concluded that RvD1 uses a PLC pathway to increase IP3 that releases Ca^{2+} from intracellular stores and causes an increase in the influx of extracellular Ca^{2+} (Fig. 3.6). The resultant increase in the cytosolic $[Ca^{2+}]_i$ stimulates secretion. The second arm of the PLC pathway production of DAG and activation of PKC is used to increase the $[Ca^{2+}]_{i,}$ but whether PKC plays a role in secretion awaits investigation of the different PKC isoforms.

PLCγ is activated by EGF as PLCγ is an adapter molecule attached to the EGFR. PLCγ can be phosphorylated and activated upon stimulation and dimerization of the EGFR. We recently found that RvD1 uses a matrix metalloproteinase ADAM17 to release EGF by ectodomain shedding and activate the EGFR to increase $[Ca^{2+}]_i$ and activate ERK1/2 to stimulate secretion [51]. Thus RvD1 could also activate PLCγ in addition to PLCβ to increase $[Ca^{2+}]_i$ and stimulate secretion. Thus two different types of PLC are used by RvD1 to stimulate goblet cell mucin secretion.

3.4.2.2 Phospholipase D and A2 Pathways

The next two pathways investigated were activation of PLD and PLA2 [47] (Fig. 3.6). These pathways were not investigated in as much detail as PLC. The role of PLD in RVD1 stimulated increase in $[Ca^{2+}]_i$ and secretion was investigated using 1-butanol the active inhibitor of PLD and tert-butanol, its inactive control. The RvD1-induced

increase in $[Ca^{2+}]_i$ and stimulation of secretion was almost completely blocked by 1-butanol. The inactive control only partially blocked the increase in $[Ca^{2+}]_i$ and did not block the stimulation of secretion. To study PLA2 aristolochic acid was used. Aristolochic acid blocked both the RvD1 caused increase in $[Ca^{2+}]_i$ and stimulation of secretion. These results are consistent with RvD1 using both PLD and PLA2 to stimulate goblet cell secretion. Additional experiments should identify the components of these pathways.

3.4.2.3 Extracellular Regulated Kinase 1/2 Pathway

RvD1 activates ERK1/2 to increase $[Ca^{2+}]_i$ and stimulate secretion as shown by inhibition of both functions by the MEK inhibitor U0126 [45] (Fig. 3.6). ERK1/2 could function as a component in several of the pathways studied. ERK1/2 could be activated by induction of the EGFR using the adapter proteins Ras, Raf, and MEK. ERK1/2 could also be downstream of PLD or PLA2.

3.4.3 Summary of Pathways Activated by RvD1

RvD1 stimulates mucin secretion from conjunctival goblet cells by a receptor specific mechanism using ALX/FPR2 in rats and GPR32 in humans. RvD1 uses multiple signaling pathways including PLC, PLD, and PLA2 that each use specific signaling components to increase $[Ca^{2+}]_i$ and could also activate ERK1/2 to stimulate secretion. In addition RvD1 transactivates the EGFR to increase $[Ca^{2+}]_i$, activate ERK1/2 and stimulate secretion. Endogenously produced RvD1 has multiple pathways available to stimulate conjunctival goblet cell mucin secretion to help maintain a normal mucous layer of tears.

3.5 Conclusion

The SPM RvD1, along neural regulation, is available to protect the ocular surface from dessicating stress, chemicals, temperature, allergens, particulate matter, and pathogens in the external environment. RvD1 is present in the tear film where it can access its receptors on the basolateral membranes of the goblet cells. RvD1 uses the ALX/FPR2 receptor in rat goblet cells and the GPR32 in human goblet cells. Activation of these receptors employs multiple intracellular pathways including PLC, PLD, PLA2 and the EGFR to increase $[Ca^{2+}]_i$ and activate ERK1/2 to stimulate secretion. The main secretory product of the goblet cells is the large, gel-forming mucin MU5AC, which is released into the innermost layer of the tear film where it is protective of the ocular surface. This mucin can trap bacteria and particulate matter and remove them from the ocular surface via the nasal lacrimal drainage. RvD1 thus preserves ocular surface homeostasis and maintains this surface in a non-inflamed, normal physiologic state of an optimum amount of mucin secretion. RvD1 forms a second important mechanism along with activation of a neural reflex pathway to regulate goblet cell mucin secretion and protect the ocular surface in health.

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References

1. DelMonte DW, Kim T (2011) Anatomy and physiology of the cornea. J Cataract Refract Surg 37(3):588–598
2. Ramphal R, McNiece MT, Polack FM (1981) Adherence of Pseudomonas aeruginosa to the injured cornea: a step in the pathogenesis of corneal infections. Ann Ophthalmol 13(4):421–425
3. Streilein JW (2003) Ocular immune privilege: therapeutic opportunities from an experiment of nature. Nat Rev Immunol 3(11):879–889
4. Dartt DA (2002) Regulation of mucin and fluid secretion by conjunctival epithelial cells. Prog Retin Eye Res 21(6):555–576
5. Gipson IK, Argueso P (2003) Role of mucins in the function of the corneal and conjunctival epithelia. Int Rev Cytol 231:1–49
6. Mantelli F, Argueso P (2008) Functions of ocular surface mucins in health and disease. Curr Opin Allergy Clin Immunol 8(5):477–483

7. Mauris J, Mantelli F, Woodward AM, Cao Z, Bertozzi CR, Panjwani N et al (2013) Modulation of ocular surface glycocalyx barrier function by a galectin-3 N-terminal deletion mutant and membrane-anchored synthetic glycopolymers. PLoS One 8(8):e72304
8. Hodges RR, Dartt DA (2013) Tear film mucins: front line defenders of the ocular surface; comparison with airway and gastrointestinal tract mucins. Exp Eye Res 117:62–78
9. Knop E, Knop N (2005) The role of eye-associated lymphoid tissue in corneal immune protection. J Anat 206(3):271–285
10. Kinoshita S, Ueta M (2010) Innate immunity of the ocular surface. Jpn J Ophthalmol 54(3):194–198
11. Li D, Hodges RR, Bispo P, Gilmore MS, Gregory-Ksander M, Dartt DA (2017) Neither non-toxigenic Staphylococcus aureus nor commensal S. epidermidi activates NLRP3 inflammasomes in human conjunctival goblet cells. BMJ Open Ophthalmol 2(1):e000101
12. McGilligan VE, Gregory-Ksander MS, Li D, Moore JE, Hodges RR, Gilmore MS et al (2013) Staphylococcus aureus activates the NLRP3 inflammasome in human and rat conjunctival goblet cells. PLoS One 8(9):e74010
13. Barbosa FL, Xiao Y, Bian F, Coursey TG, Ko BY, Clevers H et al (2017) Goblet cells contribute to ocular surface immune tolerance-implications for dry eye disease. Int J Mol Sci 18(5):978
14. Baudouin C, Rolando M, Benitez Del Castillo JM, Messmer EM, Figueiredo FC, Irkec M et al (2018) Reconsidering the central role of mucins in dry eye and ocular surface diseases. Prog Retin Eye Res. 2018, In Press.
15. Dartt DA, Masli S (2014) Conjunctival epithelial and goblet cell function in chronic inflammation and ocular allergic inflammation. Curr Opin Allergy Clin Immunol 14(5):464–470
16. Dartt DA, Kessler TL, Chung EH, Zieske JD (1996) Vasoactive intestinal peptide-stimulated glycoconjugate secretion from conjunctival goblet cells. Exp Eye Res 63(1):27–34
17. Dartt DA, McCarthy DM, Mercer HJ, Kessler TL, Chung EH, Zieske JD (1995) Localization of nerves adjacent to goblet cells in rat conjunctiva. Curr Eye Res 14(11):993–1000
18. Kessler TL, Mercer HJ, Zieske JD, McCarthy DM, Dartt DA (1995) Stimulation of goblet cell mucous secretion by activation of nerves in rat conjunctiva. Curr Eye Res 14(11):985–992
19. Candia OA, Alvarez LJ (2008) Fluid transport phenomena in ocular epithelia. Prog Retin Eye Res 27(2):197–212
20. Candia OA, Kong CW, Alvarez LJ (2008) IBMX-elicited inhibition of water permeability in the isolated rabbit conjunctival epithelium. Exp Eye Res 86(3):480–491
21. Rios JD, Zoukhri D, Rawe IM, Hodges RR, Zieske JD, Dartt DA (1999) Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion. Invest Ophthalmol Vis Sci 40(6):1102–1111
22. Kanno H, Horikawa Y, Hodges RR, Zoukhri D, Shatos MA, Rios JD et al (2003) Cholinergic agonists transactivate EGFR and stimulate MAPK to induce goblet cell secretion. Am J Physiol Cell Physiol 284(4):C988–C998
23. Li D, Jiao J, Shatos MA, Hodges RR, Dartt DA (2013) Effect of VIP on intracellular [Ca2+], extracellular regulated kinase 1/2, and secretion in cultured rat conjunctival goblet cells. Invest Ophthalmol Vis Sci 54(4):2872–2884
24. Dickson L, Finlayson K (2009) VPAC and PAC receptors: from ligands to function. Pharmacol Ther 121(3):294–316
25. Puro DG (2018) Role of ion channels in the functional response of conjunctival goblet cells to dry eye. Am J Physiol Cell Physiol 315(2):C236–CC46
26. Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510(7503):92–101
27. Williams RN, Bhattacherjee P, Eakins KE (1983) Biosynthesis of lipoxygenase products by ocular tissues. Exp Eye Res 36(3):397–402
28. Kulkarni PS, Srinivasan BD (1989) Cyclooxygenase and lipoxygenase pathways in anterior uvea and conjunctiva. Prog Clin Biol Res 312:39–52
29. Kulkarni PS, Kaufman PL, Srinivasan BD (1987) Eicosapentaenoic acid metabolism in cynomolgus and rhesus conjunctiva and eyelid. J Ocul Pharmacol 3(4):349–356
30. Gao Y, Su J, Zhang Y, Chan A, Sin JH, Wu D et al (2018) Dietary DHA amplifies LXA4 circuits in tissues and lymph node PMN and is protective in immune-driven dry eye disease. Mucosal Immunol 11(6):1674–1683
31. Gao Y, Min K, Zhang Y, Su J, Greenwood M, Gronert K (2015) Female-specific downregulation of tissue polymorphonuclear neutrophils drives impaired regulatory T cell and amplified effector T cell responses in autoimmune dry eye disease. J Immunol 195(7):3086–3099
32. Wei J, Gronert K (2017) The role of pro-resolving lipid mediators in ocular diseases. Mol Asp Med 58:37–43
33. Serhan CN, Hamberg M, Samuelsson B (1984) Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc Natl Acad Sci U S A 81(17):5335–5339
34. Barden AE, Mas E, Mori TA (2016) n-3 fatty acid supplementation and proresolving mediators of inflammation. Curr Opin Lipidol 27(1):26–32
35. Weiss GA, Troxler H, Klinke G, Rogler D, Braegger C, Hersberger M (2013) High levels of anti-inflammatory and pro-resolving lipid mediators lipoxins and resolvins and declining docosahexaenoic acid levels in human milk during the first month of lactation. Lipids Health Dis 12:89
36. Arnardottir H, Orr SK, Dalli J, Serhan CN (2016) Human milk proresolving mediators stimulate resolution of acute inflammation. Mucosal Immunol 9(3):757–766

37. Mas E, Croft KD, Zahra P, Barden A, Mori TA (2012) Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation. Clin Chem 58(10):1476–1484
38. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN (2014) Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol 307(1):C39–C54
39. Zhu M, Wang X, Hjorth E, Colas RA, Schroeder L, Granholm AC et al (2016) Pro-resolving lipid mediators improve neuronal survival and increase Abeta42 phagocytosis. Mol Neurobiol 53(4):2733–2749
40. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG (2004) Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci U S A 101(22):8491–8496
41. English JT, Norris PC, Hodges RR, Dartt DA, Serhan CN (2017) Identification and profiling of specialized pro-resolving mediators in human tears by lipid mediator metabolomics. Prostaglandins Leukot Essent Fat Acids 117:17–27
42. Lima-Garcia JF, Dutra RC, da Silva K, Motta EM, Campos MM, Calixto JB (2011) The precursor of resolvin D series and aspirin-triggered resolvin D1 display anti-hyperalgesic properties in adjuvant-induced arthritis in rats. Br J Pharmacol 164(2):278–293
43. Walter SD, Gronert K, McClellan AL, Levitt RC, Sarantopoulos KD, Galor A (2016) omega-3 tear film lipids correlate with clinical measures of dry eye. Invest Ophthalmol Vis Sci 57(6):2472–2478
44. Masoudi S, Zhao Z, Willcox M (2017) Relation between ocular comfort, arachidonic acid mediators, and histamine. Curr Eye Res 42(6):822–826
45. Li D, Hodges RR, Jiao J, Carozza RB, Shatos MA, Chiang N et al (2013) Resolvin D1 and aspirin-triggered resolvin D1 regulate histamine-stimulated conjunctival goblet cell secretion. Mucosal Immunol 6(6):1119–1130
46. Dartt DA, Hodges RR, Li D, Shatos MA, Lashkari K, Serhan CN (2011) Conjunctival goblet cell secretion stimulated by leukotrienes is reduced by resolvins D1 and E1 to promote resolution of inflammation. J Immunol 186(7):4455–4466
47. Lippestad M, Hodges RR, Utheim TP, Serhan CN, Dartt DA (2017) Resolvin D1 increases mucin secretion in cultured rat conjunctival goblet cells via multiple signaling pathways. Invest Ophthalmol Vis Sci 58(11):4530–4544
48. Hodges RR, Li D, Shatos MA, Bair JA, Lippestad M, Serhan CN et al (2017) Lipoxin A4 activates ALX/FPR2 receptor to regulate conjunctival goblet cell secretion. Mucosal Immunol 10(1):46–57
49. Hodges RR, Li D, Shatos MA, Serhan CN, Dartt DA (2016) Lipoxin A4 counter-regulates histamine-stimulated glycoconjugate secretion in conjunctival goblet cells. Sci Rep 6:36124
50. Zoukhri D, Hodges RR, Sergheraert C, Dartt DA (1998) Protein kinase C isoforms differentially control lacrimal gland functions. Adv Exp Med Biol 438:181–186
51. Kaye R, Botten N, Lippestad M, Li D, Hodges RR, Utheim TP et al (2018) Resolvin D1, but not resolvin E1, transactivates the epidermal growth factor receptor to increase intracellular calcium and glycoconjugate secretion in rat and human conjunctival goblet cells. Exp Eye Res 180:53–62

4 The Evolving Role of Specialized Pro-resolving Mediators in Modulating Neuroinflammation in Perioperative Neurocognitive Disorders

Ting Yang and Niccolò Terrando

Abstract

Surgery can be a life-saving procedure; however, significant complications may occur after routine procedures especially in older and more frail patients. Perioperative neurocognitive disorders (PNDs), including delirium and postoperative cognitive dysfunction, are the most common complications in older adults following common procedures such as orthopedic or cardiac surgery. The consequences of PNDs can be devastating, with longer in-hospital stay, poorer prognosis, and higher mortality rates. Inflammation is gaining considerable interest as a critical driver of cognitive deficits. In this regard, resolution of inflammation, once thought to be a passive process, may provide novel approaches to treat neuroinflammation and PNDs. Herein we review the role for impaired resolution after surgery and the growing role of specialized pro-resolving mediators (SPMs) in regulating postoperative neuroinflammation and neurological complications after surgery.

Keywords

Astrocytes · Blood-brain barrier · Bone · Brain · Cytokines · Delirium · Healing · Macrophages · Maresins · Memory · Microglia · Neuroinflammation · Resolvins · Surgery

Perioperative neurocognitive disorders (PNDs), previously referred to as postoperative cognitive dysfunction (POCD), include acute changes in cognitive function (i.e. delirium) and longer-lasting memory impairments [1]. PNDs have become the most common complications in older adults after common surgical procedures such as cardiac and orthopedic surgery, and negatively affect post-operative outcomes and recovery in at-risk subjects [2]. Indeed, patients who suffer from PNDs require intensive nursing care, report higher mortality rates, and become at greater risk for further complications, including permanent dementia [3, 4]. Although advanced age has been recognized as a prominent risk factor for PNDs, the pathophysiology of these complications is still poorly understood. Recent studies showed pro-inflammatory signaling molecules can be identified in the central nervous system (CNS) of

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both PND patients and animal models, implicating surgery-induced neuroinflammation as a key contributor in the pathophysiology of PNDs. The aim of this review is to discuss the role of dysregulated immunity after surgery and the therapeutic potential for specialized pro-resolving lipid mediators (SPMs) in the perioperative neurocognitive arena.

We and others have been interested in clarifying the pathogenesis of PNDs and developed preclinical translational models to study the impact of anesthesia and surgery on the CNS. During surgery, aside from direct effects of anesthetic agents on the CNS (reviewed in [5]), acute inflammation triggered by trauma and sterile injury can negatively affect brain function, contributing to sickness behavior and PND-like pathology [6]. If poorly controlled, dysregulated inflammation leads to more extensive organ dysfunction and tissue injury. Release of pro-inflammatory cytokines, alarmins, and leukotrienes appear to associate with distant long-term effects on the brain, contributing to neuroinflammation, neurotoxicity, and subsequent memory impairments. The mechanisms whereby systemic inflammation affects CNS function remain partly defined and are object of active investigations. Systemic cytokines have been shown to enter the brain through different immune-to-brain signaling pathways, which include humoral, neuronal, and cellular routes [7]. Using a well-established PND model of orthopedic surgery, which frequently leads to cognitive dysfunctions in humans, we first reported a key role for systemic pro-inflammatory cytokines, including interleukin (IL)-1β and tumor necrosis factor alpha (TNFα), in mediating surgery-induced neuroinflammation and cognitive decline following surgery [6, 8]. These initial results as well as work from other models of surgery-induced cognitive dysfunction, prompt for a prominent role of the systemic and humoral response in PND pathogenesis. However, peripheral cytokines in response to non-CNS surgery were also shown to disrupt the blood-brain barrier (BBB), thus facilitating the migration of peripheral cells, including macrophages, into the brain parenchyma through activation of TNFα/nuclear factor (NF)-κB signaling pathway [9] [10]. BBB homeostasis plays fundamental role in the communication between peripheral inflammation and neuroinflammation [11]. Using fibrinogen as a classical marker of BBB disruption, we reported a significant deposition of this blood-derived molecule in the hippocampal parenchyma [12]. Similar results were reported in other surgical models, with evident BBB opening and disruption of tight junctions after anesthesia and surgery [13]. More recently we described a role for neuronal processing, including pain signaling at the level of the spinal cord, in disrupting acute neurogenesis after orthopedic surgery [14]. These factors, together with the overall activation of innate immune pathways, have been highlighted as pathogenic mechanisms underlying cognitive decline in preclinical models and in early clinical studies. Preclinical models have evaluated multiple therapeutic strategies to modulate neuroinflammation and PND-like behavior. Selective targeting of key pro-inflammatory cytokines (such as anti-TNFα and IL-1 receptor agonist) prevent neuroinflammation and improve cognitive decline in animal models [6, 15]. Macrophage-specific deletion of IKKβ, a central coordinator of TNFα activation of NF-κB, prevents BBB disruption and macrophage infiltration in the hippocampus following surgery. Moreover, harnessing the cholinergic reflex by stimulating the α7 subtype of nicotinic acetylcholine receptors (α7 nAChR) in macrophages inhibited NF-κB activity and BBB disruption, thus preventing neuroinflammation and cognitive decline following surgery [12]. Although these and several other therapies may successfully reduce postoperative neuroinflammation and limit PNDs, they often contribute to side effects by over suppressing the immune system, increasing the risk of infection and delaying wound healing, which are all crucial in the context of postoperative recovery. Thus, it is pivotal to identify safer therapeutic strategies to prevent neuroinflammation without suppressing systemic immune functions.

Is now well appreciated that resolution of inflammation is an active process [16–18]. Synthesis and release of anti-inflammatory

molecules is required to balance and orchestrate the overall immune response [19]. The acute inflammatory response can be divided into two stages: initiation and resolution. Resolution launches shortly after the initiation of inflammatory response [20]. Seminal work pioneered by Dr. Serhan and colleagues has demonstrated how different mediators actively participate in resolving acute inflammation in a highly regulated manner (recently reviewed in [21]). Fundamental to the resolution process are SPMs, endogenous mediators generated by polyunsaturated fatty acids (PUFA) with signaling abilities to limit inflammation [22]. Omega-3 PUFAs are important catalysts for the synthesis of potent lipid mediators that can exert pro-resolving and anti-inflammatory actions. Oxygenated metabolites derived from eicosapentaenoic acid (20:5(n-3) or EPA) and docosahexaenoic acid (22:6(n-3) or DHA), enriched in fish oils, lead to structurally distinct families of "*resolution phase interaction products*" [23], which include resolvins, maresins, protectins (as well as sulfide-conjugates in tissue regeneration), and lipoxins (from arachidonic acid). All SPMs display potent anti-inflammatory actions and immunoregulatory properties now tested in several pre-clinical models of acute and chronic inflammation. Indeed, SPMs inhibit the excessive swarming of neutrophils infiltration and enhance the microbial clearance function of innate immune cells [24]. SPMs also operate as "resolution agonists" to regulate the acute inflammation and limit the development of chronic inflammation; importantly they do not show immunosuppressive effects [23, 25]. The exaggerated inflammation and non-resolution of pro-inflammatory processes are characteristic of several neurological conditions and disease states like stroke, neurodegeneration, and chronic pain. Evidence from clinical studies using dietary fish oil supplementation suggest promising effects of omega 3 fatty acid in modulating inflammation both in acute and chronic conditions [26, 27]. Yet the role of SPMs in resolution of neuroinflammation and neuroprotection is just emerging. In Alzheimer's disease (AD), circulating SPMs and receptor expression are diminished in the brain [28]. A comprehensive review on the role of SPMs in AD is provided in [29]. Here we will review the growing role of SPMs in the perioperative space, focusing on neuroinflammation in PNDs.

Microglia are the primary active immune defense in the CNS responsible to maintain brain homeostasis. However, activated microglia can cause overproduction of pro-inflammatory cytokines and reactive oxidative species that can lead to persistent inflammation and exacerbate pathological changes in CNS [30, 31]. In PND microglia respond to surgical trauma following BBB opening and monocytes infiltration. Modulation of microglial activity is an attractive therapeutic targeted for multiple conditions ranging from neurodegeneration to chronic pain [32]. *In vitro* studies using immortalised murine microglial cell line BV-2 showed pre-incubation with resolvin D1 (RvD1) and E1 (RvE1) inhibit lipopolysaccharide (LPS)-induced TNF-α, IL-6 and IL-1β gene expression via regulating of miRNAs expression and NF-κB signaling pathway [33]. Similar effects of RvD1 were demonstrated in separate studies using human glioma cells or isolated primary rat microglia [34, 35]. RvD1 and maresin-1 (MaR1) also down-regulate β-amyloid $(A\beta)_{42}$-induced CD11b and CD40 expression in human microglial cells. Moreover, MaR1 and aspirin triggered lipoxin A_4 (ATL) both exert stimulatory effect on microglial cells to uptake $A\beta_{42}$ [36, 37]. In addition to reducing the expression of M1-like markers activation of microglia, RvD1 also promotes the expression of Arg1 and Ym1 in IL-4 activated BV2 cells [38]. These *in vitro* findings demonstrate that SPMs are directly involved in regulating both classical and alternative microglia activation, thus may display potent therapeutic effects in inflammatory diseases of the CNS. The regulatory effects of SPMs on microglial cells have also been also verified *in vivo*. A study using Tg2576 mice, which overexpresses a mutant form of amyloid precursor protein (APP) and develops early AD onset, showed neuroprotective effects of ATL by shifting microglial phenotype to a more anti-inflammatory state [37]. In models of surgery-induced microglial activation, both lipoxins and resolvins have been shown to improve neuroinflammation by

attenuating release of pro-inflammatory cytokines [39–41]. Importantly, other classes of inflammatory-stop signals can modulate neuroinflammation in PND-models. Using a rat model of cardiopulmonary bypass with deep hypothermic circulatory arrest treatment with annexin A1 (ANXA1) was able to improve cognitive outcomes and modulate microglial activation by inhibiting NF-κB p65 transcriptional activity and subsequent cytokine production [42]. Recently, we demonstrated that prophylaxis with MaR1 can rescue microglial activation after orthopedic surgery, as detected by morphological changes-such as shifting to a more ramified homeostatic morphology [43]. Thus, resolution agonists including SPMs may diminish neuroinflammation in part by regulating microglial phenotype, leading to improved cognitive outcomes. To date many of the SPMs have not been evaluated in models of PND and surgical recovery, which warrants future investigations.

Aside from microglia, astrocytes also contribute a key role in maintaining CNS homeostasis, including regulating brain blood flow [44, 45], synapse function [46], extracellular ion concentration [47], and interacting with endothelial cells to support the BBB [48]. Notably, a role for immune-regulation has emerged from these cells [49]. As an active immune regulator in CNS, astrocytes also sense stimulation and danger signals, responding by releasing glia-transmitters and communicating to neurons [50]. Together with microglia they also contribute to further activating adaptive immune defense [51] [49]. Following brain injury or in neurodegenerative diseases, astrocytes undergo pronounced transformation called “astrogliosis”. Astrogliosis changes the cellular expression and morphology of astrocytes and contribute to the repair and scarring process in CNS [52]. However, this reactive phenotype has also been suggested to become detrimental by upregulating the expression of IL-17 receptor and sphingosine 1-phosphate (S1P) [53, 54]. This further triggers the production of pro-inflammatory cytokines and chemokines, which can lead to exacerbated neuroinflammation and neurodegeneration [55, 56]. During neuroinflammation, the status of astrocytes actions may be determined by the danger signals in the local environment and regulated in time-specific manner [56]. The controversial roles of astrocytes indicate that these cells may play key roles in cognitive function, both in health and diseases. The specific SPMs actions on astrocytes have not been thoroughly investigated. In pain models, Lipoxin A1 exerts anti-nociceptive effect by modulation of astrocytic activation [57]. Similarly, AT-RvD1 was shown to attenuate TNF-α release from spinal astrocytes and improve mechanical hypersensitivity in a rat model of carrageenan-induced peripheral inflammation [58]. In PND models we described changes in astrocytes morphology at 24 h after orthopedic surgery [41, 43]. The astrogliosis is associated with increased basal glutamatergic synaptic transmission, reduced short term plasticity and long-term potentiation in hippocampus [41]. These pathological changes in astrocytes can be eliminated by prophylaxis with aspirin triggered resolvin-D1 (AT-RvD1) as low as ~0.1 μg/kg [41] or MaR1 at ~4 μg/kg [43]. The precise mechanisms underlying the effects of AT-RvD1 and MaR1 on postoperative astrogliosis still require further exploration. Accumulated evidences suggest the pro-inflammatory activity of astrocytes may be regulated by microglia [59] via cytokines, chemokine, complement activation [60], growth factors, and other signaling molecules [61]. After surgery the CCL2/CCR2 axis has been implicated in the neuroinflammatory response; Xu et al. showed that increased CCL2 expression in astrocytes is sufficient to activate microglia and cause learning impairments [62]. Therefore, regulation of astrogliosis may indirectly regulate microglia activity and targeting cell-specific interactions may result into effective therapies for different neurological conditions, including PNDs (Figs. 4.1 and 4.2).

Intrinsic to the postoperative neuroinflammation is the activation of the peripheral innate immune system. Sterile injury, as during surgery, rapidly triggers the release of TNFα and alarmins into the circulation. TNFα can initiate a pro-inflammatory cytokine cascade that eventually impairs the BBB homeostasis. The BBB dys-

function in turn facilitates the migration of macrophages into the hippocampus [6, 12, 41, 43]. Inhibition of the TNFα signaling by anti-TNFα antibody or genetic abrogation of macrophage-specific IKKβ prevent postoperative BBB disruption and macrophage infiltration in the hippocampus [6, 12]. This work demonstrated the primary impact of innate immune system and systemic inflammation on the development of neuroinflammation and cognitive decline. During resolution of inflammation, SPMs stimulate the cessation of PMN influx [22, 34] and macrophage clearance of cellular and toxic debris [16, 20, 63]. The regulation of macrophage phagocytosis function by SPMs may be mediated by the switch of macrophage phenotype [16]. For example, Dalli et al. demonstrated that 10 nM of MaR1 or RvD1 lead to significant reductions in CD54 and CD80 expression and a concomitant up-regulation of CD163 and CD206 in human macrophage [64]. Moreover, M2-like macrophages are more efficient in converting DHA into MaR1 [64]. Similarly, our *in vitro* work indicated 10 nM MaR1 can inhibit LPS-induced TNFα release, NF-κB nuclear translocation, superoxide generation and M1-like phenotype surface markers expression in primary bone marrow derived macrophages [43]. These modulatory effects on macrophage function/phenotype may be a key mechanism for SPMs to prevent surgery induced BBB disruption, ensuing neuroinflammation, and cognitive decline [43]. Importantly, because SPMs exert both anti-inflammatory and pro-resolving effects, they are crucial to terminate inflammation but also stimulate tissue repair [65], which is fundamental in the context of perioperative recovery. In fact, RvD1 and maresins have shown to accelerate wound healing in diabetic patients [66, 67]. AT-RvD1 delivered through nanoparticles also enhance wound healing in a mice model of peritonitis [68]. We recently demonstrated that MaR1 pretreatment boosts systemic levels of IL-10 with a long-lasting trend up to 14 days after surgery [43]. Further, we found no difference in callus formation in mice treated with MaR1 compared to vehicle, suggesting MaR1 may be a safe option to be tested in future clinical trials.

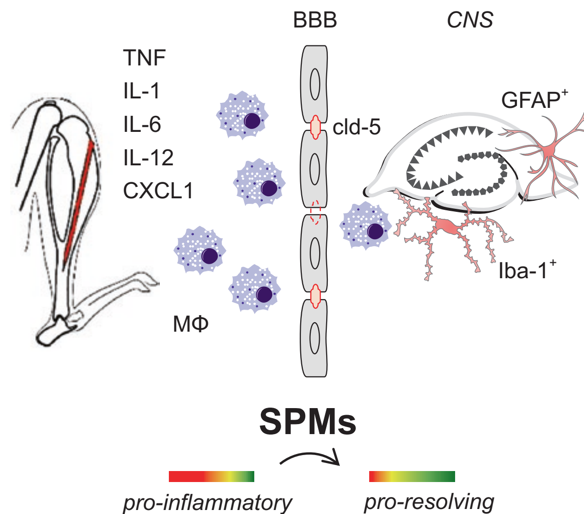

Fig. 4.1 Putative mechanisms of surgery-induced neuroinflammation and cognitive dysfunction. Surgery triggers systemic pro-inflammatory cytokines that can impair the blood-brain barrier, thus allowing peripheral cells into the brain parenchyma. Macrophage infiltration activates glia, including resident microglia and astrocytes, that acutely affect processes of synaptic plasticity and hippocampal-dependent memory function. Treatment with SPMs, such as resolvins and maresins, actively promote resolution of inflammation after surgical trauma and prevent central nervous system dysfunction through the modulation of systemic and central cell types, such as monocytes, microglia, and neurons

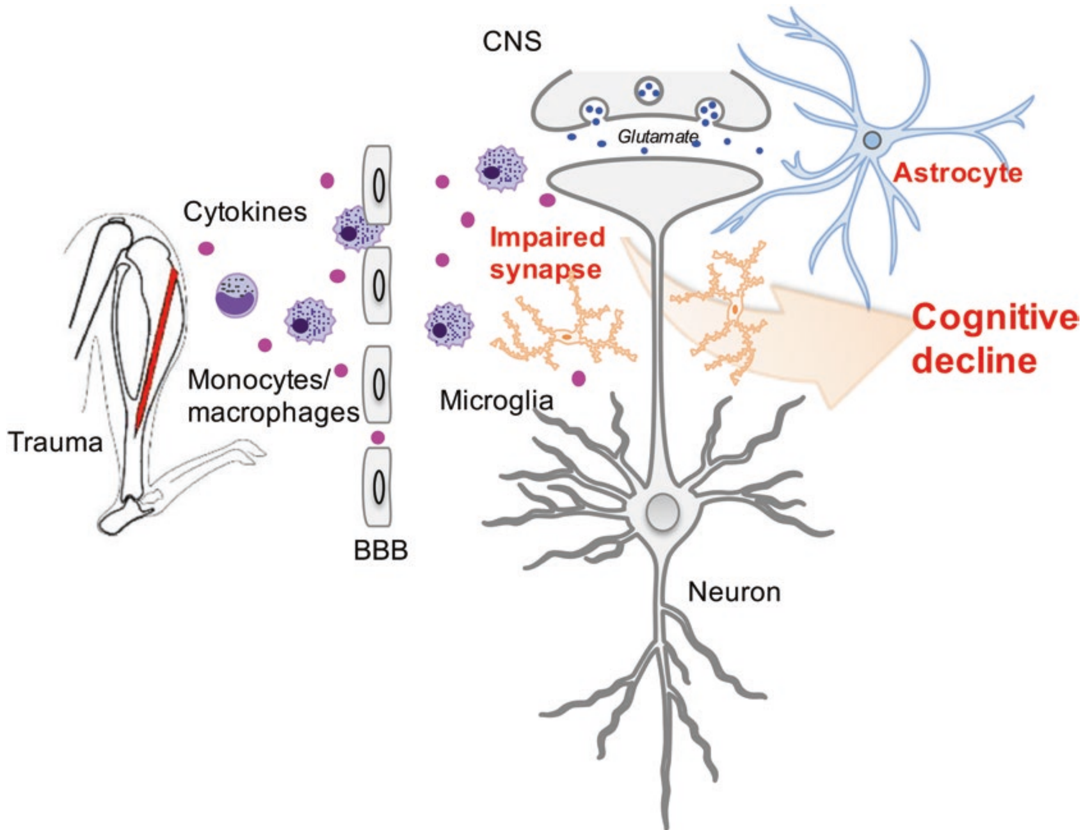

Fig. 4.2 Proposed mechanisms for neuroinflammation and surgery-induced cognitive dysfunction. Surgery has been shown to engage the innate immune system and activate a cascade of pro-inflammatory mediators, including alarmins, cytokines and eicosanoids. These molecules exert effects on the humoral and neuronal signaling overall contributing to the neuroinflammatory response. These processes are mediated not only by activation of resident microglia but also by infiltration of peripheral cells into the brain parenchyma via a disrupted blood-brain barrier. This pro-inflammatory milieu and glia dysfunction impair neuronal activity and synaptic plasticity, impinging on processes of long-term potentiation, neurotransmission, and receptor function at the synapse. In combination, these pathological hallmarks contribute to learning and memory impairments following surgical trauma

Overall, we are starting to uncover the potential for resolution agonists in the context of perioperative disorders and surgical recovery. Further research is needed to evaluate the exact mechanisms of action of different SPMs on different CNS cell types, and whether specific mediators may better target the immune response triggered by surgical trauma. SPMs have been detected in human cerebrospinal fluid, establishing proof-of-principle that may serve as biomarkers for disease progression in neurological conditions like multiple sclerosis [69] and clinical PND [43]. These mediators can also be modified by dietary interventions, for example omega-3 PUFA [70], thus providing attractive strategies to intervene before an elective surgical procedure. SPMs biomarkers may take us a step closer to personalized approaches and targeted interventions to treat common complications, like postoperative pain and PNDs. As coined by Serhan and Savill *'alpha' programs 'omega'* (i.e. the beginning programs the end) [20] could not better define the importance of the temporal events that orchestrate the acute inflammatory response in the perioperative space. Further characterizing these complex molecular events may provide novel and urgently needed approaches to safely treat PND and overall improve brain health for the millions of patients that undergo surgery every year.

References

1. Evered L et al (2018) Recommendations for the nomenclature of cognitive change associated with anaesthesia and surgery-2018. Br J Anaesth 121:1005–1012. https://doi.org/10.1016/j.bja.2017.11.087
2. American geriatrics society expert panel on postoperative delirium in older, A (2015) Postoperative delirium in older adults: best practice statement from the American Geriatrics Society. J Am Coll Surg 220(136):148–e131. https://doi.org/10.1016/j.jamcollsurg.2014.10.019
3. Chen CW et al (2014) Increased risk of dementia in people with previous exposure to general anesthesia: a nationwide population-based case-control study. Alzheimers Dement 10:196–204. https://doi.org/10.1016/j.jalz.2013.05.1766
4. Steinmetz J et al (2009) Long-term consequences of postoperative cognitive dysfunction. Anesthesiology 110:548–555. https://doi.org/10.1097/ALN.0b013e318195b569
5. Vutskits L, Xie Z (2016) Lasting impact of general anaesthesia on the brain: mechanisms and relevance. Nat Rev Neurosci 17:705–717. https://doi.org/10.1038/nrn.2016.128
6. Terrando N et al (2010) Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc Natl Acad Sci U S A 107:20518–20522. https://doi.org/10.1073/pnas.1014557107
7. Capuron L, Miller AH (2011) Immune system to brain signaling: neuropsychopharmacological implications. Pharmacol Ther 130:226–238. https://doi.org/10.1016/j.pharmthera.2011.01.014
8. Cibelli M et al (2010) Role of interleukin-1beta in postoperative cognitive dysfunction. Ann Neurol 68:360–368. https://doi.org/10.1002/ana.22082
9. Terrando N et al (2011) Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 70:986–995. https://doi.org/10.1002/ana.22664
10. Degos V et al (2013) Depletion of bone marrow-derived macrophages perturbs the innate immune response to surgery and reduces postoperative memory dysfunction. Anesthesiology 118:527–536. https://doi.org/10.1097/ALN.0b013e3182834d94
11. Sweeney MD, Sagare AP, Zlokovic BV (2018) Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol 14:133–150. https://doi.org/10.1038/nrneurol.2017.188
12. Terrando N et al (2011) Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 70:986–995. https://doi.org/10.1002/ana.22664
13. Yang S et al (2017) Anesthesia and surgery impair blood-brain barrier and cognitive function in mice. Front Immunol 8:902. https://doi.org/10.3389/fimmu.2017.00902
14. Zhang MD et al (2016) Orthopedic surgery modulates neuropeptides and BDNF expression at the spinal and hippocampal levels. Proc Natl Acad Sci USA 113:E6686–E6695. https://doi.org/10.1073/pnas.1614017113
15. Cibelli M et al (2010) Role of interleukin-1beta in postoperative cognitive dysfunction. Ann Neurol 68:360–368. https://doi.org/10.1002/ana.22082
16. Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101. https://doi.org/10.1038/nature13479
17. Serhan CN, Chiang N (2013) Resolution phase lipid mediators of inflammation: agonists of resolution. Curr Opin Pharmacol 13:632–640. https://doi.org/10.1016/j.coph.2013.05.012
18. Serhan CN, Chiang N, Dalli J (2017) New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. Mol Asp Med 64:1–17. https://doi.org/10.1016/j.mam.2017.08.002
19. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6:1191–1197. https://doi.org/10.1038/ni1276
20. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6:1191–1197. https://doi.org/10.1038/ni1276
21. Serhan CN, Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 128:2657–2669. https://doi.org/10.1172/JCI97943
22. Serhan CN et al (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192:1197–1204
23. Serhan CN et al (2007) Resolution of inflammation: state of the art, definitions and terms. FASEB J 21:325–332. https://doi.org/10.1096/fj.06-7227rev
24. Malawista SE, de Boisfleury Chevance A, van Damme J, Serhan CN (2008) Tonic inhibition of chemotaxis in human plasma. Proc Natl Acad Sci USA 105:17949–17954. https://doi.org/10.1073/pnas.0802572105
25. Recchiuti A, Serhan CN (2012) Pro-resolving lipid mediators (SPMs) and their actions in regulating miRNA in novel resolution circuits in inflammation. Front Immunol 3:298. https://doi.org/10.3389/fimmu.2012.00298
26. Langerhuus SN et al (2012) The effect of dietary fatty acids on post-operative inflammatory response in a porcine model. APMIS 120:236–248. https://doi.org/10.1111/j.1600-0463.2011.02834.x
27. Rosell M, Wesley AM, Rydin K, Klareskog L, Alfredsson L (2009) Dietary fish and fish oil and the risk of rheumatoid arthritis. Epidemiology 20:896–901. https://doi.org/10.1097/EDE.0b013e3181b5f0ce
28. Wang X et al (2015) Resolution of inflammation is altered in Alzheimer's disease. Alzheimers Dement 11(40):50 e41–50 e42. https://doi.org/10.1016/j.jalz.2013.12.024
29. Whittington RA, Planel E, Terrando N (2017) Impaired resolution of inflammation in Alzheimer's disease: a review. Front Immunol 8:1464. https://doi.org/10.3389/fimmu.2017.01464

30. Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8:57–69. https://doi.org/10.1038/nrn2038
31. Polazzi E, Contestabile A (2002) Reciprocal interactions between microglia and neurons: from survival to neuropathology. Rev Neurosci 13:221–242
32. Chen G, Zhang YQ, Qadri YJ, Serhan CN, Ji RR (2018) Microglia in pain: detrimental and protective roles in pathogenesis and resolution of pain. Neuron 100:1292–1311. https://doi.org/10.1016/j.neuron.2018.11.009
33. Rey C et al (2016) Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro. Brain Behav Immun 55:249–259. https://doi.org/10.1016/j.bbi.2015.12.013
34. Serhan CN et al (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196:1025–1037
35. Xu MX et al (2013) Resolvin D1, an endogenous lipid mediator for inactivation of inflammation-related signaling pathways in microglial cells, prevents lipopolysaccharide-induced inflammatory responses. CNS Neurosci Ther 19:235–243. https://doi.org/10.1111/cns.12069
36. Zhu M et al (2016) Pro-resolving lipid mediators improve neuronal survival and increase Abeta 42 phagocytosis. Mol Neurobiol 53:2733–2749. https://doi.org/10.1007/s12035-015-9544-0
37. Medeiros R et al (2013) Aspirin-triggered lipoxin A4 stimulates alternative activation of microglia and reduces Alzheimer disease-like pathology in mice. Am J Pathol 182:1780–1789. https://doi.org/10.1016/j.ajpath.2013.01.051
38. Li L et al (2014) Resolvin D1 promotes the interleukin-4-induced alternative activation in BV-2 microglial cells. J Neuroinflammation 11:72. https://doi.org/10.1186/1742-2094-11-72
39. Feng X et al (2017) Microglia mediate postoperative hippocampal inflammation and cognitive decline in mice. JCI Insight 2:e91229. https://doi.org/10.1172/jci.insight.91229
40. Su X et al (2012) Dysfunction of inflammation-resolving pathways is associated with exaggerated postoperative cognitive decline in a rat model of the metabolic syndrome. Mol Med 18:1481–1490. https://doi.org/10.2119/molmed.2012.00351
41. Terrando N et al (2013) Aspirin-triggered resolvin D1 prevents surgery-induced cognitive decline. FASEB J 27:3564–3571. https://doi.org/10.1096/fj.13-230276
42. Zhang Z et al (2017) Neuroprotective effects of annexin A1 tripeptide after deep hypothermic circulatory arrest in rats. Front Immunol 8:1050. https://doi.org/10.3389/fimmu.2017.01050
43. Yang T et al (2019) Maresin 1 attenuates neuroinflammation in a mouse model of perioperative neurocognitive disorders. Br J Anaesth 122:350–360. https://doi.org/10.1016/j.bja.2018.10.062
44. Parri R, Crunelli V (2003) An astrocyte bridge from synapse to blood flow. Nat Neurosci 6:5–6. https://doi.org/10.1038/nn0103-5
45. Gordon GR, Mulligan SJ, Mac Vicar BA (2007) Astro control cerebrovasculature *Glia*. Astrocyte Control Cerebrovascu 55:1214–1221. https://doi.org/10.1002/glia.20543
46. Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32:421–431. https://doi.org/10.1016/j.tins.2009.05.001
47. Walz W (2000) Role of astrocytes in the clearance of excess extracellular potassium. Neurochem Int 36:291–300
48. Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7:41–53. https://doi.org/10.1038/nrn1824
49. Farina C, Aloisi F, Meinl E (2007) Astrocytes are active players in cerebral innate immunity. Trends Immunol 28:138–145. https://doi.org/10.1016/j.it.2007.01.005
50. Fiacco TA, Agulhon C, McCarthy KD (2009) Sorting out astrocyte physiology from pharmacology. Annu Rev Pharmacol Toxicol 49:151–174. https://doi.org/10.1146/annurev.pharmtox.011008.145602
51. Sorg O, Magistretti PJ (1992) Vasoactive intestinal peptide and noradrenaline exert long-term control on glycogen levels in astrocytes: blockade by protein synthesis inhibition. J Neurosci 12:4923–4931
52. Anderson MA et al (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195–200. https://doi.org/10.1038/nature17623
53. Colombo E et al (2014) Fingolimod may support neuroprotection via blockade of astrocyte nitric oxide. Ann Neurol 76:325–337. https://doi.org/10.1002/ana.24217
54. Choi JW et al (2011) FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. Proc Natl Acad Sci U S A 108:751–756. https://doi.org/10.1073/pnas.1014154108
55. Qian Y et al (2007) The adaptor Act1 is required for interleukin 17-dependent signaling associated with autoimmune and inflammatory disease. Nat Immunol 8:247–256. https://doi.org/10.1038/ni1439
56. Colombo E, Farina C (2016) Astrocytes: Key regulators of neuroinflammation. Trends Immunol 37:608–620. https://doi.org/10.1016/j.it.2016.06.006
57. Svensson CI, Zattoni M, Serhan CN (2007) Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. J Exp Med 204:245–252. https://doi.org/10.1084/jem.20061826
58. Abdelmoaty S et al (2013) Spinal actions of lipoxin A4 and 17(R)-resolvin D1 attenuate inflammation-induced mechanical hypersensitivity and spinal TNF release. PLoS One 8:e75543. https://doi.org/10.1371/journal.pone.0075543
59. Liddelow SA, Barres BA (2017) Reactive astrocytes: production, function, and therapeutic poten-

tial. Immunity 46:957–967. https://doi.org/10.1016/j.immuni.2017.06.006
60. Liddelow SA et al (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481–487. https://doi.org/10.1038/nature21029
61. Rothhammer V et al (2018) Microglial control of astrocytes in response to microbial metabolites. Nature 557:724–728. https://doi.org/10.1038/s41586-018-0119-x
62. Xu J et al (2017) Astrocyte-derived CCL2 participates in surgery-induced cognitive dysfunction and neuroinflammation via evoking microglia activation. Behav Brain Res 332:145–153. https://doi.org/10.1016/j.bbr.2017.05.066
63. Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447:869–874. https://doi.org/10.1038/nature05877
64. Dalli J et al (2013) The novel 13S,14S-epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A4 hydrolase (LTA4H), and shifts macrophage phenotype. FASEB J 27:2573–2583. https://doi.org/10.1096/fj.13-227728
65. Serhan CN, Chiang N, Van Dyke TE (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8:349–361. https://doi.org/10.1038/nri2294
66. Tang Y et al (2013) Proresolution therapy for the treatment of delayed healing of diabetic wounds. Diabetes 62:618–627. https://doi.org/10.2337/db12-0684
67. Hong S et al (2014) Maresin-like lipid mediators are produced by leukocytes and platelets and rescue reparative function of diabetes-impaired macrophages. Chem Biol 21:1318–1329. https://doi.org/10.1016/j.chembiol.2014.06.010
68. Norling LV et al (2011) Cutting edge: Humanized nano-proresolving medicines mimic inflammation-resolution and enhance wound healing. J Immunol 186:5543–5547. https://doi.org/10.4049/jimmunol.1003865
69. Pruss H et al (2013) Proresolution lipid mediators in multiple sclerosis - differential, disease severity-dependent synthesis – a clinical pilot trial. PLoS One 8:e55859. https://doi.org/10.1371/journal.pone.0055859
70. Mas E, Croft KD, Zahra P, Barden A, Mori TA (2012) Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation. Clin Chem 58:1476–1484. https://doi.org/10.1373/clinchem.2012.190199

5 Relationship Between Specialized Pro-resolving Mediators and Inflammatory Markers in Chronic Cardiac Disorders

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Keywords
Pro-resolving mediators · Inflammation · Cardiac disorders

5.1 Introduction

The term cardiovascular diseases (CVD) refers to disorders of heart and blood vessels, and include coronary heart disease, cerebrovascular disease, peripheral vascular disease, and heart failure, among others. Atherosclerosis is a common background of these diseases. It is not infrequent that some acute diseases, such as coronary syndromes, appear superimposed on a chronic arterial disease. Acute coronary syndromes (ACS), found worldwide among the leading causes of death, can be the origin of disabling chronic CVD such as heart failure [46]. Clinical and experimental evidence associates this group of alterations with an inflammatory process that takes part in its pathophysiology. In fact, inflammation is one of the most important factors for its initiation, progression, and consolidation [6].

We can consider acute inflammation as an extreme cellular response to stress that occurs when a stimulus perceived as harmful triggers mechanisms of the innate immune system [58]. Plasmatic and cellular molecules, leukocytes, and endothelial cells take part in the inflammatory process which induces an increase of vascular permeability, cell-cell interactions, and the massive recruitment of leukocytes and molecules to the tissues [8]. Since cellular dysfunction can be an inflammatory trigger, and inflammation itself can induce dysfunction or damage, a lack of control of the process can set up a perpetuating cycle [58]. Thus, if the homeostatic mechanisms are incapable of returning to the earlier limits, chronic inflammation takes place, eventually leading to systemic diseases. Various cardiovascular and metabolic diseases are examples of this phenomenon. Indeed, current knowledge permits association of metabolic disorders which show chronic inflammation, such as obesity and its comorbidities (accumulation of visceral fat, hypertension, dyslipidemia, and glucose resistance) with CVD [42]. Hence, evidence points to inflammation as an important player in CVD. The alterations in the concentrations of inflammatory markers reflect such a response. Their serum levels predict cardiovascular risk [43], and the outcome and mortality due to acute complications of ACS or to heart failure [10, 25].

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5.2 Cells and Mediators in CVD

As with other inflammatory-related conditions, cells of the immune and inflammatory responses crucially take part in the progression of CVD. The active and coordinated inflammatory resolution process, involves changes in subpopulations of inflammatory cells and their proportions, and changes in pro- and anti-inflammatory cytokines and lipid mediators, among other factors [51]. Macrophages are key cells that take part in multiple roles in the immune response, inflammation, and in different pathologies. They respond to various stimuli and express and secrete a wide range of soluble molecules and receptors which include lipid mediators, scavenger receptors, pro- and anti-inflammatory cytokines and chemokines; macrophages also present autophagic and anti-apoptotic properties [37]. We find these cells of the innate immune system in varying proportions in healthy and inflamed tissues or tissues being repaired [41]. For example, in a healthy murine heart, they represent approximately 8% of all cardiac cells [28] whilst after a myocardial infarction, the proportion of macrophages increases significantly [17].

We can distinguish two main types of macrophages: the inflammatory phenotype M1 (also known as activated by the classical route), and the anti-inflammatory phenotype M2 or activated by the alternative route; stimuli such as gamma interferon (IFNγ) or interleukin-4 (IL-4), and the lymphocyte subpopulation responsible for those stimuli, differentially regulate their activation pathways [22, 54]. For example, some authors have observed that regulatory T cells induce the differentiation of macrophages to M2 in myocardial repair. Both subtypes of macrophages have different functions and capacities, partly related to the different profiles of inflammatory and pro-resolving molecules they synthesize. The pathogen-associated molecular patterns (PAMPs) activate the M1 macrophages, a fact that induces the release of pro-inflammatory cytokines [21]. These macrophages synthesize a pro-inflammatory lipid profile mainly derived from the omega-6 (n-6) arachidonic acid (AA) such as prostaglandin E2 (PGE2), PGF2, Thromboxane B2 (TXB2) and leukotriene B4 (LTB4) [12].

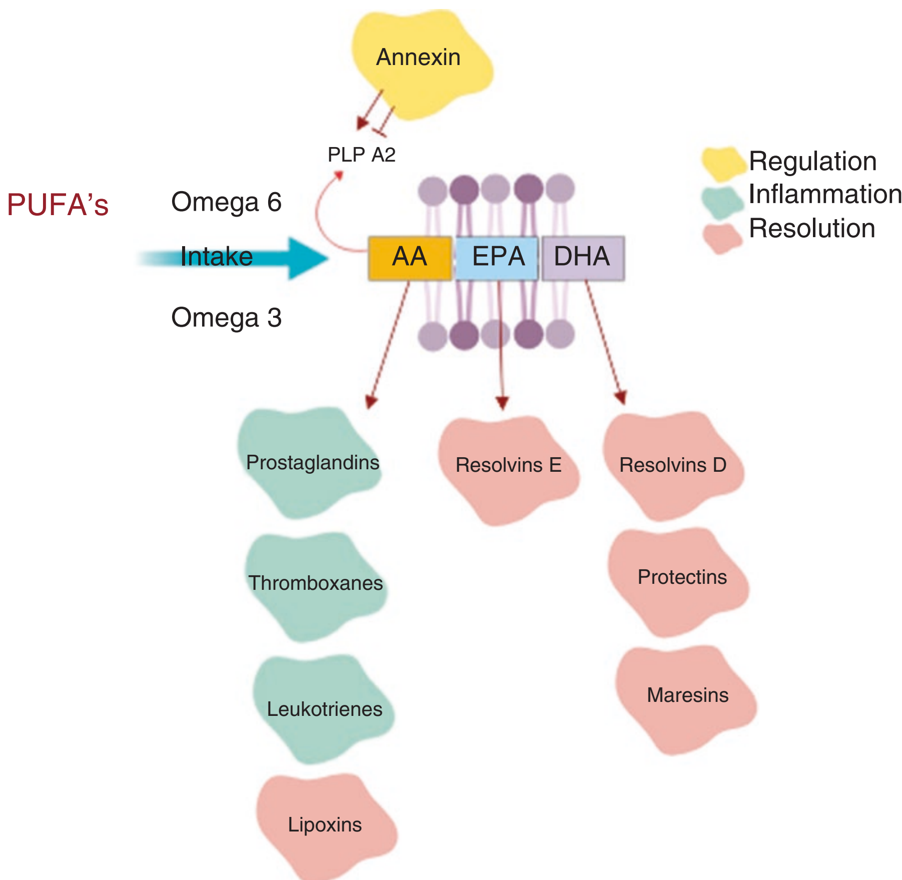
Tissue repair seems to need the lipid profile of the M2 subtype. The involved mediators include derivatives of the omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We call their derivates specialized pro-resolving lipid mediators (SPMs): resolvins (Rv), protectins (PD) and maresins (MaR). Lipoxins (LX) are also SPMs but their precursor is AA [32]. Serhan and his group have identified two classes of resolvins: that of the E series correspond to the EPA derivatives; and the class of the D series corresponds to the DHA derivatives; the latter being also the precursor of protectins and maresins. M2 produce more SPMs than the M1 macrophages, such as RvD5, MaR1, PD1, LXA4, LXB4, LXB5, and RvE2, [12]. Apoptotic neutrophils stimulate M2 macrophages: they produce lipid mediators that activate the SPM profile characteristic of M2 [12]. One of the main features of M2 macrophages is clearing apoptotic cells, a process known as epherocytosis, essential for restoring damaged tissue.

5.3 SPMs and Other Mediators

SPMs play important roles in resolving inflammation, and thus in a physiologic repair of tissues [4]. The membrane's phospholipids of activated cells are the precursors of SPMs and other lipid mediators of the inflammatory process [50]. Inflammatory stimuli such as lesions, microorganisms, and IL-8 activate phospholipase (FLP) A2 that produces free AA [8, 49]. When metabolized, this fatty acid forms a family of oxygenated products called eicosanoids, because of their 20-carbon structure [34]. Eicosanoids include prostaglandins, prostacyclins, thromboxanes, leukotrienes, lipoxins, and hydroxy and hydroperoxy fatty acids. While the enzymatic activity of cyclooxygenase (COX) synthesizes prostaglandins and thromboxanes, that of lipoxygenase (LOX) synthesizes leukotrienes and lipoxins. In a later stage of inflammation, and depending on the composition of the cell mem-

brane, EPA and DHA substitute AA as the substrates of lipid mediators. The SPMs derived from those n-3 fatty acids, exert powerful anti-inflammatory and pro-resolving actions mainly by decreasing the influx of neutrophils to tissues, by enhancing non-phlogistic recruitment of monocytes into tissues, and by decreasing the production of pro-inflammatory mediators [11]. Cell membrane surface receptors mediate their signaling [29, 33]. Annexin A1, a stress response endogenous protein, also mediates inflammatory resolution and can decrease the production of leukotrienes and prostaglandins by an inhibitory action on FLPA2 [15].

erosclerotic plaques represents a risk to develop myocardial infarction and cerebrovascular events [57]. In atherosclerosis, vascular smooth muscle cells have increased proliferative and chemotactic features, reduced expression of contractile proteins and increased production of proinflammatory cytokines [4]; they show increased inflammation and oxidative stress, and extensive areas of necrosis composed of non-clarified apoptotic cells [19]. Some authors suggest that poor resolution of inflammation merges the above [19], hence various researchers have tested diverse experimental approaches to prove this hypothesis. SPMs delay the progression of atherosclerosis



5.4 Resolution and CVD

Atherosclerosis is a disease that is a convincing example of the consequences of an inefficient inflammatory resolution. In humans, having ath-

and promote greater plaque stability [18]. A notable observation is that mouse atherosclerotic plaque treated with SPMs increases its thickness and the synthesis of collagen in the fibrous layer, thus gaining stability [18, 19].

Today there is a great interest in studying resolvins in cardiac alterations because they seem to be crucial players in restoring lesions since they reduce the response of vascular smooth muscle cells and decrease the damage through the local activation of resolution mechanisms [8]. There are reports that resolvins can reduce the risk of developing atherosclerosis and its complications by preventing platelet aggregation, inducing vasodilation [13], inducing neutrophil apoptosis and limiting the size of the infarct [8]. In humans, however, there is still a long way to fully assess their therapeutic efficiency and possible toxic doses.

Resolvin E1 was the first metabolite of EPA identified, its biosynthesis depends on the interaction between endothelial cells and leukocytes in the inflammatory process [48]. Several studies suggest that RvE1 plays an important role in the restoration and repair of tissue damage, favoring resolution by decreasing the production of pro-inflammatory proteins and platelet aggregation. Administering RvE1 reduces the size of the lesion and decreases aortic expression of tumor necrosis factor (TNF) and IFNγ in a murine model of atherosclerosis [47]. In a model of periodontitis and atherogenesis in mice, it decreases the levels of C-reactive protein (CRP) and the infiltration of macrophages to the intima [27]. RvE1 takes part in reducing platelet aggregation induced by thromboxane [20] and, in a model of ischemia-reperfusion of the myocardium in rats, its intravenous administration reduces the size of the infarct in a dose-dependent manner [31].

The mechanisms of action of resolvins of the D series in the inflammatory resolution of cardiac alterations seem to be different. In a model of arterial damage in rats, treatment with RvD1 attenuates both the levels of oxidative stress and the activation of the NFκB signaling pathway [9]. Intact human arteries incubated with DHA ex vivo, produce precursors of resolvins of the D series, which inhibit the adhesion of monocytes induced by stimulation of endothelial cells with TNF [9], suggesting that the local production of SPMs is necessary to promote inflammatory resolution. The in vitro treatment with RvD1 and RvD2 of vascular smooth muscle cells decreases the proliferation, migration, and production of superoxide anion, and the expression of proinflammatory genes [3, 59]. The phosphoinositide 3-kinase (PI3K)/serine/threonine kinase Akt (PI3K/Akt) signaling interactive pathway mediates the protective effects of RvD1 (and of lipoxins), as treating with its inhibitor can block the cardioprotective effect of RvD1 [23]. In a model of damage caused by hepatic ischemia, RvD1 inhibits inflammatory pathways, decreasing the levels of IL-6, TNF, and myeloperoxidase, and increases phosphorylation of Akt, which favors its protective effect. In addition, RvD1 reduces the expression of pro-fibrotic genes and the deposition of collagen, which reduces post-infarction fibrosis [30]. In ischemic lesions, neutrophils infiltrate and release reactive oxygen species (ROS) and other mediators that damage tissue integrity [56]. Exogenous administration of RvD1 after myocardial infarction in mice reduces both the accumulation of neutrophils and fibrosis, which induces an improvement in cardiac function [30].

RvD2 has effects on cellular recruitment and proliferation. In an animal model, RvD2 limits the recruitment of neutrophils to the left ventricle and reduces the density of macrophages in the infarcted area [8]. This lipid reduces cell proliferation and lymphocyte recruitment in a model of arterial angioplasty in rabbits [38]. Similarly, in an intimal arterial neoformation model in mice induced by carotid ligation, RvD2 reduced the proliferation of smooth muscle cells and the recruitment of neutrophils and macrophages. It also induced lower levels of TNF and of granulocyte and macrophage colony-stimulating factor (GM-CSF) [3]. Acting in a similar way of RvD2, the administration of LXA4 in rats with myocarditis reduces the infiltration of inflammatory cells [53].

Not being an SPM, but because of its role in inhibiting FLPA2, annexin A1 has been studied in atherosclerosis. Asymptomatic plaques of the carotid artery have elevated levels of the protein, and its plasmatic concentrations correlate negatively with the total areas of the plaques in atherosclerosis models, which suggests an important role of annexin in resolving cardiac inflammation

[15]. Its action on regulating the production of leukotrienes and prostaglandins may account for its capacity to inhibit the migration of leukocytes into the plaques and its early protective effects [19].

5.5 Diet PUFAs as Precursors of SPMs and CVD

Independent of the use of pure SPMs in the current approaches, there is a long history of use of their precursors n-6 and n-3 PUFAs derived from the diet, specifically by consuming seafoods or their purified compounds. Being essential in the development and function of the organism [8], the human body can synthesize only small amounts of EPA and DHA [60]. Some studies observe that consuming fatty acids changes the profile of the membrane phospholipids, and the composition of the fatty acids of different cells and tissues, affecting their availability [32]. Other studies have shown beneficial effects of n-3 on various pathological conditions [52]. The quality of life of people with chronic inflammatory diseases such as arthritis and asthma [35], the severity of chronic inflammation [7] and the risk for cardiovascular diseases [26] improve with a diet with n-3 PUFAs. The incidence of heart failure in older adults associates negatively with the total plasma concentration of n-3 [40]. Coincidently, a meta-analysis shows that supplementing with EPA and DHA has positive effects on risk factors for cardiovascular diseases because they have hypolipemic, hypotensive, antiarrhythmic and anti-inflammatory actions [1].

Until today it is not fully clear the role of diets that include DHA and EPA to protect against cardiovascular diseases. There is not a consensus despite a wide range of studies that show their beneficial effects, in part because many factors are involved and influence the outcome. For example, a diet enriched with n-6 and n-3 in a 4:1 ratio (as opposed to the 15:1 obtained with a typical occidental diet) decreases the mortality rate for cardiovascular diseases [55]. Other factors to consider include the different EPA/DHA ratios used (0.5:1–1.4:1), different lengths of consumption (few months to more than 6 years), the great variability in doses (<1–4 g/d), the diseases studied, comorbidities (diabetes, hypertension and stroke), and the interaction with drugs. [1, 2].

To reach effective blood levels to reduce the risk of CVD or prevent secondary complications, various studies have concluded that the daily consumption of EPA plus DHA must be of at least 1 g [5, 36, 44]. Consistently, in studies with lower doses of PUFAs, no protection against a possible cardiovascular event is achieved [14]. Some authors suggest higher doses to prevent complications in patients with cardiac disease, for example, consuming 3.36 g of EPA plus DHA in patients with stable coronary disease, helps to restore the levels of SPMs and promotes the phagocytosis of clots. [16]. Administering high doses of n-3 PUFAs in patients with atherosclerosis and type 2 diabetes, accompanied by adequate medical therapy, increases the serum concentrations of EPA and DHA, although it does not induce changes in the concentrations of RvD1 [45]. In rats, a diet enriched with DHA decreases pro-inflammatory proteins such as CRP, IL-6, TNF, and IL-1β, and favors an increase in the concentrations of RvD2 and RvD3 [39]. Fish oil treatment favors protection against thrombosis and damage induced by vascular remodeling in mice, reducing local inflammation and increasing resolution by increasing RvE1 [24].

5.6 Conclusions

Many studies suggest that uncontrolled inflammation and failure in the resolution response are the basis of a myriad of human diseases. SPMs seem to play a crucial role in restoring damaged tissue and in the recovery of function. In fact, it is possible that the success of the inflammatory resolution largely depends on the capacity of producing SPMs and how these mediators can contain chronic inflammation. The study of inflammatory resolution in cardiovascular diseases still represents a challenge. It is still necessary to find out if administering SPMs or their precursors will have the beneficial therapeutic use they promise. Indeed, finding successful

ways of resolving inflammation can provide important advances in preventing cardiometabolic diseases.

References

1. AbuMweis S, Jew S, Tayyem R, Agraib L (2018) Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: a meta-analysis of randomised placebo-control human clinical trials. J Hum Nutr Diet 31(1):67–84. https://doi.org/10.1111/jhn.12493
2. Ajith TA, Jayakumar TG (2019) Omega-3 fatty acids in coronary heart disease: recent updates and future perspectives. Clin Exp Pharmacol Physiol 46(1):11–18. https://doi.org/10.1111/1440-1681.13034
3. Akagi D, Chen M, Toy R, Chatterjee A, Conte MS (2015) Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. FASEB J 29(6):2504–2513. https://doi.org/10.1096/fj.14-265363
4. Bennett MR, Sinha S, Owens GK (2016) Vascular smooth muscle cells in atherosclerosis. Circ Res 118(4):692–702. https://doi.org/10.1161/CIRCRESAHA.115.306361
5. Breslow JL (2006) N−3 fatty acids and cardiovascular disease. Am J Clin Nutr 83(6):1477S–1482S. https://doi.org/10.1093/ajcn/83.6.1477S
6. Calabrò P, Golia E, Maddaloni V, Malvezzi M, Casillo B, Marotta C, Golino P (2009) Adipose tissue-mediated inflammation: the missing link between obesity and cardiovascular disease? Intern Emerg Med 4(1):25–34. https://doi.org/10.1007/s11739-008-0207-2
7. Calder PC, Grimble RF (2002) Polyunsaturated fatty acids, inflammation and immunity. Eur J Clin Nutr 56(Suppl 3):S14–S19. https://doi.org/10.1038/sj.ejcn.1601478
8. Capó X, Martorell M, Busquets-Cortés C, Tejada S, Tur JA, Pons A, Sureda A (2018) Resolvins as proresolving inflammatory mediators in cardiovascular disease. Eur J Med Chem 153:123–130. https://doi.org/10.1016/j.ejmech.2017.07.018
9. Chatterjee A, Komshian S, Sansbury BE, Wu B, Mottola G, Chen M, Conte MS (2017) Biosynthesis of proresolving lipid mediators by vascular cells and tissues. FASEB J 31(8):3393–3402. https://doi.org/10.1096/fj.201700082R
10. Chin BSP, Blann AD, Gibbs CR, Chung NAY, Conway DG, Lip GYH (2003) Prognostic value of interleukin-6, plasma viscosity, fibrinogen, von Willebrand factor, tissue factor and vascular endothelial growth factor levels in congestive heart failure. Eur J Clin Investig 33(11):941–948
11. Dalli J, Colas RA, Serhan CN (2013) Novel n-3 Immunoresolvents: structures and actions. Sci Rep 3(1):1940. https://doi.org/10.1038/srep01940
12. Dalli J, Serhan CN (2012) Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood 120(15):e60–e72. https://doi.org/10.1182/blood-2012-04-423525
13. Das UN (2008) Can endogenous lipid molecules serve as predictors and prognostic markers of coronary heart disease? Lipids Health Dis 7(1):19. https://doi.org/10.1186/1476-511X-7-19
14. de Goede J, Geleijnse JM, Boer JMA, Kromhout D, Verschuren WMM (2010) Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with low fish intake. J Nutr 140(5):1023–1028. https://doi.org/10.3945/jn.109.119271
15. Drechsler M, de Jong R, Rossaint J, Viola JR, Leoni G, Wang JM et al (2015) Annexin A1 counteracts chemokine-induced arterial myeloid cell recruitment. Circ Res 116(5):827–835. https://doi.org/10.1161/CIRCRESAHA.116.305825
16. Elajami TK, Colas RA, Dalli J, Chiang N, Serhan CN, Welty FK (2016) Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. FASEB J 30(8):2792–2801. https://doi.org/10.1096/fj.201500155R
17. Frantz S, Nahrendorf M (2014) Cardiac macrophages and their role in ischaemic heart disease. Cardiovasc Res 102(2):240–248. https://doi.org/10.1093/cvr/cvu025
18. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B et al (2016) An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun 7(1):12859. https://doi.org/10.1038/ncomms12859
19. Fredman G, Spite M (2017) Specialized pro-resolving mediators in cardiovascular diseases. Mol Asp Med 58:65–71. https://doi.org/10.1016/j.mam.2017.02.003
20. Fredman G, Van Dyke TE, Serhan CN (2010) Resolvin E1 regulates adenosine diphosphate activation of human platelets. Arterioscler Thromb Vasc Biol 30(10):2005–2013. https://doi.org/10.1161/ATVBAHA.110.209908
21. Frostegård J (2013) Immunity, atherosclerosis and cardiovascular disease. BMC Med 11(1):117. https://doi.org/10.1186/1741-7015-11-117
22. Fujiu K, Nagai R (2013) Contributions of cardiomyocyte–cardiac fibroblast–immune cell interactions in heart failure development. Basic Res Cardiol 108(4):357. https://doi.org/10.1007/s00395-013-0357-x
23. Gilbert K, Bernier J, Bourque-Riel V, Malick M, Rousseau G (2015) Resolvin D1 reduces infarct size through a phosphoinositide 3-kinase/protein kinase B mechanism. J Cardiovasc Pharmacol 66(1):72–79. https://doi.org/10.1097/FJC.0000000000000245
24. Gong Y, Lin M, Piao L, Li X, Yang F, Zhang J et al (2015) Aspirin enhances protective effect of fish oil against thrombosis and injury-induced vascular

remodelling. Br J Pharmacol 172(23):5647–5660. https://doi.org/10.1111/bph.12986
25. González-Pacheco H, Bojalil R, Amezcua-Guerra LM, Sandoval J, Eid-Lidt G, Arias-Mendoza A, Martínez-Sánchez C (2019) Derivation and validation of a simple inflammation-based risk score system for predicting in-hospital mortality in acute coronary syndrome patients. J Cardiol 73(5):416–424. https://doi.org/10.1016/j.jjcc.2018.11.010
26. Hameed I, Masoodi SR, Mir SA, Nabi M, Ghazanfar K, Ganai BA (2015) Type 2 diabetes mellitus: from a metabolic disorder to an inflammatory condition. World J Diabetes 6(4):598–612. https://doi.org/10.4239/wjd.v6.i4.598
27. Hasturk H, Abdallah R, Kantarci A, Nguyen D, Giordano N, Hamilton J, Van Dyke TE (2015) Resolvin E1 (RvE1) attenuates atherosclerotic plaque formation in diet and inflammation-induced Atherogenesis. Arterioscler Thromb Vasc Biol 35(5):1123–1133. https://doi.org/10.1161/ATVBAHA.115.305324
28. Heidt T, Courties G, Dutta P, Sager HB, Sebas M, Iwamoto Y et al (2014) Differential contribution of monocytes to heart macrophages in steady-state and after myocardial infarction. Circ Res 115(2):284–295. https://doi.org/10.1161/CIRCRESAHA.115.303567
29. Ji R-R, Xu Z-Z, Strichartz G, Serhan CN (2011) Emerging roles of resolvins in the resolution of inflammation and pain. Trends Neurosci 34(11):599–609. https://doi.org/10.1016/j.tins.2011.08.005
30. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN et al (2015) Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol 84:24–35. https://doi.org/10.1016/j.yjmcc.2015.04.003
31. Keyes KT, Ye Y, Lin Y, Zhang C, Perez-Polo JR, Gjorstrup P, Birnbaum Y (2010) Resolvin E1 protects the rat heart against reperfusion injury. Am J Phys Heart Circ Phys 299(1):H153–H164. https://doi.org/10.1152/ajpheart.01057.2009
32. Kumar A, Mastana SS, Lindley MR (2016) n-3 fatty acids and asthma. Nutr Res Rev 29(01):1–16. https://doi.org/10.1017/S0954422415000116
33. Lee CH (2012) Resolvins as new fascinating drug candidates for inflammatory diseases. Arch Pharm Res 35(1):3–7. https://doi.org/10.1007/s12272-012-0121-z
34. Leslie CC (2015) Cytosolic phospholipase a 2 : physiological function and role in disease. J Lipid Res 56(8):1386–1402. https://doi.org/10.1194/jlr.R057588
35. Lordan S, Ross RP, Stanton C (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. Mar Drugs 9(6):1056–1100. https://doi.org/10.3390/md9061056
36. Marik PE, Varon J (2009) Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. Clin Cardiol 32(7):365–372. https://doi.org/10.1002/clc.20604
37. Martinet W, Meyer I, Verheye S, Schrijvers DM, Timmermans J-P, Meyer GRY (2013) Drug-induced macrophage autophagy in atherosclerosis: for better or worse? Basic Res Cardiol 108(1):321. https://doi.org/10.1007/s00395-012-0321-1
38. Miyahara T, Runge S, Chatterjee A, Chen M, Mottola G, Fitzgerald JM et al (2013) D-series resolvin attenuates vascular smooth muscle cell activation and neointimal hyperplasia following vascular injury. FASEB J 27(6):2220–2232. https://doi.org/10.1096/fj.12-225615
39. Morin C, Blier PU, Fortin S (2015) Eicosapentaenoic acid and docosapentaenoic acid monoglycerides are more potent than docosahexaenoic acid monoglyceride to resolve inflammation in a rheumatoid arthritis model. Arthritis Res Ther 17(1):142. https://doi.org/10.1186/s13075-015-0653-y
40. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM et al (2011) Circulating long-chain ω-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study. Ann Intern Med 155(3):160–170. https://doi.org/10.7326/0003-4819-155-3-201108020-00006
41. Nahrendorf M, Swirski FK (2014) Imaging systemic inflammation in patients with acute myocardial infarction. Circ Cardiovasc Imaging 7(5):762–764. https://doi.org/10.1161/CIRCIMAGING.114.002410
42. Niiranen TJ, Vasan RS (2016) Epidemiology of cardiovascular disease: recent novel outlooks on risk factors and clinical approaches. Expert Rev Cardiovasc Ther 14(7):855–869. https://doi.org/10.1080/14779072.2016.1176528
43. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11(2):85–97. https://doi.org/10.1038/nri2921
44. Patterson AC, Chalil A, Aristizabal Henao JJ, Streit IT, Stark KD (2015) Omega-3 polyunsaturated fatty acid blood biomarkers increase linearly in men and women after tightly controlled intakes of 0.25, 0.5, and 1 g/d of EPA + DHA. Nutr Res 35(12):1040–1051. https://doi.org/10.1016/j.nutres.2015.09.016
45. Poreba M, Mostowik M, Siniarski A, Golebiowska-Wiatrak R, Malinowski KP, Haberka M, Gajos G (2017) Treatment with high-dose n-3 PUFAs has no effect on platelet function, coagulation, metabolic status or inflammation in patients with atherosclerosis and type 2 diabetes. Cardiovasc Diabetol 16(1):50. https://doi.org/10.1186/s12933-017-0523-9
46. Roifman I, Beck PL, Anderson TJ, Eisenberg MJ, Genest J (2011) Chronic inflammatory diseases and cardiovascular risk: a systematic review. Can J Cardiol 27(2):174–182. https://doi.org/10.1016/j.cjca.2010.12.040
47. Salic K, Morrison MC, Verschuren L, Wielinga PY, Wu L, Kleemann R et al (2016) Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. Atherosclerosis 250:158–165. https://doi.org/10.1016/j.atherosclerosis.2016.05.001

48. Schwanke RC, Marcon R, Bento AF, Calixto JB (2016) EPA- and DHA-derived resolvins' actions in inflammatory bowel disease. Eur J Pharmacol 785:156–164. https://doi.org/10.1016/j.ejphar.2015.08.050
49. Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510(7503):92–101. https://doi.org/10.1038/nature13479
50. Serhan CN, Chiang N, Dalli J (2015) The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution. Semin Immunol 27(3):200–215. https://doi.org/10.1016/j.smim.2015.03.004
51. Serhan CN, Chiang N, Van Dyke TE (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8(5):349–361. https://doi.org/10.1038/nri2294
52. Shaikh NA, Yantha J, Shaikh S, Rowe W, Laidlaw M, Cockerline C et al (2014) Efficacy of a unique omega-3 formulation on the correction of nutritional deficiency and its effects on cardiovascular disease risk factors in a randomized controlled VASCAZEN® REVEAL trial. Mol Cell Biochem 396(1–2):9–22. https://doi.org/10.1007/s11010-014-2132-1
53. Shi Y, Pan H, Zhang H-Z, Zhao X-Y, Jin J, Wang H-Y (2017) Lipoxin A4 mitigates experimental autoimmune myocarditis by regulating inflammatory response, NF-κB and PI3K/Akt signaling pathway in mice. Eur Rev Med Pharmacol Sci 21(8):1850–1859
54. Sica A, Mantovani A (2012) Macrophage plasticity and polarization: in vivo veritas. J Clin Investig 122(3):787–795. https://doi.org/10.1172/JCI59643
55. Simopoulos AP (2008) The importance of the Omega-6/Omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233(6):674–688. https://doi.org/10.3181/0711-MR-311
56. Swirski FK, Nahrendorf M (2013) Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 339(6116):161–166. https://doi.org/10.1126/science.1230719
57. Virmani R, Burke AP, Farb A, Kolodgie FD (2006) Pathology of the vulnerable plaque. J Am Coll Cardiol 47(8):C13–C18. https://doi.org/10.1016/j.jacc.2005.10.065
58. Wang A, Luan HH, Medzhitov R (2019) An evolutionary perspective on immunometabolism. Science 363(6423):eaar3932. https://doi.org/10.1126/science.aar3932
59. Wu B, Mottola G, Chatterjee A, Lance KD, Chen M, Siguenza IO et al (2017) Perivascular delivery of resolvin D1 inhibits neointimal hyperplasia in a rat model of arterial injury. J Vasc Surg 65(1):207–217.e3. https://doi.org/10.1016/j.jvs.2016.01.030
60. Yadav NS, Wierzbicki A, Aegerter M, Caster CS, Pérez-Grau L, Kinney AJ et al (1993) Cloning of higher plant omega-3 fatty acid desaturases. Plant Physiol 103(2):467–476

6 Specialized Pro-resolving Mediators Directs Cardiac Healing and Repair with Activation of Inflammation and Resolution Program in Heart Failure

Ganesh V. Halade and Bochra Tourki

Abstract

After myocardial infarction, splenic leukocytes direct biosynthesis of specialized pro-resolving mediators (SPMs) that are essential for the resolution of inflammation and tissue repair. In a laboratory environment, after coronary ligation of healthy risk free rodents (young adult mice) leukocytes biosynthesize SPMs with induced activity of lipoxygenases and cyclooxygenases, which facilitate cardiac repair. Activated monocytes/macrophages drive the biosynthesis of SPMs following experimental myocardial infarction in mice during the acute heart failure. In the presented review, we provided the recent updates on SPMs (resolvins, lipoxins and maresins) in cardiac repair that may serve as novel therapeutics for future heart failure therapy/management. We incorporated the underlying causes of non-resolving inflammation following cardiac injury if superimposed with obesity, hypertension, diabetes, disrupted circadian rhythm, co-medication (painkillers or oncological therapeutics), and/or aging that may delay or impair the biosynthesis of SPMs, intensifying pathological remodeling in heart failure.

Keywords

Cardiac repair · Heart failure · Leukocytes · Myocardial infarction · Resolution of inflammation · Specialized pro-resolving mediators

Abbreviations

AA	Arachidonic acid
AT-LXA_4	Aspirin-triggered LXA_4
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
H and E	hematoxylin and eosin
HF	heart failure
LV	left ventricle
LX_4	lipoxin A4
L_XB_4	lipoxin B4
MaR1	maresin 1
MaR2	maresin 2
MI	myocardial infarction
RvD1	resolvin D1
RvD4	resolvin D4

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6.1 Introduction

Heart failure (HF) is the chronic, end stage, and irreversible pathology secondary to ischemic coronary artery disease, predominantly superimposed on aging. HF is also common with non-ischemic cardiovascular diseases with preserved ejection fraction (HFpEF) [1, 2]. After myocardial infarction (MI; heart attack), inflammatory HF is categorized by maladaptive changes in size, shape, and function of the left ventricle, advancing to cardiac remodeling (Fig. 6.1) [3]. In a clinical setting, the size/area of a patient's infarct does not determine the likelihood of adverse remodeling, with or without reperfusion [4]. Therefore, the high rate of hospital admission or re-admission, as well as cardiovascular morbidity and mortality, is primarily due to HF associated with aging [5].

Currently, 5.7 million people are diagnosed with HF in the US, but the projections of this disease are expected to increase to 46% [6], with more than 8 million affected by 2030. As of today, a total of 26 million people are suffering from advanced HF worldwide [7]. During recent years, the discovery of advanced surgery tools, novel medical device technology, therapeutics, and team-based symptom management approaches have advanced in order to minimize patient discomfort. The combination of medical devices and therapeutic procedures have either helped or interfered with the majority of initial inflammatory and reparative responses to MI, but have not slowed the trajectory for disease progression to HF. Every year there are still 915,000 new cases of HF, accounting for an incidence approaching 10 in 1000 individuals over 65 years of age [7]. The continuous growth of HF patients reveals a major gap in our knowledge in how to

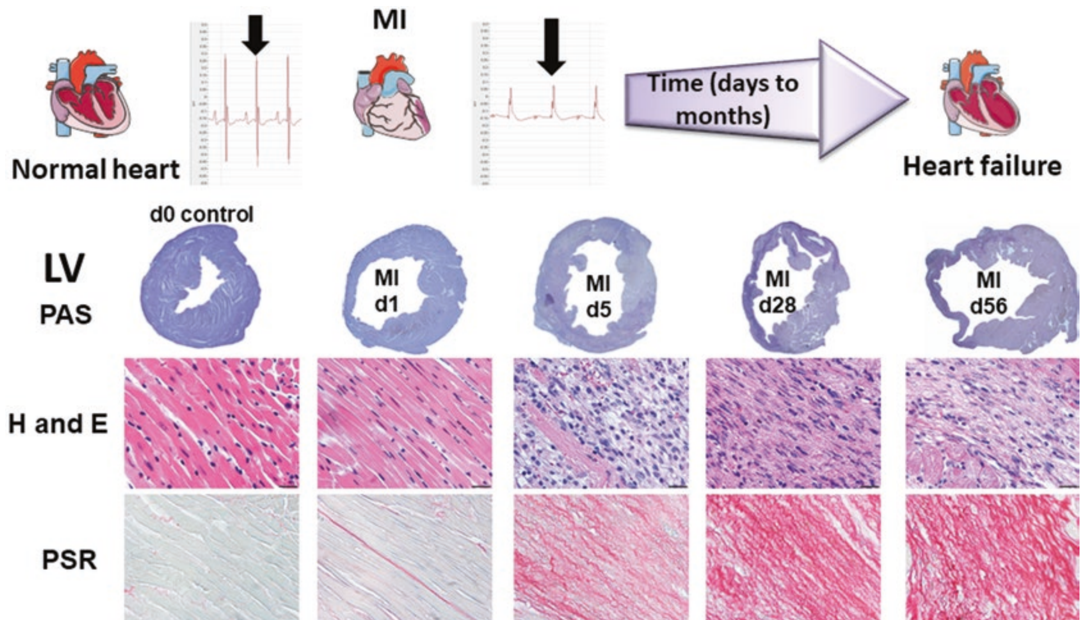

Fig. 6.1 Heart attack induced left ventricle (LV) structural and fibrotic remodeling from acute (day 1 and 5) to chronic (day 28–56) heart failure in mice model of permanent coronary ligation. From the top to the bottom: difference in ECG from normal mice to MI-induced heart after coronary ligation; Periodic acid–Schiff (PAS; 1.25X) staining of the LV after MI from day 1 to day 56 compared to day 0 naïve control. Hematoxylin and Eosin (H and E; 40X) staining of LV showing the organized myocardium structure before MI compared to necrotic and apoptotic disorganized myocardium from day 5 to day 56; Picrosirius red (PSR) staining confirmed the deposition of collagen and compact fibrotic remodeling in the infarcted area initiated at day 5 and progressive to day 56

properly treat and identify novel target signaling pathways to resolve inflammation without the adverse effects of medication or therapies, which frequently occurs with current anti-inflammatory treatments [8, 9]. Aspirin, often considered the safest anti-inflammatory treatment, has failed to limit the event of coronary heart disease and stroke in elderly individuals and is often associated with high incidences of intracranial and extracranial bleeding within the same risk group [10].

In this review, we summarized the leukocyte-directed biosynthesis of SPMs in myocardial healing and the beginning of future applications of novel SPMs to limit chronic inflammation in the setting of HF with potential enhancement to the reparative program [11]. SPMs are biosynthesized by leukocytes triggering endogenous cardiac healing as part of the acute innate response involved in the inflammation-resolution process. The successful biosynthesis of SPMs terminates inflammation and facilitates repair by stimulating distinct resolution processes necessary for tissue repair and regeneration from MI to advanced HF [12–14].

6.2 Immune-Responsive Systems in Myocardium Wound Healing

Pathological inflammation and physiological inflammation are two distinct and primary challenges in identifying and treating injury, infection, or stress (physical or neuro-hormonal mental exhaustion). Physiological inflammation, although necessary, needs to be both activated and inactivated within a specified time in order to avoid damage caused by sustained pathological inflammation. Distinct differences include the phenotype and/or biomarker of injured cells and tissues and the nature and timing of immune signals. Successful physiological inflammation depends on the infiltration of neutrophils and monocytes ('get-in signal'), phagocytic activity of leukocytes ('eat-me signal'), and the on time departure, or efferocytosis, of neutrophils ('get out signal'). The synchronicity of leukocyte entry, coordinated phagocyte activity and an on time exit prevents undesirable consequences of an excessive inflammatory process through the resolution process, which enhances the healing and tissue repair mechanism [15].

Although HF is a multifactorial and heterogeneous end stage disease, the mechanisms of initial inflammation and subsequent initiation of tissue repair are relatively undefined in the acute setting of MI and the role of these critical pathways in the chronic setting remains largely unexplored [16]. HF is predominantly superimposed on other risk factors, like aging and renal failure; however, it is not only risk factors that amplify the complexity of this disease. There is an inter-organ crosstalk between the heart and peripheral organs in HF that delays the cardiac healing program (Fig. 6.1). We defined a clear interaction between the spleen and heart, as 'the splenocardiac axis' as well as the cardio-renal axis [17]. In this context, the spleen is not acting as a leukocyte reservoir but instead as a site for the continuous activation of SPMs in cardiac injury. The spleen is considered an integrative organ in cardiac healing through the production of multiple families of SPMs to activate the cardiac resolution program because splenic leukocytes define the resolution of inflammation in HF [18, 19]. In order to prevent the progression of HF pathology from acute to chronic inflammation, the inflammatory response must be actively resolved (Fig. 6.2). In this review, we highlighted the role of leukocytes, particularly macrophages, that biosynthesize SPMs in cardiac repair and remodeling after injury.

6.3 Immune-Responsive and Leukocytes Directed Cardiac Repair

Cardiac repair is based on the timely activation of cellular and molecular pathways that delay reactive fibrosis signaling, resulting in the formation of scar tissue [20]. Both innate and adaptive

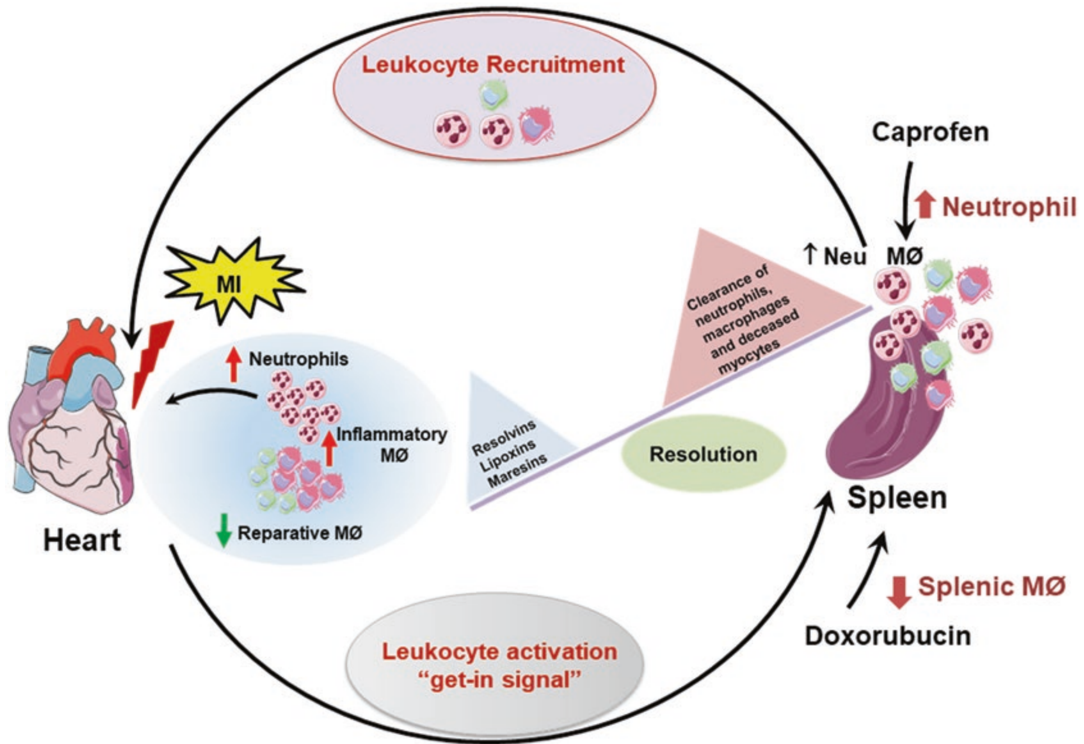

Fig. 6.2 SPMs balance or imbalance defines the inflammation and resolution outcome. Resolution of post-MI inflammation is an active process that requires a balance between proresolving mediators (SPMs) and clearance of leukocytes after acute inflammatory response. In case of heart failure associated to co-medication, this balance is impaired. For example doxorubicin, an oncological drug induced a spleen atrophy with a decrease of splenic macrophages that impairs SPMs biosynthesizing enzymes *(Jadapalli JK* et al*., AJP-HC 2018)*. Likewise, pain-killer caprofen, when administrated before MI for 2 weeks to mice, impairs the splenocardiac axis, dysregulated resolving mediators, over activates neutrophils, and decrease the reparative macrophages at the site of the injury therefore non-resolving inflammation (*Halade GV* et al*., J Leukoc Biol 2018*). *LV* Left Ventricle, *MI* Myocardial Infarction, *MØ* macrophages, *Neu* neutrophils

immune mechanisms are involved in this process [21]. Recently, the belief that activated splenic leukocytes play a critical role in both repair and remodeling has been validated. Specialists in adaptive immune responses demonstrated that the depletion of mature B lymphocytes impeded monocyte mobilization, limited myocardial injury, and improved heart function post-MI and Treg function in cardiac repair [22, 23]. However, cardiac repair alone is insufficient due to various additional risk factors, such as obesity, insulin resistance, diabetes, dyslipidemia, and hypertension. These risk factors can hinder healing, promoting defective tissue repair or unresolved inflammation that can progress to adverse ventricular remodeling thereby leading to end stage of HF [24]. Following cardiac or other tissue injury, the resolution of inflammation is a prime indicator and precursor of tissue repair [25]. In a clinical setting, the hematological profile of a HF patient shows elevated levels of leukocytes, particularly neutrophils and inflammation of the arterial wall in addition to the infarcted myocardium [26, 27]. In mice, the density of leukocytes (neutrophils/monocytes) increase in the infarcted LV in response to myocardial injury [28]. This spike is due to the depletion of splenic leukocytes that occurs while a steady flow of leukocytes are provided to the infarcted LV after MI [18]. In fact, splenic leukocytes mobilize to injured myocardium enriched with fatty acids, such as arachidonic acid (AA), docosahexaenoic acid

(DHA), eicosapentaenoic acid (EPA), and activated lipoxygenases (LOXs). These activated cells, primarily monocytes, then further differentiate into macrophages and biosynthesize SPMs during the acute inflammatory response, suggesting that the acute inflammatory response coincides with the resolving response in cardiac healing [18, 29].

The spleen, as the immune reservoir, coordinates the time-dependent healing of an infarcted LV and determines resolving and non-resolving inflammation. First, immune cells are recruited to stimulate the repair of damaged myocardium [30]. The fatty acid composition in the spleen and the enrichment of the immune system may indirectly affect the cellular events of both of these processes. The depletion of leukocytes (macrophages/neutrophils) is correlated with the lower levels of pro-resolving mediators. Macrophages and the interaction of macrophages with innate leukocyte players are essential for the biosynthesis of bioactive mediators and cardiac healing [31]. The magnitude of cardiac injury, the activation, and initiation of the inflammatory response, and its counter regulation triggers the recruitment of reparative cells that protect against adverse remodeling [32, 33]. Physiological importance of spleen is recorded in humans, since asplenic patients develop ischemic heart disease and excess risk of infection [34].

Recently, the technological advancements in molecular and cellular biology have yielded progress in biomarker discovery and functional characterization that improve our understanding of LV remodeling mechanisms. In the context of cardiac healing, the resolution process has become a frontline focus of inflammation-resolution research and SPMs have become a new strategy for chronic diseases. Many preclinical studies and some clinical reports have shown benefits of SPMs, such as limiting the second wave of tissue infiltration of neutrophils, reducing collateral tissue damage, shortening the resolution interval, enhancing macrophage phagocytosis, amplifying efferocytosis, and counter regulating pro-inflammatory chemical mediators [35, 36].

6.3.1 Neutrophils – Digest Deceased Cardiomyocyte

After cardiac injury, the clearance of deceased myocytes is the primary objective of the leukocytes-based phagocytic system. Neutrophils are important effector cells to activate resolution and cardiac healing post-MI and neutrophil depletion in early healing can lead to early remodeling and HF [15]. Neutrophils dismantle the extracellular matrix and enter the site of injury in order to access the deceased and the necrotic tissue, accelerating pro-inflammatory cytokine production [37]. Neutrophils are highly phagocytic cells containing granules filled with various defensins, elastases, and proteases (matrix matelloproteinase, MMP2, cathepsins, cathelicidins, phosphatases, oxidases, and collagenases) and their primary function is to dissolve matrix and phagocytose; however, this can amplify the inflammatory response [38]. Neutrophils are classically known as immune-responsive inflammatory cells (termed as N1 neutrophils) but recent reports indicated that, like macrophages, neutrophils undergo polarization and show anti-inflammatory properties (termed as N2). These N2 neutrophils account for 20% of the neutrophils present 7 days post-MI [39]. The phenotypes of N2 neutrophils are distinguishable by the high expression of the macrophage mannose receptor CD206 and interlukin-10 (IL-10). Neutrophils orchestrate post-MI healing by polarizing macrophages towards a reparative phenotype but can also play an active role in the resolution of inflammation by depleting the chemoattractants that initially drew them to the site of injury [40, 41]. Therefore, these cells contribute directly to the inflammation-resolution process and indirectly to cardiac repair after MI.

6.3.2 Macrophage – Programs Neutrophils Clearance and SPMs Biosynthesis

Recent findings imply a potential role of the mononuclear phagocytic system includes macrophage cells in the repair of infarcted myocardium

[42, 43]. According to macrophage biology research, many studies are devoted to the description of mouse cardiac monocyte and macrophage subsets and the refinement of macrophage classifications are ongoing [44]. With respect to recent data, the composition of macrophage subsets in the myocardium undergo dynamic changes throughout the course of a lifetime [45]. Fate mapping studies confirmed that the monocyte/macrophage systems placed in the heart prior to birth maintain self-renewal properties independently of a circulation feedback system [46]. Unfortunately, this discovery has limited translational potential since it is still unclear whether generation and differentiation of macrophages from precursors are impaired with age. However, it is well proven that macrophages become either partially or totally dysfunctional in wound healing within the context of obesity and diabetes superimposed on aging in both humans and rodents [47].

We know that macrophages have polarization profiles similar to neutrophils; within 1 day post-MI, macrophage exhibit a pro-inflammatory M1 profile [48–50]. At the post-MI "healing stage" (up to 7 days post-MI), there is a transition from predominately M1 macrophages to primarily anti-inflammatory macrophages, and then to reparative M2 cells [51, 52]. Cardiac reparative M2-like macrophages are a complicated mixture of heterogeneous subsets that have strengthened their tissue-repairing abilities by upregulating various anti-inflammatory and repair-associated genes after MI [42, 53]. These two types of cells (M1 and M2) are derived from Ly6C^{hi} monocytes and Ly6C^{low} monocytes, respectively. Reparative M2 macrophages are recruited simultaneously with inflammatory leukocytes in the post-infarct LV to mediate myocardial healing through the secretion of anti-inflammatory cytokines and growth factors, such as VEGF and TGF-ß, that contribute to myofibroblast activation and neoangiogenesis [54]. Deceased cardiac fibroblasts emit signals causing the activation of monocytes via MCP-1-mediated chemotaxis and adhesion to ICAM-1/VCAM-1, and induce their differentiation to M1 or M2 macrophages [44]. Some researches include a third macrophage subtype found in resolving exudates referred to as 'resolution phase macrophages'. These macrophages possess characteristics of both M1 and M2 cells and we believe that they represent cardiac reparative macrophages, especially with the recent clinical strategies focused on improving macrophage function to improve healing outcomes [55–57]. Macrophage biology and phagocytic systems likely vary with the magnitude of injury and therefore the monocyte and macrophage responses to injury can differ depending on the experimental model. For example, in a model of neonatal mice, the depletion of macrophages after MI impaired cardiac function and angiogenesis. The heart can fully regenerate without scarring following MI in neonatal mice; however, the regenerative capacity of macrophages is lost 7 days after birth [28]. Under other conditions, like in a model of adult mice, after MI, Ly6C^{hi} macrophages (M1 macrophages), and Ly6C^{low} (M2 macrophages) are the main effectors of cardiac remodeling.

The kinetic and detailed macrophage and neutrophil profiling studies demonstrated that after cardiac injury, splenic monocyte-enriched leukocytes mobilized to the infarct area and biosynthesized SPMs. Macrophages are a major contributor in SPMs biosynthesis [58]. In addition, lipid mediator profiling of the spleen indicates that the spleen also receives feedback signal for biosynthesis of lipid mediators, which promote self-resolution of inflammation at the left ventricular site [59]. In cardiac injury, SPMs are biosynthesized immediately by primarily activated macrophages with the initiation of inflammation [60]. In response to cardiac injury or stress, the resolution-phase activated macrophages also express ALOX-15, indicating that they may also contribute to SPMs production during resolution [61, 62]. M2 macrophages produce more pro-resolving lipid mediators than M1 macrophages [63]. SPMs initiate the switch from inflammation to resolution by reducing neutrophil recruitment and T cell cytokine production and increasing recruitment of phagocytic monocytes [64]. Resident macrophages express TGF-β1 and IL-10, presumably because of phagocytizing apoptotic neutrophils; these cells also expressed

ALOX-15 and TIMD4 (T-cell immunoglobulin domain and mucin domain 4) to facilitate the recognition and uptake of apoptotic cells [65, 66]. Recent results indicate that treatment with DHA derived pro-resolving mediators, like exogenous RvD1, stimulate a switch in macrophage phenotype from pro-inflammatory to pro-resolving, a M2-like phenotype [67, 68]. Studies found that human M2 macrophages are associated with increased MaR1 levels [61]. This increase is due to the ability of this macrophage subtype to convert the 13S, 14S epoxide intermediate to MaR1 [60]. As critical regulators of the resolution program, targeting the actions of macrophages could be an effective strategy to control inflammation and cardiac remodeling.

6.3.3 Knowledge Gap of Inflammation and Resolution as Part of Cardiac Reparative Program

After cardiac injury, the resolution phase is an active biosynthetic process that overlaps or coincides with the first response of the innate immune system. This was confirmed using structural elucidation of the SPMs in a variety of wound healing models [19]. Inflammation-resolution process-derived bioactive metabolites implicate an array of receptors that transduce pro-resolving action. Among them, the most studied receptors are GPCRs as effectors of resolution shown indirectly through use of the receptor knockout cells and mice. These GPCR receptors (ChemR23, GPR32, and FPR2) activate signals and transduce the pro-resolving signals of chemerin peptides, resolvin E1 (RvE1) and resolvin D1 (RvD1) and lipoxins [69–71].

Recent, emerging data added confirmation to previous thoughts that resolution is a primarily, active stage of the immune response to cardiac injury and not an inactive, secondary stage. This stage acts as an overlap with the innate response, adding a third phase after the initiation of inflammation and resolution, creating a post-resolution response dominated by macrophages and lymphocytes [72]. Although data on SPMs primarily focuses on innate immune cells involved in the resolution of acute inflammation, resolution creates a microenvironment conducive for the optimal development, tissue repair, and modulation of the adaptive immunity [19, 58, 73]. We conclude that a better understanding of SPMs biosynthesis and the pharmacology of SPMs receptors, especially in chronic inflammatory settings like HF, could minimize the gap between the initiation of inflammation and initiation of resolution, capitalizing the fundamental actions of these effectors of resolution.

6.4 SPMs Biosynthesis and Resolution of Inflammation in Cardiac Repair

Splenic leukocytes mobilize to the site of infarcted injury and biosynthesize SPMs to limit the excessive inflammation that can be detrimental to healthy tissues, particularly in sterile inflammation. In addition to the stop signal in acute inflammatory response, SPMs also stimulate distinct processes necessary for tissue repair and regeneration [74]. SPMs have been associated with positive feedback loops, where one pro-resolving mediator induces the biosynthesis of another [15]. The administration of RvD1 3 h after MI in mice induces lipoxin A_4 and MaR1 to facilitate the resolution of inflammation and cardiac regeneration [75]. SPMs are produced mainly by the inter-organ interactions of monocyte-derived macrophages and neutrophils via distinct enzymatic pathways from essential fatty acids, such as omega-3 and omega 6, polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [76]. Several endogenous SPMs have been discovered, including lipoxins, resolvins, protectins, and maresins [36], which are directly and indirectly involved in driving the initiation of inflammation-resolution and successfully terminating inflammation. Healthy and risk free animals, such as young or adult mice, effectively mobilize circulating neutrophils, platelets,

and monocytes/macrophages from the spleen after cardiac injury to promote left ventricle healing, which is essential to delay HF [59]. After cardiac injury, monocytes/macrophages serve as a major contributor for the biosynthesis of SPMs that have multiple cellular targets in the inflammatory response, including immune cells, platelets, and vascular cells [74]. Leukocyte-directed SPMs actively counter-regulate and control the production of pro-inflammatory mediators, including cytokines, leukotrienes, and eicosanoids, and regulate leukocyte trafficking and phenotype after both sterile and infectious challenges [77].

6.4.1 Resolvins

These are novel bioactive autacoids termed “resolvins” because they are endogenously generated by resolution phase interaction products classified as either E-series resolvins, if leukocyte directed biosynthesis is initiated from EPA, or D-series resolvins, when resolvins are biosynthesized from DHA [78, 79]. Subcutaneous administration of RvD1 limits the activation of neutrophils in the spleen and promotes the clearance of macrophages and neutrophils from the infarcted site to facilitate cardiac healing. After ischemic injury, treatment of RvD1 primarily activates the ALX/FPR2 receptor in both the spleen and left ventricle to promote effective resolution of inflammation and limit residual time of neutrophils at the site of infarction (‘get-out’ signal) [30, 80].

A reperfusion study in rats confirmed that intraventricular administration of RvD1 reduces myocardium infarct size; however, presence of excess linoleic acid attenuates the myocardial protection offered by RvD1 [81, 82]. In another experimental study, after cardiac injury using permanent coronary ligation model, RvD1 administration in mice not only resolves the inflammatory response, but also reduced the expression of fibrotic genes, such as *colla1, coll2a1* and *tnc*, in the early phase of cardiac healing. Thus, RvD1 limits fibrotic signaling and facilitate organ homeostasis [75, 83]. These changes of ECM in acute HF suggest that RvD1 orchestrates mature scar formation and stabilizes the ECM by resolving inflammation. An experimental model of hind limb ischemia (HLI) in mice, RvD2 stimulated arteriogenic revascularization suggesting that resolvins may be a novel class of mediators that both resolve inflammation and promote arteriogenesis [84] (Table 6.1). Presented experimental studies in rodent models strongly indicate that SPMs offer pharmacological action in the range of ng or pg doses suggestive of outstanding potency [85], in contrast to nonsteroidal anti-inflammatory drugs (NSAID) which effective in a range of mg/kg doses that are immune suppressive and impact the cardiovascular physiology and pathobiology [86].

SPMs are produced by the coordinated activity of lipoxygenases (LOXs) in the heart and cyclooxygenases (COXs) in the spleen; these are the key enzymes for generating potent bioactive lipid mediators, which primarily synchronizes the resolution of inflammation in pathophysiological settings [87]. Fredman et al. reported that RvD1 favors synthesis of the pro-resolving lipid mediator, LXA_4, via limiting nuclear translocation of 5-LOX in atherosclerotic inflammation [88]. RvD1 critically balanced LOX enzymes by stimulating 5-LOX expression, which is essential for wound healing post-MI [89]. RvD1 has the potential to delay HF by limiting cytoplasmic to nucleus translocation to delay atherosclerotic inflammation and facilitate neutrophil clearance. Future long-term studies focused on RvD1 are warranted in order to prove its utilization in chronic HF management in patients with heterogeneous cardiovascular pathology [75]. Another compound of the D-series resolvins family, RvD3, was used as a model of self-resolving peritonitis in aging mice. The study, performed by Arnardottir and colleagues, reported that increased levels of RvD1 and RvD3 treatment stimulated increases in monocyte-accelerated resolution of acute inflammation via efferocytosis [90]. RvD3 has been argued to be one of the most potent of the D-series resolvins [91].

Regarding the E-series resolvins, Arita et al. reported leukocyte-directed actions of RvE1 as

Table 6.1 Role of SPMs in cardiac repair in different experimental models

Molecules		Experimental Model	Mechanisms	Effect	References
Resolvins	RvD1	*In vivo*, mice model of heart failure	ALX/FPR2 receptor	↑ clearance of neutrophils from the spleen	[75]
				↑ clearance of macrophages from healing site	
				↓ expression of fibrotic genes	
	RvE1	*In vivo*, mice model of acute heart failure	ChemR23 receptor	Protected cardiomyocytes against apoptosis	[107]
			BLT1 receptor	↓ protected cardiomyocytes against apoptosis.	
				↓infiltration of dominant Ly6C^{hi} Mos/Mps	
Lipoxins	15-epi-LXA_4	*In vivo*, mice model of heart failure	ALX/FPR2 receptor	↑ clearance of neutrophils	[103]
				Improve ventricular function	
				Activate GPR120 receptor	
				Inhibit GPR40 receptor	
Maresins	MaR1	*In vitro,* human macrophages *In vivo*, model of atherosclerosis	GPR32	↑ Efferocytosis and phagocytosis	[60, 108]
			BLT1 receptor	↓PMN infiltration	
				↑reparative macrophages	

one of the local mediators of tissue homeostasis during inflammation-resolution [92, 93]. The physiological functions of RvE1, and RvE2, both biosynthesized by neutrophils via the 5LOX pathway and RvE3 biosynthesized by 12/15-LOXs in eosinophils, have been studied in the last few years [94, 95]. The myocardial protective action of RvE1 action is quite different from other resolvins and protectins. RvE1 specifically binds to ChemR23 and BLT1 to offer pro-resolving responses in human polymorphonuclear (PMN) cells [96]. This binding enhances macrophage phagocytosis via phosphoprotein-mediated signaling [97]. It blocks LTB_4 binding and signals via BLT1 to promote apoptosis of PMN for their clearance by macrophages, while LTB_4-BLT1 signals PMN survival.

6.4.2 Lipoxins

Lipoxins A_4 and B_4 are lipoxygenase-interaction products of arachidonic acid and are biosynthesized at the site of injury to facilitate resolution of inflammation. Lipoxins are endogenous that offers anti-inflammation and pro-resolution at nanomolar concentrations, inhibiting chemokine-driven recruitment of both granulocytes and monocytes [98, 99]. At the site of inflammation, lipoxins also stimulate macrophages to ingest and clear apoptotic neutrophils, exerting pro-resolving actions through the activation of ALX/FPR2 [100, 101]. In correlation with lipoxins generation, LTB_4 was proved higher in the spleen at a naïve state and increases along with lipoxins in the LV at day 1 post-MI [18]. Recent studies demonstrated that 15-epi-LXA_4 coordinates the upregulation of IL-6 and IL-1β at day 1 post-MI to facilitate an unaltered, active inflammatory phase post-MI without altering the acute inflammatory response. Liposomal delivery of 15-epi-LXA_4 showed a similar protective function as free 15-epi-LXA_4, improving ventricular function through the activation of neutrophil clearance in the LV during the resolving phase with an increase of ALX/FPR2 and Ccl2 during the acute inflammatory phase in a murine model of HF [102]. Additionally, 15-epi-LXA_4 promotes the resolution of inflammation by activating GPR120 and inhibiting GPR40 in a translational HF model (Table 6.1) [3, 102, 103].

6.4.3 Maresins

Maresins (macrophage mediators in resolving inflammation) are derived from the marine omega-3 fatty acid DHA [104]. Leukocyte-directed maresins (MaR) are produced by macrophages via initial lipoxygenation at the carbon-14 position created by the insertion of a molecular oxygen, producing a 13S,14S-epoxide-maresin intermediate that is enzymatically converted to maresin family members (MaR 1, MaR2, and MaR conjugate in tissue regeneration (MCTR) that regulate phagocytosis, and the repair and regeneration of damaged tissue [36, 102]. An recent study by Halade et al. showed that the quantification of MaR1 at day 1 post-MI in mice is significantly higher in LV than spleen. However, at the naïve state without any induced injury, MaR1 is higher in the spleen than in the LV. These findings confirmed that splenic monocyte-enriched leukocytes mobilize to the infarct area and biosynthesize SPMs [18]. In an *in vivo* study, MaR1 reduced neutrophil and macrophage recruitment and increased polarization of M2 macrophages in the arterial wall [105]. MaR1 have the ability to skew macrophage phenotypes toward resolution-like, with increased TGF-β production and secretion [106]. After an infection, activated macrophages biosynthesize MaRs that increases phagocytosis and efferocytosis, resulting in the clearance of microbes (Table 6.1) [80].

6.5 SPMs Regulation and Dysregulation

Fatty acids-derived drug (FADD) discovery exemplified the generation of SPMs from essential fatty acids [59]. This discovery is unique and novel for endogenous, bioactive pro-resolving mediators, represents a paradigm shift in our understanding of the dynamic regulation of acute inflammation, and has led to a new era of resolution physiology and resolution pharmacology. Within a single cell type, lipid mediator class switching can occur and pro-resolving lipid mediators can regulate each other. SPMs have dual roles as counter-regulators of inflammation, as anti-inflammatory without being immunosuppressive, and activators of resolution [109]. For example, RvD1 reduces nuclear localization of 5-LOX in macrophages and diverts arachidonic acid metabolism from pro-inflammatory lipid mediator LTB_4, to SPM LXA_4 [62]. RvD1, activated through ALX/FPR2, protects macrophages from oxidative stress–induced apoptosis during efferocytosis, in part, by regulating nicotinamide adenine dinucleotide phosphate oxidase activation and expression of apoptotic proteins, Bcl-XL, and Bcl-2 [110]. In the other hand, the downregulation of NF-κβ and TNF-α by RvD5 could be beneficial in the HF model, as both of these factors are increased in the heart post-cardiac injury [111]. Therefore, SPM emerge both as potent regulators of macrophage responses of interest during the resolution phase of acute inflammatory responses and effectors in macrophage mediated responses [112]. Of interest, receptors of SPMs, including ALX/FPR2 and ChemR23, are expressed in the human saphenous vein SMC, and administration of RvE1 and 15-epi-LXA_4 counter-regulate platelet-derived growth factor–stimulated VSMC migration in a dose-dependent manner [113]. These results build on previous studies showing that lipoxins counter-regulate leukocyte-mediated microvascular permeability *in vivo* during acute inflammation, stimulated by pro-inflammatory lipid mediators [114]. The actions of SPMs were recently reported in human settings where an RvE1 analog entered human clinical trials for dry eye inflammation [115]. In light of the role of SPMs in the resolution, these compounds display potent actions in the adaptive immune system including the regulation of T-cell phenotypes and responses [58, 116]. Because Treg cells are an important cell subset involved in modulating and maintaining self-regulation of the immune system, SPMs potentially induced Treg (iTreg) differentiation, with the lipids significantly enhancing Foxp3 expression compared to control iTreg cells. They affect not only Treg induction, but also specific functional properties, like TH cell polarization [58]. The actions of SPMs on T cells are mediated by GPR32 and ALX/FPR2

receptors. It has been proven that RvD1, RvD2, and MaR1 exert a non-cytotoxic regulatory role on cells; it represents a promising beginning for a new avenue of resolution physiology research.

Biosynthesized maresins counter-regulate the pro-inflammatory cytokines, such as *IL-1β*, *IL-6*, and *TNF-α*. They also regulate nuclear factor kappa B (*NF-κ B*) gene products, increase the regulation of T cell *de novo* synthesis and intracellular levels of cyclic adenosine monophosphate, regenerate tissue, and play a role in anti-nociceptive action [36, 117]. SPMs might possibly act on the balance between pathogenic TH1/TH17 and tolerogenic Treg cells, which are typically altered during chronic inflammation [58]. However, these endogenous pathways of resolution could be disrupted or dysregulated with co-medication. In fact, treatment with painkiller caprofen or oncologic drug doxorubicin facilitate splenocardiac resolution deficiency in mice with cardiac injury, creating non-resolving inflammation (Fig. 6.2) [118, 119]. Other factors, like metabolic dysfunction, are major contributors of SPMs dysregulation. For example, obesity, hypertension, diabetes, and aging are associated with HF and could give an extra rise to unresolved chronic inflammation [120]. Next, we discussed the impact of SPMs dysregulation in cardiometabolic disorder thereby impact on resolution physiology.

6.5.1 Obesity

The unprecedented, continuous rise in the prevalence of obesity and obesity-related disorders is causally linked to a chronic state of low-grade inflammation in adipose tissue and many other organs [120, 121]. In the case of obesity, the molecules and signaling pathways have dual roles as inflammatory mediators as well as regulators of energy storage and metabolism [109]. The on time resolution of inflammation and the return of this tissue to homeostasis are key components to reducing obesity-induced metabolic dysfunctions. Results indicate that in inflammatory adipose tissue, RvD1, and RvD2 are potent pro-resolving mediators that counteract both local adipokine production and monocyte accumulation in obesity-induced adipose inflammation [90]. However, it has been demonstrated, that the formation of SPMs is severely dysregulated in inflamed, obese adipose tissue [120]. A deficiency in pro-resolving mediators in obese adipose tissue within the setting of obesity could be the consequence of a structural deficiency in the tissue content of omega-3-PUFAs, which are established substrates for SPM biosynthesis [122]. Alternatively, the loss of SPMs in obesity may reflect accelerated tissue SPMs conversion and clearance to inactive further metabolites because 15-PG-dehydrogenase/eicosanoid oxidoreductase, the key enzyme in SPM inactivation, is markedly up-regulated in obese adipose tissue [123]. Moreover, not only is activated lipoxygenase essential for SPM biosynthesis, but also the substrate product of these enzymes is essential for precise immune responses. A study involving mice found lipidomic analysis showed higher levels of arachidonic acid and 12(S)-hydroxyeicosatetraenoic acid (12-HETE) at day 1 post-MI an obese group compared with the non-obese group (Fig. 6.3) [124]. However, studies involving the obesity paradox recently provided evidence that both mouse and human adipose tissue have the capacity to generate LXA_4 [111].

6.5.2 Diabetes

Uncontrolled inflammation plays an essential role in the pathogenesis of diabetes and its associated pathologies, like HF [125]. Also, the key sequela of adipose tissue inflammation is insulin resistance leading to type 2 diabetes (T2D) [126–128]. In this setting, a known complication of T2D is impaired wound healing; the administration of RvD1 to diabetic mice enhances wound healing, compared with control or vehicle treatment [129]. Therefore, SPMs may limit factors associated with T2D via its ability to quell inflammation and promote adipose and liver tissue homeostasis. RvD1 improved insulin sensitivity, reduced adipose inflammation, and reduced steatosis in mouse models of T2D [130, 131]. In

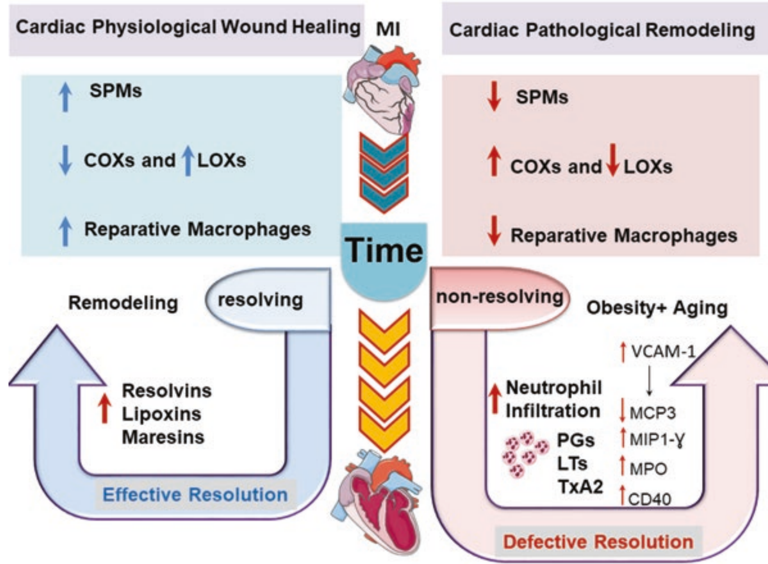

Fig. 6.3 Physiological and pathological healing in mice delineates the effective and defective resolution in heart failure. Physiological or pathological healing depends on resolution of inflammation process. Physiological cardiac remodeling is a consequence of an effective resolution, with the production of necessary SPMs at the right time by reparative macrophages. However, many factors can lead to a defective resolution like obesity and aging that induce a low-grade chronic state of inflammation with a high levels of COXs and LOXs-derived pro-inflammatory mediators with low levels of SPMs and reparative macrophages along with the increase of VCAM-1 (vascular cell adhesion molecule 1), MPO (myeloperoxidase), CD40 (cluster of differentiation 40), PGs (prostaglandins), LTs (leukotrienes), and TxA_2 (thromboxane A_2)

addition to resolvins, lipoxins also showed prominent results in both mice and human and may have therapeutic potential in the context of diabetes associated vascular complications [132]. However, it is unknown if T2D affects the resolution of inflammation and whether treatment with pro-resolving mediators would stimulate resolution and ameliorate clinical complications of T2D such as impaired wound healing.

6.5.3 Hypertension

Hypertension remains a significant risk factor for the development of HF with various mechanisms contributing to both systolic and diastolic dysfunctions [133]. Much less is known about the role of anti-inflammatory molecules in the regulation of blood pressure in general or in the development of hypertension [134]. In contrast, higher levels of pro-inflammatory mediators, 5-HETE, 12-HETE and 15-HETE, were observed in preeclamptic placentae, a disorder of a multifactorial etiology that compromise maternal and fetal well-being as well as cardiovascular health later in life [135]. Endothelial and vascular inflammation also contribute in sustaining "low-grade inflammation" and have shown to be more associated with pulmonary hypertension (PH) [136]. Recent data demonstrated that *in vitro* RvD1 prevents arterial wall over-activity in human (HPH). This effect appears to be related to a decrease in Ca^{2+} sensitivity in HPA smooth muscle cells. RvD1 also decreases the expression of transmembrane protein member 16A (TMEM16A), a specific marker of PH [137]. In the same context, Jannaway et al. demonstrated that low nanomolar concentrations of RvE1, RvD1, and RvD2 can prevent constriction in rat and human arteries induced by a thromboxane mimetic [138].

6.5.4 Disruption of Circadian Clock

Many physiological functions, including leukocyte and platelet responses, are responsive to day and light schedules in order to regulate cell and organ specific circadian clocks. Results from several recent studies demonstrate that many metabolic functions are controlled by circadian rhythm genes, enzymes, and proteins [139]. Disturbances to various aspects of these fundamental mechanisms are thought to be responsible for many of the diseases that affect modern societies, including cardiovascular and metabolic disorders [140, 141]. In context to cardiac biology, the direct influence of circadian rhythms on SPMs and endogenous mechanisms in the resolution of inflammation remains largely unexplored. A study demonstrated that cardiomyocte-specific deletion of the circadian clock component, Bmal1, leads to age-dependent dilated cardiomyopathy and decreased lifespan in mice [142, 143]. Additionally, the circadian release of glucocorticoids and their link to downstream anti-inflammatory and pro-resolution mediator annexin A1 facilitates the return to homeostasis [140]. A recent study suggests that patients with risk of coronary vascular disease (CVD) have defective diurnal regulation of vascular n-3 docosapentaenoic acid (DPA) derived resolvins. RvDn-3 DPA is involved in regulating peripheral blood cell responses and tissue protection. The marked reductions in plasma RvDn-3 DPA during the early morning hours indicate that alterations in biosynthesis due to dysregulation of lipoxygenase and may contribute to CVD onset and propagation. This finding can be explained by the increase of adenosine, a regulator of 5-LOX activity in plasma from patients with CVD [144].

6.5.5 Exercise

Exercise limitation is one of the hallmarks of heart failure, and an increasing degree of intolerance is associated with a poor prognosis [145]. One study measured SPMs in human blood during post-exercise recovery. It was demonstrated that lipoxins (LXA_4 and LXB_4), resolvins (RvE1 and RvD1), and the protectin D1 isomer increased in human serum during the early hours of post-exercise recovery (0–3 h post-exercise). However, ibuprofen treatment with exercise reduced the response of pro-resolving lipid mediators to exercise [146]. These results are consistent with those reported earlier by Gangemi et al. in which LXA_4 in human urine was found to increase with treadmill exercise [147]. In contrast to the former belief, exercise training in HF patients has proven to be safe and has no adverse effect on left ventricular remodeling [148]. However, an exercise paradox exists, and recently a study showed that exercise alters β-alanine, augments histidyl dipeptide levels, and scavenges lipid peroxidation products in human skeletal muscle [149].

6.5.6 Aging and Microbiome

Aging and age-associated chronic and unresolved low-grade inflammation, “inflammaging,” are progressive and all-time predominant factors for CVD [150, 151]. The underlying mechanisms of inflammaging remain of interest, but a plausible hypothesis for the non-resolving inflammation in the elderly is a defect in the inflammation–resolution process. The baseline pre- and post-MI environments are also altered with age [94, 152, 153]. A recent study in a context of muscle generation showed a correlation between aging and CCR2. The data explained that in young WT $CCR2^{-/-}$, both dysfunctional macrophages and a pro-inflammatory environment were observed. Moreover, this study concludes that $CCR2^{-/-}$ in mice have a positive feedback loop that promotes inflammaging in young mice when compared to aging WT mice [154]. In humans, urinary lipoxins (LXs) were decreased in the elderly, resulting in a profound imbalance between pro-resolving LXs and LTs [147]. In line with these results, a study demonstrated that aging mice dysregulate the formation

of pro-inflammatory and pro-resolving molecules with a decrease in LOXs expression, depending on the influx or availability of substrates that temper acute inflammatory-resolving phases, leading to the promotion or repression of inflammation post-MI (Fig. 6.3) [155]. Aged mice have increased inflammation, LTs, and decreased SPMs compared to young mice [91]. A recent, aging, metabolome human study described that RvD6 levels were decreased in an aging individuals, indicating the role of lipid metabolism in progressive aging [156]. Thus, there is evidence of defective SPMs in aging but the mechanisms remain of interest. Compared to evidence related to aging and inflammation, less is known regarding associations between aging and the microbiome; however, along with age related HF, current evidence has found links between HF in general and specifically HF in elderly women with alterations in microbial composition and function [157]. Since HF has been associated with impaired intestinal barrier function and bacterial translocation, leading to inflammatory and immune responses [158]. Studies have shown that significant alterations of the intestinal bacterial microbiome, like a significant decrease of *Coriobacteriaceae*, *Erysipelotrichaceae* and *Ruminococcaceae* in the familiar level and *Blautia*, *Collinsella*, *uncl. Erysipelotrichaceae* and *uncl. Ruminococcaceae* at the genus level [159]. Recently, a study showed that resolvins biosynthesizing substrates improved gut microbiome in elderly women [160].

6.6 Conclusion

After cardiac injury, leukocyte-directed biosynthesis of SPMs is necessary for cardiac healing in order to return to a hemostatic state. Leukocyte-derived SPM actions successfully operate the transition from inflammation to resolution. However, the dysregulation due to physical inactivity, co-medication, aging or cardiometabolic disorders like obesity, hypertension, and diabetes, or even disruption of sleep/wake up cycles impact the resolution process and the production of pro-resolving mediators and therefore non-resolving inflammation in cardiovascular diseases. Future studies are warranted to determine the mechanism of action, additional receptors for SPMs, and interactions with lifestyles or medications in the cardiac repair program.

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References

1. Keulenaer GWB DL (2008) Heart failure with preserved ejection fraction. Eur Cardiol 4(1):31–33
2. Tannenbaum S, Sayer GT (2015) Advances in the pathophysiology and treatment of heart failure with preserved ejection fraction. Curr Opin Cardiol 30(3):250–258
3. Halade GV, Kain V, Ingle KA (2018) Heart functional and structural compendium of cardiosplenic and cardiorenal networks in acute and chronic heart failure pathology. Am J Physiol Heart Circ Physiol 314(2):H255–Hh67
4. Westman PC, Lipinski MJ, Luger D, Waksman R, Bonow RO, Wu E et al (2016) Inflammation as a driver of adverse left ventricular remodeling after acute myocardial infarction. J Am Coll Cardiol 67(17):2050–2060
5. Duarte Vera YC, Caceres Vinueza SV, Daher Nader JE, Lara Teran JF (2018) A novel agent in the treatment of heart failure with depressed systolic function. Archivos de cardiologia de Mexico 88(4):287–297
6. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M et al (2016) Executive summary: heart disease and stroke statistics--2016 update: a report from the American Heart Association. Circulation 133(4):447–454
7. Savarese G, Lund LH (2017) Global public health burden of heart failure. Card Fail Rev 3(1):7–11
8. Halade GV, Kain V, Wright GM, Jadapalli JK (2018) Subacute treatment of carprofen facilitate splenocardiac resolution deficit in cardiac injury. J Leukoc Biol 104(6):1173–1186
9. Krishnan V, Booker D, Cunningham G, Jadapalli JK, Kain V, Pullen AB et al (2019) Pretreatment of carprofen impaired initiation of inflammatory- and overlapping resolution response and promoted cardiorenal syndrome in heart failure. Life Sci 218:224–232
10. McNeil JJ, Wolfe R, Woods RL, Tonkin AM, Donnan GA, Nelson MR et al (2018) Effect of aspirin on

cardiovascular events and bleeding in the healthy elderly. N Engl J Med 379(16):1509–1518
11. Banovic M, Pusnik-Vrckovnik M, Nakou E, Vardas P (2018) Myocardial regeneration therapy in heart failure: current status and future therapeutic implications in clinical practice. Int J Cardiol 260:124–130
12. Fredman G, Spite M (2017) Specialized pro-resolving mediators in cardiovascular diseases. Mol Asp Med 58:65–71
13. Buckley CD, Gilroy DW, Serhan CN (2014) Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity 40(3):315–327
14. Kohli P, Levy BD (2009) Resolvins and protectins: mediating solutions to inflammation. Br J Pharmacol 158(4):960–971
15. Sugimoto MA, Sousa LP, Pinho V, Perretti M, Teixeira MM (2016) Resolution of inflammation: what controls its onset? Front Immunol 7:160
16. Dick SA, Epelman S (2016) Chronic heart failure and inflammation: what do we really know? Circ Res 119(1):159–176
17. Jahng JW, Song E, Sweeney G (2016) Crosstalk between the heart and peripheral organs in heart failure. Exp Mol Med 48:e217
18. Halade GV, Norris PC, Kain V, Serhan CN, Ingle KA (2018) Splenic leukocytes define the resolution of inflammation in heart failure. Sci Signal 11(520):eaao1818
19. Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. Nature 510(7503):92–101
20. Frangogiannis NG (2006) The mechanistic basis of infarct healing. Antioxid Redox Signal 8(11–12):1907–1939
21. Epelman S, Liu PP, Mann DL (2015) Role of innate and adaptive immune mechanisms in cardiac injury and repair. Nat Rev Immunol 15(2):117–129
22. Zouggari Y, Ait-Oufella H, Bonnin P, Simon T, Sage AP, Guerin C et al (2013) B lymphocytes trigger monocyte mobilization and impair heart function after acute myocardial infarction. Nat Med 19(10):1273–1280
23. Lai SL, Marin-Juez R, Stainier DYR (2018) Immune responses in cardiac repair and regeneration: a comparative point of view. Cell Mol Life Sci CMLS76(7):1365–1380
24. Shinde AV, Frangogiannis NG (2014) Fibroblasts in myocardial infarction: a role in inflammation and repair. J Mol Cell Cardiol 70:74–82
25. Aurora AB, Olson EN (2014) Immune modulation of stem cells and regeneration. Cell Stem Cell 15(1):14–25
26. Madjid M, Fatemi O (2013) Components of the complete blood count as risk predictors for coronary heart disease: in-depth review and update. Tex Heart Inst J 40(1):17–29
27. Swirski FK, Nahrendorf M (2013) Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science (New York, NY) 339(6116):161–166
28. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R et al (2014) Macrophages are required for neonatal heart regeneration. J Clin Invest 124(3):1382–1392
29. Halade GV, Dorbane A, Ingle KA, Kain V, Schmitter JM, Rhourri-Frih B (2018) Comprehensive targeted and non-targeted lipidomics analyses in failing and non-failing heart. Anal Bioanal Chem 410(7):1965–1976
30. Tourki B, Halade G (2017) Leukocyte diversity in resolving and nonresolving mechanisms of cardiac remodeling. FASEB J 31(10):4226–4239
31. Jadapalli JK, Halade GV (2018) Unified nexus of macrophages and maresins in cardiac reparative mechanisms. FASEB J 32(10):5227–5237
32. Frangogiannis NG (2014) The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol 11(5):255–265
33. Frangogiannis NG (2012) Regulation of the inflammatory response in cardiac repair. Circ Res 110(1):159–173
34. Robinette CD, Fraumeni JF Jr (1977) Splenectomy and subsequent mortality in veterans of the 1939-45 war. Lancet (London, UK) 2(8029):127–129
35. Elajami TK, Colas RA, Dalli J, Chiang N, Serhan CN, Welty FK (2016) Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. FASEB J 30(8):2792–2801
36. Serhan CN (2017) Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. FASEB J 31(4):1273–1288
37. Frodermann V, Nahrendorf M (2017) Neutrophil-macrophage cross-talk in acute myocardial infarction. Eur Heart J 38(3):198–200
38. Swirski FK (2015) Inflammation and repair in the ischaemic myocardium. Hamostaseologie 35(1):34–36
39. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M et al (2017) Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. Eur Heart J 38(3):187–197
40. Ma Y, Yabluchanskiy A, Iyer RP, Cannon PL, Flynn ER, Jung M et al (2016) Temporal neutrophil polarization following myocardial infarction. Cardiovasc Res 110(1):51–61
41. Eming SA, Wynn TA, Martin P (2017) Inflammation and metabolism in tissue repair and regeneration. Science (New York, NY) 356(6342):1026–1030
42. Heidt T, Courties G, Dutta P, Sager HB, Sebas M, Iwamoto Y et al (2014) Differential contribution of monocytes to heart macrophages in steady-state and after myocardial infarction. Circ Res 115(2):284–295
43. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B et al (2014) Embryonic

and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. Immunity 40(1):91–104
44. Gombozhapova A, Rogovskaya Y, Shurupov V, Rebenkova M, Kzhyshkowska J, Popov SV et al (2017) Macrophage activation and polarization in post-infarction cardiac remodeling. J Biomed Sci 24(1):13
45. Weinberger T, Schulz C (2015) Myocardial infarction: a critical role of macrophages in cardiac remodeling. Front Physiol 6:107
46. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M et al (2013) Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 38(1):79–91
47. Linehan E, Fitzgerald DC (2015) Ageing and the immune system: focus on macrophages. Eu J Microbiol immunol 5(1):14–24
48. Chen B, Frangogiannis NG (2016) Macrophages in the remodeling failing heart. Circ Res 119(7):776–778
49. Frangogiannis NG (2015) Emerging roles for macrophages in cardiac injury: cytoprotection, repair, and regeneration. J Clin Invest 125(8):2927–2930
50. Honold L, Nahrendorf M (2018) Resident and monocyte-derived macrophages in cardiovascular disease. Circ Res 122(1):113–127
51. Lindsey ML, Saucerman JJ, DeLeon-Pennell KY (2016) Knowledge gaps to understanding cardiac macrophage polarization following myocardial infarction. Biochim Biophys Acta 1862(12):2288–2292
52. Ma Y, Mouton AJ, Lindsey ML (2018) Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction. Trans Res J Lab Clin Med 191:15–28
53. Shiraishi M, Shintani Y, Shintani Y, Ishida H, Saba R, Yamaguchi A et al (2016) Alternatively activated macrophages determine repair of the infarcted adult murine heart. J Clin Invest 126(6):2151–2166
54. Fraccarollo D, Galuppo P, Bauersachs J (2012) Novel therapeutic approaches to post-infarction remodelling. Cardiovasc Res 94(2):293–303
55. Ariel A, Serhan CN (2012) New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. Front Immunol 3:4
56. Stables MJ, Shah S, Camon EB, Lovering RC, Newson J, Bystrom J et al (2011) Transcriptomic analyses of murine resolution-phase macrophages. Blood 118(26):e192–e208
57. Koh TJ, DiPietro LA (2011) Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med 13:e23
58. Chiurchiu V, Leuti A, Dalli J, Jacobsson A, Battistini L, Maccarrone M et al (2016) Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. Sci Transl Med 8(353):353ra111
59. Halade GV, Black LM, Verma MK (2018) Paradigm shift – metabolic transformation of docosahexaenoic and eicosapentaenoic acids to bioactives exemplify the promise of fatty acid drug discovery. Biotechnol Adv 36(4):935–953
60. Dalli J, Zhu M, Vlasenko NA, Deng B, Haeggstrom JZ, Petasis NA et al (2013) The novel 13S,14S-epoxy-maresin is converted by human macrophages to maresin 1 (MaR1), inhibits leukotriene A4 hydrolase (LTA4H), and shifts macrophage phenotype. FASEB J 27(7):2573–2583
61. Dalli J, Serhan CN (2012) Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood 120(15):e60–e72
62. Sansbury BE, Spite M (2016) Resolution of acute inflammation and the role of Resolvins in immunity, thrombosis, and vascular biology. Circ Res 119(1):113–130
63. Marcon R, Bento AF, Dutra RC, Bicca MA, Leite DF, Calixto JB (2013) Maresin 1, a proresolving lipid mediator derived from omega-3 polyunsaturated fatty acids, exerts protective actions in murine models of colitis. J Immunol 191(8):4288–4298
64. Lannan KL, Spinelli SL, Blumberg N, Phipps RP (2017) Maresin 1 induces a novel pro-resolving phenotype in human platelets. J Thromb haemost: JTH 15(4):802–813
65. Uderhardt S, Herrmann M, Oskolkova OV, Aschermann S, Bicker W, Ipseiz N et al (2012) 12/15-lipoxygenase orchestrates the clearance of apoptotic cells and maintains immunologic tolerance. Immunity 36(5):834–846
66. Kobayashi N, Karisola P, Pena-Cruz V, Dorfman DM, Jinushi M, Umetsu SE et al (2007) TIM-1 and TIM-4 glycoproteins bind phosphatidylserine and mediate uptake of apoptotic cells. Immunity 27(6):927–940
67. Hellmann J, Tang Y, Kosuri M, Bhatnagar A, Spite M (2011) Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese-diabetic mice. FASEB J 25(7):2399–2407
68. Titos E, Rius B, Gonzalez-Periz A, Lopez-Vicario C, Moran-Salvador E, Martinez-Clemente M et al (2011) Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. J Immunol 187(10):5408–5418
69. Cash JL, Norling LV, Perretti M (2014) Resolution of inflammation: targeting GPCRs that interact with lipids and peptides. Drug Discov Today 19(8):1186–1192
70. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G et al (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196(8):1025–1037

71. Serhan CN, Hamberg M, Samuelsson B (1984) Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc Natl Acad Sci U S A 81(17):5335–5339
72. Newson J, Stables M, Karra E, Arce-Vargas F, Quezada S, Motwani M et al (2014) Resolution of acute inflammation bridges the gap between innate and adaptive immunity. Blood 124(11):1748–1764
73. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192(8):1197–1204
74. Sansbury BE, Spite M (2016) Resolution of acute inflammation and the role of Resolvins in immunity, thrombosis, and vascular biology. Circ Res 119(1):113–130
75. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN et al (2015) Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol 84:24–35
76. Serhan CN, Chiang N, Van Dyke TE (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8(5):349–361
77. Colas RA, Souza PR, Walker ME, Burton M, Zaslona Z, Curtis AM et al (2018) Impaired production and diurnal regulation of vascular RvDn-3 DPA increase systemic inflammation and cardiovascular disease. Circ Res 122(6):855–863
78. Serhan CN, Yacoubian S, Yang R (2008) Anti-inflammatory and proresolving lipid mediators. Annu Rev Pathol 3:279–312
79. Hong S, Lu Y, Yang R, Gotlinger KH, Petasis NA, Serhan CN (2007) Resolvin D1, protectin D1, and related docosahexaenoic acid-derived products: analysis via electrospray/low energy tandem mass spectrometry based on spectra and fragmentation mechanisms. J Am Soc Mass Spectrom 18(1):128–144
80. Jadapalli JK, Halade GV (2018) Unified nexus of macrophages and maresins in cardiac reparative mechanisms. FASEB J 32(10):5227–5237
81. Gilbert K, Bernier J, Bourque-Riel V, Malick M, Rousseau G (2015) Resolvin D1 reduces infarct size through a phosphoinositide 3-kinase/protein kinase B mechanism. J Cardiovasc Pharmacol 66(1):72–79
82. Gilbert K, Malick M, Madingou N, Bourque-Riel V, Touchette C, Rousseau G (2016) Linoleic acid attenuates cardioprotection induced by resolvin D1. J Nutr Biochem 31:122–126
83. Halade GV, Kain V, Serhan CN (2018) Immune responsive resolvin D1 programs myocardial infarction-induced cardiorenal syndrome in heart failure. FASEB J 32(7):3717–3729
84. Zhang MJ, Sansbury BE, Hellmann J, Baker JF, Guo L, Parmer CM et al (2016) Resolvin D2 enhances Postischemic revascularization while resolving inflammation. Circulation 134(9):666–680
85. Serhan CN, Petasis NA (2011) Resolvins and protectins in inflammation resolution. Chem Rev 111(10):5922–5943
86. Varga Z, Sabzwari SRA, Vargova V (2017) Cardiovascular risk of nonsteroidal anti-inflammatory drugs: an under-recognized public health issue. Cureus 9(4):e1144
87. Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T et al (2002) Role of prostacyclin in the cardiovascular response to thromboxane A2. Science (New York, NY) 296(5567):539–541
88. Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J et al (2014) Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B4 synthesis by inhibiting a calcium-activated kinase pathway. Proc Natl Acad Sci USA 111(40):14530–14535
89. Blomer N, Pachel C, Hofmann U, Nordbeck P, Bauer W, Mathes D et al (2013) 5-lipoxygenase facilitates healing after myocardial infarction. Basic Res Cardiol 108(4):367
90. Clària J, Dalli J, Yacoubian S, Gao F, Serhan CN (2012) Resolvin D1 and resolvin D2 govern local inflammatory tone in obese fat. J Immunol 189(5):2597–2605
91. Arnardottir HH, Dalli J, Colas RA, Shinohara M, Serhan CN (2014) Aging delays resolution of acute inflammation in mice: reprogramming the host response with novel nano-proresolving medicines. J Immunol 193(8):4235–4244
92. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S et al (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med 201(5):713–722
93. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S et al (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med 201(5):713–722
94. Schwab JM, Chiang N, Arita M, Serhan CN (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature 447(7146):869–874
95. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN (2012) Resolvin E2 formation and impact in inflammation resolution. J Immunol 188(9):4527–4534
96. Levy BD (2010) Resolvins and protectins: natural pharmacophores for resolution biology. Prostaglandins Leukot Essent Fatty Acids 82(4–6):327–332
97. Chiang N, de la Rosa X, Libreros S, Serhan CN (2017) Novel Resolvin D2 receptor axis in infectious inflammation. J Immunol 198(2):842–851
98. Serhan CN (2007) Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 25:101–137

99. Samuelsson B, Hammarstrom S, Hamberg M, Serhan CN (1985) Structural determination of leukotrienes and lipoxins. Adv Prostaglandin Thromboxane Leukot Res 14:45–71
100. Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M (2005) A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. J Biol Chem 280(15):15267–15278
101. Stables MJ, Gilroy DW (2011) Old and new generation lipid mediators in acute inflammation and resolution. Prog Lipid Res 50(1):35–51
102. Kain V, Liu F, Kozlovskaya V, Ingle KA, Bolisetty S, Agarwal A et al (2017) Resolution agonist 15-epi-Lipoxin A_4 programs early activation of resolving phase in post-myocardial infarction healing. Sci Rep 7(1):9999
103. Kain V, Liu F, Kozlovskaya V, Ingle KA, Bolisetty S, Agarwal A et al (2017) Resolution agonist 15-epi-Lipoxin A_4 programs early activation of resolving phase in post-myocardial infarction healing. Sci Rep 7(1):9999
104. Maderna P, Cottell DC, Toivonen T, Dufton N, Dalli J, Perretti M et al (2010) FPR2/ALX receptor expression and internalization are critical for lipoxin A_4 and annexin-derived peptide-stimulated phagocytosis. FASEB J 24(11):4240–4249
105. Akagi D, Chen M, Toy R, Chatterjee A, Conte MS (2015) Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. FASEB J 29(6):2504–2513
106. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF et al (2009) Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med 206(1):15–23
107. Liu G, Liu Q, Shen Y, Kong D, Gong Y, Tao B et al (2018) Early treatment with Resolvin E1 facilitates myocardial recovery from ischaemia in mice. Br J Pharmacol 175(8):1205–1216
108. Colas RA, Dalli J, Chiang N, Vlasakov I, Sanger JM, Riley IR et al (2016) Identification and Actions of the Maresin 1 Metabolome in Infectious Inflammation. J Immunol 197(11):4444–4452
109. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C et al (2016) Resolving lipid mediators Maresin 1 and Resolvin D2 prevent Atheroprogression in mice. Circ Res 119(9):1030–1038
110. Tang S, Wan M, Huang W, Stanton RC, Xu Y (2018) Maresins: specialized Proresolving lipid mediators and their potential role in inflammatory-related diseases. Mediat Inflamm 2018:2380319
111. Claria J, Lopez-Vicario C, Rius B, Titos E (2017) Pro-resolving actions of SPM in adipose tissue biology. Mol Asp Med 58:83–92
112. Lee HN, Surh YJ (2013) Resolvin D1-mediated NOX2 inactivation rescues macrophages undertaking efferocytosis from oxidative stress-induced apoptosis. Biochem Pharmacol 86(6):759–769
113. Jove M, Mate I, Naudi A, Mota-Martorell N, Portero-Otin M, De la Fuente M et al (2016) Human aging is a metabolome-related matter of gender. J Gerontol A Biol Sci Med Sci 71(5):578–585
114. Dalli J, Serhan CN (2017) Pro-resolving mediators in regulating and conferring macrophage function. Front Immunol 8:1400
115. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AHK, Pande R et al (2010) Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. Am J Pathol 177(4):2116–2123
116. Serhan CN, Takano T, Clish CB, Gronert K, Petasis N (1999) Aspirin-triggered 15-epi-lipoxin A4 and novel lipoxin B4 stable analogs inhibit neutrophil-mediated changes in vascular permeability. Adv Exp Med Biol 469:287–293
117. Lee CH (2012) Resolvins as new fascinating drug candidates for inflammatory diseases. Arch Pharm Res 35(1):3–7
118. Halade GV, Kain V, Wright GM, Jadeapalli JK (2018) Subacute treatment of carprofen facilitate splenocardiac resolution deficit in cardiac injury. J Leukoc Biol 104(6):1173–1186
119. Jadapalli JK, Wright GW, Kain V, Sherwani MA, Sonkar R, Yusuf N et al (2018) Doxorubicin triggers splenic contraction and irreversible dysregulation of COX and LOX that alters the inflammation-resolution program in the myocardium. Am J Physiol Heart Circ Physiol 315(5):H1091–Hh100
120. Halade GV, Kain V (2017) Obesity and Cardiometabolic defects in heart failure pathology. Compr Physiol 7(4):1463–1477
121. Tourki B, Halade GV (2018) The failing of the obesity paradox in the failing heart. Am J Physiol Heart Circ Physiol 315:H1353–H1355
122. Spite M, Clària J, Serhan CN (2014) Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. Cell Metab 19(1):21–36
123. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee C-H, Yang R et al (2010) Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proc Natl Acad Sci U S A 107(4):1660–1665
124. Lopez EF, Kabarowski JH, Ingle KA, Kain V, Barnes S, Crossman DK et al (2015) Obesity superimposed on aging magnifies inflammation and delays the resolving response after myocardial infarction. Am J Physiol Heart Circ Physiol 308(4):H269–H280
125. Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, Del Cañizo-Gómez FJ (2014) Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? World J Diabetes 5(4):444–470
126. Freire MO, Dalli J, Serhan CN, Van Dyke TE (2017) Neutrophil Resolvin E1 receptor expression and function in type 2 diabetes. J Immunol 198(2):718–728

127. Ferrante AW Jr (2007) Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. J Intern Med 262(4):408–414
128. Ouchi N, Parker JL, Lugus JJ, Walsh K (2011) Adipokines in inflammation and metabolic disease. Nat Rev Immunol 11(2):85–97
129. Hotamisligil GS (2006) Inflammation and metabolic disorders. Nature 444(7121):860–867
130. Hellmann J, Tang Y, Kosuri M, Bhatnagar A, Spite M (2011) Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese-diabetic mice. FASEB J 25(7):2399–2407
131. Titos E, Rius B, González-Périz A, López-Vicario C, Morán-Salvador E, Martínez-Clemente M et al (2011) Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. J Immunol 187(10):5408–5418
132. Brennan EP, Mohan M, McClelland A, de Gaetano M, Tikellis C, Marai M et al (2018) Lipoxins protect against inflammation in diabetes-associated atherosclerosis. Diabetes 67(12):2657–2667
133. Tang Y, Zhang MJ, Hellmann J, Kosuri M, Bhatnagar A, Spite M (2013) Proresolution therapy for the treatment of delayed healing of diabetic wounds. Diabetes 62(2):618–627
134. Kannan A, Janardhanan R (2014) Hypertension as a risk factor for heart failure. Curr Hypertens Rep 16(7):447
135. Pearson T, Zhang J, Arya P, Warren AY, Ortori C, Fakis A et al (2010) Measurement of vasoactive metabolites (hydroxyeicosatetraenoic and epoxyeicosatrienoic acids) in uterine tissues of normal and compromised human pregnancy. J Hypertens 28(12):2429–2437
136. Didion SP (2017) Unraveling the role and complexities of inflammation in hypertension, Hypertension (Dallas, Tex: 1979). 70(4):700–702
137. Pullamsetti SS, Savai R, Janssen W, Dahal BK, Seeger W, Grimminger F et al (2011) Inflammation, immunological reaction and role of infection in pulmonary hypertension. Clin Microbiol Infect 17(1):7–14
138. Hiram R, Rizcallah E, Sirois C, Sirois M, Morin C, Fortin S et al (2014) Resolvin D1 reverses reactivity and Ca2+ sensitivity induced by ET-1, TNF-alpha, and IL-6 in the human pulmonary artery. Am J Physiol Heart Circ Physiol 307(11):H1547–H1558
139. McAlpine CS, Swirski FK (2016) Circadian influence on metabolism and inflammation in atherosclerosis. Circ Res 119(1):131–141
140. Perretti M, D'Acquisto F (2009) Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol 9(1):62–70
141. Krump E, Picard S, Mancini J, Borgeat P (1997) Suppression of leukotriene B4 biosynthesis by endogenous adenosine in ligand-activated human neutrophils. J Exp Med 186(8):1401–1406
142. Ingle KA, Kain V, Goel M, Prabhu SD, Young ME, Halade GV (2015) Cardiomyocyte-specific Bmal1 deletion in mice triggers diastolic dysfunction, extracellular matrix response, and impaired resolution of inflammation. Am J Physiol Heart Circ Physiol 309(11):H1827–H1836
143. Young ME, Brewer RA, Peliciari-Garcia RA, Collins HE, He L, Birky TL et al (2014) Cardiomyocyte-specific BMAL1 plays critical roles in metabolism, signaling, and maintenance of contractile function of the heart. J Biol Rhythm 29(4):257–276
144. Colas RA, Souza PR, Walker ME, Burton M, Zasłona Z, Curtis AM et al (2018) Impaired production and diurnal regulation of vascular $RvD_{n-3\ DPA}$ increase systemic inflammation and cardiovascular disease. Circ Res 122(6):855–863
145. Alvarez P, Hannawi B, Guha A (2016) Exercise and heart failure: advancing knowledge and improving care. Methodist Debakey Cardiovasc J 12(2):110–115
146. Markworth JF, Vella L, Lingard BS, Tull DL, Rupasinghe TW, Sinclair AJ et al (2013) Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. Am J Physiol Regul Integr Comp Physiol 305(11):R1281–R1296
147. Gangemi S, Pescara L, D'Urbano E, Basile G, Nicita-Mauro V, Davì G et al (2005) Aging is characterized by a profound reduction in anti-inflammatory lipoxin A4 levels. Exp Gerontol 40(7):612–614
148. Taylor RS, Sagar VA, Davies EJ, Briscoe S, Coats AJ, Dalal H et al (2014) Exercise-based rehabilitation for heart failure. Cochrane Database Syst Rev (4):Cd003331
149. Hoetker D, Chung W, Zhang D, Zhao J, Schmidtke VK, Riggs DW et al (2018) Exercise alters and beta-alanine combined with exercise augments histidyl dipeptide levels and scavenges lipid peroxidation products in human skeletal muscle. J Appl Physiol (1985). https://doi.org/10.1152/japplphysiol.00007.2018
150. Meschiari CA, Ero OK, Pan H, Finkel T, Lindsey ML (2017) The impact of aging on cardiac extracellular matrix. GeroScience 39(1):7–18
151. Franceschi C, Campisi J (2014) Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 69(Suppl 1):S4–S9
152. Yabluchanskiy A, Ma Y, DeLeon-Pennell KY, Altara R, Halade GV, Voorhees AP et al (2016) Myocardial infarction superimposed on aging: MMP-9 deletion promotes M2 macrophage polarization. J Gerontol A Biol Sci Med Sci 71(4):475–483
153. Yabluchanskiy A, Ma Y, Chiao YA, Lopez EF, Voorhees AP, Toba H et al (2014) Cardiac aging is initiated by matrix metalloproteinase-9-mediated endothelial dysfunction. Am J Physiol Heart Circ Physiol 306(10):H1398–H1407

154. Melton DW, Roberts AC, Wang H, Sarwar Z, Wetzel MD, Wells JT et al (2016) Absence of CCR2 results in an inflammaging environment in young mice with age-independent impairments in muscle regeneration. J Leukoc Biol 100(5):1011–1025
155. Halade GV, Kain V, Black LM, Prabhu SD, Ingle KA (2016) Aging dysregulates D- and E-series resolvins to modulate cardiosplenic and cardiorenal network following myocardial infarction. Aging 8(11):2611–2634
156. Jové M, Maté I, Naudí A, Mota-Martorell N, Portero-Otín M, De la Fuente M et al (2016) Human aging is a metabolome-related matter of gender. J Gerontol A Biol Sci Med Sci 71(5):578–585
157. Buford TW. (Dis)Trust your gut: the gut microbiome in age-related inflammation, health, and disease. Microbiome. 2017;5(\):80
158. Zabell A, Tang WHW (2017) Targeting the microbiome in heart failure. Curr Treat Options Cardiovasc Med 19(4):27
159. Luedde M, Winkler T, Heinsen F-A, Rühlemann MC, Spehlmann ME, Bajrovic A et al (2017) Heart failure is associated with depletion of core intestinal microbiota. ESC Heart Fail 4(3):282–290
160. Menni C, Zierer J, Pallister T, Jackson MA, Long T, Mohney RP et al (2017) Omega-3 fatty acids correlate with gut microbiome diversity and production of N-carbamylglutamate in middle aged and elderly women. Sci Rep 7(1):11079

7 Novel n-3 Docosapentaneoic Acid-Derived Pro-resolving Mediators Are Vasculoprotective and Mediate the Actions of Statins in Controlling Inflammation

Jesmond Dalli, Kimberly Pistorius, and Mary E. Walker

Abstract

Inflammation is a fundamentally protective process that guards the host from invading pathogens and is central in the repair and regeneration of damaged tissue. However, when uncontrolled, the overzealous response leads to tissue damage and malaise. Indeed, this process is now appreciated to be at the center of many chronic inflammatory diseases including vascular disease and arthritis. Studies investigating the mechanisms through which acute inflammation is actively turned off allowing tissues to regain function demonstrated that the essential fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are enzymatically converted to bioactive mediators. These autacoids carry distinct structures and biological actions, actively reprogramming the inflammatory reaction to promote its termination by counter-regulating the production of pro-inflammatory mediators and regulate leukocyte trafficking as well as phenotype. Recently we found that n-3 docosapentaenoic acid (DPA), which was until then only regarded as a biosynthetic intermediate in the formation of DHA from EPA, is also converted to structurally distinct bioactive mediators that reprogram the host immune response. In the present review we will discuss the evidence underpinning the biological actions of these novel n-3 DPA-derived autacoids in particular as they pertain to the vascular system.

Keywords

n-3 docosapentaenoic acid · Resolution · Inflammation · Lipid mediators · Vascular inflammation · Leukocytes · Statins · Biomarkers

7.1 Introduction

Success in the evolution of multicellular organisms was, at least in part, reliant on the development of a system to repair and regenerate

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damaged tissues as well as to defend the organism from invading microbial pathogens [1]. This defense system is embodied in the inflammatory process which when self-limited is fundamentally protective and coordinates both the killing and disposal of invading pathogens as well as the repair and regeneration of damaged tissues [2]. However, when this process becomes dysregulated it leads to disease [3–7]. Pioneering studies investigating mechanisms that regulate the termination of inflammation uncovered a new genus of mediators produced via the stereoselective conversion of essential fatty acids, termed as specialized pro-resolving mediators (SPM) [8]. This superfamily includes the arachidonic acid (AA)-derived lipoxins, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) resolvins [9], and the DHA-derived protectins [10] and maresins [11]. These mediators share select biological actions that include i) limiting neutrophil recruitment to the site of inflammation, ii) they counter-regulate the production of pro-inflammatory mediators including prostaglandins, leukotrienes, cytokines and chemokines (e.g. Tumor necrosis factor alpha), iii) promote the uptake and killing of bacteria and iv) increase the uptake and clearance of apoptotic cells [3–6, 8]. In addition, each of the mediators exerts unique biological actions for example, the EPA-derived resolvin E1 regulates platelet activation [12], the DHA-derived protectins regulate viral replication [13] and the DHA-derived maresins promote tissue regeneration [3, 14].

SPM exert their potent biological actions via the activation of specific G-protein coupled receptors which include the Lipoxin A_4 receptor (ALX/FPR2), GPR32/DRV1, GPR18/DRV2 and the chemerin receptor ChemR23/ERV1 [15]. In addition to activating cognate receptors, these protective mediators also regulate the onset and propagation of inflammatory responses by acting as partial agonists or antagonists to receptors of inflammatory mediators including BLT-1, the Leukotriene (LT)B_4 (5S,12R-dihydroxy- 6Z,8E,10E,14Z- eicosatetraenoic acid) receptor [16], and cysLT1 the receptor for LTC_4 (5S-hydroxy-6R-(S-glutathionyl)-7E,9E,11Z,14Z-eicosatetraenoic acid) and LTD_4 (5S-hydroxy-6R-(S-cysteinylglycinyl)-7E,9E,11Z,14Z-eicosatetraenoic acid) [16]. Studies investigating mechanisms involved in the onset and propagation of inflammatory disorders indicate defects in the production of the pro-resolving mediators, their enhanced further metabolism or an impairment in their ability to activate downstream signaling via their cognate receptors are linked with disease onset and/or progression [3–6, 8].

In mammals, the essential fatty acid alpha-linolenic acid (9Z, 12Z, 15Z-octadecatrienoic acid; ALA) is enzymatically elongated and desaturated to produce EPA and subsequently to DHA with n-3 docosapentaenoic acid (7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid; n-3 DPA) being the biosynthetic intermediate in this process [17–19]. n-3 DPA contains a 22-carbon chain with 5 double bonds and differs from DHA as it lacks a cis-double bond at carbon 4, which, although a small change, provides its own specific biologically relevant actions [19, 20]. These actions have been identified in a variety of mammalian tissues, including plasma, brain, retina and heart. Genome-wide association studies in humans uncovered a correlation between increases in peripheral blood n-3 DPA concentrations and single nucleotide polymorphisms in the gene encoding for the fatty acid elongase 2 (ELOVL2, 21]. We recently found that, in addition to EPA and DHA, n-3 DPA is substrate for conversion to novel families of bioactive mediators [20]. The aim of the present review is to discuss the actions of these novel families of mediators in regulating key leukocyte responses.

7.2 n-3 DPA-Derived SPM Are Novel Resolution Agonists

Events that occur in the early phases of an inflammatory reaction are suggested to determine whether the response is self-limited or perpetuates and becomes chronic [22]. Assessment of events occurring within the circulation during acute self-limited inflammation demonstrated that the concentrations of n-3 DPA were rapidly

upregulated during acute inflammation, to an extent that was comparable to essential fatty acids that are involved in the biosynthesis of lipid mediators, including arachidonic acid and DHA. Using a systematic approach coupling structure elucidation with functional readouts we found that endogenous n-3 DPA is converted to bioactive mediators in both mice and human leukocytes that carried pro-resolving properties [20]. These mediators are congenerous with DHA products, namely D-series resolvins ($RvD_{n-3\ DPA}$), protectins ($PD_{n-3\ DPA}$) and maresins ($MaR_{n-3\ DPA}$), with unique stereochemistries [20]. In self resolving exudates, the production of these molecules was temporally regulated where for example $RvD1_{n-3\ DPA}$ (7S,8R,17S-trihydroxy-9E,11E,13Z,15E,19Z -docosapentaenoic acid) and $PD2_{n-3\ DPA}$ (16,17-dihydroxy-7Z,10,13, 14,19Z- docosapentaenoic acid) displayed a bi-phasic profile, reaching a maximum during peak neutrophil infiltration and late into resolution. $PD1_{n-3\ DPA}$ (10R,17S-dihydroxy-7Z,11E,13E,15Z,19Z-docosapentaenoic acid), $MaR2_{n-3\ DPA}$ (13,14-dihydroxy-7Z,9,11, 16Z, 19Z-docosapentaenoic acid) and $MaR3_{n-3\ DPA}$ (4, 21-dihydroxy-7Z,10Z,12E,16Z,19Z-docosapentaenoic acid) levels were each found to reach a maximum at the 4 h interval and gradually decreased over the next 20 h. The peak in exudate $RvD2_{n-3\ DPA}$ (7S,16,17S-trihydroxy-8,10Z,12,14,19Z-docosapentaenoic acid) levels coincided with the onset of resolution (the point where PMN levels reach ~50% of maximim neutrophil counts). $RvD5_{n-3\ DPA}$ (7S,17S-dihydroxy-4Z,8,10Z,13Z,15,19Z-docosahexaenoic acid) levels were found to gradually increase over the course of inflammation-resolution, with a maximum being reached late in the resolution phase. The n-3 DPA product corresponding to $MaR1_{n-3\ DPA}$ (7S,14S-dihydroxy-8E, 10E, 12Z, 16Z, 19Z-docosapentaenoic acid) gave levels that were elevated in the peritoneum of naive mice, where upon challenge with zymosan these levels drastically decreased [20]. Of note, each of these molecules displayed leukocyte directed actions whereby incubation of human neutrophils with $RvD5_{n-3\ DPA}$ or $PD1_{n-3\ DPA}$ markedly reduced neutrophil adhesion to TNF-α activated endothelial cells and chemotaxis towards IL-8, to a similar extent as the DHA-derived RvD2 (7S,16R,17S-trihydroxy- 4Z,8E,10Z,12E,14E,19Z- docosahexaenoic acid) [20, 23, 24]. $RvD2_{n-3\ DPA}$ and $RvD5_{n-3\ DPA}$ also regulate the expression of adhesion molecules on peripheral blood leukocytes and platelets including the expression of CD11b on neutrophils and monocytes as well as CD62P and CD63 on platelets [25]. Furthermore, they also regulate the formation of leukocyte-platelet heterotypic aggregates in both human and mouse peripheral blood. The biological actions of these molecules extend beyond the regulation of mechanisms in leukocyte trafficking. Indeed, n-3 DPA-derived SPMs also regulate the uptake of apoptotic cells by macrophages, a key biological action in the resolution of inflammation, with increases in macrophage efferocytosis of up to 70% at doses as low as 1 nM [20, 23]. These mediators also display endothelial directed actions, counteracting the TNF-α- mediated upregulation of adhesion molecules, such as Intercellular Adhesion Molecule 1 (ICAM-1/CD54), on endothelial cells [20].

7.3 Diurnal Regulation of $RvD_{n-3\ DPA}$ Controls Vascular Leukocyte and Platelet Activation

Circadian mechanisms are at the heart of a number of physiological functions, including leukocyte and platelet responses [26, 27]. Disturbances to various aspects of these fundamental mechanisms are thought to be responsible for many of the diseases that afflict modern societies, including cardiovascular and metabolic disorders [26–28]. These conditions are characterized by a dysregulated inflammatory response, although the exact mechanisms that underlie this inflammatory state remain of interest. Recent studies demonstrate that the production of $RvD_{n-3\ DPA}$ are diurnally regulated in the peripheral blood of healthy volunteers [25] (Fig. 7.1). Multivariate analysis of plasma lipid mediator profiles demonstrated a diurnal shift in plasma LM-SPM concentrations. This shift was associated with an

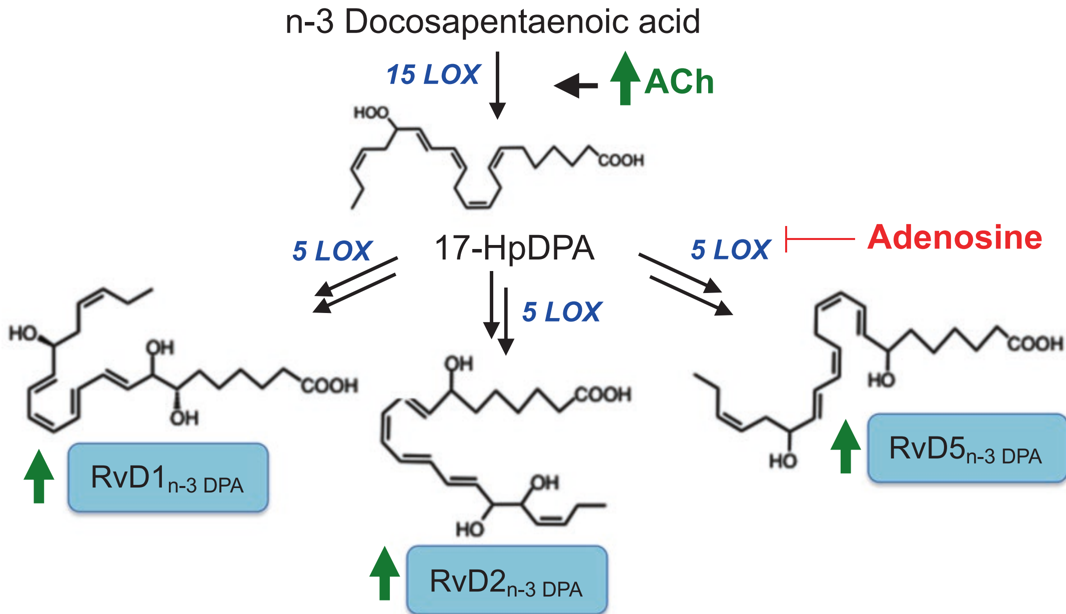


Fig. 7.1 Diurnal changes in RvD$_{n\text{-}3\ DPA}$ regulates peripheral blood leukocyte and platelet activation protecting from cardiovascular disease. In peripheral blood diurnal changes in acetylcholine (ACh) upregulates 15-lipoxygenase (ALOX15) activity promoting RvD$_{n\text{-}3\ DPA}$ biosynthesis that limit the physiological activation of monocytes, neutrophils and platelets during the early morning hours. Increases in circulating adenosine concentrations in patients with cardiovascular disease inhibit ALOX5 activity disrupting the diurnal changes in plasma RvD$_{n\text{-}3\ DPA}$ and increasing peripheral blood leukocyte and platelet activation

increase in the amounts of n-3 DPA derived mediators, including RvD1$_{n\text{-}3\ DPA}$ and RvD5$_{n\text{-}3\ DPA}$ from the evening (18:00 h) to morning intervals (7:00 and 9:00 h). These diurnal changes in peripheral blood RvD$_{n\text{-}3\ DPA}$ concentrations were abrogated in mice lacking the main orchestrator of the molecular clock, Aryl hydrocarbon receptor nuclear translocator-like protein 1, in myeloid cells. Of note, the fluctuations in plasma RvD$_{n\text{-}3\ DPA}$ were associated with a regulation of leukocyte and platelet activation that reaches a maximum between 7:00 and 9:00 h coincident with an increase in RvD$_{n\text{-}3\ DPA}$ concentrations. The production of these mediators was found to be under the control of acetylcholine (ACh), with peripheral blood concentrations of this neurotransmitter also reaching a maximum during the early hours of the morning (i.e. 7:00 h). Furthermore, incubation of whole blood with ACh increased RvD$_{n\text{-}3\ DPA}$ concentrations, including RvD2$_{n\text{-}3\ DPA}$, under both static and flow conditions.

Assessment of the production of these mediators in patients with cardiovascular disease (CVD) demonstrated significant decreases in plasma RvD$_{n\text{-}3\ DPA}$ concentrations and a marked impairment in their diurnal regulation when compared with healthy volunteers. Flow cytometric analysis of peripheral blood leukocyte from patients with CVD demonstrated increases in the expression of CD11b on both neutrophils and monocytes when compared with healthy volunteers. This was coupled with increases in platelet–neutrophil and platelet–monocyte aggregates in peripheral blood from patients with CVD [25]. In addition, we found a significant relationship between peripheral blood RvD$_{n\text{-}3\ DPA}$

concentrations and leukocyte and platelet activation, as demonstrated by a negative correlation between $RvD_{n\text{-}3\ DPA}$ and neutrophil CD41, monocyte CD41, and platelet CD63 and CD42b expression. Investigations into mechanisms that lead to the downregulation of peripheral blood $RvD_{n\text{-}3\ DPA}$ in patients with CVD demonstrated a link between peripheral blood adenosine concentrations and the activity of one of the $RvD_{n\text{-}3\ DPA}$ biosynthetic enzymes, ALOX5. Adenosine, which via the activation of the A2a receptor, downregulates the activity of ALOX5 [29], was increased in peripheral blood from patients with CVD. The role of adenosine in downregulating $RvD_{n\text{-}3\ DPA}$ concentrations was further underscored by experiments where peripheral blood from CVD patients was incubated with adenosine deaminase, leding to the restoration of peripheral blood $RvD_{n\text{-}3\ DPA}$ concentrations [25].

These findings led us to propose $RvD_{n\text{-}3\ DPA}$ as endogenous protective signals that control physiological platelet and leukocyte activation. This is further supported by observations made in Apolipoprotein E deficient mice ($ApoE^{-/-}$) mice fed a western diet. Treatment of these mice with $RvD5_{n\text{-}3\ DPA}$ reduced platelet-leukocytes aggregates *in vivo* and modulated vascular lipid mediator profiles reducing concentrations of the pro-thrombogenic mediator Thromboxane (Tx) A_2 (measured as its metabolite TxB_2) and upregulating the formation of pro-resolving mediators including MaR1 (7S,14S-dihydroxy-4Z, 8E, 10E, 12Z, 16Z, 19Z-docosahexaenoic acid) and aspirin triggered (AT)-LXA_4 (5S,6R,15R-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid). Furthermore $RvD5_{n\text{-}3\ DPA}$ also decreased early aortic lesions in $ApoE^{-/-}$ mice. The present findings are in line with published findings demonstrating an altered production of vascular DHA-derived SPMs including RvD2 and MaR1 and impaired resolution responses in the pathogenesis of atherosclerosis [30, 31]. Together these findings demonstrate that alterations in the diurnal regulation of vascular $RvD_{n\text{-}3\ DPA}$ may occur early in the pathogenesis of cardiovascular diseases that results in vascular inflammation and impaired biosynthesis of DHA derived SPM.

7.4 $PD_{n\text{-}3\ DPA}$ Regulates Macrophage Phenotype and Function During Monocyte to Macrophage Differentiation

It is now well appreciated that in chronic inflammatory conditions, such as atherosclerosis and rheumatoid arthritis, monocytes play a central role in the initiation, propagation and termination of inflammation [32, 33]. Upon recruitment to the site of inflammation, these cells either differentiate to inflammatory macrophages that propagate the inflammatory response or to resolution phase macrophages that promote the termination of inflammation and restitution of tissue function. Studies investigating mechanism that regulate the differentiation of macrophages demonstrate a role for the $PD_{n\text{-}3\ DPA}$ pathway in regulating the phenotype and function of monocyte-derived macrophages [34] (Fig. 7.2). Using a total organic synthetic approach coupled with lipid mediator profiling, human ALOX15 and ALOX15B were identified as the enzymes that catalyse the first two steps in the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway yielding an allylic epoxide. Using total organic synthesis we established the absolute stereochemistry of this epoxide as 16*S*,17*S*-epoxy-7*Z*,10*Z*,12*E*,14*E*,19*Z*-docosapentaenoic acid [34, 35]. This intermediate was in turn converted to $PD1_{n\text{-}3\ DPA}$ and $PD2_{n\text{-}3\ DPA}$ by distinct epoxide hydrolase enzymes, where in human cells epoxide hydrolase 2 (EPHX2) was found to catalyse the conversion of 16*S*,17*S*-$ePD_{n\text{-}3\ DPA}$ to $PD2_{n\text{-}3\ DPA}$. Experiments investigating the expression of the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway during monocyte to macrophage differentiation found the expression of all three enzymes was upregulated during monocyte to macrophage differentiation. Of note, ALOX15 expression was higher in M2 differentiated cells, whereas the expression of ALOX15B was higher in M1 cells [34].

Pharmacological inhibition and genetic deletion of ALOX15 enzymes in monocytes led to phenotypic and functional changes in monocyte-derived macrophages. In cells where ALOX15

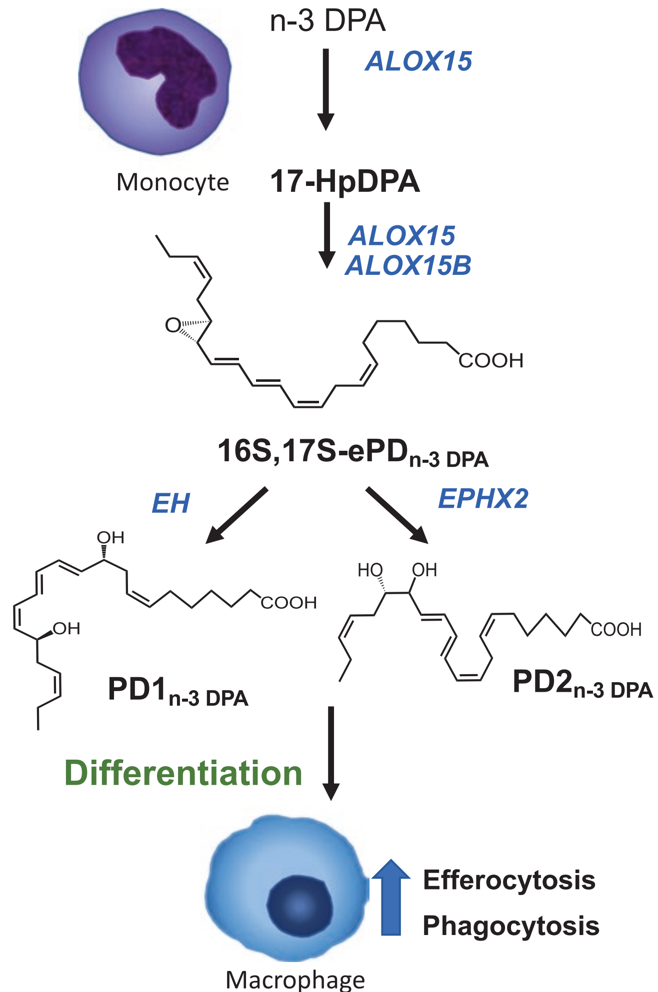


Fig. 7.2 **$PD_{n\text{-}3\ DPA}$ biosynthetic pathway and its regulation of monocyte-derived macrophage phenotype and function.** In the $PD_{n\text{-}3\ DPA}$ pathway n-3 DPA is converted to 17-HpDHA and then to 16S, 17S-epoxy-$PD_{n\text{-}3\ DPA}$ by either ALOX15 or ALOX15B. This is then hydrolyzed by epoxide hydrolase activity to $PD1_{n\text{-}3\ DPA}$ and $PD2_{n\text{-}3\ DPA}$. This pathway regulates macrophage phenotype and function during monocyte-to-macrophage differentiation

was inhibited there was a downregulation of several lineage markers including CD206, CD163 and CD64 and a shift in macrophage phenotype [34]. This downregulation in phagocytic receptors was of functional consequence since inhibition of the $PD_{n\text{-}3\ DPA}$ biosynthetic pathway also significantly downregulated the ability of human macrophages to uptake apoptotic cells, a key pro-resolving action [36, 37]. Of note, this alteration in macrophage phenotype and function was recovered with the reconstitution of components within the $PD_{n\text{-}3\ DPA}$ pathway. Incubation of human monocyte-derived macrophages with either $PD1_{n\text{-}3\ DPA}$ or 16S, 17S-epoxy-$PD_{n\text{-}3\ DPA}$ led to a restoration of several phagocytic receptors and increased macrophage efferocytosis. Furthermore, administration of $PD1_{n\text{-}3\ DPA}$ to ALOX15 deficient mice also restored the phenotype and efferocytic activity of macrophages *in vivo* [34]. Thus, these findings identify the $PD_{n\text{-}3\ DPA}$ pathway as a component in the monocyte-to-macrophage differentiation program that regulates their phenotype and function.

7.5 RvTs Are Produced During the Early Stages of Acute Inflammation and Temper Host Immune Responses

Recent studies have described and characterized a new family of bioactive mediators produced from n-3 DPA. This new family of four resolvins

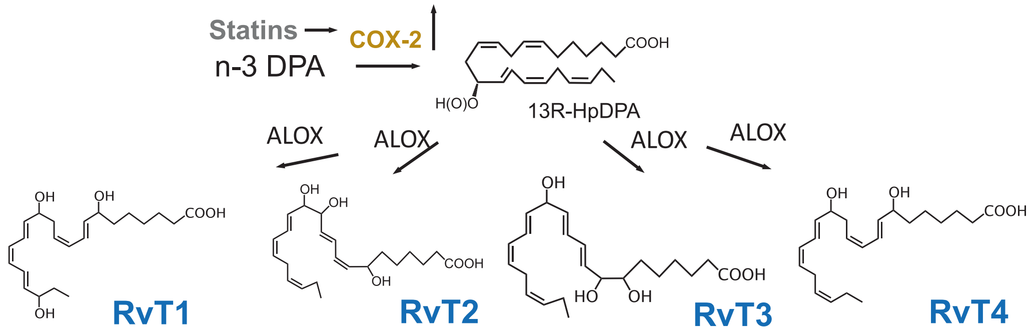
is termed the 13-series resolvins (RvT) given that all four molecules display potent host protective actions and carry a hydroxyl group on carbon 13 [38]. RvTs are biosynthesized from n-3 DPA in a process that requires both endothelial cell COX-2 and neutrophil lipoxygenase (ALOX) activity. The role for ALOX enzymes in the biosynthesis of RvT was established using heavy oxygen incorporation [38] with the identity of the ALOX enzymes catalyzing this reaction remaining of interest.

During acute inflammation, cytokines such as TNF-α and IL-1β are released, activating endothelial cells that upregulate the expression of COX-2. Endothelial COX-2 converts n-3 DPA to 13R-hydro(peroxy)-docosa-7Z,10Z,14E,16Z,19Z-pentaenoic acid (13R-HpDPA); 13R-HpDPA or its reduced alcohol form 13R-hydroxy-docosa-7Z,10Z,14E,16Z,19Z-pentaenoic acid (13R-HDPA) is then donated to neutrophils during neutrophil-endothelial cell interactions, whereby it is first converted to 7-hydro(peroxy)-13R-hydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid [38]. This molecule can then undergo a second lipoxygenation reaction to yield 7,13R,20-trihydroxy-docosa-8,10,14E,16Z,18-pentaenoic acid that was coined as RvT1. The hydroperoxide can also, in a lipoxygenase-dependent manner, undergo an epoxidation reaction to yield the allylic epoxide 7,8-epoxy-13-hydroxy-docosa-9,11,14E,16Z,19Z-pentaenoic acid. This is then enzymatically hydrolysed to 7,12,13-trihydroxy-docosa-8,10,14E,16Z,19Z-pentaenoic acid and 7,8,13-trihydroxy-docosa-9,11,14E,16Z,19Z-pentaenoic acid, coined RvT2 and RvT3, respectively. Finally, 7-hydro(peroxy)-13R-hydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid is also reduced to 7,13R-dihydroxy-docosa-8,10Z,14E,16Z,19Z-pentaenoic acid that is coined RvT4 (Fig. 7.3).

RvTs are produced in humans during exercise, that is now seen as a self-resolving inflammatory state, marked by an increase in neutrophil-endothelial interactions with upregulation of plasma RvT [38]. These mediators are also produced during infection when RvT levels are upregulated in plasma from septic patients when compared to healthy volunteers. RvTs are protective in mice during acute inflammation where a mixture of RvT1, RvT2, RvT3 and RvT4 immediately before or 2 h after intraperitoneal *Escherichia coli* inoculation resulted in host-protection, increasing survival by >60%. Indeed, RvT limited neutrophil recruitment to the site of inflammation, increased phagocytosis and intracellular ROS levels and upregulated macrophage efferocytosis. Of note, the protective actions of RvTs resulted from the reprogramming of the innate host response since RvT did not display direct bactericidal actions at biologically-relevant concentrations. Additionally, RvT reduced monocyte and macrophage expression of inflammasome components, decreasing caspase-1 and IL-1β expression and lactate dehydrogenase activity, a marker of pyroptosis. RvT also reduced peripheral blood platelet-leukocyte aggregates, an observation associated with reduced systemic inflammation. Exudate macrophage efferocytosis was also increased and a significant reduction in local and systemic eicosanoid levels was found in mice given RvT [38].

Using a total organic synthetic approach, we recently established the complete stereochemistry and biosynthetic role of 13R-HDPA in the RvT pathway [39]. Chirally pure precursors were used in conjunction with stereoselective reactions that installed the configuration at the carbon 13 atom as R and formed geometrically pure double-bond moieties.

Synthetic 13R-HDPA was matched with biogenic 13-HDPA, obtained by incubating n-3 DPA with human recombinant COX-2. Structural evaluation of synthetic 13R-HDPA and biogenic 13-HDPA was carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS) to attain MRM chromatograms to match retention times and MS-MS spectra to identify matching daughter ions. Chiral LC-MS/MS was used to confirm the stereochemistry around carbon 13, and UV-Vis spectrophotometry was used to match the double bond conjugation system. Incubation of the synthetic material with human leukocytes demonstrated that this was rapidly converted to all four RvTs [39]. These findings



Bacterial Infections

- Limit neutrophil activation and recruitment
- Increase vascular PGI_2
- Downregulate inflammasome activation
- Counter-regulate the production of inflammatory mediators
- Enhance neutrophil and macrophage phagocytosis of bacteria
- Upregulate efferocytosis
- Increase survival from lethal infections

Inflammatory Arthritis

- Limit neutrophil activation and recruitment to the joint
- Regulate joint monocyte and macrophage phenotype
- Reduce peripheral blood monocyte, neutrophil and platelet activation
- Counter-regulate the production of inflammatory mediators
- Reduce joint damage

Fig. 7.3 RvT biosynthesis and actions in mediating the protective actions of statins in infections and inflammatory arthritis. Production of RvT is initiated via the conversion of n-3 DPA by the endothelial COX-2 expression yielding 13-HpDHA, that can then be converted to 13-HDPA and donated to neutrophils where via ALOX activity this is converted to RvT1–4. Statin mediated S-nitrosylation of COX-2 upregulates 13-HDPA production contributing to the upregulation of RvTs

confirmed that the stereochemistry around the hydroxyl group on carbon 13 was in the R orientation and the double bond geometry around the conjugated double bond system was found to be in *E, Z* with the complete stereochemistry established as 13(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z-docosapentaenoic acid as well as the role of this intermediate in the RvT biosynthetic pathway.

7.6 RvT Mediate the Biological Actions of Statins in Infectious-Inflammation and Inflammatory Arthritis

SPMs are implicated in mediating the protective actions of a number of clinically relevant therapeutics including aspirin and statins, whereby aspirin initiates the biosynthesis of epimeric forms of SPMs [40], while lovastatin, for example, upregulates the biosynthesis of 15-epi-LXA_4 [41]. In addition, atorvastatin was recently found to increase RvT formation during human neutrophil-endothelial cell interactions, as well as in mice during infections. This increase in RvT production resulted from the S-nitrosylation of COX-2 leading to increased 13R-HDPA levels, suggesting that the S-nitrosylation of COX-2 increased catalytic activity of the enzyme. This finding was in concordance with the S-nitrosylation of COX-2 cysteine residues in the presence of atorvastatin [38]. Inhibition of inducible nitric oxide synthase (iNOS) by L-NG-nitroarginine (L-NAME) reduced the atorvastatin-mediated increases in plasma RvT levels after *E. coli* inoculation. A similar reduction was observed when mice were given celecoxib, a COX-2 specific inhibitor. This highlights the complex regulatory axis of COX-2, as post-translational modification of the enzyme may

yield mediators with distinct biological activities to the classic eicosanoids.

This mechanism was recently also found to be protective in arthritic inflammation where administration of atorvastatin upregulated RvT concentrations during inflammatory arthritis in both peripheral blood and joints [42]. Of note, this protective mechanism was not unique to atorvastatin and was shared with pravastatin. Atorvastatin administration during ongoing arthritis led to a 43% increase in total RvT amounts in arthritic paws compared to vehicle-treated mice. Pravastatin also increased paw RvT by ~20% with increases in RvT1 and RvT2.

These increases in joint RvT concentrations were also linked with decreases in tissue prostanoids and LTB_4 concentrations. Prostaglandins were reduced by 20–40% by all statins tested when compared to vehicle. Exceptionally, $PGF_{2\alpha}$ was reduced by ~75% in mice given pravastatin. LTB_4 concentrations were reduced ~50% by atorvastatin and ~15% by pravastatin. Additionally, TxB_2 was reduced 20–50% both statins [42]. The upregulation in RvT concentrations were linked with a reduction in disease severity where in mice administered atorvastatin, disease progression was dampened at day 4 post disease initiation, with disease scores reaching a maximum of 9.1 ± 1.2 at day 5 which was sustained until day 7. When mice were administered pravastatin, disease activity at day 5 was lower compared with mice administered vehicle, with a reduction in disease activity maintained until day 7 measured both as reduction in of clinical score and edema. In addition, both statins also lead to a reduction in joint damage at a histological level.

In inflammatory arthritis atorvastatin and pravastatin administration also regulates both circulating and tissue resident leukocyte responses. In non-classical monocytes, atorvastatin reduced the expression of CD11b by ~18% and platelet-monocyte aggregation (measured by a decrease in CD62P) was reduced by ~24% compared to mice given vehicle [42]. Pravastatin significantly reduced platelet-monocyte aggregation by ~35%, and decreased CD11b expression by ~10%. In classical monocytes, expression of CD11b and CD62P were significantly reduced by ~42% and ~34% respectively in mice given atorvastatin compared to mice given vehicle. In mice given pravastatin, CD11b expression was decreased by ~40% and platelet-monocyte aggregation reduced by ~35%. Compared to vehicle, neutrophil activation markers were significantly reduced by atorvastatin and pravastatin, reducing CD11b expression by ~30% and platelet-neutrophil aggregation by ~24%. Of note, administration of celecoxib that inhibits the upregulation of RvT by pravastatin and atorvastatin reverses the protective actions of these statins on both disease severity and leukocyte responses [42].

7.7 Conclusion

The identification of n-3 DPA as a substrate to novel, structurally distinct, mediators that display potent host protective activities demonstrates that complex mediator networks become activated during acute inflammation to ensure tissue homeostasis. This is further underscored by the observation of a selective regulation of distinct lipid mediator pathways in a tissue and cell specific manner. In addition, mounting evidence suggests that some of the beneficial actions of a number of widely used drugs, including statins, is mediated via the regulation of these protective pathways. Given the potent actions of n-3 DPA-derived SPMs in regulating systemic and peripheral inflammatory responses, utilizing analogues and mimetics may be useful therapeutics in the prevention and treatment of chronic inflammatory diseases. In addition, strategies to boost their endogenous production potentially via supplementation with n-3 DPA may also be useful in controlling inflammation. While the clinical evidence for this approach is currently limited, recent studies in healthy volunteers provide evidence for its effectiveness, whereby administration of n-3 DPA upregulates peripheral blood concentrations of $RvD5_{n\text{-}3\ DPA}$ [43]. Future studies will need to identify the patient populations that will be responsive to this approach and the supplement forms that will be effective in regulating n-3 DPA-derived SPM concentrations. Furthermore, changes in the observation that

tissue concentrations of these SPM are altered in disease and that their levels can be upregulated by certain therapeutics (e.g. atorvastatin and pravastatin) suggest that these pathways may also be useful as biomarkers in both patient stratification and measuring the effectiveness of treatment efficacy.

References

1. Malagoli D (2016) The evolution of the immune system: conservation and diversification. Elsevier, Amsterdam
2. Majno G (1991) The ancient riddle of sigma eta psi iota sigma (sepsis). J Infect Dis 163(5):937–945
3. Dalli J, Serhan CN (2018) Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology. Br J Pharmacol 176:1024–1037. https://doi.org/10.1111/bph.14336
4. de Gaetano M, McEvoy C, Andrews D, Cacace A, Hunter J, Brennan E, Godson C (2018) Specialized pro-resolving lipid mediators: modulation of diabetes-associated cardio-, reno-, and retino-vascular complications. Front Pharmacol 9:1488. https://doi.org/10.3389/fphar.2018.01488
5. Perretti M, Norling LV (2017) Actions of SPM in regulating host responses in arthritis. Mol Asp Med 58:57–64. https://doi.org/10.1016/j.mam.2017.04.005
6. Quiros M, Nusrat A (2019) Saving problematic mucosae: SPMs in intestinal mucosal inflammation and repair. Trends Mol Med 25:124–135. https://doi.org/10.1016/j.molmed.2018.12.004
7. Serhan CN, Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 128(7):2657–2669. https://doi.org/10.1172/JCI97943
8. Serhan CN (2017) Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. Mol Asp Med 58:1–11. https://doi.org/10.1016/j.mam.2017.03.001
9. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K (2000) Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 192(8):1197–1204
10. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL (2002) Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med 196(8):1025–1037
11. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M (2009) Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med 206(1):15–23. https://doi.org/10.1084/jem.20081880
12. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN (2008) Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. Blood 112(3):848–855. https://doi.org/10.1182/blood-2007-11-122598
13. Morita M, Kuba K, Ichikawa A, Nakayama M, Katahira J, Iwamoto R, Watanebe T, Sakabe S, Daidoji T, Nakamura S, Kadowaki A, Ohto T, Nakanishi H, Taguchi R, Nakaya T, Murakami M, Yoneda Y, Arai H, Kawaoka Y, Penninger JM, Arita M, Imai Y (2013) The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. Cell 153(1):112–125. https://doi.org/10.1016/j.cell.2013.02.027
14. Dalli J, Vlasakov I, Riley IR, Rodriguez AR, Spur BW, Petasis NA, Chiang N, Serhan CN (2016) Maresin conjugates in tissue regeneration biosynthesis enzymes in human macrophages. Proc Natl Acad Sci U S A 113(43):12232–12237. https://doi.org/10.1073/pnas.1607003113
15. Chiang N, Serhan CN (2017) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Asp Med 58:114–129. https://doi.org/10.1016/j.mam.2017.03.005
16. Colas RA, Dalli J, Chiang N, Vlasakov I, Sanger JM, Riley IR, Serhan CN (2016) Identification and actions of the Maresin 1 metabolome in infectious inflammation. J Immunol 197(11):4444–4452. https://doi.org/10.4049/jimmunol.1600837
17. Calder PC (2011) Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol 668(Suppl 1):S50–S58. https://doi.org/10.1016/j.ejphar.2011.05.085
18. De Caterina R (2011) n-3 fatty acids in cardiovascular disease. N Engl J Med 364(25):2439–2450. https://doi.org/10.1056/NEJMra1008153
19. Crawford MA, Broadhurst CL, Guest M, Nagar A, Wang Y, Ghebremeskel K, Schmidt WF (2013) A quantum theory for the irreplaceable role of docosahexaenoic acid in neural cell signalling throughout evolution. Prostaglandins Leukot Essent Fatty Acids 88(1):5–13. https://doi.org/10.1016/j.plefa.2012.08.005
20. Dalli J, Colas RA, Serhan CN (2013) Novel n-3 immunoresolvents: structures and actions. Sci Rep 3:1940. https://doi.org/10.1038/srep01940
21. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, Nettleton JA, King IB, Weng LC, Bhattacharya S, Bandinelli S, Bis JC, Rich SS, Jacobs DR Jr, Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Chen YD, Friedlander Y, Djousse L, Wu JH, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L, Fornage M, Tsai MY, Mozaffarian D, Steffen LM (2011) Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide

association studies from the CHARGE consortium. PLoS Genet 7(7):e1002193. https://doi.org/10.1371/journal.pgen.1002193
22. Serhan CN, Savill J (2005) Resolution of inflammation: the beginning programs the end. Nat Immunol 6(12):1191–1197. https://doi.org/10.1038/ni1276
23. Aursnes M, Tungen JE, Vik A, Colas R, Cheng CY, Dalli J, Serhan CN, Hansen TV (2014) Total synthesis of the lipid mediator PD1n-3 DPA: configurational assignments and anti-inflammatory and pro-resolving actions. J Nat Prod 77(4):910–916. https://doi.org/10.1021/np4009865
24. Gobbetti T, Dalli J, Colas RA, Federici Canova D, Aursnes M, Bonnet D, Alric L, Vergnolle N, Deraison C, Hansen TV, Serhan CN, Perretti M (2017) Protectin D1n-3 DPA and resolvin D5n-3 DPA are effectors of intestinal protection. Proc Natl Acad Sci U S A 114(15):3963–3968. https://doi.org/10.1073/pnas.1617290114
25. Colas RA, Souza PR, Walker ME, Burton M, Zaslona Z, Curtis AM, Marques RM, Dalli J (2018) Impaired production and diurnal regulation of vascular RvDn-3 DPA increase systemic inflammation and cardiovascular disease. Circ Res 122(6):855–863. https://doi.org/10.1161/CIRCRESAHA.117.312472
26. Ingle KA, Kain V, Goel M, Prabhu SD, Young ME, Halade GV (2015) Cardiomyocyte-specific Bmal1 deletion in mice triggers diastolic dysfunction, extracellular matrix response, and impaired resolution of inflammation. Am J Physiol Heart Circ Physiol 309(11):H1827–H1836. https://doi.org/10.1152/ajpheart.00608.2015
27. McAlpine CS, Swirski FK (2016) Circadian influence on metabolism and inflammation in atherosclerosis. Circ Res 119(1):131–141. https://doi.org/10.1161/CIRCRESAHA.116.308034
28. Puttonen S, Oksanen T, Vahtera J, Pentti J, Virtanen M, Salo P, Kivimaki M (2010) Is shift work a risk factor for rheumatoid arthritis? The Finnish public sector study. Ann Rheum Dis 69(4):779–780. https://doi.org/10.1136/ard.2008.099184
29. Krump E, Picard S, Mancini J, Borgeat P (1997) Suppression of leukotriene B4 biosynthesis by endogenous adenosine in ligand-activated human neutrophils. J Exp Med 186(8):1401–1406
30. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C, Silvestre-Roig C, Dittmar G, Doring Y, Drechsler M, Weber C, Zimmer R, Cenac N, Soehnlein O (2016) Resolving lipid mediators Maresin 1 and Resolvin D2 prevent atheroprogression in mice. Circ Res 119(9):1030–1038. https://doi.org/10.1161/CIRCRESAHA.116.309492
31. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, Connolly ES, Solomon R, Jones DM, Heyer EJ, Spite M, Tabas I (2016) An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun 7:12859. https://doi.org/10.1038/ncomms12859
32. Buckley CD, McGettrick HM (2018) Leukocyte trafficking between stromal compartments: lessons from rheumatoid arthritis. Nat Rev Rheumatol 14(8):476–487. https://doi.org/10.1038/s41584-018-0042-4
33. Tabas I, Lichtman AH (2017) Monocyte-macrophages and T cells in atherosclerosis. Immunity 47(4):621–634. https://doi.org/10.1016/j.immuni.2017.09.008
34. Pistorius K, Souza PR, De Matteis R, Austin-Williams S, Primdahl KG, Vik A, Mazzacuva F, Colas RA, Marques RM, Hansen TV, Dalli J (2018) PDn-3 DPA pathway regulates human monocyte differentiation and macrophage function. Cell Chem Biol 25(6):749–760 e749. https://doi.org/10.1016/j.chembiol.2018.04.017
35. Primdahl KG, Tungen JE, De Souza PRS, Colas RA, Dalli J, Hansen TV, Vik A (2017) Stereocontrolled synthesis and investigation of the biosynthetic transformations of 16(S),17(S)-epoxy-PDn-3 DPA. Org Biomol Chem 15(40):8606–8613. https://doi.org/10.1039/c7ob02113e
36. Chiang N, Serhan CN (2017) Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Asp Med 58:114–129. https://doi.org/10.1016/j.mam.2017.03.005
37. Dalli J, Serhan C (2016) Macrophage proresolving mediators-the when and where. Microbiol Spectr 4 (3). https://doi.org/10.1128/microbiolspec.MCHD-0001-2014
38. Dalli J, Chiang N, Serhan CN (2015) Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med 21(9):1071–1075. https://doi.org/10.1038/nm.3911
39. Primdahl KG, Aursnes M, Walker ME, Colas RA, Serhan CN, Dalli J, Hansen TV, Vik A (2016) Synthesis of 13(R)-Hydroxy-7Z,10Z,13R,14E,16Z,19Z Docosapentaenoic acid (13R-HDPA) and its biosynthetic conversion to the 13-series Resolvins. J Nat Prod 79(10):2693–2702. https://doi.org/10.1021/acs.jnatprod.6b00634
40. Claria J, Serhan CN (1995) Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A 92(21):9475–9479
41. Planaguma A, Pfeffer MA, Rubin G, Croze R, Uddin M, Serhan CN, Levy BD (2010) Lovastatin decreases acute mucosal inflammation via 15-epi-lipoxin A4. Mucosal Immunol 3(3):270–279. https://doi.org/10.1038/mi.2009.141
42. Walker ME, Souza PR, Colas RA, Dalli J (2017) 13-series resolvins mediate the leukocyte-platelet actions of atorvastatin and pravastatin in inflammatory arthritis. FASEB J 31(8):3636–3648. https://doi.org/10.1096/fj.201700268
43. Markworth JF, Kaur G, Miller EG, Larsen AE, Sinclair AJ, Maddipati KR, Cameron-Smith D (2016) Divergent shifts in lipid mediator profile following supplementation with n-3 docosapentaenoic acid and eicosapentaenoic acid. FASEB J 30(11):3714–3725. https://doi.org/10.1096/fj.201600360R

8 Aspects of Prostaglandin Glycerol Ester Biology

Philip J. Kingsley, Carol A. Rouzer, Amanda J. Morgan, Sachin Patel, and Lawrence J. Marnett

Abstract

The Cyclooxygenase enzymes (COX-1 and COX-2) incorporate 2 molecules of O_2 into arachidonic acid (AA), resulting in an array of bioactive prostaglandins. However, much work has been done showing that COX-2 will perform this reaction on several different AA-containing molecules, most importantly, the endocannabinoid 2-arachidonoylglycerol (2-AG). The products of 2-AG oxygenation, prostaglandin glycerol esters (PG-Gs), are analogous to canonical prostaglandins. This chapter reviews the literature detailing the production, metabolism, and bioactivity of these compounds, as well as their detection in intact animals.

Keywords

Endocannabinoid · Cannabinoid · Cyclooxygenase · COX-2 · Inflammation · Prostaglandin · Prostaglandin glyceryl ester · Transgenic mouse · Anandamide · 2-Arachidonoyl glycerol

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8.1 Introduction

The cyclooxygenase (COX) enzymes, which exist as two isoforms (COX-1 and COX-2), incorporate two molecules of O_2 into arachidonic acid (AA), generating the intermediate prostaglandin H_2 (PGH_2). PGH_2 is further transformed by various prostaglandin synthases to produce the array of canonical prostaglandins familiar to many researchers (Scheme 8.1). Yu [1] and Kozak [2] reported that the arachidonoyl-containing endogenous lipids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are efficient substrates for COX-2, but not COX-1. These

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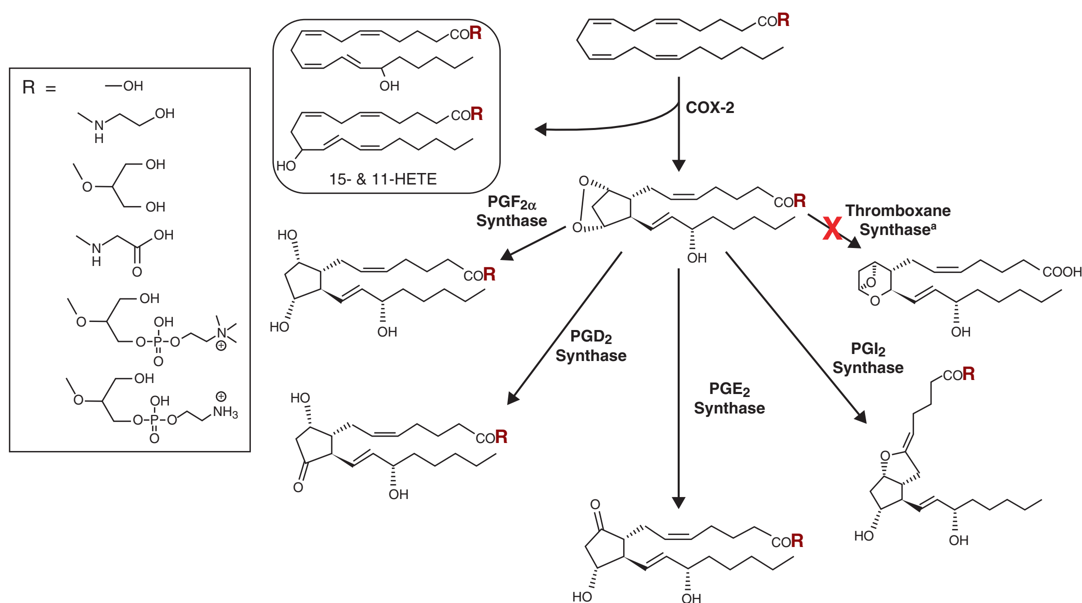

Scheme 8.1 Schematic of COX-2-mediated oxygenation of various arachidonoyl-containing lipid species. (*a* – PGH_2 derived from AEA or 2-AG is not a substrate for thromboxane synthase. It is unknown if PGH_2-LPC or PGH_2-LPE are acted upon by this enzyme)

compounds – referred to as endocannabinoids because they are ligands for the cannabinoid receptors CB1 and CB2 – are processed by COX-2 analogously to AA, generating the corresponding PGH_2 ethanolamide (PG-EA) or glyceryl ester (PG-G). These intermediates are acted upon by most of the prostaglandin synthases, leading to an array of prostaglandin derivatives (Scheme 8.1). Curiously, thromboxane synthase does not accept PGH_2 derivatives; thus, there are no thromboxane analogs produced from endocannabinoids (2). Subsequent to these reports, other researchers identified additional arachidonic acid analogs that are selective substrates for COX-2. These compounds include *N*-arachidonylglycine [3], 2-O-arachidonoyl ether [4], arachidonoyl-lysophosphatidylethanolamine (AA-LPE), and arachidonyl-lysophosphatidylcholine (AA-LPC) [5].

Our laboratory has a long-standing interest in exploring the biology of these putative COX-2 products, especially those of 2-AG. The hypothesis that PG-Gs are relevant bioactive lipids is mainly based on three observations: (*1*) 2-AG is present at high levels in many mammalian tissues (*i.e.*, nmol/g in brain tissue); (*2*) kinetic studies indicate 2-AG and AA are utilized by COX-2 with similar efficiencies *in vitro* [2]; and (*3*) PG-Gs have been shown to possess significant biological activity. This chapter will review research on (*i*) production of PG-Gs in enzymatic and cellular systems, (*ii*) biological activity of PG-Gs, (*iii*) analytical methodology for detection and measurement of PG-Gs, (*iv*) the presence of PG-Gs in intact animals, and (*v*) recent work from the Patel and Marnett laboratories involving PG-Gs in the murine central nervous system (CNS). It should be noted that the biochemistry, enzymology, and pharmacology of the PG-EAs, formed from the oxygenation of AEA by COX-2, are distinct from those of the PG-Gs but are beyond the scope of this review. Several groups have published interesting work elucidating the biochemistry of PG-EAs, and recent reviews and original research in this area can be found here [6–9].

8.2 PG-Gs in *in Vitro* and Cellular Systems

The majority of reports regarding non-AA-derived products of COX-2 oxygenation used both reconstituted enzymatic and cellular systems to characterize the oxygenated products as well as the mechanism of their production. In the initial report of PG-G formation, Kozak et al. [2] used purified murine and human COX-2 for assessment of the kinetics (via O_2 uptake) of 2-AG oxygenation and the identification of COX-2 reaction products. These studies established that, when assayed individually, COX-2 utilizes AA and 2-AG with similar efficiencies (k_{cat}/K_m = 2.4 s^{-1} μM^{-1} and 4.0 s^{-1} μM^{-1} for AA and 2-AG, respectively, for human COX-2 and 2.5 s^{-1} μM^{-1} and 2.3 s^{-1} μM^{-1} for AA and 2-AG, respectively, for murine COX-2). The authors used the RAW264.7 murine macrophage-like cell line to confirm that the observed enzymatic reactions could also take place in a cellular setting, where 2-AG would be subject to metabolism by several different pathways. Since RAW264.7 cells do not express COX-2 under basal conditions, they were pretreated with interferon-γ (INF-γ) and bacterial lipopolysaccharide (LPS) to induce the COX-2 enzyme. The investigators established that exogenous 2-AG can be taken up by cells, processed to PGD_2-G via COX-2, and secreted into the extracellular medium. They further demonstrated the formation of PGD_2-G from endogenous 2-AG following stimulation of LPS- and IFN-γ-pretreated RAW264.7 cells with ionomycin. The formation of PGD_2-G by the cells was consistent with the fact that they produce predominantly PGD_2 from AA. In subsequent studies, Kozak et al. demonstrated the formation of PGE_2-G and $PGF_{2\alpha}$-G from exogenous 2-AG by human HCA-7 colon adenocarcinoma cells, which constitutively express COX-2 [10]. In addition, Siegmund et al. reported the synthesis of PGD_2-G from exogenously provided 2-AG by hepatic stellate cells isolated from mice pretreated with bile duct ligation to induce liver fibrosis. The investigators confirmed that bile duct ligation led to induction of COX-2 expression in the hepatic stellate cells [11].

Rouzer and Marnett [12] further investigated the cellular production of PG-Gs in a more physiologically relevant setting by isolating murine resident peritoneal macrophages (RPMs). These cells are known to have a relatively high level of AA in their phospholipid pool and are able to synthesize COX-2 and generate large amounts of PGs in response to a variety of inflammatory stimuli. Here, RPMs were pretreated with LPS to induce COX-2 expression and then challenged with zymosan, which stimulated the release of 2-AG. The authors showed that PGE_2-G and PGI_2-G were produced by RPMs without the addition of exogenous 2-AG, and these PG-Gs were released from the cells. As in the case of RAW264.7 cells, the primary PG-G products in RPMs were consistent with the primary PG products, which were PGE_2 and PGI_2.

Based on kinetic analyses of COX-2-mediated oxygenation of AA and 2-AG *in vitro*, which had shown similar enzymatic efficiency for the two substrates, one would predict that the ratio of PG-Gs to PGs produced in cells should be similar to the ratio of available 2-AG to AA. However, in zymosan-stimulated RPMs, the ratio of total PG to PG-G production was roughly 1000:1, whereas the ratio of free AA to 2-AG in these cells was 10:1. The much lower than expected production of PG-Gs relative to PGs based on substrate availability could not be explained on the basis of PG-G hydrolysis [12]. These observations led to more extensive kinetic analyses of 2-AG and AA oxygenation by COX-2 via experiments in which both substrates were present. Results of these kinetic studies were consistent with previously published data suggesting that the homodimeric COX-2 enzyme behaves as a heterodimer with one subunit acting as the catalytic site and the other serving an allosteric function. Specifically, the results supported the hypothesis that AA and 2-AG compete with each other for both the catalytic and allosteric sites and that binding of either substrate in the allosteric site suppresses 2-AG oxygenation and promotes AA oxygenation. Consequently, when both substrates are present, oxygenation of AA is favored over that of 2-AG [13]. It is not clear whether this phenomenon fully explains the

observed relatively low yield of PG-Gs produced by zymosan-stimulated RPMs. In fact, COX-2 requires peroxide-dependent activation before oxygenation of any substrate can occur, and a report from Musee and Marnett indicates that higher levels of peroxide are required to activate 2-AG oxygenation compared to AA oxygenation [14]. Other factors, such as the actual availability of free 2-AG and AA at the COX-2 active site within the cell, may also play a role.

While investigating the phenomenon of substrate-selective inhibition, in which an inhibitor blocks COX-2-dependent oxygenation of 2-AG more strongly than that of AA, Duggan et al. [15] employed a model utilizing dorsal root ganglia (DRG). DRGs were harvested, plated, and treated with a cocktail of compounds that included LPS and IFN-γ to induce COX-2. Ionomycin was subsequently added to stimulate release of 2-AG, resulting in the production of PG-Gs. The predominant products of 2-AG oxygenation were PGE_2-G and $PGF_{2\alpha}$-G. Interestingly, ethanolamide derivatives of PGs were also produced by the cells, in addition to free acid PGs. The absolute quantities of the various products were not reported. With this model, the authors showed that within the physiological system of DRGs, the *R*-profen class of inhibitors acted as substrate-selective inhibitors.

It should be noted that other reports have demonstrated COX-2-dependent effects of 2-AG in a variety of cell systems, suggesting that these effects may be due to conversion of the endocannabinoid to a PG-G. However, in many of these studies, the actual synthesis of a PG-G in the tissue or cells of interest was not confirmed. We have limited this discussion to cases in which PG-G formation was demonstrated analytically.

8.3 Biological Activity of PG-Gs

Concurrent with the elucidation of the production and control of PG-Gs, several investigators have reported interesting and potent biological activities of PG-Gs, both in cellular systems and in intact mammals. However, the discovery of any PG-G-associated biological activity immediately led to the question of whether or not the effects were mediated by known endocannabinoid or prostanoid receptors as opposed to distinct receptors. An initial pharmacologic study ruled out significant activity of PGE_2-G at the four EP receptors that modulate the effects of PGE_2, as well as the FP, DP, TP, and IP receptors [16]. More recently, Woodward et al. used the more metabolically and chemically stable amide derivatives of the 1(3)- and 2-glyceryl esters of PGE_2 and $PGF_{2\alpha}$ to show that neither compound has significant activity at any of the prostanoid receptors. The highest activity was noted in the case of PGE_2-serinolamide (the stable analog of the 2-glyceryl ester of PGE_2) at the EP3 receptor, for which an EC_{50} of 500 nM was observed. EC_{50} values were > 3000 nM for all tested analogs at all other receptors [17].

The earliest report of PG-G-mediated biological activity came from Nirodi et al., who found that PGE_2-G, but not $PGF_{2\alpha}$-G or PGD_2-G, caused Ca^{2+} mobilization in the murine RAW264.7 macrophage-like cell line at picomolar concentrations. Ca^{2+} mobilization was secondary to increases in intracellular inositol 1,4,5-trisphospate, and it resulted in the activation of protein kinase C and, subsequently, extracellular signal regulated kinases (ERKs) 1 and 2. Importantly, the authors also showed that PGE_2 did not elucidate these effects [16]. In follow-up studies, Richie-Jannetta et al. demonstrated PGE_2-G-mediated Ca^{2+} mobilization in the H1819 human non-small-cell lung cancer cell line. In these studies, similar activity was observed regardless of whether the PGE_2 moiety was attached at the *sn*-1 or *sn*-2 position of the glycerol, and $PGF_{2\alpha}$-G exhibited activity similar to that of PGE_2-G. In contrast, the corresponding free acid prostanoids were inactive [18].

A number of studies have suggested a potential role for PG-Gs in the nervous system. Sang et al. [19] demonstrated that PGE_2-G, $PGF_{2\alpha}$-G, and PGD_2-G increased miniature inhibitory postsynaptic currents (mIPSCs) in murine hippocampal neurons. Shortly thereafter, the same group reported that these three PG-Gs enhanced miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neurons, an indication of enhanced

glutamatergic synaptic transmission [20]. In both cases, 2-AG had the opposite effect, a role for the CB1 receptor was ruled out, and the involvement of both IP_3 and ERKs was implicated. The PGE_2-G-mediated enhancement of mEPSCs was also found to induce neuronal injury and/or death. Yang et al. also reported an effect of PGE_2-G, $PGF_{2\alpha}$-G, and PGD_2-G on hippocampal neurotransmission. In their case, an increase in basal synaptic transmission and long-term potentiation in hippocampal slices was observed that, consistent with prior findings, was dependent on IP_3-mediated Ca^{2+} mobilization and ERK signaling [21]. Commensurate with the neurotoxic effects noted by Sang et al., Valdeolivas and colleagues reported that PGE_2-G was neurotoxic to the M-213-2O fetal rat striatum-derived cell line. Their studies suggested that PGE_2-G is a mediator of malonate-dependent neurotoxicity, which in M-213-2O cells, serves as a model of Huntington's disease [22]. In studies involving tissues other than the hippocampus, Lindgren and colleagues reported that PGE_2-G induces an enhancement of stimulus-evoked neurotransmitter release by the presynaptic neuron at the neuromuscular junction. They further demonstrated that the effect is dependent on production of nitric oxide and does not involve the EP1 or EP2 receptors for PGE_2 [23]. Woodward et al. demonstrated that the amide derivatives of the 1(3)- and 2-glycerol esters of PGE_2 lower intraocular pressure in canine eyes, whereas the comparable derivatives of $PGF_{2\alpha}$ are much less effective. In the monkey eye, the derivative of the 1(3)-glycerol ester of PGE_2 was more effective than the 2-glycerol ester [17].

Most other actions of PG-Gs have been identified in various models of immune or inflammatory responses. Alhouayek et al. [24] demonstrated that PGD_2-G suppresses while PGE_2-G and $PGF_{2\alpha}$-G increase IL-1β secretion by murine J774 macrophage-like cells. Their accompanying exploration of LPS-stimulated 2-AG metabolism by the cells suggested that 2-AG-dependent decreases in macrophage activation in response to LPS is mediated by PGD_2-G. In later work, Alhouayek et al. reported that PGD_2-G reduced symptoms of inflammation in a murine dextran sodium sulfate-induced colitis model; the related compounds PGD_2 and PGD_2-EA did not have similar effects [25]. Raman and colleagues demonstrated that the nonenzymatic breakdown product of PGD_2-G, 15-deoxy-$\Delta^{12,14}$-PGJ_2-G (15d-PGJ_2-G), activates the PPARγ receptor leading to decreased transcriptional activity of nuclear factor of activated T cells (NFAT), and ultimately, reduced IL-2 expression in the human Jurkat T lymphoblastoid cell line. The researchers suggest that 15d-PGJ_2-G mediates the 2-AG-dependent suppression of IL-2 production in these cells [26, 27]. In one of the few studies in intact animals, Hu et al. reported that intraplantar administration of PGE_2-G in rats produced hyperalgesia and allodynia (8). In this study, PGE_2 induced hyperalgesia in a manner similar to PGE_2-G. However, while $EP_{1–4}$ receptor antagonists blocked the hyperalgesic effect of PGE_2, they did not block the hyperalgesic effects of PGE_2-G, suggesting that the PGE_2-G response is mediated by a pathway distinct from that of PGE_2. Nevertheless, this study highlights one of the major challenges of elucidating the role of PG-Gs *in vivo*. The compounds are subject to hydrolysis by multiple esterases such that the half-life of PG-Gs in biological tissues is short (see below). Consequently, some reports of PG-G-mediated biological effects are actually due to free PGs obtained following hydrolysis. We have limited this discussion to cases in which the observed pharmacology appears to be due directly to the action of PG-Gs.

A common finding in these studies of the biological activities of PG-Gs is that neither 2-AG nor the free acid prostaglandins mediate the same effects, and in many cases, a role for CB or prostanoid receptors was specifically ruled out. This strongly implies that there is one (or several) receptors specific for PG-Gs. In support of this hypothesis, Bruser et al. recently reported that PGE_2-G is a potent ligand of the $P2Y_6$ receptor [28]. In this study, the authors identified G protein-coupled receptors (GPCRs) in PGE_2-G response-positive cells via RNA sequence analysis. From a pool of these candidate GPCRs, the authors established that the UDP receptor $P2Y_6$ is also a receptor for PGE_2-G. In fact, they report that PGE_2-G

activates $P2Y_6$ with an EC_{50} of 1.2 pM, compared to an EC_{50} of 78 nM for the canonical ligand, UDP.

8.4 Analytical Methodology for PG-G Quantitation

While several types of analytical methodologies have been applied to lipid detection and quantitation in the last several decades, reports of PG-G quantitation have employed almost exclusively LC-MS techniques. Previously, our group produced protocols [29] and reviews [30] regarding this type of analysis, and other researchers have employed similar methodologies. The typical work flow involves homogenization of cells or animal tissue followed by a secondary purification step (often solid phase extraction (SPE)). Finally, an aliquot of the dried and reconstituted sample is injected onto an LC-MS system where the analytes are chromatographed on a reverse-phase column and detected by mass spectrometry via selected reaction monitoring (SRM) (Note: sometimes researchers use the term "multiple reaction monitoring" (MRM), which is equivalent to SRM).

Our most recent report [31] employed homogenization of brain tissue via sonication in acetonitrile. No secondary purification was employed. Phospholipids are known to be less soluble than eicosanoids in acetonitrile, and it has been our experience that little interference is experienced in the analysis of PG-Gs and related lipids with this method. PG-Gs were chromatographed on a C18 column with ammonium acetate in the mobile phase to promote the generation of the $[M + NH_4]^+$ ion. For unknown reasons, PG-Gs preferentially co-ordinate with the ammonium cation, and this complex provides several candidate fragment ions (Fig. 8.1), giving the researcher several options for SRM analysis. PG-Gs will also coordinate with Na^+, but typically sodium complexes give rise to relatively weak product spectra compared to those of H^+ and NH_4^+ complexes, rendering this ionization method not useful for SRM detection.

In one of the few studies of PG-G detection in samples from an intact animal, Hu et al. (8) measured PGE_2-G in rat hind paws by homogenizing the tissues in methanol, then subjecting the diluted homogenates to purification via C18 SPE. Samples were finally analyzed on a LC-MS/MS system, where PGE_2-G was ionized via

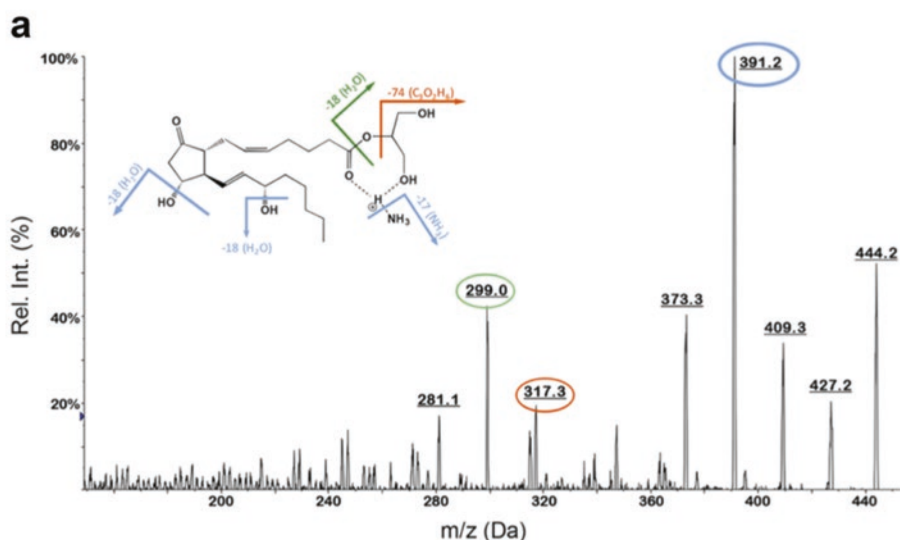

Fig. 8.1 Product ion spectrum of PGE_2-G complexed with an ammonium cation (*m/z* of $[M + NH_4]^+ = 444.2$). SRM analysis may be accomplished by setting the Q1 *m/z* to 444 and the Q3 *m/z* to any of the several product masses observed

co-ordination with the ammonium ion ($[M + NH_4]^+$) and detected on a triple quadrupole mass spectrometer operating in SRM mode. For analyte confirmation, the authors used an LC system coupled to a quadrupole/time-of-flight mass spectrometer and compared the product ion spectra of the putative, endogenous PGE_2-G and an authentic standard.

Similarly, Alhouayek et al. [25] homogenized tissues of interest in chloroform and subsequently performed a modified Folch-type extraction. Samples were further purified via reverse-phase SPE, then subjected to LC-MS/MS analysis on a triple quadrupole instrument, again operating the mass spectrometer in SRM mode.

Researchers have quantitated PG-EAs using similar techniques. Gatta et al. [6] quantified $PGF_{2\alpha}$-EA in murine spinal cord by homogenizing the tissue and extracting with acetone. The samples were further purified by open-bed chromatography on silica gel. The eluted fraction containing PG-EAs was analyzed on a reverse-phase LC-MS system (using a C18 column). Interestingly, the analytes were detected as $[M + Na]^+$ ions by a high-resolution mass spectrometer (an IT-TOF instrument manufactured by Shimadzu). Uraquhart et al. [32] examined rabbit cornea and cornea homogenates for PG-EAs. A Folch-type extraction was used to purify the analytes from the homogenized tissue, and resultant samples were quantified via LC-MS/MS, where several SRM transitions were employed for $PGF_{2\alpha}$-EA, PGE_2-EA, and PGD_2-EA detection. The authors utilized precursor ion $[M + H\text{-}H_2O]^+$ for all PG-EA species.

8.5 PG-Gs in *in Vivo* Systems

Despite the relative abundance of reports of PG-G generation in both *in vitro* and cellular experimental models, non-traditional COX-2 products (both PG-Gs and other classes) have proven difficult to detect *in vivo*, and few such reports exist in the literature. As previously mentioned, Hu reported the presence of PGE_2-G in the hindpaw and brain of rats treated with carrageenan [33]. Gatta reported the presence of $PGF_{2\alpha}$-ethanolamide ($PGF_{2\alpha}$-EA) in the spinal cords of mice subjected to kaolin/λ-carrageenan inflammation of the knee [6]. More recently, Liu reported the presence of several COX-2 derivatives in human myocardium and murine liver [5]. Here, the authors detail the discovery of PGE_2-LPE and -LPC, as well as the 11-HETE- and 15-HETE- derivatives of AA LPE and AA LPC, which are also COX-2 products.

One potential factor for the relative lack of detectable levels of PG-Gs *in vivo* is a high susceptibility of PG-Gs to enzymatic hydrolysis, resulting in the generation of the corresponding free PG and glycerol. Kozak et al. reported that the half-life of PGE_2-G in rat plasma is 14 s, and the half-life for appearance of PGE_2 is 16 s [34]. Additionally, these authors reported that no starting material was detectable in rat plasma 5 min after dosing the animal with 2 mg/kg of PGE_2-G. Vila reported that monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) both hydrolyze PG-Gs [35], whereas Manna reported that LYPLA2 does this as well [36]. Indeed, several other enzymes have been reported to carry out this reaction, among them ABHD6, ABHD12, CES-1, and PPT1 [37, 38].

As noted above, there also appear to be kinetic limitations on the generation of PG-Gs via COX-2. AA is typically present at higher concentrations in mammalian tissue, with the excess of up to an order of magnitude. This, combined with the AA-dependent allosteric suppression of 2-AG oxygenation by COX-2 [13] and the lower peroxide tone required for AA oxygenation as opposed to 2-AG oxygenation [14] suggests that AA will likely be the preferred substrate of the enzyme under most *in vivo* conditions.

8.5.1 PG-Gs in Transgenic Mouse Brain Tissue

Historically, and possibly for the reasons detailed above, in analyses of murine CNS tissue, PG-Gs have proven to be below the limit of detection for both our methods and those of other researchers [33, 39]. We hypothesized that increased levels of the COX-2 enzyme would generate PG-Gs in a

mouse brain setting. Towards this end, we acquired the Thy-1 hCOX-2 mouse. These mice overexpress human COX-2 (hCOX-2) primarily in the neurons of the striatum, amygdala, cerebral cortex, and hippocampus [40]. Litters resulting from the breeding of these mice contain both wild-type and transgenic (Tg) overexpressor animals. The experimental data shown below were generated with 60–90 day-old mice that were obtained in this way [31].

Western blotting analysis verified the presence of hCOX-2 in the brain tissue (prefontal cortex and hippocampus) of the Tg animals (Fig. 8.2) [31]. However, PG-Gs were not detected in analyses of Tg or WT brain tissue (Fig. 8.3). We hypothesized that an increase in substrate levels (i.e., 2-AG) may afford detectable PG-G generation. Thus, WT and Tg mice were dosed with the monoacylglycerol lipase (MAGL) inhibitor, JZL184 [41]. In agreement with previous literature reports, 2-AG was increased in the JZL184-dosed subjects (data not shown), and PG-Gs were detected (Fig. 8.3). Levels of $PGF_{2\alpha}$-G, PGE_2-G, and PGD_2-G were found to be in the range of 5–50 pmol per g of tissue (wet weight).

The COX-2 dependence of PG-G production was tested by dosing the Tg + JZL184 animals concurrently with the highly selective COX-2 inhibitor lumiracoxib (LMX) [42]. The LMX-treated animals showed LMX levels in brain tissue of 200–400 pmol/g and an almost complete elimination of PG-G species (Fig. 8.4). Interestingly, PGE_2 and PGD_2 were also markedly reduced.

8.5.2 PG-Gs in Response to Systemic Inflammation

Having established a model system in which robust PG-Gs can be observed with Tg mice, we wanted to explore the possibility of observing PG-Gs without genetic manipulation. As previous reports of COX-2 products *in vivo* involved the administration of inflammatory stimuli, we treated WT mice with LPS (3 mg/kg once per day for 2 days) with and without concurrent administration of JZL184. Some of the LPS-treated mice produced PG-Gs, albeit at levels 1–2 orders of magnitude lower than those of the Tg + JZL184 subjects (Fig. 8.5). Note that tissues from animals treated with LPS alone exhibited a trend toward increased PG-G levels, though this did not reach

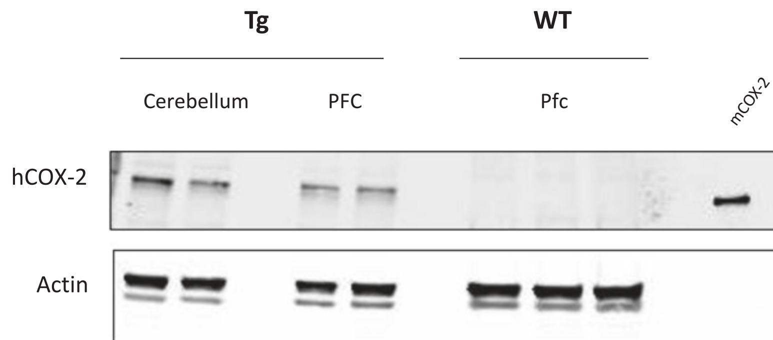

Fig. 8.2 Western blot of selected brain regions of Tg and WT mice (ref 17). (Figure reproduced by permission from Morgan et al. [31]. Copyright 2018, American Chemical Society)

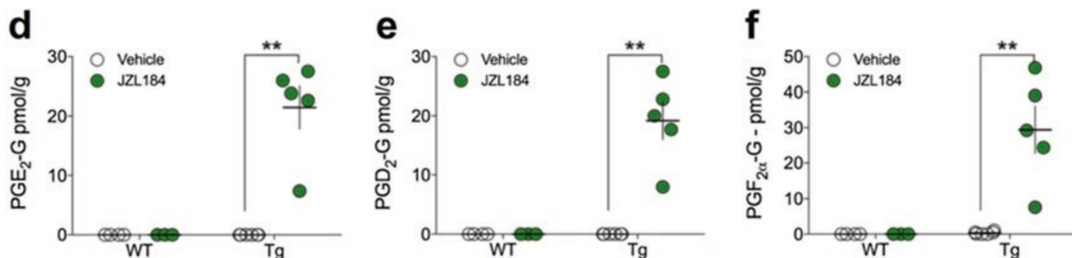

Fig. 8.3 Detection and quantitation of PG-G species in Tg mice. PG-Gs are observed only in the Tg + JZL184 subjects (∗∗ indicated $p < 0.01$). (Figure reproduced by permission from Morgan et al. [31]. Copyright 2018, American Chemical Society)

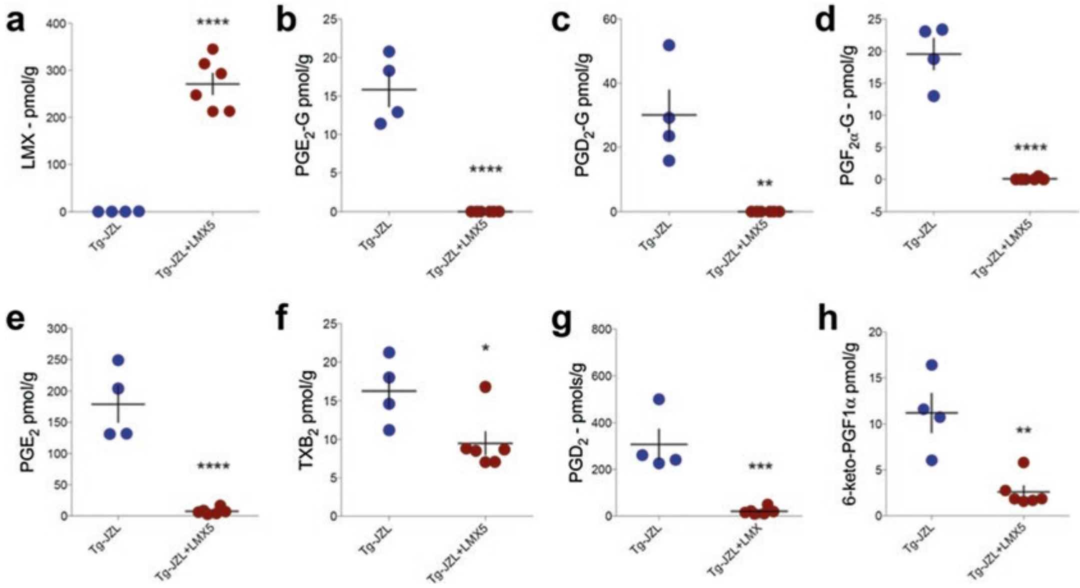

Fig. 8.4 Effect of LMX on lipid species in brain tissue of Tg + JZL184 animals. ($* \ p < 0.05$, $** \ p < 0.01$, $*** \ p < 0.001$, $* \ p < 0.0001$). *Note*: Figure **d** has a non-zero Y-axis. (Figure reproduced by permission from Morgan et al. [31]. Copyright 2018, American Chemical Society)

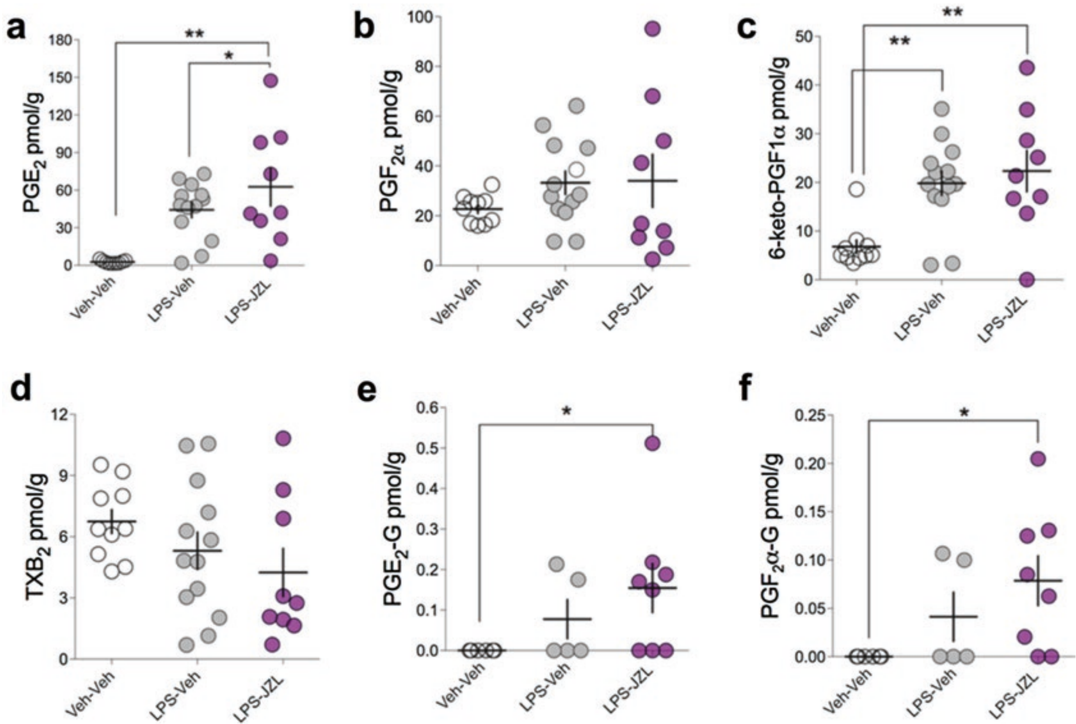

Fig. 8.5 Effect of systemic LPS treatment on brain lipid levels of Tg and Tg + JZL184 subjects ($*p < 0.05$, $** \ p < 0.01$). (Figure reproduced by permission from Morgan et al. [31]. Copyright 2018, American Chemical Society)

statistical significance when compared to controls. It is interesting to note, however, that there was no statistical significance between PG-G levels in the brains of animals treated with LPS alone and those treated with LPS and JZL184, although levels in the latter animals were statistically different from those in the controls. These findings suggest that LPS treatment increased PG-G levels in the brains of the mice even in the absence of JZL184; however, a larger study will be necessary to test this hypothesis.

8.6 Conclusions and Future Directions

The ability of COX-2 to metabolize 2-AG and other ester and amide derivatives of AA is now well established. However, the physiological relevance of this phenomenon remains unsettled. It is clear that PG-Gs exert interesting biological activities in tissues relevant to their site of production. Furthermore, their high potencies in many systems (with EC_{50}'s in the pM range) suggest that only very low levels of these lipid mediators may be necessary to exert important physiological functions. Clearly, a major impediment to elucidating the potential relevance of PG-G formation is our inability to detect these compounds *in vivo*. One should note that this does not preclude relevance. For example, the highly relevant eicosanoids thromboxane A_2 and prostacyclin (PGI_2) are not detected in tissue or cellular samples. Rather, their production is monitored through the detection of their nonenzymatic breakdown products, thromboxane B_2 and 6-keto-$PGF_{1\alpha}$. Unfortunately, in the case of PG-Gs, their hydrolysis products are free acid PGs. Thus, it is impossible to know whether PGs detected in a biological sample may have initially been synthesized from 2-AG or AA.

Given these challenges, the Tg mice described here offer an intriguing model that can be used to detect PG-G formation *in vivo*, albeit under conditions that are not totally physiological. Of perhaps greater interest are our results from LPS-stimulated animals suggesting that, eventually, models may be found in which PG-G biosynthesis can be detected and monitored under conditions that will allow us to assess their role in physiological or pathophysiological processes. Such models will also allow further study of additional aspects of COX-2 biology, such as the *in vivo* relevance of substrate selective inhibition.

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References

1. Yu M, Ives D, Ramesha CS (1997) Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. J Biol Chem 272(34):21181–21186
2. Kozak KR, Rowlinson SW, Marnett LJ (2000) Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. J Biol Chem 275(43):33744–33749. https://doi.org/10.1074/jbc.M007088200
3. Prusakiewicz JJ, Kingsley PJ, Kozak KR, Marnett LJ (2002) Selective oxygenation of N-arachidonylglycine by cyclooxygenase-2. Biochem Biophys Res Commun 296(3):612–617
4. Dong L, Zou H, Yuan C, Hong YH, Uhlson CL, Murphy RC, Smith WL (2016) Interactions of 2-O-arachidonylglycerol ether and ibuprofen with the allosteric and catalytic subunits of human COX-2. J Lipid Res 57(6):1043–1050. https://doi.org/10.1194/jlr.M067512
5. Liu X, Moon SH, Jenkins CM, Sims HF, Gross RW (2016) Cyclooxygenase-2 mediated oxidation of 2-Arachidonoyl-Lysophospholipids identifies unknown lipid signaling pathways. Cell Chem Biol 23(10):1217–1227. https://doi.org/10.1016/j.chembiol.2016.08.009
6. Gatta L, Piscitelli F, Giordano C, Boccella S, Lichtman A, Maione S, Di Marzo V (2012) Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. PLoS One 7(2):e31111. https://doi.org/10.1371/journal.pone.0031111
7. Silvestri C, Martella A, Poloso NJ, Piscitelli F, Capasso R, Izzo A, Woodward DF, Di Marzo V (2013) Anandamide-derived prostamide F2alpha negatively regulates adipogenesis. J Biol Chem 288(32):23307–23321. https://doi.org/10.1074/jbc.M113.489906
8. Woodward DF, Liang Y, Krauss AH (2008) Prostamides (prostaglandin-ethanolamides) and their pharmacology. Br J Pharmacol 153(3):410–419. https://doi.org/10.1038/sj.bjp.0707434
9. Woodward DF, Wang JW, Poloso NJ (2013) Recent progress in prostaglandin F2alpha ethanolamide (prostamide F2alpha) research and therapeutics.

Pharmacol Rev 65(4):1135–1147. https://doi.org/10.1124/pr.112.007088
10. Kozak KR, Crews BC, Morrow JD, Wang LH, Ma YH, Weinander R, Jakobsson PJ, Marnett LJ (2002) Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. J Biol Chem 277(47):44877–44885. https://doi.org/10.1074/jbc.M206788200
11. Siegmund SV, Wojtalla A, Schlosser M, Schildberg FA, Knolle PA, Nusing RM, Zimmer A, Strassburg CP, Singer MV (2016) Cyclooxygenase-2 contributes to the selective induction of cell death by the endocannabinoid 2-arachidonoyl glycerol in hepatic stellate cells. Biochem Biophys Res Commun 470(3):678–684. https://doi.org/10.1016/j.bbrc.2016.01.083
12. Rouzer CA, Marnett LJ (2005) Glycerylprostaglandin synthesis by resident peritoneal macrophages in response to a zymosan stimulus. J Biol Chem 280(29):26690–26700. https://doi.org/10.1074/jbc.M501021200
13. Mitchener MM, Hermanson DJ, Shockley EM, Brown HA, Lindsley CW, Reese J, Rouzer CA, Lopez CF, Marnett LJ (2015) Competition and allostery govern substrate selectivity of cyclooxygenase-2. Proc Natl Acad Sci U S A 112(40):12366–12371. https://doi.org/10.1073/pnas.1507307112
14. Musee J, Marnett LJ (2012) Prostaglandin H synthase-2-catalyzed oxygenation of 2-arachidonoylglycerol is more sensitive to peroxide tone than oxygenation of arachidonic acid. J Biol Chem 287(44):37383–37394. https://doi.org/10.1074/jbc.M112.381202
15. Duggan KC, Hermanson DJ, Musee J, Prusakiewicz JJ, Scheib JL, Carter BD, Banerjee S, Oates JA, Marnett LJ (2011) (R)-Profens are substrate-selective inhibitors of endocannabinoid oxygenation by COX-2. Nat Chem Biol 7(11):803–809
16. Nirodi CS, Crews BC, Kozak KR, Morrow JD, Marnett LJ (2004) The glyceryl ester of prostaglandin E2 mobilizes calcium and activates signal transduction in RAW264.7 cells. Proc Natl Acad Sci U S A 101(7):1840–1845. https://doi.org/10.1073/pnas.0303950101
17. Woodward DF, Poloso NJ, Wang JW (2016) Prostaglandin E2-glyceryl Ester: in vivo evidence for a distinct pharmacological identity from intraocular pressure studies. J Pharmacol Exp Ther 358(2):173–180. https://doi.org/10.1124/jpet.116.232512
18. Richie-Jannetta R, Nirodi CS, Crews BC, Woodward DF, Wang JW, Duff PT, Marnett LJ (2010) Structural determinants for calcium mobilization by prostaglandin E2 and prostaglandin F2alpha glyceryl esters in RAW 264.7 cells and H1819 cells. Prostaglandins Other Lipid Mediat 92(1–4):19–24. https://doi.org/10.1016/j.prostaglandins.2010.01.003
19. Sang N, Zhang J, Chen C (2007) COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity. J Neurochem 102(6):1966–1977. https://doi.org/10.1111/j.1471-4159.2007.04668.x
20. Sang N, Zhang J, Chen C (2006) PGE2 glycerol ester, a COX-2 oxidative metabolite of 2-arachidonoyl glycerol, modulates inhibitory synaptic transmission in mouse hippocampal neurons. J Physiol 572. (Pt 3:735–745. https://doi.org/10.1113/jphysiol.2006.105569
21. Yang H, Zhang J, Andreasson K, Chen C (2008) COX-2 oxidative metabolism of endocannabinoids augments hippocampal synaptic plasticity. Mol Cell Neurosci 37(4):682–695. https://doi.org/10.1016/j.mcn.2007.12.019
22. Valdeolivas S, Pazos MR, Bisogno T, Piscitelli F, Iannotti FA, Allara M, Sagredo O, Di Marzo V, Fernandez-Ruiz J (2013) The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis, rather than enhancing striatal damage, protects striatal neurons from malonate-induced death: a potential role of cyclooxygenase-2-dependent metabolism of 2-AG. Cell Death Dis 4:e862. https://doi.org/10.1038/cddis.2013.387
23. Lindgren CA, Newman ZL, Morford JJ, Ryan SB, Battani KA, Su Z (2013) Cyclooxygenase-2, prostaglandin E2 glycerol ester and nitric oxide are involved in muscarine-induced presynaptic enhancement at the vertebrate neuromuscular junction. J Physiol 591(19):4749–4764. https://doi.org/10.1113/jphysiol.2013.256727
24. Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG (2013) Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. Proc Natl Acad Sci U S A 110(43):17558–17563. https://doi.org/10.1073/pnas.1314017110
25. Alhouayek M, Buisseret B, Paquot A, Guillemot-Legris O, Muccioli GG (2018) The endogenous bioactive lipid prostaglandin D2-glycerol ester reduces murine colitis via DP1 and PPARgamma receptors. FASEB J 32(9):5000–5011. https://doi.org/10.1096/fj.201701205R
26. Raman P, Kaplan BL, Kaminski NE (2012) 15-Deoxy-Delta(1)(2),(1)(4)-prostaglandin J(2)-glycerol, a putative metabolite of 2-arachidonyl glycerol and a peroxisome proliferator-activated receptor gamma ligand, modulates nuclear factor of activated T cells. J Pharmacol Exp Ther 342(3):816–826. https://doi.org/10.1124/jpet.112.193003
27. Raman P, Kaplan BL, Thompson JT, Vanden Heuvel JP, Kaminski NE (2011) 15-deoxy-delta12,14-prostaglandin J2-glycerol ester, a putative metabolite of 2-arachidonyl glycerol, activates peroxisome proliferator activated receptor. Mol Pharmacol 80(1):201–209. https://doi.org/10.1124/mol.110.070441
28. Bruser A, Zimmermann A, Crews BC, Sliwoski G, Meiler J, Konig GM, Kostenis E, Lede V, Marnett LJ, Schoneberg T (2017) Prostaglandin E2 glyceryl ester is an endogenous agonist of the nucleotide receptor P2Y6. Sci Rep 7(1):2380. https://doi.org/10.1038/s41598-017-02414-8
29. Kingsley PJ, Rouzer CA, Saleh S, Marnett LJ (2005) Simultaneous analysis of prostaglandin glyceryl

esters and prostaglandins by electrospray tandem mass spectrometry. Anal Biochem 343(2):203–211. https://doi.org/10.1016/j.ab.2005.05.005
30. Kudalkar SNKPJ, Marnett LJ (2016) Assay of endocannabinoid oxidation by Cyclooxygenase-2. Methods Mol Biol 1412:205–216
31. Morgan AJ, Kingsley PJ, Mitchener MM, Altemus M, Patrick TA, Gaulden AD, Marnett LJ, Patel S (2018) Detection of Cyclooxygenase-2-derived oxygenation products of the endogenous cannabinoid 2-Arachidonoylglycerol in mouse brain. ACS Chem Neurosci 9(7):1552–1559. https://doi.org/10.1021/acschemneuro.7b00499
32. Urquhart P, Wang J, Woodward DF, Nicolaou A (2015) Identification of prostamides, fatty acyl ethanolamines, and their biosynthetic precursors in rabbit cornea. J Lipid Res 56(8):1419–1433. https://doi.org/10.1194/jlr.M055772
33. Hu SS, Bradshaw HB, Chen JS, Tan B, Walker JM (2008) Prostaglandin E2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NFkappaB activity. Br J Pharmacol 153(7):1538–1549. https://doi.org/10.1038/bjp.2008.33
34. Kozak KR, Crews BC, Ray JL, Tai HH, Morrow JD, Marnett LJ (2001) Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides in vitro and in vivo. J Biol Chem 276(40):36993–36998. https://doi.org/10.1074/jbc.M105854200
35. Vila A, Rosengarth A, Piomelli D, Cravatt B, Marnett LJ (2007) Hydrolysis of prostaglandin glycerol esters by the endocannabinoid-hydrolyzing enzymes, monoacylglycerol lipase and fatty acid amide hydrolase. Biochemistry 46(33):9578–9585. https://doi.org/10.1021/bi7005898
36. Manna JD, Wepy JA, Hsu KL, Chang JW, Cravatt BF, Marnett LJ (2014) Identification of the major prostaglandin glycerol ester hydrolase in human cancer cells. J Biol Chem 289(49):33741–33753. https://doi.org/10.1074/jbc.M114.582353
37. Savinainen JR, Kansanen E, Pantsar T, Navia-Paldanius D, Parkkari T, Lehtonen M, Laitinen T, Nevalainen T, Poso A, Levonen AL, Laitinen JT (2014) Robust hydrolysis of prostaglandin glycerol esters by human monoacylglycerol lipase (MAGL). Mol Pharmacol 86(5):522–535. https://doi.org/10.1124/mol.114.094284
38. Xie S, Borazjani A, Hatfield MJ, Edwards CC, Potter PM, Ross MK (2010) Inactivation of lipid glyceryl ester metabolism in human THP1 monocytes/macrophages by activated organophosphorus insecticides: role of carboxylesterases 1 and 2. Chem Res Toxicol 23(12):1890–1904. https://doi.org/10.1021/tx1002194
39. Chicca A, Gachet MS, Petrucci V, Schuehly W, Charles RP, Gertsch J (2015) 4′-O-methylhonokiol increases levels of 2-arachidonoyl glycerol in mouse brain via selective inhibition of its COX-2-mediated oxygenation. J Neuroinflammation 12:89. https://doi.org/10.1186/s12974-015-0307-7
40. Andreasson KI, Savonenko A, Vidensky S, Goellner JJ, Zhang Y, Shaffer A, Kaufmann WE, Worley PF, Isakson P, Markowska AL (2001) Age-dependent cognitive deficits and neuronal apoptosis in cyclooxygenase-2 transgenic mice. J Neurosci 21(20):8198–8209
41. Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF (2009) Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. Nat Chem Biol 5(1):37–44. https://doi.org/10.1038/nchembio.129
42. Marshall PJ BJ, Wasvary J, van Duzer J, Du Z, Scott G, Rordorf C, Milosavljev S, and, RA, F (2002) The in vitro and in vivo selectivity of COX189, A new and highlyselective inhibitor of COX-2. Ann Rheum Dis 61 (259)

Targeting the COX/mPGES-1/PGE_2 Pathway in Neuroblastoma

9

Karin Larsson, Anna Kock, Per Kogner, and Per-Johan Jakobsson

Abstract

The importance of prostaglandin E_2 in cancer progression is well established, but research on its role in cancer has so far mostly been focused on epithelial cancer in adults while the knowledge about the contribution of prostaglandin E_2 to childhood malignancies is limited. Neuroblastoma, an extracranial solid tumor of the sympathetic nervous system, mainly affects young children. Patients with tumors classified as high-risk have poor survival despite receiving intensive treatment, illustrating a need for new treatments complimenting existing ones. The basis of neuroblastoma treatment e.g. chemotherapy and radiation therapy, target the proliferating genetically unstable tumor cells leading to treatment resistance and relapses. The tumor microenvironment is an avenue, still to a great extent, unexplored and lacking effective targeted therapies. Cancer-associated fibroblasts is the main source of prostaglandin E_2 in neuroblastoma contributing to angiogenesis, immunosuppression and tumor growth. Prostaglandin E_2 is formed from its precursor arachidonic acid in a two-step enzymatic reaction. Arachidonic acid is first converted by cyclooxygenases into prostaglandin H_2 and then further converted by microsomal prostaglandin E synthase-1 into prostaglandin E_2. We believe targeting of microsomal prostaglandin E synthase-1 in cancer-associated fibroblasts will be an effective future therapeutic strategy in fighting neuroblastoma.

Keywords

Microsomal prostaglandin E synthase-1 · Prostaglandin E_2 · mPGES-1 inhibition · COX-inhibition · Neuroblastoma · Cancer-associated fibroblasts · Tumor microenvironment · Targeted therapy · Tumor-promoting inflammation · Cancer

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9.1 Introduction

Prostaglandin E_2 (PGE_2) is a pro-inflammatory mediator belonging to the eicosanoid family of bioactive lipids and the most abundant lipid mediator in tumor tissue. Numerous studies

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describe upregulation of PGE_2 in different tumors [1–5] and the contribution of PGE_2 to tumor progression is linked to several of the hallmarks of cancer including increased proliferation of tumor cells [6, 7], withstanding apoptosis signals [8], increased angiogenesis [9], invasion and metastasis [10], and evasion of host immune response [11, 12].

Neuroblastoma is a neural crest derived childhood malignancy of the sympathetic nervous system. Neuroblastoma accounts for approximately 6–10% of the childhood cancer cases and 9–15% of the pediatric cancer deaths making it the single most common and deadly tumor of childhood [13, 14]. The disease ranges from localized tumors with favorable biology and high survival to metastatic tumors with aggressive biology and poor prognosis. Neuroblastoma is stratified into three risk-groups, low-risk, intermediate-risk and high-risk groups, depending on clinical age, metastatic stage and histologic appearance with/without differentiation and biology including genetic aberrations [15]. Two of the most common genetic aberration in the unfavorable high-risk neuroblastoma is amplification of the *MYCN* oncogene (*MYCN*-amplified) and loss of the long arm of chromosome 11 (11q-deletion) [16]. Children with high-risk tumors only have a survival rate of 40–50% despite intensive multimodal treatment including intense induction chemotherapy, ablative therapy with stem cell rescue, radiation therapy, immune therapy and attempts to complete surgical removal [17]. The remarkable progression in pediatric oncology with increasing survival of most childhood malignancies during the second half of the twentieth century has stagnated in the last decade highlighting the need for new therapeutic targets and strategies to further increase survival. Intensification of current treatments is not an option since life long side effects and secondary malignancies already pose an increasing problem in surviving children [18].

Chemotherapy and radiation therapy hit the fast proliferating tumor cells that are genetically unstable resulting in clonal evolution and the development of therapy resistance [19]. Stromal cells that are not targeted by chemotherapy and radiation, continue to produce important mediators such as cytokines, chemokines, growth factors and PGE_2 that sustains inflammation and promote tumor repopulation [20]. It has therefore lately been suggested that therapies targeting genetically stable cells in the stromal compartment will be a promising alternative or an additional therapeutic strategy [21, 22]. This review will address PGE_2-driven inflammation, its contribution to neuroblastoma progression and inhibition of PGE_2 as a new therapeutic strategy in neuroblastoma treatment.

9.2 Prostaglandin E_2 Biosynthesis and Signaling

Arachidonic acid (AA), the initial precursor for prostanoid (prostaglandins and thromboxane) biosynthesis, is an omega-6 fatty acid and released from cellular membranes by phospholipases A_2 (PLA_2). PGE_2 is synthesized from AA in a two-step enzymatic reaction. In the first step AA is converted by cyclooxygenases (COX)-1 and − 2 into a reactive intermediate, prostaglandin (PG)H_2 via a transition molecule PGG. PGH_2 is then converted into PGE_2, PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane (TXA_2) by specific terminal synthases i.e. PGE synthases, PGD synthases, PGF synthase, PGI synthase and TX synthase respectively. The key PGE_2 producing synthase is microsomal prostaglandin E synthase-1 (mPGES-1) [23]. During normal conditions mPGES-1 is expressed at low levels but then rapidly induced upon stimuli with pro-inflammatory cytokines (IL-1β, TNF-α) [24, 25], endotoxins (LPS) [26], growth factors (EGF) [27] and hypoxia [28]. There is two additional PGE_2 synthases reported, namely cytosolic prostaglandin E synthase (cPGES) and microsomal prostaglandin E synthase-2 (mPGES-2), with no structural relationship to mPGES-1 [29, 30]. While the function of mPGES-2 as a PGE_2 synthase has been shown both for recombinantly expressed enzyme [31] and in mammalian cells [29], the function of mPGES-2 as a PGE_2 synthase in physiological and pathological condi-

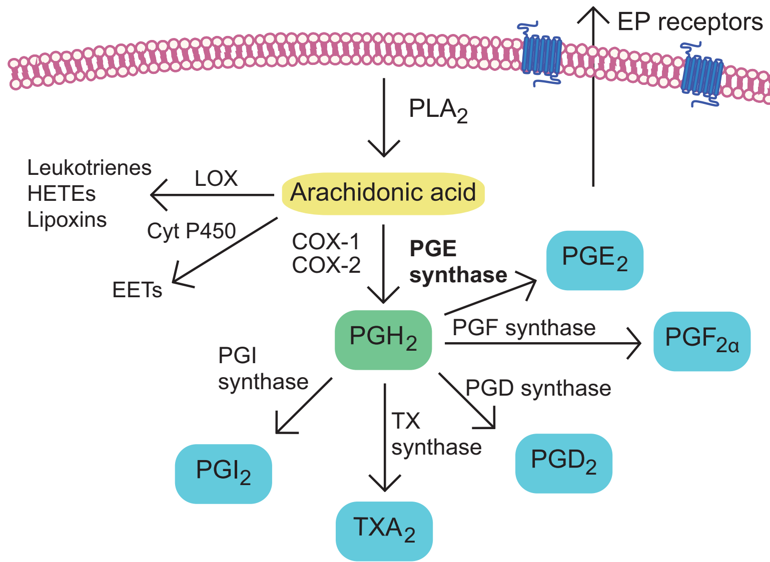

Fig. 9.1 Prostaglandin E_2 biosynthesis. Arachidonic acid (AA) released from cellular membranes is converted to prostaglandin (PG)E_2 in a two-step enzymatic reaction. In the first step AA is oxidized by cyclooxygenase (COX) -1 or -2 into the short lived intermediate PGH_2. In the second step microsomal prostaglandin E synthase-1 (mPGES-1) converts PGH_2 into PGE_2. PGH_2 can also be converted into other prostanoids (PGD_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) or thromboxane (TXA_2)) by respective terminal synthases. PLA_2, phospholipase A_2; EP, E prostanoid; HETEs, hydroxyeicosatetraenoic acids; EETs, epoxyeicosatrienoic acids; LOX, lipoxygenase; Cyt, Cytochrome

tions remains to be proven [32, 33]. PGE synthase activity was found in cPGES purified from rat brain and in mammalian cells transfected with cPGES cDNA [30]. cPGES (also known as p23 or cPGES/p23) is a co-chaperone to Hsp90 and has been linked to cancer but not coupled to any PGE synthase activity *in vivo* [34, 35]. PGE_2 can also be metabolized non-enzymatically from PGH_2 but at a much slower rate than enzymatic conversion by mPGES-1 [36]. The biosynthesis of prostanoids is summarized in Fig. 9.1.

PGE_2 elicit its response by four G-protein coupled receptors, the E prostanoid receptors EP1, EP2, EP3 and EP4. The EP receptors are coupled to Gα proteins with stimulatory or inhibitory subunits leading to the activation of a diverse panel of downstream signaling pathways (Fig. 9.2). EP2 and EP4 are coupled with Gαs that activates adenylyl cyclase (AC) that in turn leads to cAMP production. EP3 is coupled to Gαi that instead inhibits AC. EP1 is coupled to Gαq that activates phospholipase C leading to increased Ca^{2+} influx [37].

9.3 PGE_2 in the Tumor Microenvironment

Solid tumors are not only composed of malignant cells but a complex mix of cellular components including cancer cells, immune cells, endothelial cells, cancer-associated fibroblasts (CAFs) and the non-cellular extracellular matrix (ECM) contributing to the production of growth factors and pro-inflammatory mediators, thus providing the prerequisites for tumor progression. PGE_2 play an important role in the orchestration of the many processes involved in the developing tumor microenvironment [38]. By downregulating Th1 cytokines (IFNγ, TNFα and IL-2) and upregulating Th2 cytokines (IL-10, IL-4 and IL-6) PGE_2 shift the balance from the anti-tumor Th1 response towards immunosuppressive Th2 response [39–41]. PGE_2 both directly and via the shift in cytokine production suppress the cytotoxic activity of cytotoxic T lymphocytes and natural killer cells, inhibits maturation of dendritic cells and promotes immunosuppressive cells like T regulatory cells and myeloid derived

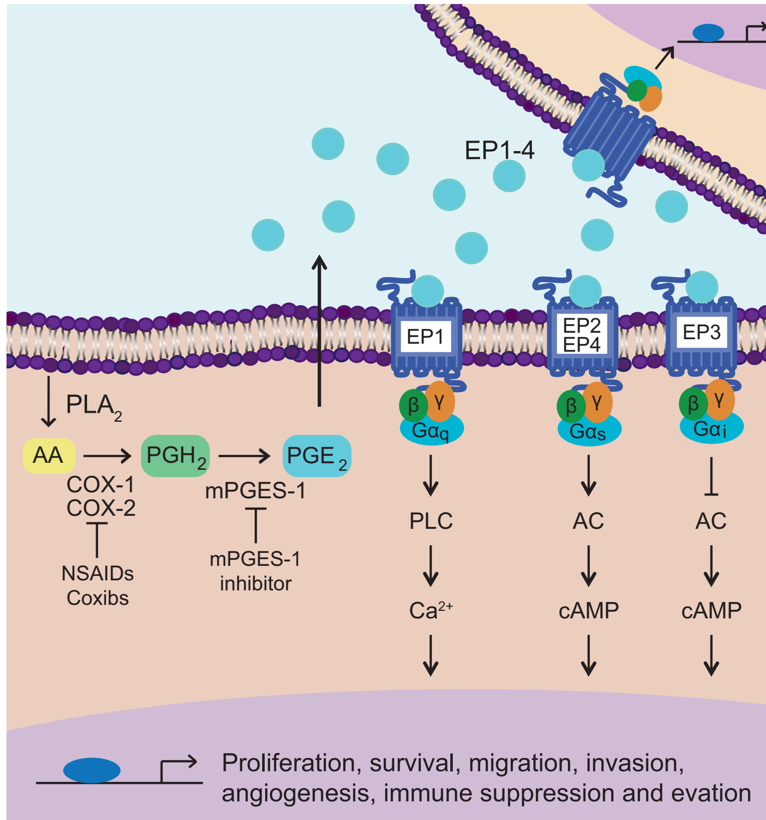

Fig. 9.2 PGE_2 signaling. PGE_2 formed via COXs and mPGES-1 is released from cells and confer downstream signaling via four E prostanoid (EP) receptors, EP1-EP4, either on the same cell (autocrine) or neighboring cells (paracrine). The EP receptors are G-protein coupled receptors that leads to changes in intracellular Ca^{2+} or cyclic adenosine monophosphate (cAMP) levels. EP1 results in increased Ca^{2+} flux mediated through phospholipase C (PLC), EP2 and EP4 leads to increased cAMP levels and EP3 in decrease levels of cAMP via activation or inhibition of adenylyl cyclase (AC) respectively. To inhibit PGE_2 signaling, either COXs or mPGES-1 can be inhibited or EP receptor antagonists can be used. NSAIDs, nonsteroidal anti-inflammatory drugs

suppressor cells [42–46]. PGE_2 also promotes the polarization of tumor-associated macrophages (TAMs) towards a phenotype close to M2 polarized macrophages with surface expression of CD163 and CD206 [47]. The M2-like TAMs in turn contribute to tumor progression and immune suppression by the production of cytokines and chemokines e.g. IL-6 and IL-10 [48]. CAFs, one of the most abundant cell type within the tumor microenvironment, are recruited and activated by tumor cells and influence several important processes in tumor progression including ECM remodeling, migration and metastasis, immune modulation and angiogenesis via secretion of a plethora of soluble factors, including PGE_2 [49]. PGE_2 produced by CAFs, TAMs and tumor cells can stimulate vascular endothelial growth factor (VEGF) production in both CAFs and tumor cells and thereby promote angiogenesis [50, 51].

9.4 Tumor-Promoting Inflammation in Neuroblastoma

Lately, several investigations about the stromal compartment of neuroblastoma tumors have improved our knowledge about the role of non-malignant cells in the tumor microenvironment to neuroblastoma progression [21]. In a study by

Carlson and coworkers, a shift was found during tumor progression in an experimental MYCN-driven neuroblastoma model, from an adaptive immune response in early lesions to a state of immature innate immune response in larger tumors. Also, a change in the population of infiltrating macrophages, the TAMs, was detected from an M1 macrophage phenotype in early neoplastic lesions to an M2-like macrophage phenotype in the established tumors leading to a gradually increasing immunosuppressive microenvironment [52]. Asgharzadeh and colleagues also illuminated the importance of TAMs in metastatic spread of neuroblastoma. Metastatic *MYCN* non-amplified tumors had a higher degree of TAM infiltration than non-metastatic tumors and by calculating an inflammation-related gene score, survival rate could be predicted [53]. We also found an increased infiltration of M2-like TAMs (CD163 and CD206 expression) in high-risk neuroblastoma compared to low-risk neuroblastoma [2]. In addition to TAMs, other cell types were also infiltrating the neuroblastoma tumors e.g. dendritic cells, T cells, B cells and CAFs. The CAFs were found in proximity to the dendritic cells and T-cells indicating an interaction between these cell types [2]. The numbers of TAMs and area of CAFs were found to correlate with clinical stage, in a study by Hashimoto et al. They also observed that TAMs resided close to the CAFs, suggesting an interaction also between CAFs and macrophages in the neuroblastoma tumor microenvironment and that these cell together promote neuroblastoma progression [54]. These results were recently confimed in a study by Borriello et al., where they demonstrated a correlation between CD163 expressing cells and CAFs in neuroblastoma tumors and also in this study it was found that the cell types resided in proximity to each other indicating an interaction [55]. In addition to promoting cancer cell growth and interacting with immune cells, CAFs also promote angiogenesis [56]. Zeine et al. found an association between high levels of α-SMA positive CAFs and microvascular proliferation in neuroblastoma tumors suggesting a role of CAFs in promoting angiogenesis also in neuroblastoma [57].

9.5 Prostaglandin E_2 in Neuroblastoma

9.5.1 Expression of PGE_2 Producing Enzymes and Receptors in Neuroblastoma

As reported by us previously, mPGES-1 is expressed in human neuroblastoma tumors [2] as well as in experimental neuroblastoma tumors [58]. The highest levels of mPGES-1 was found in a subset of high-risk tumors with 11q-deletion but without *MYCN*-amplification, compared to high-risk tumors with *MYCN*-amplification and low-risk tumors without poor prognosis biological features. Colocalization of mPGES-1 with markers such as vimentin, fibroblast specific protein (FSP-1), fibroblast activating protein (FAP) and platelet derived growth factor receptor β (PDGFRβ) in the tumors suggested that the major source of mPGES-1 expression was cancer-associated fibroblasts [2]. In experimental tumors we also found colocalization of mPGES-1 with PDGFRβ indicating the validity of these models to study mPGES-1 inhibition in neuroblastoma. Even though we did not find mPGES-1 expression in tumor cells in primary human or experimental neuroblastoma tumors there are reports about mPGES-1 expression in IL-1β stimulated SK-N-SH cells [59, 60], and PGE_2 production in SK-N-SH and SK-N-BE(2) cells when treated with IL-1β and AA [61].

The source of PGH_2, the substrate for PGE_2 biosynthesis, in neuroblastoma tumors is not yet fully understood. In a study by Johnsen et al., extensive COX-2 expression was found both in neuroblastoma tumors and cell lines that further responded to NSAID therapy *in vitro* and *in vivo* [62]. In a more recent study, we on the other hand primarily found COX-1 expression in the neuroblastoma tumors both on mRNA level and protein level, with the exception of *MYCN*-amplified tumors where COX-2 was the dominant isoform. Interestingly, there were not many cells co-expressing COX-1 and mPGES-1, suggesting transcellular transfer of PGH_2 [2].

The levels of PGE_2 not only depend on COX and mPGES-1 expression but also on the PGE_2 degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH). In the subset of high mPGES-1 expressing neuroblastoma tumors with 11q-deletion only low levels of 15-PGDH was detected whereas in the low mPGES-1 expressing tumors (*MYCN*-amplified tumors and low-risk tumors) high expression of 15-PGDH was detected. The low expression of 15-PGDH probably contributed to the increased levels of PGE_2 found in the 11q-deleted tumors [2].

In a comprehensive study by Rasmuson et al., an abundant expression of all EP receptors in the tumor tissue irrespective of tumor subset was detected. Expression of the EP receptors was found in tumor cells and in the stromal vasculature [61]. In a following study, we also found expression of all EP receptors in neuroblastoma tumors with *MYCN*-amplification and 11q-deletion. EP1, EP2 and EP3 was found in both tumor cells and stromal cells whereas EP4 showed a clear stromal localization [58]. The reason for this discrepancy could be that in the previous study paraffin embedded material was used while in the latter frozen tumor material was used.

9.5.2 COX-Inhibitors in Experimental Neuroblastoma Models *in vivo*

The benefits of COX-inhibition to reduced tumor growth in neuroblastoma experimental models have been demonstrated in several studies, summarized in Table 9.1. Johnsen et al. showed a significantly reduced tumor growth and tumor weight at sacrifice, in nude rats with SK-SY5Y neuroblastoma xenografts, treated with either celecoxib (10 mg/day for 10 days) or diclofenac (250 g/l drinking water for 11 days). They also demonstrated increased apoptosis in the tumors from diclofenac treated rats [62]. In another study, transgenic *MYCN*$^{+/+}$ mice were treated with low-dose aspirin for 10 days (10 mg/kg/day), leading to reduced tumor growth and a decrease in tumor-promoting inflammation [52]. Additionally, a COX-2 specific inhibitor NS-398 (15 mg/kg/day for 4.5 weeks) was found to reduce growth of subcutaneous SK-N-AS xenografts as well as bone metastasis in nude mice. Treatment with NS-398 also reduced expression of VEGF-A and microvessel density [63]. We also showed that diclofenac (250 g/l drinking water for 9 days) significantly reduced tumor growth in mice carrying SK-N-AS neuroblas-

Table 9.1 Overview of neuroblastoma *in vivo* studies targeting PGE_2 biosynthesis

Inhibitor	Target	*In vivo* model	Treatment		Effects	References
			Dose	Time		
Celecoxib	COX-2	rat xeno, SK-SY5Y	10 mg, *p.o.*	10 days	Tumor growth↓	[62]
Diclofenac[a]	COX-1/2	rat xeno, SK-SY5Y	250 g/l	11 days	Inhibit growth	
					Apoptosis↑	
Aspirin[b]	COX-1	mice, tg-*MYCN*$^{+/+}$	10 mg/kg, *p.o.*	10 days	Tumor growth↓	[52]
					Inflammation↓	
NS-398	COX-2	mice xeno, SK-N-AS	15 mg/kg, *i.p.*	4.5 weeks	Tumor growth↓	[63]
					Bone metastasis↓	
					Angiogenesis↓	
Diclofenac[a]	COX-1/2	mice xeno, SK-N-AS	250 g/l	9 days	Tumor growth↓	[2]
Compound III	mPGES-1	mice xeno, SK-N-AS	50 mg/kg, *i.p.*	8 days	Tumor growth↓	[58]
		mice, tg-*MYCN*$^{+/+}$	50 mg/kg, *i.p.*	10 days	Tumor growth↓	
					Angiogenesis↓	
					CAF infiltration↓	
					M1/M2↑	

[a]Mice had free access to drinking water supplemented with diclofenac at the indicated concentration
[b]Dose of aspirin administered equivalent to low-dose aspirin in humans. *Xeno*, xenograft; *tg*, transgenic; *p.o.*, oral gavage, *i.p.*, intraperitoneal. In all experiments, inhibitors were administered daily during the indicated time

toma xenografts, and that the reduction of tumor growth was associated with a reduction of PGE_2 in the tumor tissue [2].

9.5.3 Selective Inhibition of mPGES-1 in Experimental Neuroblastoma Models *in vivo*

In a recently published paper, we investigated the effect of selective mPGES-1 inhibition on neuroblastoma growth [58]. We used two experimental neuroblastoma mouse models, a xenograft model reflecting the 11q-deleted subset of high-risk neuroblastoma and an inflammatory MYCN-driven transgenic model [52, 64]. In both models, daily intraperitoneal injections of the cross-species human/murine mPGES-1 inhibitor Compound III (CIII, 50 mg/kg) for 8–10 days, resulted in reduced tumor growth. We also found interesting changes in the tumor microenvironment upon pharmacological mPGES-1 inhibition with CIII, highlighting the importance of PGE_2 in several aspects of the tumor microenvironment all of which probably contributed to the resulting decrease in tumor growth. Firstly, tumors from CIII treated mice were less vascularized compared to controls, assessed by immunohistochemical (IHC) analysis with an antibody towards the endothelial marker CD31. Secondly, IHC analysis of macrophage polarization markers revealed less pro-tumorigenic M2 phenotype of the TAMs (CD206) in tumors from CIII treated mice compared to controls. A concomitant increase in antitumorigenic M1 phenotype of the TAMs, detected with flow cytometry, confirmed a shift towards a less immunosuppressive tumor microenvironment in CIII treated mice compared to controls. Thirdly, host-derived PGE_2 producing CAFs infiltrating the tumors were positive for PDGFRβ, EP4 and the IL-1 receptor type I as well as phosphorylated STAT3, describing a pro-inflammatory pro-tumorigenic CAF phenotype. In an *in vitro* migration assay, IL-1β treatment induced migration of fibroblasts towards tumor cells, a migration that was inhibited either by CIII or an EP4 antagonist, suggesting a role of PGE_2 in recruiting stromal cells to the tumor microenvironment. Inhibition of mPGES-1 *in vivo* also resulted in decreased presence of PDGFRβ expressing CAFs, thus not only inhibiting mPGES-1 enzyme activity in CAFs already infiltrating the tumor but, consistent with the *in vitro* data, reducing further recruitment of CAFs. These data together provide strong evidence that mPGES-1/PGE_2 play an important role in the developing tumor microenvironment in neuroblastoma creating an immunosuppressive milieu and contributing to CAF infiltration and angiogenesis.

9.6 Clinical Benefit of PGE_2 Blocking Therapy in Cancer Treatment

All anti-tumor treatments strive to induce apoptosis or cell death, either by directly targeting the tumor cells or via the immune system. The cellular stress that follow massive cell death, induced by cytotoxic treatment or irradiation of cells, also leads to induction and release of PGE_2, stimulating tumor repopulation [65–67]. Proposed mechanisms how PGE_2 support tumor cell survival and repopulation include changing the apoptotic threshold [68], suppression of inflammatory responses [69] and by promotion of cancer stem cells [70, 71]. Several studies have illuminated the benefits of PGE_2 inhibition in combination with chemotherapy. Recently, Kurtova et al. showed that the cytotoxic treatment-induced apoptosis and the accompanied PGE_2 release promoted cancer stem cells repopulation and chemoresistance. Inhibition of PGE_2 with celecoxib (5 mg/kg/day, starting 2 days before cytotoxic treatment and then continuing throughout the experiment) in combination with gemcitabine/cisplatin (GC) administered in several treatment cycles reduced chemotherapy resistance compared to GC treatment alone in a bladder cancer xenograft model [20]. Also in neuroblastoma *in vivo* studies, there are reports about beneficial combination effects with celecoxib and cytotoxic drugs. Celecoxib (10 mg/day for 12 days) was found to potentiate the antitumor effect of irinotecan and doxorubicin in neuroblastoma (SH-SY5Y) xenografted rats [72]. Additional studies confirmed an enhanced anti-tumor effect of irinotecan when adminis-

tered in combination with low-dose celecoxib (5 mg/kg/day for 20 consecutive days) in three patient-derived neuroblastoma xenografts [73]. Celecoxib (250 mg/kg/day for 20 days) and a PGE_2 neutralizing antibody was also found to enhance the effect of radiation in a head and neck squamous carcinoma xenograft model [74]. Several recent studies also indicate that inhibition of PGE_2 could be a strategy to enhance the clinical outcome of immune-based therapies [75–77]. Weather mPGES-1 inhibitors will have the same beneficial combinatorial effects as celecoxib remains to be proven.

9.7 A Niche for mPGES-1 Inhibition in Cancer Treatment

Although NSAIDs and Coxibs have proved anti-tumor efficiency in mouse models and epidemiology data supports the benefits of regular NSAID intake to prevent cancer, the severe side effects on the gastrointestinal tract and cardiovascular system has hampered their use as chemo-preventing and chemopotentiating agents. In children, there is also a fear of Reye's syndrome that has been associated with viral induced fever and intake of aspirin, which is why the use of aspirin in children below 16 years of age is usually not recommended [78, 79]. In addition, some cytostatic drugs like the anthracyclines are cardiotoxic and therefore may increase the risk of cardiovascular adverse effects seen with Coxibs in adults, also in children [80].

An mPGES-1 inhibitor would potentially have the same anti-tumor properties seen with COX inhibitors without the severe side effects on the gastrointestinal tract and the cardiovascular system, resulting from the inhibition of all prostaglandins. Still, there are no mPGES-1 inhibitors available in the clinic to date. There are three main reasons why the development of mPGES-1 inhibitors has failed to reach the clinic. (*i*) 'Guilt of association' to COX-2 inhibitors. Since mPGES-1 preferentially couples with COX-2 there are concerns that an mPGES-1 inhibitor would have the same deleterious effect on the cardiovascular system. (*ii*) A three amino acid phylogenetic difference between human mPGES-1 and murine mPGES-1 in the catalytic cleft renders most candidate drugs developed to human mPGES-1 inefficient towards murine mPGES-1 and thus obstructing pre-clinical testing [81]. (*iii*) Lack of differentiation to NSAIDs. Researchers have failed to provide compelling evidence of the potential benefits of mPGES-1 inhibition compared to COX-inhibition.

In contrast to COX-2 inhibitors, mPGES-1 inhibitors would not affect the thromboxane/prostacyclin balance in favor of thromboxane production and would thereby not have the same effect on the cardiovascular system. Indeed, pharmacological inhibition of mPGES-1 with CIII [82], a cross-species human/murine mPGES-1 inhibitor, was even shown to increase production of prostacyclin and reduce vasoconstriction of blood vessels [83]. There are also studies showing chemoprotective effects of prostacyclin and PGD_2, effects that would be lost upon COX inhibition but would be spared or even enhanced by mPGES-1 inhibition and the potential shunting to prostacyclin and PGD_2. For comprehensive reviews of pre-clinical mPGES-1 inhibitors see references [84, 85]. For a summary of the advantages and disadvantages with mPGES-1 inhibition versus COX inhibition see Table 9.2.

Table 9.2 Advantages and disadvantages with mPGES-1 inhibition vs COX inhibition in cancer treatment

	mPGES-1 inhibition
+	Anti-tumor efficiency *in vivo*.
+	Selective PGE_2 inhibition.
+	Cardiovascular safe.
–	No available inhibitor in the clinic.
–	Unknown shunting effects.
–	Poor efficiency of human inhibitors in mouse models.
	COX inhibition
+	Anti-tumor efficiency *in vivo*.
+	Supportive epidemiologic data.
–	Inhibit chemoprotective prostacyclin and PGD_2.
–	Gastrointestinal side effects.
–	Cardiovascular side effects.
–	Fear that aspirin causes Reye's syndrome in children.
–	Could increase cardiotoxicity of cytotoxic drugs.

9.8 Conclusions

A link between tumor-promoting inflammation and prostaglandin E_2 is well established in adult tumors [41]. There are recent studies highlighting the contribution of the tumor microenvironment to neuroblastoma progression, but less studies exist that cover the role of PGE_2 in the neuroblastoma microenvironment. We have suggested a role of PGE_2-driven inflammation in neuroblastoma with CAFs as the main source of mPGES-1 expression [2, 58]. In experimental neuroblastoma tumors, inhibition of CAF-derived PGE_2 slows down tumor growth and alters several parameters of the tumor microenvironment in favor of the host; however, further investigations are warranted elucidating underlying mechanism behind the role of PGE_2 in the microenvironment of neuroblastoma.

COX-inhibitors are widely used drugs and have shown promising results in experimental models of neuroblastoma and in occasional clinical reports, but they are with a few exceptions not used in the regular clinical treatment today as adjuvant neuroblastoma therapy. The reason for this could be attributed to the risk of adverse effects with prolonged use of NSAIDs in the adult population and that these risks are not well documented in children. Even though inhibition of PGE_2, using COX inhibitors or mPGES-1 inhibitors, reduces tumor growth it will not be used as monotherapy in cancer treatment. Instead the great benefit of reducing PGE_2 in the tumor microenvironment lay in sensitizing tumor cells to killing [86]. As an adjuvant treatment, inhibition of PGE_2 have the potential to improve the effects of established therapies, e.g. chemotherapy, radiation therapy and immune therapy leading to increased survival or lowering the doses of these treatments and their associated side effects. Thus, we envision that inhibition of mPGES-1/PGE_2 has the potential to be widely used as a future adjuvant anti-tumor therapy.

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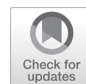
References

1. Cohen EG, Almahmeed T, Du BH et al (2003) Microsomal prostaglandin E synthase-1 is overexpressed in head and neck squamous cell carcinoma. Clin Cancer Res 9(9):3425–3430
2. Larsson K, Kock A, Idborg H et al (2015) COX/mPGES-1/PGE2 pathway depicts an inflammatory-dependent high-risk neuroblastoma subset. Proc Natl Acad Sci U S A 112(26):8070–8075. https://doi.org/10.1073/pnas.1424355112
3. van Rees BP, Sivula A, Thorén S et al (2003) Expression of microsomal prostaglandin E synthase-1 in intestinal type gastric adenocarcinoma and in gastric cancer cell lines. Int J Cancer 107(4):551–556. https://doi.org/10.1002/ijc.11422
4. Yoshimatsu K, Golijanin D, Paty PB et al (2001) Inducible microsomal prostaglandin E synthase is overexpressed in colorectal adenomas and cancer. Clin Cancer Res 7(12):3971–3976
5. Yoshimatsu K, Altorki NK, Golijanin D et al (2001) Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. Clin Cancer Res 7(9):2669–2674
6. Pai R, Soreghan BA, Szabo IL et al (2002) Prostaglandin E-2 directly promotes human colon cancer growth by triggering activation of ERK2, c-fos gene and cell proliferation. Gastroenterology 122(4):A240–A240
7. Sheng H, Shao J, Washington MK, DuBois RN (2001) Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. J Biol Chem 276(21):18075–18081. https://doi.org/10.1074/jbc.M009689200
8. Sheng H, Shao J, Morrow JD, Beauchamp RD, DuBois RN (1998) Modulation of apoptosis and Bcl-2 expression by prostaglandin E2 in human colon cancer cells. Cancer Res 58(2):362–366
9. Pai R, Szabo IL, Soreghan BA et al (2001) PGE(2) stimulates VEGF expression in endothelial cells via ERK2/JNK1 signaling pathways. Biochem Biophys Res Commun 286(5):923–928. https://doi.org/10.1006/bbrc.2001.5494
10. Buchanan FG, Wang D, Bargiacchi F, DuBois RN (2003) Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 278(37):35451–35457. https://doi.org/10.1074/jbc.M302474200
11. Kalinski P (2012) Regulation of immune responses by prostaglandin E2. J Immunol 188(1):21–28. https://doi.org/10.4049/jimmunol.1101029
12. Obermajer N, Wong JL, Edwards RP et al (2012) PGE(2)-driven induction and maintenance of cancer-associated myeloid-derived suppressor cells. Immunol

Investig 41(6–7):635–657. https://doi.org/10.3109/08820139.2012.695417
13. Brodeur GM (2003) Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 3(3):203–216. https://doi.org/10.1038/nrc1014
14. Johnsen JI, Kogner P, Albihn A, Henriksson MA (2009) Embryonal neural tumours and cell death. Apoptosis 14(4):424–438. https://doi.org/10.1007/s10495-009-0325-y
15. Cohn SL, Pearson AD, London WB et al (2009) The International Neuroblastoma Risk Group (INRG) classification system: an INRG task force report. J Clin Oncol 27(2):289–297. https://doi.org/10.1200/JCO.2008.16.6785
16. Carén H, Kryh H, Nethander M et al (2010) High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. Proc Natl Acad Sci U S A 107(9):4323–4328. https://doi.org/10.1073/pnas.0910684107
17. Ladenstein R, Potschger U, Pearson ADJ et al (2017) Busulfan and melphalan versus carboplatin, etoposide, and melphalan as high-dose chemotherapy for high-risk neuroblastoma (HR-NBL1/SIOPEN): an international, randomised, multi-arm, open-label, phase 3 trial. Lancet Oncol 18(4):500–514. https://doi.org/10.1016/S1470-2045(17)30070-0
18. Moreno L, Vaidya SJ, Pinkerton CR et al (2013) Long-term follow-up of children with high-risk neuroblastoma: the ENSG5 trial experience. Pediatr Blood Cancer 60(7):1135–1140. https://doi.org/10.1002/pbc.24452
19. Friedman R (2016) Drug resistance in cancer: molecular evolution and compensatory proliferation. Oncotarget 7(11):11746–11755. https://doi.org/10.18632/oncotarget.7459
20. Kurtova AV, Xiao J, Mo Q et al (2015) Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. Nature 517(7533):209–213. https://doi.org/10.1038/nature14034
21. Borriello L, Seeger RC, Asgharzadeh S, DeClerck YA (2015) More than the genes, the tumor microenvironment in neuroblastoma. Cancer Lett 380(1):304–314. https://doi.org/10.1016/j.canlet.2015.11.017
22. Matthay KK, Maris JM, Schleiermacher G et al (2016) Neuroblastoma. Nat Rev Dis Primers 2:Article:16078. https://doi.org/10.1038/nrdp.2016.78
23. Jakobsson PJ, Thorén S, Morgenstern R, Samuelsson B (1999) Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A 96(13):7220–7225
24. Subbaramaiah K, Yoshimatsu K, Scherl E et al (2004) Microsomal prostaglandin E synthase-1 is overexpressed in inflammatory bowel disease. Evidence for involvement of the transcription factor Egr-1. J Biol Chem 279(13):12647–12658. https://doi.org/10.1074/jbc.M312972200
25. Uracz W, Uracz D, Olszanecki R, Gryglewski RJ (2002) Interleukin 1beta induces functional prostaglandin E synthase in cultured human umbilical vein endothelial cells. J Physiol Pharmacol 53(4 Pt 1):643–654
26. Xiao L, Ornatowska M, Zhao G et al (2012) Lipopolysaccharide-induced expression of microsomal prostaglandin E synthase-1 mediates late-phase PGE2 production in bone marrow derived macrophages. PLoS One 7(11):e50244. https://doi.org/10.1371/journal.pone.0050244
27. Donnini S, Finetti F, Terzuoli E et al (2012) EGFR signaling upregulates expression of microsomal prostaglandin E synthase-1 in cancer cells leading to enhanced tumorigenicity. Oncogene 31(29):3457–3466. https://doi.org/10.1038/onc.2011.503
28. Xue X, Shah YM (2013) Hypoxia-inducible factor-2alpha is essential in activating the COX2/mPGES-1/PGE2 signaling axis in colon cancer. Carcinogenesis 34(1):163–169. https://doi.org/10.1093/carcin/bgs313
29. Murakami M, Nakashima K, Kamei D et al (2003) Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J Biol Chem 278(39):37937–37947. https://doi.org/10.1074/jbc.M305108200
30. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I (2000) Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem 275(42):32775–32782. https://doi.org/10.1074/jbc.M003504200
31. Tanikawa N, Ohmiya Y, Ohkubo H et al (2002) Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem Biophys Res Commun 291(4):884–889. https://doi.org/10.1006/bbrc.2002.6531
32. Jania LA, Chandrasekharan S, Backlund MG et al (2009) Microsomal prostaglandin E synthase-2 is not essential for in vivo prostaglandin E-2 biosynthesis. Prostaglandins Other Lipid Mediat 88(3–4):73–81. https://doi.org/10.1016/j.prostaglandins.2008.10.003
33. Hara S (2017) Prostaglandin terminal synthases as novel therapeutic targets. Proc Jpn Acad Ser B Phys Biol Sci 93(9):703–723. https://doi.org/10.2183/pjab.93.044
34. Cano LQ, Lavery DN, Sin S et al (2015) The co-chaperone p23 promotes prostate cancer motility and metastasis. Mol Oncol 9(1):295–308. https://doi.org/10.1016/j.molonc.2014.08.014
35. Simpson NE, Gertz J, Imberg K, Myers RM, Garabedian MJ (2012) Research resource: enhanced genome-wide occupancy of estrogen receptor alpha by the cochaperone p23 in breast cancer cells. Mol Endocrinol 26(1):194–202. https://doi.org/10.1210/me.2011-1068
36. Yu R, Xiao L, Zhao GQ, Christman JW, van Breemen RB (2011) Competitive enzymatic interactions determine the relative amounts of prostaglandins E-2 and D-2. J Pharmacol Exp Ther 339(2):716–725. https://doi.org/10.1124/jpet.111.185405

37. Sugimoto Y, Narumiya S (2007) Prostaglandin E receptors. J Biol Chem 282(16):11613–11617. https://doi.org/10.1074/jbc.R600038200
38. Nakanishi M, Rosenberg DW (2013) Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol 35(2):123–137. https://doi.org/10.1007/s00281-012-0342-8
39. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP (2002) Prostaglandins as modulators of immunity. Trends Immunol 23(3):144–150. https://doi.org/10.1016/S1471-4906(01)02154-8
40. Snijdewint FGM, Kalinski P, Wierenga EA, Bos JD, Kapsenberg ML (1993) Prostaglandin-E(2) differentially modulates cytokine secretion profiles of human T-helper lymphocytes. J Immunol 150(12):5321–5329
41. Wang D, Dubois RN (2010) Eicosanoids and cancer. Nat Rev Cancer 10(3):181–193. https://doi.org/10.1038/nrc2809
42. Obermajer N, Kalinski P (2012) Key role of the positive feedback between PGE(2) and COX2 in the biology of myeloid-derived suppressor cells. Oncoimmunology 1(5):762–764. https://doi.org/10.4161/onci.19681
43. Baratelli F, Lin Y, Zhu L et al (2005) Prostaglandin E-2 induces FOXP3 gene expression and T regulatory cell function in human CD4(+) T cells. J Immunol 175(3):1483–1490. https://doi.org/10.4049/jimmunol.175.3.1483
44. Miao J, Lu X, Hu YF et al (2017) Prostaglandin E-2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor microenvironment. Oncotarget 8(52):89802–89810. https://doi.org/10.18632/oncotarget.21155
45. Goto T, Herberman RB, Maluish A, Strong DM (1983) Cyclic-Amp as a mediator of prostaglandin-E-induced suppression of human natural-killer cell-activity. J Immunol 130(3):1350–1355
46. Kalinski P, Hilkens CMU, Snijders A, Snijdewint FGM, Kapsenberg ML (1997) IL-12-deficient dendritic cells, generated in the presence of prostaglandin E-2, promote type 2 cytokine production in maturing human naive T helper cells. J Immunol 159(1):28–35
47. Liu LX, Ge DX, Ma L et al (2012) Interleukin-17 and prostaglandin E2 are involved in formation of an M2 macrophage-dominant microenvironment in lung cancer. J Thorac Oncol 7(7):1091–1100. https://doi.org/10.1097/JTO.0b013e3182542752
48. Wan SS, Zhao ED, Kryczek I et al (2014) Tumor-associated macrophages produce interleukin 6 and signal via STAT3 to promote expansion of human hepatocellular carcinoma stem cells. Gastroenterology 147(6):1393–1404. https://doi.org/10.1053/j.gastro.2014.08.039
49. Öhlund D, Elyada E, Tuveson D (2014) Fibroblast heterogeneity in the cancer wound. J Exp Med 211(8):1503–1523. https://doi.org/10.1084/jem.20140692
50. Guo XY, Oshima H, Kitmura T, Taketo MM, Oshima M (2008) Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. J Biol Chem 283(28):19864–19871. https://doi.org/10.1074/jbc.M800798200
51. Wang XY, Klein RD (2007) Prostaglandin E-2 induces vascular endothelial growth factor secretion in prostate cancer cells through EP2 receptor-mediated cAMP pathway. Mol Carcinog 46(11):912–923. https://doi.org/10.1002/mc.20320
52. Carlson LM, Rasmuson A, Idborg H et al (2013) Low-dose aspirin delays an inflammatory tumor progression in vivo in a transgenic mouse model of neuroblastoma. Carcinogenesis 34(5):1081–1088. https://doi.org/10.1093/carcin/bgt009
53. Asgharzadeh S, Salo JA, Ji L et al (2012) Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. J Clin Oncol 30(28):3525–3532. https://doi.org/10.1200/JCO.2011.40.9169
54. Hashimoto O, Yoshida M, Koma Y et al (2016) Collaboration of cancer-associated fibroblasts and tumour-associated macrophages for neuroblastoma development. J Pathol 240(2):211–223. https://doi.org/10.1002/path.4769
55. Borriello L, Nakata R, Sheard MA et al (2017) Cancer-associated fibroblasts share characteristics and protumorigenic activity with mesenchymal stromal cells. Cancer Res 77(18):5142–5157. https://doi.org/10.1158/0008-5472.CAN-16-2586
56. Pietras K, Östman A (2010) Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res 316(8):1324–1331. https://doi.org/10.1016/j.yexcr.2010.02.045
57. Zeine R, Salwen HR, Peddinti R et al (2009) Presence of cancer-associated fibroblasts inversely correlates with Schwannian stroma in neuroblastoma tumors. Mod Pathol 22(7):950–958. https://doi.org/10.1038/modpathol.2009.52
58. Kock A, Larsson K, Bergqvist F et al (2018) Inhibition of microsomal prostaglandin E synthase-1 in cancer-associated fibroblasts suppresses neuroblastoma tumor growth. EBioMedicine 32:84–92. https://doi.org/10.1016/j.ebiom.2018.05.008
59. Olajide OA, Velagapudi R, Okorji UP, Sarker SD, Fiebich BL (2014) Picralima nitida seeds suppress PGE(2) production by interfering with multiple signalling pathways in IL-1 beta-stimulated SK-N-SH neuronal cells. J Ethnopharmacol 152(2):377–383. https://doi.org/10.1016/j.jep.2014.01.027
60. Wendeburg L, de Oliveira ACP, Bhatia HS, Candelario-Jalil E, Fiebich BL (2009) Resveratrol inhibits prostaglandin formation in IL-1 beta-stimulated SK-N-SH neuronal cells. J Neuroinflammation 6:Artn 26. https://doi.org/10.1186/1742-2094-6-26
61. Rasmuson A, Kock A, Fuskevåg OM et al (2012) Autocrine prostaglandin E2 signaling promotes tumor cell survival and proliferation in childhood neuroblastoma. PLoS One 7(1):e29331. https://doi.org/10.1371/journal.pone.0029331
62. Johnsen JI, Lindskog M, Ponthan F et al (2004) Cyclooxygenase-2 is expressed in neuroblastoma, and nonsteroidal anti-inflammatory drugs induce apoptosis and inhibit tumor growth in vivo. Cancer

Res 64(20):7210–7215. https://doi.org/10.1158/0008-5472.CAN-04-1795
63. Tsutsumimoto T, Williams P, Yoneda T (2014) The SK-N-AS human neuroblastoma cell line develops osteolytic bone metastases with increased angiogenesis and COX-2 expression. J Bone Oncol 3(3–4):67–76. https://doi.org/10.1016/j.jbo.2014.10.002
64. Weiss WA, Aldape K, Mohapatra G, Feuerstein BG, Bishop JM (1997) Targeted expression of MYCN causes neuroblastoma in transgenic mice. EMBO J 16(11):2985–2995. https://doi.org/10.1093/emboj/16.11.2985
65. Allen CP, Tinganelli W, Sharma N et al. (2015) DNA damage response proteins and oxygen modulate prostaglandin E-2 growth factor release in response to low and high LET ionizing radiation. Front Oncol 5:UNSP 260. https://doi.org/10.3389/fonc.2015.00260
66. Huang Q, Li F, Liu XJ et al (2011) Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. Nat Med 17(7):860–U231. https://doi.org/10.1038/nm.2385
67. Trifan OC, Durham WF, Salazar VS et al (2002) Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. Cancer Res 62(20):5778–5784
68. Greenhough A, Smartt HJM, Moore AE et al (2009) The COX-2/PGE(2) pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis 30(3):377–386. https://doi.org/10.1093/carcin/bgp014
69. Hangai S, Ao T, Kimura Y et al (2016) PGE2 induced in and released by dying cells functions as an inhibitory DAMP. Proc Natl Acad Sci U S A 113(14):3844–3849. https://doi.org/10.1073/pnas.1602023113
70. Liu QL, Yuan WQ, Tong DL et al (2016) Metformin represses bladder cancer progression by inhibiting stem cell repopulation via COX2/PGE2/STAT3 axis. Oncotarget 7(19):28235–28246. https://doi.org/10.18632/oncotarget.8595
71. Pang LY, Hurst EA, Argyle DJ (2016) Cyclooxygenase-2: a role in cancer stem cell survival and repopulation of cancer cells during therapy. Stem Cells Int 2016:1–11. Artn 2048731. https://doi.org/10.1155/2016/2048731
72. Ponthan F, Wickström M, Gleissman H et al (2007) Celecoxib prevents neuroblastoma tumor development and potentiates the effect of chemotherapeutic drugs in vitro and in vivo. Clin Cancer Res 13(3):1036–1044. https://doi.org/10.1158/1078-0432.CCR-06-1908
73. Kaneko M, Kaneko S, Suzuki K (2009) Prolonged low-dose administration of the cyclooxygenase-2 inhibitor celecoxib enhances the antitumor activity of irinotecan against neuroblastoma xenografts. Cancer Sci 100(11):2193–2201. https://doi.org/10.1111/j.1349-7006.2009.01280.x
74. Davis TW, O'Neal JM, Pagel MD et al (2004) Synergy between celecoxib and radiotherapy results from inhibition of cyclooxygenase-2-derived prostaglandin E-2, a survival factor for tumor and associated vasculature. Cancer Res 64(1):279–285. https://doi.org/10.1158/0008-5472.Can-03-1168
75. Hennequart M, Pilotte L, Cane S et al (2017) Constitutive IDO1 expression in human tumors is driven by cyclooxygenase-2 and mediates intrinsic immune resistance. Cancer Immunol Res 5(8):695–709. https://doi.org/10.1158/2326-6066.CIR-16-0400
76. Hou W, Sampath P, Rojas JJ, Thorne SH (2016) Oncolytic virus-mediated targeting of PGE2 in the tumor alters the immune status and sensitizes established and resistant tumors to immunotherapy. Cancer Cell 30(1):108–119. https://doi.org/10.1016/j.ccell.2016.05.012
77. Zelenay S, van der Veen AG, Bottcher JP et al (2015) Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell 162(6):1257–1270. https://doi.org/10.1016/j.cell.2015.08.015
78. Casteels-Van Daele M, Van Geet C, Wouters C, Eggermont E (2000) Reye syndrome revisited: a descriptive term covering a group of heterogeneous disorders. Eur J Pediatr 159(9):641–648
79. Pugliese A, Beltramo T, Torre D (2008) Reye's and Reye's-like syndromes. Cell Biochem Funct 26(7):741–746. https://doi.org/10.1002/cbf.1465
80. Franco VI, Lipshultz SE (2015) Cardiac complications in childhood cancer survivors treated with anthracyclines. Cardiol Young 25:107–116. https://doi.org/10.1017/S1047951115000906
81. Pawelzik SC, Uda NR, Spahiu L et al (2010) Identification of key residues determining species differences in inhibitor binding of microsomal prostaglandin E synthase-1. J Biol Chem 285(38):29254–29261. https://doi.org/10.1074/jbc.M110.114454
82. Leclerc P, Idborg H, Spahiu L et al (2013) Characterization of a human and murine mPGES-1 inhibitor and comparison to mPGES-1 genetic deletion in mouse models of inflammation. Prostaglandins Other Lipid Mediat 107:26–34. https://doi.org/10.1016/j.prostaglandins.2013.09.001
83. Ozen G, Gomez I, Daci A et al (2017) Inhibition of microsomal PGE synthase-1 reduces human vascular tone by increasing PGI2: a safer alternative to COX-2 inhibition. Br J Pharmacol 174:4087–4098. https://doi.org/10.1111/bph.13939
84. Koeberle A, Werz O (2015) Perspective of microsomal prostaglandin E-2 synthase-1 as drug target in inflammation-related disorders. Biochem Pharmacol 98(1):1–15. https://doi.org/10.1016/j.bcp.2015.06.022
85. Psarra A, Nikolaou A, Kokotou MG, Limnios D, Kokotos G (2017) Microsomal prostaglandin E-2 synthase-1 inhibitors: a patent review. Expert Opin Ther Pat 27(9):1047–1059. https://doi.org/10.1080/13543776.2017.1344218
86. Bergqvist F, Ossipova E, Idborg H et al (2019) Inhibition of mPGES-1 or COX-2 results in different proteomic and lipidomic profiles in A549 lung cancer cells. Front Pharmacol. https://doi.org/10.3389/fphar.2019.00636



10 Metabolomics Biomarkers for Precision Psychiatry

Pei-an (Betty) Shih

Abstract

The treatment of psychiatric disorders remains a significant challenge in part due to imprecise diagnostic criteria and incomplete understanding of the molecular pathology involved. Current diagnostic and pharmacological treatment guidelines use a uniform approach to address each disorder even though psychiatric clinical presentation and prognosis within a disorder are known to be heterogeneous. Limited therapeutic success highlights the need for a precision medicine approach in psychiatry, termed precision psychiatry. To practice precision psychiatry, it is essential to research and develop multiple omics-based biomarkers that consider environmental factors and careful phenotype determination. Metabolomics, which lies at the endpoint of the "omics cascade," allows for detection of alterations in systems-level metabolites within biological pathways, thereby providing insights into the mechanisms that underlie various physiological conditions and pathologies. The eicosanoids, a family of metabolites derived from oxygenated polyunsaturated fatty acids, play a key role in inflammatory mechanisms and have been implicated in psychiatric disorders such as anorexia nervosa and depression. This review (1) provides background on the current clinical challenges of psychiatric disorders, (2) gives an overview of metabolomics application as a tool to develop improved biomarkers for precision psychiatry, and (3) summarizes current knowledge on metabolomics and lipidomic findings in common psychiatric disorders, with a focus on eicosanoids. Metabolomics is a promising tool for precision psychiatry. This research has great potential for both discovering biomarkers and elucidating molecular mechanisms underlying psychiatric disorders.

Keywords

Systematic review · Psychiatric disorders · Metabolomics · Eicosanoids · Polyunsaturated fatty acids · Biomarkers

10.1 Challenges in Clinical Psychiatry

Psychiatric disorders can impair one's thinking, perceptions, emotions, and behaviors, resulting in significant distress or impairment of personal functioning [1]. The five most common categories of psychiatric disorders are *anxiety disorders,* including generalized anxiety disorder and

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post-traumatic stress disorder; *mood disorders,* such as depression and bipolar disorder; *schizophrenia and psychotic disorders*; *dementia*; and *eating disorders,* including anorexia nervosa and binge-eating disorder. Psychiatric disorders are prevalent, with an astonishing 46.4% lifetime prevalence of having at least one major psychiatric disorders in the United States [2]. Psychiatric conditions represent a major public health problem due to their associated disabilities [3] and mortality [4]. The estimated global burden of psychiatric disorders accounts for up to 32% of years lived with disability, and more than 13% of disability-adjusted life-years [5]. Moreover, psychiatric disorders are significant predictors of the onset and severity of subsequent serious medical illnesses such as heart disease [6].

Diagnosis in psychiatry is based on a classification system that includes clinical nosologies such as the International Classification of Diseases [7] and the Diagnostic and Statistical Manual of Mental Disorders [8]. The current diagnostic system is universally applied, not only clinically, but also in research and policy settings such as drug-approval and insurance-reimbursement systems. Although these diagnostic criteria are regularly revised to improve validity, more disagreements about diagnosis fundamentals are found in psychiatry than in any other branch of medicine [9, 10]. The heterogeneous presentation of psychiatric disorders is a result not only of phenotypic, biological, and genetic heterogeneity, but also the outcome of complex interactions between environmental and biological factors. A lack of clear understanding about the complex psychopathology contributing to each disorder leads to inadequate or ineffective treatment strategy. Taking depression as an example, although antidepressants provide substantial benefits for many, issues including lack of efficacy, intolerance, delayed therapeutic onset, and risk of relapse are frequently reported. In fact, results from one of the largest randomized trials involving 4041 patients from 41 clinical sites around the country showed that the remission rate from the first line of treatment was only 28% [11]. Clearly, there is a lot of room for improvement in clinical psychiatry.

10.2 Omics-Based Strategies in Precision Psychiatry

A promising strategy to overcome obstacles in clinical psychiatry is "precision medicine," an emerging approach that aims to improve health and advance individualized care by taking into account "each person's variability in genes, environment, and lifestyle" [12]. This ambitious initiative requires collecting dense data from a large number of cohort studies, including studies of psychiatric disorders [13]. The rise of biotechnologies that simultaneously measure thousands of data points has been timely in meeting the needs of precision medicine. These high-throughput technologies yield multifaceted data, including genomics, epigenomics, transcriptomics, proteomics, metabolomics and are collectively referred to as "multi-omics" [14]. Used effectively, multi-omics investigation enables exploration of complex interactions in biological systems and their roles in health and disorders.

Among individual "omics" disciplines, the most frequently published in psychiatry are genome-wide association studies (GWAS) [15, 16]. GWAS have revealed evidence of substantial pleiotropy or shared genetic etiology among several psychiatric disorders [17]. Due to the polygenic, multi-factorial nature of psychiatric disorders and the inherent limitations of GWAS design, the genetic loci identified are typically small in effect size and of questionable clinical significance [18]. By itself, GWAS likely remain limited in yielding significant translational advances to improve diagnostic accuracy and treatment effectiveness [19].

A multidisciplinary approach that combines multiple omics data– integrated multi-dimensional omics– is much more likely to offer complementary vantage points to enrich our knowledge of expression and functions of genomic factors associated with a disorder, thus improving diagnosis, prognosis, and treatment development [20]. For example, a recent GWAS meta-analysis revealed a high degree of correlation [average genetic correlation (rg) = 0.40] among bipolar disorder, major depressive disorder (MDD), and schizophrenia [21]. On the other hand, a molecular pro-

filing approach characterizing 181 proteins and small molecules in serum showed excellent potential to distinguish schizophrenia from healthy controls, as well as from subjects with MDD, bipolar disorder, and Asperger syndrome [22]. Studies incorporating both investigation methods in the same study cohort likely will lead to significant improvement in diagnostic accuracy.

Biomarkers are *objective* surrogates of genetic, tissue-specific, and environmental factors, as well as their interactions [23]. An effective biomarker system such as integrated multi-dimensional omics will thus serve as one of the most informative research and clinical tools and move the practice of psychiatry closer to the goal of precision psychiatry [24].

10.3 Unique Role of Metabolomics Biomarkers

While GWAS provide information on genomic risk factors that are often unmodifiable, metabolomics studies measure our metabolic state, determined not only by genomic factors but also modified by diet, environmental factors, and host factors such as the childhood experiences and gut microbiome. The metabolic profile serves as a quantifiable, dynamic readout of biochemical state that can inform underlying molecular mechanisms of the disorder or phenotype. As such, metabolomics data have higher relevance to the "disordered state" and may serve well as predictive, prognostic, diagnostic biomarkers [25] for psychiatric disorders. The remainder of this chapter provides a brief summary of the analytical techniques most commonly used in metabolomics studies, and reports on and discusses a selection of psychiatric metabolomics and lipidomic studies. In particular, it highlights a specific class of metabolites called eicosanoids and their unique role in unraveling how disorders are influenced by the interactive relationship between genes and diet [26].

10.4 Overview of Metabolomics Analytical Techniques and Methodologies

The likelihood of success for precision psychiatry lies in the accuracy and comprehensive dimensionality of the data. Analytical techniques for metabolomics have come a long way. Nuclear magnetic resonance (NMR), mass spectrometry (MS), and electrochemical detection are commonly used techniques to identify and quantify metabolites [27]. NMR is less sensitive than MS-based methods, yet it is favorable due to the absence of detection bias and is useful in identifying novel metabolite structures [28]. Compared to NMR, MS is superior in mass analysis capabilities and is usually used together with other separation instruments such as gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE). GC and LC have traditionally been used in metabolomics studies; CE has gained popularity in recent years [27]. In clinical laboratories, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is known for high specificity and sensitivity [29]. LC-MS/MS can detect compounds with low molecular weight (such as eicosanoids) with better sensitivity, selectivity, and higher throughput than high performance liquid chromatography or GC-MS [29].

Equally as important as choosing the right instrument for metabolomics measurement are the design and analysis aspects of metabolomics. Metabolomics analysis methods can broadly be categorized in two ways. "Untargeted metabolomics" (global) analysis captures a wide array of detectable metabolites, including those with unknown functions or that have not been seen previously [30]. Untargeted metabolomics offers the unique advantage of discovering novel perturbations and detecting the relationship between interconnected metabolites from multiple pathways in an unbiased fashion [30]. In contrast, a "targeted metabolomics" approach focuses on a narrower, prespecified cluster of metabolites that have been hypothesized to play a role in the disorder studied. Internal standards allow for quantification

of analytes in targeted metabolomics, offering better control of sensitivity, stability, and reproducibility of each targeted metabolite. Having some prior knowledge of these metabolites and biochemical pathways means associations identified in targeted analysis can move more quickly to other molecular or translational studies to further define mechanisms underlying the phenotype associations.

Recent advances in these complementary approaches have helped elucidate informative metabolomics biomarkers relevant in psychiatric disorders such as eicosanoids with inflammation regulatory functions. These biomarkers show promise for capturing early biochemical changes in the disease state [31] and enabling early diagnosis of psychiatric disorders. While analytical considerations for generating metabolomics data are beyond the scope of this chapter, it is important to note that metabolites are volatile, with a short half-life, making rigorous quality control of biological samples and assay preparation necessary to ensure validity of the findings.

10.5 Metabolomics Studies of Common Psychiatric Disorders

Using an untargeted metabolomics approach and proton NMR (1H-NMR) system, a serum metabolite profile was effective in separating schizophrenia from healthy controls [32]. Moreover, the results seem to reinforce the importance of the glycolysis pathway and a "hyperglutamate hypothesis" previously proposed [33] in schizophrenia. Examining both serum and urinary metabolites, 1H-NMR and gas chromatography with two-dimensional gas chromatography (GC-TOFMS) platforms revealed several pathways implicated in schizophrenia including fatty acid metabolism, carbohydrate metabolism, and amino acid metabolism [34].

Metabolic profiles of cerebrospinal fluid samples from drug-naïve (or minimally treated) first-onset schizophrenia and controls suggest brain-specific alterations in glucoregulatory processes were intrinsic to disease because these dysregulations normalized after treatment with atypical antipsychotic medications in half of schizophrenia patients [35]. Patients with schizophrenia and other psychiatric disorders often experience significant weight gain during their course of treatment [36]. Lipidomic and metabolomic analyses have identified lipids associated with medication-associated weight gain [37] and metabolic predictors of future weight gain [38]. These results emphasize the added usefulness of a metabolomics approach in identifying psychiatric patients at risk of developing metabolic comorbidities [38]. To begin to address the variability in treatment response commonly found in psychiatric disorders, serum metabolites were investigated in 8 schizophrenia patients before and after risperidone monotherapy together with healthy controls. Although the sample size was small, partial least squares discriminant analysis model derived from GC-MS spectra revealed clear separations not only between schizophrenia and controls, but also between risperidone responders and non-responders [39]. These data suggest a global change of metabolites after risperidone treatment, and disturbances of energy metabolism, antioxidant defense systems, neurotransmitter metabolism, fatty acid biosynthesis, and phospholipid metabolism in schizophrenia, which could be partially normalized by risperidone therapy [39].

In major depressive disorder, at least 17 peripheral blood mononuclear cell -derived metabolites identified in the GC-MC platform were significantly altered when compared with controls, indicating disturbances of energy and neurotransmitter metabolism [40]. In a urinary metabolomics study, the NMR- and GC-MS–based methods identified two sets of metabolites that effectively discriminate "moderate" and "severe" patients from healthy controls, respectively [41]. These metabolites implicate involvement of gut microbial metabolites, glycine biosynthesis, and cell death and survival in MDD [41].

Depression is heterogeneous in its presentation and pathophysiology, affecting people of all ages, including those with medical diseases. Metabolomic analysis of plasma from older adults with and without depression revealed lower levels of several neurotransmitters and medium chain fatty acids in depression. Also, the profile of those with remission from depression was more similar to non-depressed controls than to the depressed individuals [42]. In a medical cohort of patients with heart failure with and without depression, GC-MS and LC-MS platforms revealed higher concentrations of several amino acids and dicarboxylic fatty acids [43], consistent with prior findings in neurotransmitter systems and fatty acid metabolism dysregulation. These results suggest that metabolomics biomarkers might be useful as objective diagnostic tests for depressive disorder. Based on findings in several untargeted metabolomics studies of depression and pharmacometabolomic studies [44, 45], a new study investigating whether these metabolites (sphingomyelins, lysophosphatidylcholines, phosphatidylcholines, and acylcarnitines) could act as predictors of depression recovery found that the addition of metabolites in all predictive models outperformed models without these metabolites [46].

In bipolar disorder, 1H-NMR-based analysis revealed lipids, lipid metabolism-related molecules, and some amino acids that distinguished bipolar subjects, with 7 specific markers as “key metabolites” [47]. A study using dual platform (NMR spectroscopy and GC-MS) revealed 5 urinary metabolite biomarkers with higher accuracy than single-platform derived markers in discriminating bipolar disorder from healthy controls [48]. In another study, an increased proportion of serum sphingolipids and glycerolipids and a decreased proportion of glycerophospholipids were found in bipolar disorder patients when UltraPerformance LC coupled with high-resolution MS was used [49]. However, of the top 5 most differential lipids identified, 3 had unknown biology and could not be identified in any databases [49].

10.6 Polyunsaturated Fatty Acids in Psychiatric Disorders

While advances in mass spectrometry have expanded our knowledge of the patterns of metabolomic perturbation in psychiatric disorders, nongenetic risk factors such as diet play a major role in neuronal fitness [50–52]. Essential fatty acids represent a modifiable risk factor for neuropathophysiological processes [53, 54]. While a number of hypotheses exist for etiology of psychiatric disorders, inflammation has recently been shown to play a role in common mental disorders such as depression [55] and schizophrenia [56].

Bioactive lipid mediators are a class of underappreciated, under-utilized molecules in studies of inflammation. Specifically, the bioactive metabolites derived from fatty acids, termed eicosanoids, participate in modulation of inflammation [57, 58] and pain [59], and have been shown to affect risks of hypertension [60], cardiovascular diseases [61], cancer [62], anorexia nervosa [63], and schizophrenia [64]. To more comprehensively assess how dietary-based intervention [65] may affect inflammation and psychiatric outcomes [66–68], MS technology has been extended to lipidomics analysis for polyunsaturated fatty acids (PUFA) [69, 70]. These lipids include the 18-carbon “essential” PUFA such as linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3), 20-carbon PUFA arachidonic acid (ARA; 20:4n-6) and eicosapentaenoic acid (EPA; 20:5n-3), and 22-carbon PUFA such as docosapentaenoic acid (DPA; 22:5) and docosahexaenoic acid (DHA; 22:6n-3).

The clinical benefits observed when supplementing n-3 PUFA in inflammation-driven diseases were the initial clues that n-3 PUFA may similarly yield symptom relief in psychiatric disorders.

Rheumatoid arthritis patients who took n-3 fatty acids supplementation showed significant clinical benefits compared with those who did not receive n-3 supplementation [71]. Patients with colon cancer who received fish oil supplementation had a significant reduction in IL-6 and TNF-alpha levels and an increase in the

percentages of CD3+ and CD+ lymphocytes when compared with the control group [72]. These data suggest that n-3 PUFA might also benefit symptoms in psychiatric disorders [73], which are now recognized as disorders of neuronal inflammation [74–76].

Peripheral levels of PUFA and n-6:n-3 PUFA ratios have been investigated in depressive disorder and bipolar disorder, the two most common mood disorders. The erythrocyte membrane ARA and DHA were significantly reduced in Taiwanese patients with bipolar disorder patients when compared with healthy controls, while no differences in total PUFAs were observed [77]. Similarly, in a sample of Italian patients with bipolar disorder, plasma DHA was significantly lower than that of healthy controls; however, EPA, AA, ALA, and ARA appeared to be elevated. Based on this result, the authors suggest that DHA may be a useful adjuvant for bipolar disorder [78].

To account for the competitive biology between the n-3 and n-6 classes of PUFA, another group analyzed a broad panel of serum lipids including PUFA in individuals with euthymic bipolar, depressive bipolar, major depressive disorder, and non-psychiatric controls. They found that higher AA:EPA and AA:EPA + DHA ratios were consistently found in the group with bipolar depression. Moreover, the AA:EPA + DHA ratio was positively correlated with depression severity in all groups, despite a lack of control on the fasting status of the subjects [79]. In another study, a high n-6:n-3 ratio and low DHA were found to be predictive of suicide risk among depressed patients [80], highlighting the potential prognostic role PUFA markers may play in depression. Lastly, several eloquent meta-analyses have examined evidence of the efficacy of treating depression or depressive symptoms with n-3 PUFA. Some took a conservative position, stating that the antidepressant efficacy of n-3 PUFAs in unipolar and bipolar depression cannot be confirmed until further replication with more "homogeneous" and larger samples [81]. Two independent meta-analyses reported n-3 PUFA supplementation, especially EPA, to be effective in treating major depression disorder and depressive symptoms [54, 82].

Schizophrenia is a serious psychiatric disorder that has been proposed as "neurodevelopmental" based on early subclinical brain imaging characteristics [83]. Decreased levels of AA and DHA were observed in never-medicated first-episode schizophrenia patients compared with medicated patients and healthy controls [84]. In another study using established schizophrenia cases and carefully selected matched controls, DHA, DPA, and DHA:ARA ratio were reduced in patients compared to controls [85].

For another psychiatric disorder, anorexia nervosa, we found the ARA:EPA ratio to be *lowered* in anorexia nervosa compared with healthy controls [63]. To determine if PUFA levels were "risk factors" or "consequences" of schizophrenia, ultra-high risk individuals (for developing schizophrenia) were followed and dietary intake was assessed. Those who later developed psychosis were found to consume more dietary n-6 PUFAs (LA, AA) and had higher AA:EPA + DHA ratios than those who did not develop psychosis [86]. Similarly, n-3 PUFA supplementation was associated with beneficial effects for anorexia nervosa in several studies [87, 88]. Together, these data support a possible protective role n-3 PUFAs play in preventing onset or worsening of psychotic symptoms. Several mechanisms have been proposed to explain n-3 PUFAs' favorable effect in psychiatric disorders, including anti-neuronal inflammation [89] and neuronal protection [90].

Although the data in psychiatric literature largely paint a favorable view for supplementation of n-3 PUFA, the verdict on the supplements' therapeutic role in psychiatric disorders cannot be established without randomized clinical trials with well-characterized longitudinal data and large sample size. While many independent studies in the past have supported the benefits of n-3 PUFAs for serious medical disorders such as cardiovascular disease and cancer, the most recent meta-analysis showed that n-3 fatty acids did not reduce the incidence of cancer and cardiovascular events (e.g., stroke, myocardial infarction, and cardiovascular event-related death) [91]. In a large randomized trial including 15,480 diabetic patients without cardiovascular disease, no

significant difference was found in the risk of serious vascular events between those who were assigned to n-3 PUFA supplementation and those who were assigned to a placebo [92]. It is thus imperative to conduct further research not only to confirm the effectiveness of n-3 PUFAs in psychiatric disorders but also to explore molecular mechanisms driving clinical benefits in patients and develop biomarkers to classify individuals with high likelihood of benefiting from PUFA supplementation. To achieve these goals, the logical next step is to take advantage of targeted metabolomics technology to focus on a specific class of metabolites, termed eicosanoids [93, 94].

10.7 Eicosanoids as Biomarkers for Psychiatric Disorders

While the pattern of association between PUFA and psychiatric disorders may at first seem straightforward, one must understand the functions and mechanisms underlying beneficial effects of any compound to use such benefits clinically. The bioactive lipids family represents the next logical class of molecules to study to elucidate PUFA mechanisms, and to establish a biomarker system that is biologically and clinically informative to guide precision psychiatry.

Major n-6 and n-3 PUFA can be oxygenated by at least 3 different enzymes to synthesize over 120 heterogeneous and pleiotropic bioactive molecules termed eicosanoids [59, 95]. While the word eicosanoid was derived from the Greek word “eikosa,” meaning “20,” based on the derivatives of the 20-carbon ARA, here “eicosanoid” is applied to also include the oxygenated products of other PUFA including LA, ALA, DHA, and DPA. The 3 enzymatic families that affect PUFA are cyclooxygenases 1 and 2 (COX-1/2); 5-, 12-, and 15-lypooxygenases (5/12/15-LOX); and P450 epoxygenase. The COX-1/2 are known to drive the synthesis of prostanoids such as prostaglandins and thromboxanes, while 5/12/15-LOX produce leukotrienes, lipoxins, and hydroxyeicosatetraenoids, and P450 synthesize HETEs and epoxyeicosatrienoids [95]. The eicosanoids most well-studied for their link to inflammation biology include prostaglandin E2 (PGE_2), a pro-inflammatory molecule stimulated by COX, and 5-LOX-produced leukotrienes, which contribute to potent inflammation in asthma and other allergic diseases [96]. CYP regulates inflammation by oxidizing ARA with its active heme iron to form anti-inflammatory HETE or epoxy-eicosatrienoic acid (EETs), which is then hydrolyzed into pro-inflammatory dihydroxy-eicosatrienoic acids (DHET) by soluble epoxide hydrolase (sEH) [97].

We have demonstrated the effectiveness of a combined use of lipidomics and targeted metabolomics in investigating anorexia nervosa, an illness characterized by rapid weight loss and reduction in food consumption [63]. Higher ratios of dihydroxy to epoxy fatty acids were found in anorexia nervosa patients than in controls, suggesting an upregulation of sEH activity, an elevation in pro-inflammatory eicosanoid profile, and a reduction in anti-inflammatory epoxy fatty acids [63]. Additionally, recovered anorexia nervosa patients showed a partial normalization in PUFA and eicosanoids, implying the resolution of inflammation and that it may be achieved by dietary intervention [26]. This is clinically relevant as well for patients with other types of psychiatric disorders because medication non-adherence rate is notoriously high, up to 80% in schizophrenia [98]. Dietary intervention may be an important alternative treatment modality for patients refusing medications.

The results of the anorexia nervosa study suggest that psychopathology and inflammatory processes in eating disorders are affected by interactions between dietary PUFA and genetically driven metabolism. With additional empirical research, food-based treatment or a nutraceutical strategy may be employed to improve outcomes in clinical psychiatry. Furthermore, as eicosanoid variation reflects *in vivo* cellular inflammation, targeted metabolomics can be applied to develop improved prognosis biomarkers.

Untargeted metabolomics emerged as a useful tool to uncover unsuspected pathways involved in psychiatric disorders, and a targeted metabolomics approach is particularly helpful in

characterizing the specificity, direction and magnitude of disease-associated metabolites, which provide molecular insight helpful to develop new treatments. In a pilot study of adolescent major depressive disorder, we characterized eicosanoids in fasting plasma at the baseline visit and final visit after a 2 year follow-up period. While all subjects displayed no difference in depression severity or profile of depression risk factors at the baseline visit, half of the subjects had progressed to significantly worse depression (refractory group) while the other half remitted. Strikingly, the eicosanoids profile in the refractory group revealed a pattern very similar to that found in patients with anorexia nervosa [99], implicating an epoxy fatty acid catalyzing enzyme, soluble epoxide hydrolase (sEH), as a common risk factor for depression and anorexia nervosa.In a study of seasonal major depression [31], quantitative changes of CYP450 pathway eicosanoids during the winter season (when subjects experienced severe depression symptoms) were similar in pattern to the eicosanoids profile we found in the refractory adolescent depression group [99], suggesting that sEH-mediated metabolism of PUFA eicosanoids underlies the psychopathology of depressive disorders [31].

sEH is known as a regulator of inflammatory resolution due to its potent and complex mechanisms in the formation/catabolism of epoxy- and diol eicosanoids [100], but its involvement with psychiatric phenotypes was only recently uncovered through MS-based discovery [31, 99] and sequencing [101]. Another group has since demonstrated that sEH inhibition showed antidepressant effects in both inflammation and social defeat stress models of depression [102] and attenuated behavioral abnormalities (i.e., hyperlocomotion and prepulse inhibition deficits) in an animal model of schizophrenia [103]. Moreover, a higher level of sEH was found in postmortem brain samples from patients with depression, schizophrenia, and bipolar disorder compared with control samples [102], strengthening the role sEH plays in psychiatric pathology.

The discovery of an association between cytochrome P450-associated bioactive lipid mediators and psychiatric disorders is made possible in part because of advances in technology, but the involvement of eicosanoids in psychiatric disorders was reported as early as the 1980s. Using low throughput techniques such as radioimmunoassay, elevated levels of PGE and PGE2 were identified in schizophrenia [104, 105], whereas PGD2, PGE2, and PGF2α and TXB2 were found to be elevated in major depressive disorder [106–109]. Almost 40 years later, the field can now take advantage of both untargeted and targeted liquid chromatography-mass spectrometry-based methods to monitor a much larger number of potential markers. A recent schizophrenia study that investigated 158 markers including PUFA, eicosanoids, and related mediators from enzyme-dependent or independent pathways uncovered 23 metabolites that were significantly altered in patients compared with healthy controls [64]. While some abnormal markers were reversed after antipsychotic treatment, anandamide, oleoylethanolamine, and ARA were identified as having the best potential for differentiating patients from controls [64].

Leveraging what is already known about the biology of bioactive lipids and the plethora of physiological and homeostatic processes they participate in, several drugs have already been developed to inhibit the production of pro-inflammatory mediators, including nonsteroidal anti-inflammatory drugs (NSAIDS) that reduce the activity of both COX-1 and COX-2 [110], cysteinyl leukotriene (cysLT) receptor antagonists that reduce bronchoconstriction caused by cycLT and pro-inflammatory cytokines in the pulmonary system [111], and COX-2 inhibitors [112]. In fact, administration of COX-2 inhibitor celecoxib has been shown to improve symptoms in schizophrenia [113], possibly through inhibiting conversion of ARA into prostanoids. Additionally, COX-2 inhibitors may be effective as an adjunctive treatment by accelerating the onset of antidepressant effects for bipolar depression and refractory major depression [114, 115].

10.8 Conclusions

While an untargeted metabolomics strategy has gained popularity for its ability to screen new and unsuspected pathways involved in psychiatric disorders, evidence of a role for eicosanoids in psychiatry is accumulating. Eicosanoids participate in the modulation of inflammatory processes and affect the risk of a number of neuropsychiatric disorders. Characterizing the eicosanoid signature in major psychiatric disorders and subtypes within can lay the foundation for individualized treatment approaches. Much work is needed to develop psychiatric multi-omics biomarkers that would not only predict risk, but could also offer an individual-specific course of disorder and responses to therapeutics. For example, studies identifying metabolomic changes during the course of psychiatric disorders are lacking. Additionally, almost all studies used biospecimens taken from blood or urine and not from the organ of disease origin, the brain. This limits researchers' ability to identify brain region-specific metabolite changes and mechanisms in human samples. Follow-up studies using model animals are critical to further research metabolome read-out and neuronal mechanisms to better define pathophysiology of psychiatric disorders. That being said, when coupled with other omics strategies, metabolomics provides a platform for clarifying the relationship among host factors (e.g., genetic variation), substrates (e.g., dietary profile), and downstream metabolomic perturbation and implicated biology. The end knowledge will improve the clinical utility of a multi-omics biomarker system on diagnostic, prognostic, and therapeutic fronts.

While emerging data already indicate beneficial effects of pharmacological agents such as COX-2 and sEH inhibitors, another unique characteristic of eicosanoids is that their substrate availability required for synthesis can be altered by dietary intake or supplementation of PUFA. This opens the door for development of a nutraceutical approach in psychiatric therapeutics. Although there are still many challenges to be addressed and further studies are required to elucidate the complex role of eicosanoids in the psychopathology of psychiatric disorders, metabolomics coupled with other multi-omics approaches can (1) provide deeper insights into the biological underpinnings of psychiatric disorders, (2) be used as powerful diagnostic, disease-monitoring, and treatment response biomarkers, and (3) bring precision psychiatry closer to reality by enabling improved drug discovery and development processes, thereby advancing pharmacometabolomics, nutrigenomics, and metabolomic engineering technologies.

References

1. Wakefield JC (2007) The concept of mental disorder: diagnostic implications of the harmful dysfunction analysis. World Psychiat 6(3):149–156
2. Kessler RC, Wang PS (2008) The descriptive epidemiology of commonly occurring mental disorders in the United States. Annu Rev Public Health 29:115–129
3. Bruffaerts R, Vilagut G, Demyttenaere K, Alonso J, Alhamzawi A, Andrade LH et al (2012) Role of common mental and physical disorders in partial disability around the world. Br J Psychiat 200(6):454–461
4. Walker ER, McGee RE, Druss BG (2015) Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA Psychiat 72(4):334–341
5. Vigo D, Thornicroft G, Atun R (2016) Estimating the true global burden of mental illness. Lancet Psychiatry 3(2):171–178
6. Ormel J, Von Korff M, Burger H, Scott K, Demyttenaere K, Huang YQ et al (2007) Mental disorders among persons with heart disease – results from World Mental Health surveys. Gen Hosp Psychiatry 29(4):325–334
7. Goldberg DP, Prisciandaro JJ, Williams P (2012) The primary health care version of ICD-11: the detection of common mental disorders in general medical settings. Gen Hosp Psychiatry 34(6):665–670
8. American Psychiatric Association (ed) (2013) American psychiatric association, diagnostic and statistical manual of mental disorders, 5th edn. Washington, DC
9. Kessler RC (2007) Psychiatric epidemiology: challenges and opportunities. Int Rev Psychiatry 19(5):509–521
10. Clark LA, Cuthbert B, Lewis-Fernandez R, Narrow WE, Reed GM (2017) Three approaches to understanding and classifying mental disorder: ICD-11, DSM-5, and the National Institute of Mental Health's Research Domain Criteria (RDoC). Psychol Sci Public Interest 18(2):72–145

11. Howland RH (2008) Sequenced treatment alternatives to relieve depression (STAR∗D). Part 2: study outcomes. J Psychosoc Nurs Ment Health Serv 46(10):21–24
12. National research council committee on a framework for developing a new taxonomy of disease (2011) National Academies Press, Washington, DC
13. Collins FS, Varmus H (2015) A new initiative on precision medicine. N Engl J Med 372(9):793–795
14. Hasin Y, Seldin M, Lusis A (2017) Multi-omics approaches to disease. Genome Biol 18(1):83
15. Wray NR, Ripke S, Mattheisen M, Trzaskowski M, Byrne EM, Abdellaoui A et al (2018) Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat Genet 50(5):668–681
16. Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) Biological insights from 108 schizophrenia-associated genetic loci. Nature 511(7510):421–427
17. Cross-Disorder Group of the Psychiatric Genomics C, Lee SH, Ripke S, Neale BM, Faraone SV, Purcell SM et al (2013) Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 45(9):984–994
18. Ward ET, Kostick KM, Lazaro-Munoz G (2019) Integrating genomics into psychiatric practice: ethical and legal challenges for clinicians. Harv Rev Psychiatry 27(1):53–64
19. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA et al (2017) 10 years of GWAS discovery: biology, function, and translation. Am J Hum Genet 101(1):5–22
20. Sun YV, Hu YJ (2016) Integrative analysis of multi-omics data for discovery and functional studies of complex human diseases. Adv Genet 93:147–190
21. Brainstorm C, Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J et al (2018) Analysis of shared heritability in common disorders of the brain. Science 360(6395)
22. Schwarz E, Guest PC, Rahmoune H, Harris LW, Wang L, Leweke FM et al (2012) Identification of a biological signature for schizophrenia in serum. Mol Psychiatry 17(5):494–502
23. Biomarkers Definitions Working G (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
24. Dalvie S, Koen N, McGregor N, O'Connell K, Warnich L, Ramesar R et al (2016) Toward a global roadmap for precision medicine in psychiatry: challenges and opportunities. OMICS 20(10):557–564
25. Beger RD, Dunn W, Schmidt MA, Gross SS, Kirwan JA, Cascante M et al (2016) Metabolomics enables precision medicine: "a white paper, community perspective". Metabolomics 12(10):149
26. Shih PB (2017) Integrating multi-omics biomarkers and postprandial metabolism to develop personalized treatment for anorexia nervosa. Prostaglandins Other Lipid Mediat 132:69–76
27. Gowda GA, Djukovic D (2014) Overview of mass spectrometry-based metabolomics: opportunities and challenges. Methods Mol Biol 1198:3–12
28. Markley JL, Bruschweiler R, Edison AS, Eghbalnia HR, Powers R, Raftery D et al (2017) The future of NMR-based metabolomics. Curr Opin Biotechnol 43:34–40
29. Grebe SK, Singh RJ (2011) LC-MS/MS in the clinical laboratory – where to from here? Clinical Biochem Rev 32(1):5–31
30. Johnson CH, Ivanisevic J, Siuzdak G (2016) Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol 17(7):451–459
31. Hennebelle M, Otoki Y, Yang J, Hammock BD, Levitt AJ, Taha AY et al (2017) Altered soluble epoxide hydrolase-derived oxylipins in patients with seasonal major depression: an exploratory study. Psychiatry Res 252:94–101
32. Tasic L, Pontes JGM, Carvalho MS, Cruz G, Dal Mas C, Sethi S et al (2017) Metabolomics and lipidomics analyses by (1)H nuclear magnetic resonance of schizophrenia patient serum reveal potential peripheral biomarkers for diagnosis. Schizophr Res 185:182–189
33. Meltzer HY, Rajagopal L, Huang M, Oyamada Y, Kwon S, Horiguchi M (2013) Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia. Int J Neuropsychopharmacol 16(10):2181–2194
34. Yang J, Chen T, Sun L, Zhao Z, Qi X, Zhou K et al (2013) Potential metabolite markers of schizophrenia. Mol Psychiatry 18(1):67–78
35. Holmes E, Tsang TM, Huang JT, Leweke FM, Koethe D, Gerth CW et al (2006) Metabolic profiling of CSF: evidence that early intervention may impact on disease progression and outcome in schizophrenia. PLoS Med 3(8):e327
36. Jin H, Shih P-aB, Golshan S, Mudaliar S, Henry R, Glorioso DK et al (2013) Comparison of longer-term safety and effectiveness of 4 atypical antipsychotics in patients over age 40: a trial using equipoise-stratified randomization. J Clin Psychiatry 74(1):11–19
37. McEvoy J, Baillie RA, Zhu H, Buckley P, Keshavan MS, Nasrallah HA et al (2013) Lipidomics reveals early metabolic changes in subjects with schizophrenia: effects of atypical antipsychotics. PLoS One 8(7):e68717
38. Suvitaival T, Mantere O, Kieseppa T, Mattila I, Poho P, Hyotylainen T et al (2016) Serum metabolite profile associates with the development of metabolic co-morbidities in first-episode psychosis. Transl Psychiatry 6(11):e951
39. Xuan J, Pan G, Qiu Y, Yang L, Su M, Liu Y et al (2011) Metabolomic profiling to identify potential serum biomarkers for schizophrenia and risperidone action. J Proteome Res 10(12):5433–5443
40. Zheng P, Fang Z, Xu XJ, Liu ML, Du X, Zhang X et al (2016) Metabolite signature for diagnosing

major depressive disorder in peripheral blood mononuclear cells. J Affect Disord 195:75–81
41. Chen JJ, Zhou CJ, Zheng P, Cheng K, Wang HY, Li J et al (2017) Differential urinary metabolites related with the severity of major depressive disorder. Behav Brain Res 332:280–287
42. Paige LA, Mitchell MW, Krishnan KR, Kaddurah-Daouk R, Steffens DC (2007) A preliminary metabolomic analysis of older adults with and without depression. Int J Geriatr Psychiatry 22(5):418–423
43. Steffens DC, Wei J, Krishnan KR, Karoly ED, Mitchell MW, O'Connor CM et al (2010) Metabolomic differences in heart failure patients with and without major depression. J Geriatr Psychiatry Neurol 23(2):138–146
44. Zhu H, Bogdanov MB, Boyle SH, Matson W, Sharma S, Matson S et al (2013) Pharmacometabolomics of response to sertraline and to placebo in major depressive disorder – possible role for methoxyindole pathway. PLoS One 8(7):e68283
45. Gupta M, Neavin D, Liu D, Biernacka J, Hall-Flavin D, Bobo WV et al (2016) TSPAN5, ERICH3 and selective serotonin reuptake inhibitors in major depressive disorder: pharmacometabolomics-informed pharmacogenomics. Mol Psychiatry 21(12):1717–1725
46. Czysz AH, South C, Gadad BS, Arning E, Soyombo A, Bottiglieri T et al (2019) Can targeted metabolomics predict depression recovery? Results from the CO-MED trial. Transl Psychiatry 9(1):11
47. Sethi S, Pedrini M, Rizzo LB, Zeni-Graiff M, Mas CD, Cassinelli AC et al (2017) (1)H-NMR, (1) H-NMR T2-edited, and 2D-NMR in bipolar disorder metabolic profiling. Int J Bipolar Disord 5(1):23
48. Chen JJ, Liu Z, Fan SH, Yang DY, Zheng P, Shao WH et al (2014) Combined application of NMR- and GC-MS-based metabonomics yields a superior urinary biomarker panel for bipolar disorder. Sci Rep 4:5855
49. Ribeiro HC, Klassen A, Pedrini M, Carvalho MS, Rizzo LB, Noto MN et al (2017) A preliminary study of bipolar disorder type I by mass spectrometry-based serum lipidomics. Psychiatry Res 258:268–273
50. Young SN (1991) The 1989 Borden Award Lecture. Some effects of dietary components (amino acids, carbohydrate, folic acid) on brain serotonin synthesis, mood, and behavior. Can J Physiol Pharmacol 69(7):893–903
51. Conklin SM, Gianaros PJ, Brown SM, Yao JK, Hariri AR, Manuck SB et al (2007) Long-chain omega-3 fatty acid intake is associated positively with corticolimbic gray matter volume in healthy adults. Neurosci Lett 421(3):209–212
52. Pottala JV, Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS (2014) Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI study. Neurology 82(5):435–442
53. Messamore E, Almeida DM, Jandacek RJ, McNamara RK (2017) Polyunsaturated fatty acids and recurrent mood disorders: phenomenology, mechanisms, and clinical application. Prog Lipid Res 66:1–13
54. Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C et al (2014) Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PLoS One 9(5):e96905
55. Young JJ, Bruno D, Pomara N (2014) A review of the relationship between proinflammatory cytokines and major depressive disorder. J Affect Disord 169:15–20
56. Tanaka T, Matsuda T, Hayes LN, Yang S, Rodriguez K, Severance EG et al (2017) Infection and inflammation in schizophrenia and bipolar disorder. Neurosci Res 115:59–63
57. Valdes AM, Ravipati S, Pousinis P, Menni C, Mangino M, Abhishek A et al (2018) Omega-6 oxylipins generated by soluble epoxide hydrolase are associated with knee osteoarthritis. J Lipid Res 59(9):1763–1770
58. Serhan CN, Chiang N, Van Dyke TE (2008) Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8(5):349–361
59. Spector AA, Kim HY (2015) Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. Biochim Biophys Acta 1851(4):356–365
60. Imig JD (2015) Epoxyeicosatrienoic acids, hypertension, and kidney injury. Hypertension 65(3):476–482
61. Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D et al (2009) Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: insight gained using metabolomic approaches. J Mol Cell Cardiol 47(6):835–845
62. Panigrahy D, Kaipainen A, Greene ER, Huang S (2010) Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. Cancer Metastasis Rev 29(4):723–735
63. Shih PB, Yang J, Morisseau C, German JB, Zeeland AA, Armando AM et al (2016) Dysregulation of soluble epoxide hydrolase and lipidomic profiles in anorexia nervosa. Mol Psychiatry 21(4):537–546
64. Wang D, Sun X, Yan J, Ren B, Cao B, Lu Q et al (2018) Alterations of eicosanoids and related mediators in patients with schizophrenia. J Psychiatr Res 102:168–178
65. Zulyniak MA, Perreault M, Gerling C, Spriet LL, Mutch DM (2013) Fish oil supplementation alters circulating eicosanoid concentrations in young healthy men. Metabolism 62(8):1107–1113
66. Zivkovic AM, Telis N, German JB, Hammock BD (2011) Dietary omega-3 fatty acids aid in the modulation of inflammation and metabolic health. Calif Agric (Berkeley) 65(3):106–111
67. Thomas J, Thomas CJ, Radcliffe J, Itsiopoulos C (2015) Omega-3 fatty acids in early prevention of inflammatory neurodegenerative disease: a focus on Alzheimer's disease. Biomed Res Int 2015:172801

68. Sears B, Ricordi C (2012) Role of fatty acids and polyphenols in inflammatory gene transcription and their impact on obesity, metabolic syndrome and diabetes. Eur Rev Med Pharmacol Sci 16(9):1137–1154
69. Lee HC, Yokomizo T (2018) Applications of mass spectrometry-based targeted and non-targeted lipidomics. Biochem Biophys Res Commun 504(3):576–581
70. Quehenberger O, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH et al (2010) Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res 51(11):3299–3305
71. Veselinovic M, Vasiljevic D, Vucic V, Arsic A, Petrovic S, Tomic-Lucic A et al (2017) Clinical benefits of n-3 PUFA and -linolenic acid in patients with rheumatoid arthritis. Nutrients 9(4)
72. Magee P, Pearson S, Allen J (2008) The omega-3 fatty acid, eicosapentaenoic acid (EPA), prevents the damaging effects of tumour necrosis factor (TNF)-alpha during murine skeletal muscle cell differentiation. Lipids Health Dis 7:24
73. Orr SK, Trepanier MO, Bazinet RP (2013) n-3 Polyunsaturated fatty acids in animal models with neuroinflammation. Prostaglandins Leukot Essent Fatty Acids 88(1):97–103
74. Alam R, Abdolmaleky HM, Zhou JR (2017) Microbiome, inflammation, epigenetic alterations, and mental diseases. Am J Med Genet B Neuropsychiatr Genet 174(6):651–660
75. Das UN (2013) Autism as a disorder of deficiency of brain-derived neurotrophic factor and altered metabolism of polyunsaturated fatty acids. Nutrition 29(10):1175–1185
76. Das UN (2013) Polyunsaturated fatty acids and their metabolites in the pathobiology of schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry 42:122–134
77. Chiu CC, Huang SY, Su KP, Lu ML, Huang MC, Chen CC et al (2003) Polyunsaturated fatty acid deficit in patients with bipolar mania. Eur Neuropsychopharmacol 13(2):99–103
78. Pomponi M, Janiri L, La Torre G, Di Stasio E, Di Nicola M, Mazza M et al (2013) Plasma levels of n-3 fatty acids in bipolar patients: deficit restricted to DHA. J Psychiatr Res 47(3):337–342
79. Scola G, Versace A, Metherel AH, Monsalve-Castro LA, Phillips ML, Bazinet RP et al (2018) Alterations in peripheral fatty acid composition in bipolar and unipolar depression. J Affect Disord 233:86–91
80. Sublette ME, Hibbeln JR, Galfalvy H, Oquendo MA, Mann JJ (2006) Omega-3 polyunsaturated essential fatty acid status as a predictor of future suicide risk. Am J Psychiatry 163(6):1100–1102
81. Ciappolino V, Delvecchio G, Agostoni C, Mazzocchi A, Altamura AC, Brambilla P (2017) The role of n-3 polyunsaturated fatty acids (n-3PUFAs) in affective disorders. J Affect Disord 224:32–47
82. Sarris J, Murphy J, Mischoulon D, Papakostas GI, Fava M, Berk M et al (2016) Adjunctive nutraceuticals for depression: a systematic review and meta-analyses. Am J Psychiatry 173(6):575–587
83. Owen MJ, O'Donovan MC, Thapar A, Craddock N (2011) Neurodevelopmental hypothesis of schizophrenia. Br J Psychiatry 198(3):173–175
84. Khan MM, Evans DR, Gunna V, Scheffer RE, Parikh VV, Mahadik SP (2002) Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. Schizophr Res 58(1):1–10
85. Assies J, Lieverse R, Vreken P, Wanders RJ, Dingemans PM, Linszen DH (2001) Significantly reduced docosahexaenoic and docosapentaenoic acid concentrations in erythrocyte membranes from schizophrenic patients compared with a carefully matched control group. Biol Psychiatry 49(6):510–522
86. Pawelczyk T, Trafalska E, Kotlicka-Antczak M, Pawelczyk A (2016) The association between polyunsaturated fatty acid consumption and the transition to psychosis in ultra-high risk individuals. Prostaglandins Leukot Essent Fatty Acids 108:30–37
87. Shih PB, Morisseau C, Le T, Woodside B, German JB (2017) Personalized polyunsaturated fatty acids as a potential adjunctive treatment for anorexia nervosa. Prostaglandins Other Lipid Mediat 133:11–19
88. Satogami K, Tseng PT, Su KP, Takahashi S, Ukai S, Li DJ et al (2019) Relationship between polyunsaturated fatty acid and eating disorders: systematic review and meta-analysis. Prostaglandins Leukot Essent Fatty Acids 142:11–19
89. Song C (2013) Essential fatty acids as potential anti-inflammatory agents in the treatment of affective disorders. Mod Trends Pharmacopsychiatry 28:75–89
90. Moffett JR, Arun P, Ariyannur PS, Namboodiri AM (2013) N-Acetylaspartate reductions in brain injury: impact on post-injury neuroenergetics, lipid synthesis, and protein acetylation. Front Neuroenerg 5:11
91. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S et al (2019) Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. N Eng J Med 380(1):23–32
92. Group ASC, Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G et al (2018) Effects of n-3 fatty acid supplements in diabetes mellitus. N Engl J Med 379(16):1540–1550
93. Yang J, Schmelzer K, Georgi K, Hammock BD (2009) Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry. Anal Chem 81(19):8085–8093
94. Astarita G, Kendall AC, Dennis EA, Nicolaou A (2015) Targeted lipidomic strategies for oxygenated metabolites of polyunsaturated fatty acids. Biochim Biophys Acta 1851(4):456–468
95. Chiurchiu V, Leuti A, Maccarrone M (2018) Bioactive lipids and chronic inflammation: managing the fire within. Front Immunol 9:38

96. Lammermann T, Afonso PV, Angermann BR, Wang JM, Kastenmuller W, Parent CA et al (2013) Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. Nature 498(7454):371–375
97. Buczynski MW, Dumlao DS, Dennis EA (2009) Thematic review series: proteomics. An integrated omics analysis of eicosanoid biology. J Lipid Res 50(6):1015–1038
98. Latha KS (2010) The noncompliant patient in psychiatry: the case for and against covert/surreptitious medication. Mens Sana Monogr 8(1):96–121
99. Shih PB, Yang J, Morisseau C, Calarge C (2017) American College of Neuropsychopharmacology annual meeting. Palm Springs, CA
100. Morisseau C, Hammock BD (2013) Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. Annu Rev Pharmacol Toxicol 53:37–58
101. Zeeland AAS-V, Bloss CS, Tewhey R, Bansal V, Torkamani A, Libiger O et al (2014) Evidence for the role of EPHX2 gene variants in anorexia nervosa. Mol Psychiatry 19(6):724–732
102. Ren Q, Ma M, Ishima T, Morisseau C, Yang J, Wagner KM et al (2016) Gene deficiency and pharmacological inhibition of soluble epoxide hydrolase confers resilience to repeated social defeat stress. Proc Natl Acad Sci U S A 113(13):E1944–E1952
103. Ma M, Ren Q, Fujita Y, Ishima T, Zhang JC, Hashimoto K (2013) Effects of AS2586114, a soluble epoxide hydrolase inhibitor, on hyperlocomotion and prepulse inhibition deficits in mice after administration of phencyclidine. Pharmacol Biochem Behav 110:98–103
104. Mathe AA, Sedvall G, Wiesel FA, Nyback H (1980) Increased content of immunoreactive prostaglandin E in cerebrospinal fluid of patients with schizophrenia. Lancet 1(8158):16–18
105. Kaiya H, Uematsu M, Ofuji M, Nishida A, Takeuchi K, Nozaki M et al (1989) Elevated plasma prostaglandin E2 levels in schizophrenia. J Neural Transm 77(1):39–46
106. Nishino S, Ueno R, Ohishi K, Sakai T, Hayaishi O (1989) Salivary prostaglandin concentrations: possible state indicators for major depression. Am J Psychiatry 146(3):365–368
107. Ohishi K, Ueno R, Nishino S, Sakai T, Hayaishi O (1988) Increased level of salivary prostaglandins in patients with major depression. Biol Psychiatry 23(4):326–334
108. Calabrese JR, Skwerer RG, Barna B, Gulledge AD, Valenzuela R, Butkus A et al (1986) Depression, immunocompetence, and prostaglandins of the E series. Psychiatry Res 17(1):41–47
109. Lieb J, Karmali R, Horrobin D (1983) Elevated levels of prostaglandin E2 and thromboxane B2 in depression. Prostaglandins Leukot Med 10(4):361–367
110. Vane JR (2002) Biomedicine. Back to an aspirin a day? Science 296(5567):474–475
111. Aharony D (1998) Pharmacology of leukotriene receptor antagonists. Am J Respir Crit Care Med 157(6 Pt 2):S214–S218. discussion S8–9, S47–8
112. Muller N, Ulmschneider M, Scheppach C, Schwarz MJ, Ackenheil M, Moller HJ et al (2004) COX-2 inhibition as a treatment approach in schizophrenia: immunological considerations and clinical effects of celecoxib add-on therapy. Eur Arch Psychiatry Clin Neurosci 254(1):14–22
113. Muller N, Krause D, Dehning S, Musil R, Schennach-Wolff R, Obermeier M et al (2010) Celecoxib treatment in an early stage of schizophrenia: results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. Schizophr Res 121(1–3):118–124
114. Muller N, Riedel M, Schwarz MJ (2004) Psychotropic effects of COX-2 inhibitors--a possible new approach for the treatment of psychiatric disorders. Pharmacopsychiatry 37(6):266–269
115. Nery FG, Monkul ES, Hatch JP, Fonseca M, Zunta-Soares GB, Frey BN et al (2008) Celecoxib as an adjunct in the treatment of depressive or mixed episodes of bipolar disorder: a double-blind, randomized, placebo-controlled study. Hum Psychopharmacol 23(2):87–94

11 Cytochrome P450 Eicosanoid Signaling Pathway in Colorectal Tumorigenesis

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Abstract

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death in the United States. It is important to discover novel cellular targets which are crucial in the pathogenesis of CRC, which could facilitate development of mechanism-based strategies to reduce the risks of CRC. Emerging studies support that the cytochrome P450 (CYP) monooxygenase/soluble epoxide hydrolase (sEH) pathway and their eicosanoid metabolites play critical roles in colonic inflammation and CRC, and could be therapeutically explored for treating or preventing CRC. Here in this review, we discuss recent studies about the roles of the CYP/sEH eicosanoid pathway in the pathogenesis of colonic inflammation and CRC.

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Keywords

Colorectal cancer · Colonic inflammation · Cytochrome P450 · Soluble epoxide hydrolase · Eicosanoids

11.1 Introduction

Colorectal cancer (CRC) is a serious health problem: there were ~140,250 new cases and ~50,630 deaths in the United States in 2018 [1], emphasizing the need for discovering novel cellular targets which are crucial in the pathogenesis of CRC. Colonic inflammation is a major risk factor for developing CRC, therefore, targeting the pathological components involved in colonic inflammation is a promising strategy to reduce the risks of CRC [2]. Eicosanoids, which are endogenous lipid signaling molecules produced from enzymatic metabolism of polyunsaturated fatty acids (PUFAs), play essential roles in inflammatory responses and were recently implicated in cancer [3, 4]. The most prominent CRC-associated eicosanoids are prostaglandins, which are produced by the cyclooxygenase-2 (COX-2) enzyme that is overexpressed in most human CRC samples [4]. Genetic knockout of *Cox-2* reduces polyp formation in azoxymethane (AOM)- or *Apc* mutation-induced CRC models [5, 6]. Furthermore, clinical and epidemiological

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studies support that pharmacological inhibitors of COX-2, such as nonsteroidal anti-inflammatory drugs (NSAIDs), are effective in reducing the risk of CRC [4]. These results support the critical importance of eicosanoid signaling in CRC. However, the gastrointestinal and cardiovascular toxicities induced by the COX-2 inhibitors have limited their clinical applications [7]. Besides COX-2, the roles of other eicosanoid pathways in colonic inflammation and CRC are not well understood [8]. It is therefore important to discover novel eicosanoid signaling pathways involved in the pathogenesis of CRC.

Besides being substrates of COX-2, PUFAs are also substrates of cytochrome P450 (CYP) monooxygenases (predominately the CYP2C and CYP2J isoforms), which convert them to epoxygenated fatty acids (EpFAs). EpFAs include epoxyeicosatrienoic acids (EETs) produced from arachidonic acid (ARA, 20:4ω-6), epoxyoctadecenoic acids (EpOMEs) from linoleic acid (LA, 18:2ω-6), epoxyoctadecadienoic acids (EpODEs) from α-linolenic acid (ALA, 18:3ω-3), and epoxydocosapentaenoic acids (EDPs) from docosahexaenoic acid (DHA, 22:6ω-3) [9]. EpFAs are metabolically unstable with a half-life of several seconds *in vivo*, in part because they could be rapidly metabolized by soluble epoxide hydrolase (sEH) to generate the corresponding fatty acid diols [10]. Currently, the CYP/sEH eicosanoid pathway is being explored by academic laboratories and pharmaceutical companies for clinical applications. For example, GlaxoSmithKline is conducting human clinical trials to test an sEH inhibitor GSK2256294; the company has found that this drug candidate is well-tolerated and causes sustained inhibition of sEH in humans [11]. Other novel classes of sEH inhibitors are also being considered for human trials [12]. In addition, recent studies have shown that some FDA-approved drugs are potent inhibitors of CYP monooxygenases [13].

Emerging research supports that the CYP/sEH pathway plays critical roles in regulating inflammation, angiogenesis, tumor growth, and tumor metastasis [14–17], and could be involved in the pathogenesis of colonic inflammation and CRC [18–21]. A better understanding of the roles of this previously unappreciated pathway in the pathogenesis of CRC could help to develop new strategies for cancer treatment or prevention. In this review, we will discuss the roles of the CYP/sEH eicosanoid pathway in the pathogenesis of colonic inflammation and CRC, as well as obesity-associated colonic inflammation and CRC.

11.2 Expression of CYP Monooxygenases and sEH in Colonic Inflammation and CRC

Our recent study has shown that CYP monooxygenases are overexpressed in colon tumor tissues and colon cancer cells [21]. Compared with control healthy mice, the expressions of mouse *CYP monooxygenases*, such as *Cyp2c38, Cyp2c39, Cyp2c55, Cyp2c65, Cyp2c70*, *Cyp2j6, Cyp2j9,* and *Cyp2j13*, are increased in colon tissues of AOM/dextran sulfate sodium (DSS)-induced CRC mice [21]. In addition, the concentrations of CYP-produced EpFAs are increased in both the plasma and colon tissues of AOM/DSS-induced CRC mice [21]. Furthermore, we find that compared with normal human colon cells (CCD-18co), the expression of *CYP2C8, CYP2C9, CYP2C19,* and *CYP2J2* is increased in human CRC cells (HCT116 and Caco-2) [21]. These results support that the CYP monooxygenase pathway is upregulated in mouse and cell culture models.

The expressions of CYP monooxygenases in human CRC are more complicated. We analyzed gene expression of *CYP monooxygenases* (*CYP2C8*, *CYP2C9*, *CYP2C19*, and *CYP2J2*) in the Cancer Genome Atlas (TCGA) database, and found that their expressions were not increased in colorectal adenocarcinoma [21]. However, we must note that the expression and activity of the CYP enzymes are regulated by multiple mechanisms, including transcription, translation, and post-translational modification, not only the mRNA expression levels [22]. Indeed, Enayetallah et al. report that CYP2C9 is detected in 13 out of 17 human colon tumor samples,

while it is not detected in matched benign samples [23]. In addition to CRC, previous studies have shown that CYP monooxygenases are overexpressed in other tumor tissues, such as breast, liver, and stomach tumors [16, 23]. There could be many mechanisms by which CYP monooxygenases are overexpressed in tumor tissues. The expression of CYP monooxygenases has been shown to be elevated by hypoxia [10], which is a common feature of tumor tissues [24]. It is feasible that the hypoxic tumor microenvironment could contribute to the increased expression of CYP monooxygenases in tumor tissues.

In humans, the expression of sEH is increased in colonic dysplasia and adenocarcinomas in ulcerative colitis (UC) patients [18]. sEH has been detected in ~40% of human colon adenocarcinomas (7 out of 17 samples) with relatively high expression levels, but is not detected in matched benign samples (0 out of 4 samples) [23]. More studies are needed to characterize the expression of sEH and the concentrations of CYP/sEH-produced eicosanoid metabolites in colonic inflammation and CRC.

11.3 Roles of CYP/sEH Eicosanoid Pathway in Colonic Inflammation and CRC

Recent research has shown that sEH plays a critical role in colonic inflammation and CRC. In the DSS-induced mouse model, sEH$^{-/-}$ mice have reduced colonic inflammation (as assessed by mucosal erosion and lymphoplasmocytosis) and carcinogenesis (tumor incidence and volume) compared with wild-type mice [18]. Similarly, in an interleukin 10 (IL-10) deficiency-induced CRC model, sEH$^{-/-}$ IL-10$^{-/-}$ mice have reduced colonic expression of pro-inflammatory cytokines and formation of ulcers and carcinomas compared with sEH$^{+/+}$ IL-10$^{-/-}$ mice [19, 20]. Together, these results support that sEH could contribute to colonic inflammation and inflammation-associated CRC. This is largely in agreement with previous studies which show that inhibition of sEH reduces inflammatory responses in various disease models. For example, in obese mice, pharmacological inhibition of sEH reduces the infiltration of macrophages and expression of pro-inflammatory cytokines in epididymal fat and liver [25, 26]. Deletion of sEH also leads to reduced infiltration of neutrophils, decreased levels of pro-inflammatory cytokines and less neuronal damage in an intracerebral hemorrhage mouse model [27]. Together, these studies demonstrate sEH is involved in many inflammation-associated diseases.

More studies are needed to better characterize the roles of sEH in cancer. Panigrahy et al. have shown that pharmacological inhibition or genetic deletion of sEH increases tumor growth and metastasis in xenograft models by stimulating tumor angiogenesis [15]. However, this finding is different from the results observed in the DSS and IL-10$^{-/-}$ mouse models [18–20]. The different results could be, at least in part, due to the differences in mouse models. The phenotypes in the DSS and IL-10$^{-/-}$ mouse models are strongly associated with inflammation, therefore, inhibition of sEH would reduce inflammatory response and attenuate inflammation-associated CRC [18–20]. In other models such as xenograft models, inhibition of sEH could upregulate angiogenesis and increase tumorigenesis [15]. However, the pro-angiogenic effects of sEH inhibition were mainly observed in mouse models [15]. A recent human clinical trial has shown that even at 100% inhibition of sEH, there is no change of the plasma concentration of vascular endothelial growth factor (VEGF), which is an important biomarker of angiogenesis [11]. Since sEH inhibitors are currently being evaluated in human clinical trials [11, 12], it is of critical importance to better understand the roles of sEH and sEH inhibitors in tumorigenesis.

Recent studies also support the critical roles of CYP monooxygenases in tumorigenesis. In a xenograft tumor model, overexpression of human CYP2C8 or CYP2J2 in endothelial cells led to increased xenograft tumor growth of B16F10 melanoma and T241 fibrosarcoma [15]. Using a Lewis lung carcinoma (LLC) resection-induced tumor metastasis model, endothelial expression of human CYP2C8 or CYP2J2 increases lung metastases [15]. Together, these results support

the pro-tumorigenic and pro-metastatic effect of CYP monooxygenases.

Our recent studies also support a potential role of CYP monooxygenases in the tumorigenesis of CRC [21]. Compared with AOM/DSS-induced *Cyp2c*$^{+/+}$ mice, the AOM/DSS-induced *Cyp2c*$^{+/-}$ mice have lower tumor numbers and total tumor burden, as well as reduced expression of CYP monooxygenases and concentrations of CYP-derived fatty acid epoxides in colon tissues, supporting the roles of Cyp2c monooxygenases in colon tumorigenesis [21]. Consistent with the results observed in transgenic mouse models, we also find that pharmacological inhibition of CYP monooxygenases suppresses AOM/DSS-induced colon tumorigenesis in mice [21]. Together, these results support that CYP monooxygenases contribute to the tumorigenesis of CRC.

11.4 Effects of CYP-Produced Eicosanoids on Inflammation and CRC

The metabolism of PUFAs by CYP monooxygenases leads to the formation of EpFAs [9]. The major EpFAs in tissues and plasma include EpOMEs produced from LA, EETs from ARA, and EDPs from DHA. Emerging research supports that these eicosanoid metabolites have potent effects on inflammation and tumorigenesis with the details discussed below.

Previous studies show that EpOMEs have a series of detrimental actions such as inducing chemotaxis, inflammation, cardiovascular diseases, and pulmonary injury [28–33]. In human studies, EpOMEs, which are termed "leukotoxins," are associated with multiple organ failures and adult respiratory distress syndrome in severe burn patients [29, 31, 34]. Notably, at a concentration as low as 10 nM, EpOMEs enhance neutrophil chemotaxis, suggesting potent pro-inflammatory effects of EpOMEs [33]. Regarding CRC, our recent study shows that treatment with 12,13-EpOME exaggerates AOM/DSS-induced CRC in mice [21], supporting the pro-CRC actions of EpOMEs. EpOMEs can be further metabolized by sEH to form the corresponding fatty acid diols termed dihydroxyoctadecenoic acids (DiHOMEs) [10]. Similar to EpOMEs, DiHOMEs have also been shown to induce chemotaxis, tissue injury, and cause mortality in animal models [34, 35].

The biological actions of EETs are more complicated, as they have been shown to have anti-inflammatory effects (negatively associated with tumorigenesis) and pro-angiogenic effects (positively associated with tumorigenesis). Indeed, many studies have shown that EETs have potent anti-inflammatory effects. In a murine carotid artery model, treatment with 11,12-EET decreases tumor necrosis factor-α (TNF-α)–induced mononuclear cell adhesion to the arterial endothelium [36]. Treatment with 14,15-EET inhibits TNF-induced degradation of IκBα in primary human lung tissue [37] and reduces LPS-activated IL-1β and TNF-α expression in mouse macrophages [18]. Together, these results support the anti-inflammatory effects of EETs. The effects of EETs on tumor inflammation are not well characterized and require more studies. Previous studies have shown that inhibition or deletion of sEH attenuates DSS- or IL-10 knockout-induced CRC [18–20]; these results could be, at least in part, due to the anti-inflammatory effects of EETs. On the other hand, EETs have pro-angiogenic effects, and therefore could promote tumor growth and metastasis. In an orthotopic cancer model, treatment of 14,15-EET at a dose as low as 15 μg/kg/day increases orthotopic PC3M-LN4 prostate tumor growth in SCID mice [15]. In transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, administration of 14,15-EET at a dose of 15 μg/kg/day promotes prostate tumor growth, suggesting that EETs increase tumor growth *in vivo* [15]. In addition, treatment with 14,15-EET increases tumor metastasis in a LLC resection model [15]. More studies are needed to further characterize the role of EETs in tumorigenesis.

Opposite to the effects of EpOMEs and EETs, our studies have shown that ω-3 PUFA-produced EDPs inhibit angiogenesis, tumor growth, and

tumor metastasis [38]. 19,20-EDP inhibits tube formation, migration, and production of matrix metalloproteinases (MMPs) in endothelial cells, and suppresses VEGF- and basic fibroblast growth factor (bFGF)-induced angiogenesis in a Matrigel plug assay in mice, demonstrating its anti-angiogenic effect [38]. Treatment with stabilized 19,20-EDP also suppresses primary tumor growth and tumor metastasis in mice [38].

Furthermore, our recent study showed that treatment with EDPs suppresses growth of MC38 xenograft colon tumors, supporting the anti-CRC effects of EDPs [39]. Consistent with our results, a recent study by Yanai et al. has shown that systematic treatment with EDPs inhibits pathological angiogenesis in a mouse model of macular degeneration [14].

To date, the molecular mechanisms for the biological actions of EpFAs are not well understood. Many eicosanoids act by binding to specific cellular targets. For example, COX-2-produced prostaglandin E_2 (PGE_2) and lipoxygenase (LOX)-produced leukotriene B_4 (LTB_4) act by binding to their G-protein coupled receptors [40]. The direct cellular target(s) of CYP monooxygenase metabolites are not well understood, hampering our understanding of their mechanisms of action. Previous studies show that EETs bind to cell membrane-bound proteins in a high-affinity, specific, and saturable manner [41–43], and some of the biological actions of EETs are G protein-dependent [43–46]. These results support that the CYP monooxygenase metabolites also act via interactions with specific cellular proteins; however, the identities of the cellular targets are not well understood. Beyond EETs, no study has investigated the cellular targets of other CYP monooxygenase metabolites (such as EpOMEs). Identification of their direct cellular targets could greatly enhance our understanding for the molecular mechanisms of the CYP monooxygenase pathway. In addition, the identified cellular targets could also serve as novel molecular targets for preventing or treating cancer and other human diseases.

Besides the EpFAs, their down-stream metabolites, termed fatty acid diols, have also been shown to be biologically active and could contribute to the biological actions of CYP monooxygenases and sEH. Recent studies have shown that dihydroxyeicosatrienoic acids (DHETs), which are metabolites of EETs produced by sEH, have pro-inflammatory effects [10, 47]. Treatment of 5,6-DHET, 8,9-DHET, 11,12-DHET, or 14,15-DHET at a dose of 3 μM stimulates primary human monocyte migration *in vitro* [50]. Similar to the pro-inflammatory effects of ARA-produced DHETs, recent studies show the DHA-produced dihydroxydocosapentaenoic acids (DHDPs) promote progression of retinopathy in mice [48, 49]. Treatment of 19,20-DHDP induces retinal angiogenesis by increasing tip cell, sprouting, and filopodia numbers in mice, all of which contributes to proliferative retinopathy [48]. Moreover, in an *ex vivo* whole mount retina model, treatment with 19,20-DHDP increases vascular endothelial cell permeability and pericyte migration into extravascular space, both of which are disease markers for diabetic retinopathy [49]. In cultured murine brain microvascular endothelial cells, treatment of 19,20-DHDP decreases junction formation between cells and reduces expression of N-cadherin, which could contribute to effect of 19,20-DHDP on vascular permeability during diabetic retinopathy [49]. Overall, these studies demonstrate that fatty acid diols are bioactive eicosanoids which could contribute to the pathogenesis of many diseases.

Together, these results support that CYP-produced eicosanoid metabolites, including EpOMEs, EETs, and EDPs, have potent effects on tumorigenesis. These results could help to establish a novel mechanistic linkage between fatty acid intake and cancer risks. For example, animal experiments have shown that a high dietary intake of LA increases AOM-induced colon tumorigenesis, suggesting its potential adverse effect on CRC [50–54]. Here our study about the promoting effects of LA-produced EpOMEs on CRC suggests that the formation of EpOMEs could contribute to the promoting effects of LA on the risks of CRC [21].

11.5 Roles of CYP/sEH Eicosanoid Pathway in Obesity-Induced Colonic Inflammation

More than one-third of US adults (34.9% or 78.6 million) are obese [55], and obese individuals have a 30–60% higher risk of developing CRC [56, 57]. Considering the obesity epidemic and the potential lethal consequence of CRC, obesity-enhanced CRC is a serious health problem in the US. However, the mechanism by which obesity increases the risks of CRC is not well understood, and there are few effective strategies to prevent obesity-enhanced CRC [58]. Using LC-MS/MS-based metabolomics, our recent research suggests that sEH could be a novel therapeutic target of obesity-induced colonic inflammation [59]. In a high fat diet (HFD)-induced obesity model in C57BL/6 mice, we find that the expression of sEH and the concentrations of sEH-produced fatty acid diols are significantly increased in the colon tissues of HFD-induced obese mice [59]. Furthermore, pharmacological inhibition or genetic ablation of sEH abolishes HFD-induced colonic inflammation in mice, with reduced expression of pro-inflammatory cytokines (*Il-1β* and *Tnf-α*) and/or decreased infiltration of immune cells in colon tissues [59]. Furthermore, we find that the inhibition or ablation of sEH attenuates HFD-induced activation of Wnt signaling pathway in colon tissues [59].

Considering the critical roles of colonic inflammation and Wnt signaling in the pathogenesis of CRC, these results support that sEH could be a potential therapeutic target of obesity-enhanced CRC. This notion is supported by previous studies, which show that inhibition of sEH has beneficial effects on colonic inflammation, CRC, and obesity, supporting that targeting sEH is a promising strategy to reduce the risks of obesity-enhanced CRC. Previous studies have shown that: [1] compared with normal colon tissues, the expression of sEH is increased in human CRC samples [18, 23]; [2] pharmacological inhibition or genetic ablation of sEH attenuates colonic inflammation and CRC [18–20]; and [3] sEH is overexpressed in the liver and adipose tissues of obese mice [60, 61]. In addition, pharmacological inhibition or genetic ablation of sEH attenuated various adverse consequences of obesity, including endoplasmic reticulum stress, metabolic syndrome, fatty liver, hepatic steatosis, inflammation, endothelial dysfunction, and diabetes [25, 26, 60, 62–68]. Together, these results support that targeting sEH could be a promising strategy to reduce the risks of CRC in obese individuals. Further studies are needed to characterize the roles of sEH in obesity-enhanced CRC.

In our studies, we find that the colonic expressions of CYP2C and CYP2J monooxygenases are not altered in HFD-treated mice [59]. However, considering that the fatty acid diols are down-stream metabolites of CYP monooxygenases, it is feasible that inhibition of CYP monooxygenases could also reduce obesity-induced colonic inflammation and associated tumorigenesis. Targeting CYP monooxygenases could be an alternative approach to reduce the risks of obesity-enhanced CRC. This approach could minimize potential adverse effects of sEH inhibition on angiogenesis [15], and further studies are needed to better characterize the roles of the CYP/sEH eicosanoid pathway in obesity-enhanced CRC.

11.6 Conclusion

CRC is the third most common cancer and the second leading cause of cancer-related death in the US [1], emphasizing the need for discovery of novel cellular targets which are crucial in the pathogenesis of colon cancer. Recent research by us and others support that CYP monooxygenases, sEH, and associated eicosanoid metabolites (EpFAs and fatty acid diols) play critical roles in regulating angiogenesis, tumor growth, and tumor metastasis [14–17], and could be involved in the pathogenesis of colonic inflammation and CRC [18–21] (Fig. 11.1). Currently pharmaceutical companies and academic laboratories are targeting the CYP/sEH eicosanoid pathway to develop therapeutic drugs. Further understanding about the roles of the CYP/sEH eicosanoid pathway in colonic inflammation and CRC could facilitate

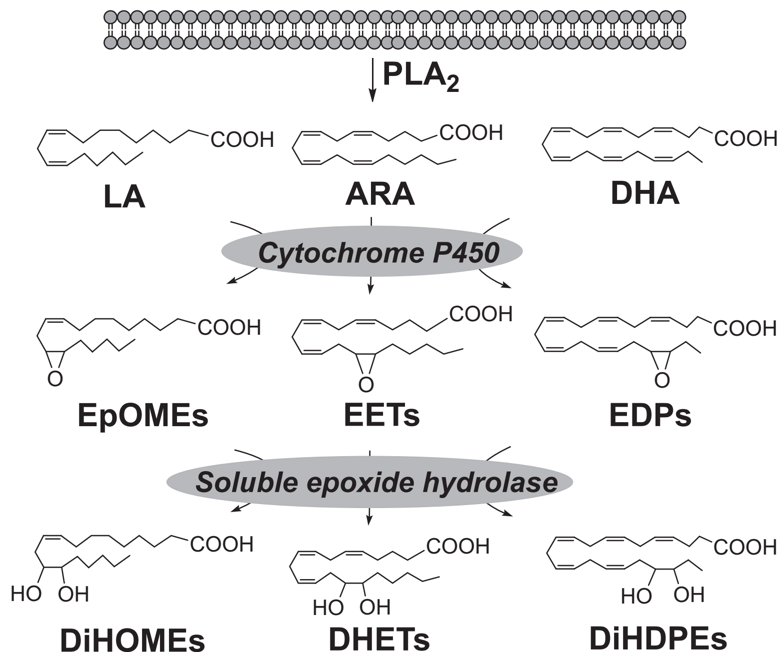

Fig. 11.1 Biochemistry of the CYP/sEH eicosanoid pathway. The metabolism of PUFAs by cytochrome P450 monooxygenases (largely CYP2C and CYP2J isoforms) leads to formation of epoxygenated fatty acids (EpFAs), which are further metabolized by soluble epoxide hydrolase (sEH) to form the corresponding fatty acid diols. Recent research shows that the CYP and sEH enzymes contribute to the pathogenesis of colonic inflammation and CRC

the development of novel therapeutic strategies in the prevention or treatment of CRC. In addition, our research supports that the omega-6-series EpFAs (e.g. EpOMEs) are associated with increased inflammation and CRC [21], while the omega-3-series EpFAs (e.g. EDPs) are associated with decreased CRC [39] (Fig. 11.1), providing a potential mechanism-based nutritional strategy to reduce the risks of CRC.

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References

1. Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. CA Cancer J Clin 68:7–30
2. Terzic J, Grivennikov S, Karin E, Karin M (2010) Inflammation and colon cancer. Gastroenterology 138:2101–2114. e2105
3. Greene ER, Huang S, Serhan CN, Panigrahy D (2011) Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat 96:27–36
4. Wang D, Dubois RN (2010) Eicosanoids and cancer. Nat Rev Cancer 10:181–193
5. Chulada PC et al (2000) Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in min mice. Cancer Res 60:4705–4708
6. Ishikawa TO, Herschman HR (2010) Tumor formation in a mouse model of colitis-associated colon cancer does not require COX-1 or COX-2 expression. Carcinogenesis 31:729–736
7. Grosser T, Fries S, FitzGerald GA (2006) Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest 116:4–15
8. Wang Y et al (2018) Eicosanoid signaling in carcinogenesis of colorectal cancer. Cancer Metastasis Rev 37:257–267
9. Zeldin DC (2001) Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem 276:36059–36062
10. Zhang G, Kodani S, Hammock BD (2014) Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer. Prog Lipid Res 53:108–123
11. Lazaar AL et al (2016) Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor. Br J Clin Pharmacol 81:971–979
12. McReynolds C, Schmidt WK, Wagner K, Hammock BD (2016) Advancing soluble epoxide hydrolase inhibitors through the valley of death into phase 1 clinical trials for treating painful diabetic neuropathy by utilizing university partnerships, collaborations, and NIH support. FASEB J 30:1272–1276

13. Veith H et al (2009) Comprehensive characterization of cytochrome P450 isozyme selectivity across chemical libraries. Nat Biotechnol 27:1050
14. Yanai R et al (2014) Cytochrome P450-generated metabolites derived from omega-3 fatty acids attenuate neovascularization. Proc Natl Acad Sci U S A 111:9603–9608
15. Panigrahy D et al (2012) Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice. J Clin Invest 122:178–191
16. Jiang JG et al (2005) Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. Cancer Res 65:4707–4715
17. Pozzi A et al (2010) The anti-tumorigenic properties of peroxisomal proliferator-activated receptor alpha are arachidonic acid epoxygenase-mediated. J Biol Chem 285:12840–12850
18. Zhang W et al (2013) Soluble epoxide hydrolase deficiency inhibits dextran sulfate sodium-induced colitis and carcinogenesis in mice. Anticancer Res 33:5261–5271
19. Zhang W et al (2012) Soluble epoxide hydrolase gene deficiency or inhibition attenuates chronic active inflammatory bowel disease in IL-10(−/−) mice. Dig Dis Sci 57:2580–2591
20. Zhang W et al (2013) Reduction of inflammatory bowel disease-induced tumor development in IL-10 knockout mice with soluble epoxide hydrolase gene deficiency. Mol Carcinog 52:726–738
21. Wang W et al (2019) Targeted metabolomics identifies cytochrome P450 monooxygenase eicosanoid pathway as novel therapeutic target of colon tumorigenesis. Cancer Res 79(8):1822–1830
22. Aguiar M, Masse R, Gibbs BF (2005) Regulation of cytochrome P450 by posttranslational modification. Drug Metab Rev 37:379–404
23. Enayetallah AE, French RA, Grant DF (2006) Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms. J Mol Histol 37:133–141
24. Vaupel P, Mayer A (2007) Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 26:225–239
25. Liu Y et al (2012) Inhibition of soluble epoxide hydrolase attenuates high-fat-diet-induced hepatic steatosis by reduced systemic inflammatory status in mice. PLoS One 7:e39165
26. Lopez-Vicario C et al (2015) Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. Proc Natl Acad Sci U S A 112:536–541
27. Wu CH et al (2017) Genetic deletion or pharmacological inhibition of soluble epoxide hydrolase reduces brain damage and attenuates neuroinflammation after intracerebral hemorrhage. J Neuroinflammation 14:230
28. Hanaki Y et al (1991) Leukotoxin, 9, 10-epoxy-12-octadecenoate: a possible responsible factor in circulatory shock and disseminated intravascular coagulation. Jpn J Med 30:224–228
29. Hayakawa M et al (1990) Proposal of leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin. Biochem Int 21:573–579
30. Hu JN et al (1988) Neutrophil-derived epoxide, 9,10-epoxy-12-octadecenoate, induces pulmonary edema. Lung 166:327–337
31. Kosaka K, Suzuki K, Hayakawa M, Sugiyama S, Ozawa T (1994) Leukotoxin, a linoleate epoxide: its implication in the late death of patients with extensive burns. Mol Cell Biochem 139:141–148
32. Ozawa T et al (1988) Cytotoxic activity of leukotoxin, a neutrophil-derived fatty acid epoxide, on cultured human cells. Biochem Int 16:369–373
33. Totani Y et al (2000) Leukotoxin and its diol induce neutrophil chemotaxis through signal transduction different from that of fMLP. Eur Respir J 15:75–79
34. Zheng J, Plopper CG, Lakritz J, Storms DH, Hammock BD (2001) Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome. Am J Respir Cell Mol Biol 25:434–438
35. Moghaddam MF et al (1997) Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. Nat Med 3:562–566
36. Node K et al (1999) Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 285:1276–1279
37. Morin C, Sirois M, Echave V, Gomes MM, Rousseau E (2008) EET displays anti-inflammatory effects in TNF-alpha stimulated human bronchi: putative role of CPI-17. Am J Respir Cell Mol Biol 38:192–201
38. Zhang G et al (2013) Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proc Natl Acad Sci U S A 110:6530–6535
39. Wang W et al (2017) Omega-3 polyunsaturated fatty acids and their cytochrome P450-derived metabolites suppress colorectal tumor development in mice. J Nutr Biochem 48:29–35
40. Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871–1875
41. Yang W et al (2008) Characterization of epoxyeicosatrienoic acid binding site in U937 membranes using a novel radiolabeled agonist, 20-125i-14,15-epoxyeicosa-8(Z)-enoic acid. J Pharmacol Exp Ther 324:1019–1027
42. Chen Y, Falck JR, Manthati VL, Jat JL, Campbell WB (2011) 20-Iodo-14,15-epoxyeicosa-8(Z)-enoyl-3-azidophenylsulfonamide: photoaffinity labeling of a 14,15-epoxyeicosatrienoic acid receptor. Biochemistry 50:3840–3848
43. Park SK et al (2018) GPR40 is a low-affinity epoxyeicosatrienoic acid receptor in vascular cells. J Biol Chem 293:10675–10691
44. Liu X et al (2017) Functional screening for G protein-coupled receptor targets of 14,15-epoxyeicosatrienoic acid. Prostaglandins Other Lipid Mediat 132:31–40

45. Li P et al (2015) Epoxyeicosatrienoic acids enhance embryonic haematopoiesis and adult marrow engraftment. Nature 523:468–471
46. Ding Y et al (2014) The biological actions of 11,12-epoxyeicosatrienoic acid in endothelial cells are specific to the R/S-enantiomer and require the G(s) protein. J Pharmacol Exp Ther 350:14–21
47. Kundu S et al (2013) Metabolic products of soluble epoxide hydrolase are essential for monocyte chemotaxis to MCP-1 in vitro and in vivo. J Lipid Res 54:436–447
48. Hu J et al (2014) Muller glia cells regulate notch signaling and retinal angiogenesis via the generation of 19,20-dihydroxydocosapentaenoic acid. J Exp Med 211:281–295
49. Hu J et al (2017) Inhibition of soluble epoxide hydrolase prevents diabetic retinopathy. Nature 552:248–252
50. Enos RT et al (2016) High-fat diets rich in saturated fat protect against azoxymethane/dextran sulfate sodium-induced colon cancer. Am J Physiol Gastrointest Liver Physiol 310:G906–G919
51. Wu B et al (2004) Dietary corn oil promotes colon cancer by inhibiting mitochondria-dependent apoptosis in azoxymethane-treated rats. Exp Biol Med (Maywood) 229:1017–1025
52. Fujise T et al (2007) Long-term feeding of various fat diets modulates azoxymethane-induced colon carcinogenesis through Wnt/beta-catenin signaling in rats. Am J Physiol Gastrointest Liver Physiol 292:G1150–G1156
53. Reddy BS, Tanaka T, Simi B (1985) Effect of different levels of dietary trans fat or corn oil on azoxymethane-induced colon carcinogenesis in F344 rats. J Natl Cancer Inst 75:791–798
54. Pot GK et al (2008) Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study. Int J Cancer 123:1974–1977
55. Ogden CL, Carroll MD, Fryar CD, Flegal KM (2015) Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS Data Brief:1–8
56. Moghaddam AA, Woodward M, Huxley R (2007) Obesity and risk of colorectal cancer: a meta-analysis of 31 studies with 70,000 events. Cancer Epidemiol Biomark Prev 16:2533–2547
57. Ma Y et al (2013) Obesity and risk of colorectal cancer: a systematic review of prospective studies. PLoS One 8:e53916
58. Roberts DL, Dive C, Renehan AG (2010) Biological mechanisms linking obesity and cancer risk: new perspectives. Annu Rev Med 61:301–316
59. Wang W et al (2018) Lipidomic profiling reveals soluble epoxide hydrolase as a therapeutic target of obesity-induced colonic inflammation. Proc Natl Acad Sci U S A 115:5283–5288
60. Bettaieb A et al (2013) Soluble epoxide hydrolase deficiency or inhibition attenuates diet-induced endoplasmic reticulum stress in liver and adipose tissue. J Biol Chem 288:14189–14199
61. De Taeye BM et al (2010) Expression and regulation of soluble epoxide hydrolase in adipose tissue. Obesity (Silver Spring) 18:489–498
62. do Carmo JM et al (2012) Inhibition of soluble epoxide hydrolase reduces food intake and increases metabolic rate in obese mice. Nutr Metab Cardiovasc Dis 22:598–604
63. Imig JD et al (2012) Soluble epoxide hydrolase inhibition and peroxisome proliferator activated receptor gamma agonist improve vascular function and decrease renal injury in hypertensive obese rats. Exp Biol Med (Maywood) 237:1402–1412
64. Iyer A et al (2012) Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats. Exp Diabetes Res 2012:758614
65. Roche C et al (2015) Soluble epoxide hydrolase inhibition improves coronary endothelial function and prevents the development of cardiac alterations in obese insulin-resistant mice. Am J Physiol Heart Circ Physiol 308:H1020–H1029
66. Zha W et al (2014) Functional characterization of cytochrome P450-derived epoxyeicosatrienoic acids in adipogenesis and obesity. J Lipid Res 55:2124–2136
67. Zhang LN et al (2011) Inhibition of soluble epoxide hydrolase attenuates endothelial dysfunction in animal models of diabetes, obesity and hypertension. Eur J Pharmacol 654:68–74
68. Luria A et al (2011) Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. Proc Natl Acad Sci U S A 108:9038–9043

12 Contributions of 12/15-Lipoxygenase to Bleeding in the Brain Following Ischemic Stroke

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Abstract

Ischemic strokes are caused by one or more blood clots that typically obstruct one of the major arteries in the brain, but frequently also result in leakage of the blood-brain barrier and subsequent hemorrhage. While it has long been known that the enzyme 12/15-lipoxygenase (12/15-LOX) is up-regulated following ischemic strokes and contributes to neuronal cell death, recent research has shown an additional major role for 12/15-LOX in causing this hemorrhagic transformation. These findings have important implications for the use of 12/15-LOX inhibitors in the treatment of stroke.

Keywords

Lipoxygenase · Eicosanoid · 12-HETE · 15-HETE · Ischemic stroke · Hemorrhage · Hemorrhagic transformation · Ischemia · Blood-brain barrier · Tissue plasminogen activator · tPA · Neuroprotection · Warfarin · STAT6

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Strokes are typically classified into two major subtypes. Of these, ischemic strokes caused by blockage of a major artery account for around 85% of cases, while the remainder are caused by hemorrhage which can be either intracerebral or subarachnoid, depending on the location of the vessel rupture. However, even among the ischemic strokes a substantial number go on to include subsequent bleeding, leading to increased brain injury. This hemorrhagic transformation frequently occurs when tissue plasminogen activator (tPA) is used to lyse the obstructive blood clot, contributing to catastrophically low usage of this potentially lifesaving therapy - only a minor percentage of ischemic stroke patients receive thrombolytic treatment. Mechanisms involving the enzyme 12/15-lipoxygenase (12/15-LOX) contribute to the hemorrhagic transformation of

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ischemic strokes both in the presence and absence of tPA, as will be discussed in this mini-review.

The liberation of polyunsaturated fatty acids including arachidonic acid in the brain following a stroke has been recognized since the early 1970s [1]. These give rise to a dizzying spectrum of eicosanoids and related compounds produced by lipoxygenases, cyclooxygenases, and cytochromes P450, including prostaglandins, leukotrienes, and hydroxyeicosatetraenoic acids (HETEs). Increased levels of 12-HETE were found along with leukotrienes C4 and D4 in gerbil brains following an experimental stroke [2]. We have similarly seen massively increased levels of 12-HETE specifically on the infarcted side of the brain in mice both 12 and 24 h after onset of ischemia (Fig. 12.1). Therapeutically, initially 5-LOX was seen as the most promising target, mostly due to a much better understanding of leukotriene biology compared to the much less studied 12/15-LOX. However, two independent findings changed this perception: In 2004, a Japanese study found that *Alox5* knockout mice developed the same level of ischemic injury following experimental stroke as matched wild type mice [3]. Around the same time, the group of Chandan Sen at Ohio State University [4] and our group [5, 6] found that *Alox15* knockout mice were protected against the consequences of an experimental stroke, developing smaller infarct sizes (Sen group 90% reduction 72 h after stroke onset, van Leyen group 40% infarct size reduction measured 24 h after initiation of experimental stroke). Moreover, subsequent studies from our lab found that the mRNA encoding 12/15-LOX was up-regulated 2.2 fold in mice 24 h after an experimental stroke [7]. Immunohistochemistry showed the increased 12/15-LOX signal both in neurons, and in endothelial cells [8]. Early work focused on injury to neurons, initially with the discovery that 12/15-LOX contributes to a form of cell death termed oxidative glutamate toxicity or oxytosis in neuronal cells [9]. More recently, a related redox pathway termed ferroptosis was introduced, which is characterized by the loss of glutathione peroxidase 4 (Gpx-4) activity [10] and in which 12/15-LOX is also involved. The commonalities and differences between these two pathways have yet to be clearly defined [10–12]. Glutathione as the major intracellular antioxidant in neurons is clearly important for both pathways, and glutathione levels also drop on the ischemic side of the brain following stroke, which presumably contributes to the activation of 12/15-LOX. The function of 12/15-LOX in this neuronal cell death pathway is to damage mitochondria and other organelles, for which the 12/15-LOX is uniquely qualified: in stress reticulocytes produced during severe anemia, as well as when

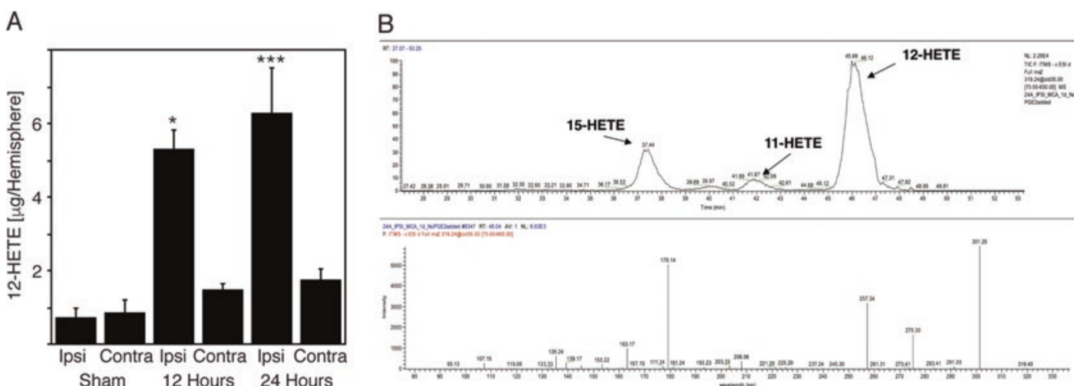

Fig. 12.1 (**a**) 12-HETE was significantly increased in the infarcted ipsilateral hemisphere of mice both 12 and 24 hours after transient focal ischemia, compared to sham-operated mice (*$p < 0.05$, ***$p < 0.001$; sham, n = 6 brains; 12 h, n = 3 brains; 24 h, n = 5 brains). (**b**) The identity of 12-HETE was confirmed by high-performance liquid chromatography (HPLC)/mass spectrometry analysis. The smaller peak for 15-HETE in the HPLC profile (top panel) is also a 12/15-LOX product. (Reprinted with permission from reference Rai et al. [24])

incubated in vitro, the enzyme attacks mitochondrial membranes [13–16], priming the mitochondria for further degradation via the ubiquitin/proteasome system. This is one of three pathways by which reticulocytes lose their organelles during maturation to become functioning erythrocytes, the others being degradation via autophagic vacuoles and exosome formation [17, 18]. The redundancy of these pathways may be the reason why no outright defects in erythropoiesis were found in Alox15 knockout mice [19].

Beyond causing cell death in neurons, however, it has in recent years become clear that 12/15-LOX also contributes to vessel injury in rodent models of stroke. Alox15 knockout mice develop 51% less edema following experimental stroke than their wild type counterparts, and 30% less immunoglobulin G (representative of blood proteins) extravasates into the brain parenchyma [8]. Several years later, we made the striking observation in a mouse model of thrombotic stroke that tPA infusion intended to lyse the occluding thrombus led to massive brain hemorrhage in these mice, which was reduced by 82% through simultaneous administration of a 12/15-LOX inhibitor, LOXBlock-1 [20] (Fig. 12.2). We have expanded on these results by investigating the effects of 12/15-LOX inhibition on both bleeding and infarct size in this thrombosis model, and found that LOXBlock-1 also improved behavioral scores in the mice [21].

This finding led us to systematically study mouse models of stroke where reperfusion following the ischemic event is associated with increased bleeding. In the classical filament model of transient focal ischemia, a filament is inserted into the internal carotid artery to partially block the middle cerebral artery on one side of the brain, which leads to a reduction of blood flow and ischemia in the striatum and cortex. The filament is then removed after a pre-specified time to allow for reperfusion. When the mouse is sacrificed after 24 h an infarct is detected, the size of which is determined by the duration of the ischemia. Typically, this model does not lead to significant bleeding, but so called hemorrhagic transformation of the ischemic stroke can be induced, for example when mice are fed with the anticoagulant warfarin [22, 23]. Warfarin is a vitamin K inhibitor that is frequently given to patients with atrial fibrillation to reduce their risk of blood clot formation and subsequent stroke. While warfarin reduces the risk of stroke in these patients, this anticoagulant can cause excessive bleeding leading to increased injury when a stroke does occur. Moreover, thrombolysis with the clotbuster tissue plasminogen activator (tPA) is contraindicated in effectively anticoagulated patients on warfarin (international normalized ratio of coagulation time (INR) > 1.7) because tPA itself has bleeding as a side effect, thus eliminating the only drug currently approved by the FDA as a treatment option. Mice pretreated with warfarin via their drinking water for 24 h prior to experimental stroke develop severe hemorrhage both when the mice receive a tPA infusion following removal of the filament, or in the absence of tPA when the stroke is severe enough (3 h of filament occlusion; Fig. 12.2) [23]. Along with the increased hemorrhage, we also found 25% higher levels of 12/15-LOX in the brains of the warfarin-treated mice. The increase was seen mostly in the vasculature, consistent with the idea that the increased vessel leakage following warfarin pretreatment is due to 12/15-LOX.

Consistent with the idea of 12/15-LOX as contributor to hemorrhage, 41% less bleeding was seen following warfarin pretreatment in Alox15 knockout mice [23]. In wild type mice, a similarly drastic reduction in hemorrhage by 38% was detected when the mice were treated with the second generation 12/15-LOX inhibitor ML351 [24], administered intraperitoneally at the time of reperfusion, 3 hours after onset of ischemia. The reduction in bleeding remained significant even when the results were adjusted to account for the reduced infarct size in the ML351-treated mice, confirming that there is a specific effect on hemorrhage. When ML351 was administered along with tPA in warfarin pretreated mice, hemorrhage was similarly reduced by 59%. Taken together, these results demonstrated that increased 12/15-LOX in the brain vasculature can contribute to excessive bleeding in the brain, which is reduced by treatment with a 12/15-LOX inhibitor.

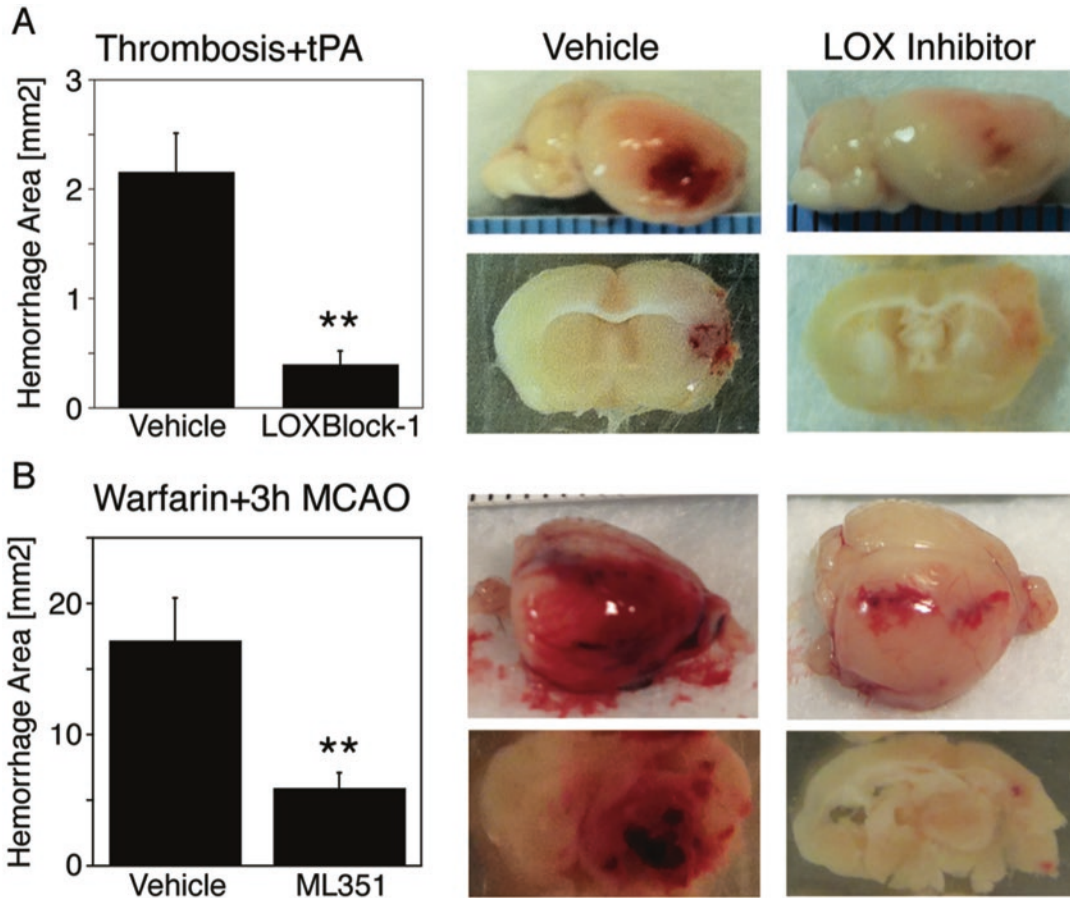

Fig. 12.2 Examples of stroke models associated with increased hemorrhage. (**a**) Thrombosis was induced when 10% ferric chloride solution was topically applied to the brain. An infusion of tissue plasminogen activator (tPA) 2 h later led to distinct hemorrhage in vehicle-treated mice after 24 h, visible both on the surface of the brain (top) and in sections (below). In contrast, when mice were intraperitoneally injected with the 12/15-LOX inhibitor LOXBlock-1 (50 mg/kg), significantly less hemorrhage was detected in the brain. (**b**) Pretreatment of mice for 24 h with the anticoagulant warfarin added to the drinking water causes massive hemorrhage following a severe form of experimental ischemic stroke with 3 h of occlusion of the middle cerebral artery. This is again visible both on the surface, as well as in brain sections. Mice treated with the 12/15-LOX inhibitor ML351 (50 mg/kg) develop far less hemorrhage (bottom right). Quantitation graphs represent hemorrhage area measured in brain sections and are reprinted with permission from references [24] (top) and [23] (bottom)

Much work remains to be done to elucidate the mechanism by which 12/15-LOX contributes to increased hemorrhage after an ischemic stroke. Important open questions include the selective up-regulation of 12/15-LOX in brain vascular endothelial cells following warfarin administration, both with and without subsequent tPA infusion. Is this a direct effect of warfarin and/or tPA, or are intermediate factors involved? Also, in neurons signal transducers and activators of transcription (STATs), specifically STAT1 and STAT6 are involved in up-regulating 12/15-LOX under ischemic conditions [7]. Are the same STATs active here, or is a different form of regulation relevant? Finally, what happens after 12/15-LOX is up-regulated in the endothelial cells and how does this lead to vessel rupture? In addition to destroying endothelial cells of the brain vasculature by damaging mitochondria, there may be a second injury mechanism induced by the signaling function of 12/15-LOX via metabolites of arachidonic acid. Both 12-HETE

and its immediate precursor 12-HPETE are known as second messengers [25], activated along the semaphorin pathway [26, 27]. In neurons, this can lead to axon retraction [28, 29], but under some conditions also to cell death [30]. Semaphorin 3A has also been reported to increase vascular permeability in experimental stroke models [31]. Downstream of 12-HETE and 12-HPETE, secretion of destabilizing matrix metalloproteinases (MMPs) may play a role [32]. Both the molecular details of this signaling pathway, and the relative contributions of both pathways to vascular injury require further study.

Because 12/15-LOX contributes to both neuronal cell death and to vessel leakage following a stroke, 12/15-LOX inhibition appears to be a particularly promising approach to stroke therapy by targeting two separate modes of injury, killing two birds with one stone. Both our group [20, 23, 24, 33, 34] and others [35, 36] have in recent years focused on developing improved inhibitors of 12/15-LOX, and it will be exciting to see if these novel molecules can turn the tide in the seemingly endless war to combat stroke. The finding that we can reduce bleeding subsequent to an ischemic stroke in the rodent model broadens the spectrum of patients that could be treated with a 12/15-LOX inhibitor. In addition to its use as a stand-alone neuroprotective treatment that could already be given in the ambulance on the way to the hospital, 12/15-LOX inhibition could also be combined with tPA thrombolysis to make the use of tPA safer. By removing the most serious side effect of tPA, this approach may lead to significantly more patients receiving tPA treatment. Furthermore, the recently introduced endovascular treatment in which a stent retriever is used to remove the obstructing blood clot could also be rendered more effective by adding a 12/15-LOX inhibitor, because even after thrombus removal cognitive deficits are seen in many patients who could benefit from the added neuroprotection[37]. Finally, besides the more common ischemic strokes there may also be a place for 12/15-LOX inhibition in the treatment of hemorrhagic strokes. We have recently completed a study to investigate the function of 12/15-LOX in subarachnoid hemorrhage, where we found increased 12/15-LOX in the brains of mice 24 h after hemorrhage induction (92 ± 60 12/15-LOX positive cells/field vs. 2 ± 2 in sham-operated controls, $p < 0.05$) [38]. In this case, 12/15-LOX expression was detected mostly in macrophages however, rather than in neurons and endothelial cells; the injury mechanism may thus differ from that in ischemic stroke. Regardless, Alox15 knockout mice developed 72% less injury than wild type mice, and 12/15-LOX inhibition also reduced injury by 55% compared to vehicle-treated mice in this model of hemorrhagic stroke.

In conclusion, despite different triggers – in the presence or absence of anticoagulant, with or without tPA treatment – 12/15-LOX is activated in various models of stroke-related hemorrhage. In addition to its benefits in infarct size reduction, 12/15-LOX inhibition may thus independently reduce hemorrhagic conversion of ischemic strokes by protecting the vasculature.

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References

1. Bazan NG Jr (1970) Effects of ischemia and electroconvulsive shock on free fatty acid pool in the brain. Biochim Biophys Acta 218(1):1–10. Epub 1970/10/06. PubMed PMID: 5473492
2. Moskowitz MA, Kiwak KJ, Hekimian K, Levine L (1984) Synthesis of compounds with properties of leukotrienes C4 and D4 in gerbil brains after ischemia and reperfusion. Science 224(4651):886–889. Epub 1984/05/25. PubMed PMID: 6719118
3. Kitagawa K, Matsumoto M, Hori M (2004) Cerebral ischemia in 5-lipoxygenase knockout mice. Brain Res 1004(1–2):198–202. Epub 2004/03/23, https://doi.org/10.1016/j.brainres.2004.01.018. S0006899304000587 [pii], PubMed PMID: 15033436
4. Khanna S, Roy S, Slivka A, Craft TK, Chaki S, Rink C et al (2005) Neuroprotective properties of the natural vitamin E alpha-tocotrienol. Stroke 36(10):2258–2264. Epub 2005/09/17, doi: 01.STR.0000181082.70763.22 [pii], https://doi.org/10.1161/01.STR.0000181082.70763.22. PubMed PMID: 16166580; PubMed Central PMCID: PMC1829173

5. van Leyen K, Kim HY, Lee SR, Jin G, Arai K, Lo EH (2006) Baicalein and 12/15-lipoxygenase in the ischemic brain. Stroke 37(12):3014–3018. Epub 2006/10/21, 01.STR.0000249004.25444.a5 [pii], https://doi.org/10.1161/01.STR.0000249004.25444.a5. PubMed PMID: 17053180
6. van Leyen K, Lee SR, Siddiq A, Ratan RR, Lo EH (2004) 12/15-lipoxygenase and the proteasome as mediators of neuronal oxidative stress and stroke. Program No 1356, 2004 abstract viewer/itinerary planner. Society for Neuroscience, Washington, DC
7. Jung JE, Karatas H, Liu Y, Yalcin A, Montaner J, Lo EH et al (2015) STAT-dependent upregulation of 12/15-lipoxygenase contributes to neuronal injury after stroke. J Cereb Blood Flow Metab. 35(12):2043–2051. Epub 2015/07/16. https://doi.org/10.1038/jcbfm.2015.169. PubMed PMID: 26174325; PubMed Central PMCID: PMCPMC4671126
8. Jin G, Arai K, Murata Y, Wang S, Stins MF, Lo EH et al (2008) Protecting against cerebrovascular injury: contributions of 12/15-lipoxygenase to edema formation after transient focal ischemia. Stroke 39(9):2538–2543. Epub 2008/07/19. doi: STROKEAHA.108.514927 [pii], https://doi.org/10.1161/STROKEAHA.108.514927. PubMed PMID: 18635843; PubMed Central PMCID: PMC2754072
9. Li Y, Maher P, Schubert D (1997) A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron 19(2):453–463. Epub 1997/08/01. S0896-6273(00)80953-8 [pii]. PubMed PMID: 9292733.
10. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al (2018) Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018 . Cell Death Differ. 25(3):486–541. Epub 2018/01/25. https://doi.org/10.1038/s41418-017-0012-4. PubMed PMID: 29362479; PubMed Central PMCID: PMCPMC5864239
11. Maher P, van Leyen K, Dey PN, Honrath B, Dolga A, Methner A. (2018) The role of Ca(2+) in cell death caused by oxidative glutamate toxicity and ferroptosis. Cell Calcium 70:47–55. Epub 2017/05/27. https://doi.org/10.1016/j.ceca.2017.05.007. PubMed PMID: 28545724; PubMed Central PMCID: PMCPMC5682235
12. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ et al (2017) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell. 171(2):273–285. Epub 2017/10/07. https://doi.org/10.1016/j.cell.2017.09.021. PubMed PMID: 28985560; PubMed Central PMCID: PMCPMC5685180
13. Schewe T, Halangk W, Hiebsch C, Rapoport SM (1975) A lipoxygenase in rabbit reticulocytes which attacks phospholipids and intact mitochondria. FEBS Lett 60(1):149–152. Epub 1975/12/01. 0014-5793(75)80439-X [pii]. PubMed PMID: 6318.
14. Kuhn H, Banthiya S, van Leyen K (2015) Mammalian lipoxygenases and their biological relevance. Biochim Biophys Acta. 1851(4):308–330. Epub 2014/10/16. https://doi.org/10.1016/j.bbalip.2014.10.002. PubMed PMID: 25316652; PubMed Central PMCID: PMCPMC4370320
15. Grüllich C, Duvoisin RM, Wiedmann M, van Leyen K (2001) Inhibition of 15-lipoxygenase leads to delayed organelle degradation in the reticulocyte. FEBS Lett 489(1):51–54. Epub 2001/03/07. S0014-5793(01)02080-4 [pii]. PubMed PMID: 11231012.
16. van Leyen K, Duvoisin RM, Engelhardt H, Wiedmann M (1998) A function for lipoxygenase in programmed organelle degradation. Nature 395(6700):392–395. Epub 1998/10/06. https://doi.org/10.1038/26500. PubMed PMID: 9759730.
17. Koury MJ, Sawyer ST, Brandt SJ (2002) New insights into erythropoiesis. Curr Opin Hematol 9(2):93–100. Epub 2002/02/15, PubMed PMID: 11844990.
18. Blanc L, Barres C, Bette-Bobillo P, Vidal M (2007) Reticulocyte-secreted exosomes bind natural IgM antibodies: involvement of a ROS-activatable endosomal phospholipase iPLA2. Blood 110(9):3407–3416. Epub 2007/08/02. https://doi.org/10.1182/blood-2007-04-085845. PubMed PMID: 17666570.
19. Sun D, Funk CD (1996) Disruption of 12/15-lipoxygenase expression in peritoneal macrophages. Enhanced utilization of the 5-lipoxygenase pathway and diminished oxidation of low density lipoprotein. J Biol Chem 271(39):24055–24062. Epub 1996/09/27, PubMed PMID: 8798642
20. Yigitkanli K, Pekcec A, Karatas H, Pallast S, Mandeville E, Joshi N et al (2013) Inhibition of 12/15-lipoxygenase as therapeutic strategy to treat stroke. Ann Neurol 73(1):129–135. https://doi.org/10.1002/ana.23734. PubMed PMID: 23192915; PubMed Central PMCID: PMC3563836
21. Karatas H, Eun Jung J, Lo EH, van Leyen K (2018) Inhibiting 12/15-lipoxygenase to treat acute stroke in permanent and tPA induced thrombolysis models. Brain Res 1678:123–128. https://doi.org/10.1016/j.brainres.2017.10.024. PubMed PMID: 29079502; PubMed Central PMCID: PMC5714685
22. Pfeilschifter W, Spitzer D, Czech-Zechmeister B, Steinmetz H, Foerch C (2011) Increased risk of hemorrhagic transformation in ischemic stroke occurring during warfarin anticoagulation: an experimental study in mice. Stroke 42(4):1116–1121. Epub 2011/02/19. https://doi.org/10.1161/STROKEAHA.110.604652. PubMed PMID: 21330626.
23. Liu Y, Zheng Y, Karatas H, Wang X, Foerch C, Lo EH et al (2017) 12/15-Lipoxygenase inhibition or Knockout Reduces Warfarin-associated hemorrhagic transformation after experimental stroke. Stroke. 48(2):445–451. Epub 2017/01/07. https://doi.org/10.1161/STROKEAHA.116.014790. PubMed PMID: 28057806; PubMed Central PMCID: PMCPMC5263178

24. Rai G, Joshi N, Jung JE, Liu Y, Schultz L, Yasgar A et al (2014) Potent and selective inhibitors of human reticulocyte 12/15-lipoxygenase as anti-stroke therapies. J Med Chem 57(10):4035–4048. https://doi.org/10.1021/jm401915r. PubMed PMID: 24684213; PubMed Central PMCID: PMC4033661
25. Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH et al (1987) Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of Aplysia sensory cells. Nature 328(6125):38–43. Epub 1987/07/02. https://doi.org/10.1038/328038a0. PubMed PMID: 2439918.
26. Mikule K, Gatlin JC, de la Houssaye BA, Pfenninger KH (2002) Growth cone collapse induced by semaphorin 3A requires 12/15-lipoxygenase. J Neurosci 22(12):4932–4941. Epub 2002/06/22. doi: 22/12/4932 [pii] PubMed PMID: 12077190.
27. Pekcec A, Yigitkanli K, Jung JE, Pallast S, Xing C, Antipenko A et al (2013) Following experimental stroke, the recovering brain is vulnerable to lipoxygenase-dependent semaphorin signaling. FASEB J 27(2):437–445. https://doi.org/10.1096/fj.12-206896. PubMed PMID: 23070608; PubMed Central PMCID: PMC3545523
28. He Z, Wang KC, Koprivica V, Ming G, Song HJ (2002) Knowing how to navigate: mechanisms of semaphorin signaling in the nervous system. Sci STKE 2002(119):re1. Epub 2002/02/14. https://doi.org/10.1126/stke.2002.119.re1. PubMed PMID: 11842242.
29. Kolodkin AL, Tessier-Lavigne M (2011) Mechanisms and molecules of neuronal wiring: a primer. Cold Spring Harb Perspect Biol. 3(6). Epub 2010/12/03. https://doi.org/10.1101/cshperspect.a001727. PubMed PMID: 21123392; PubMed Central PMCID: PMCPMC3098670
30. Jiang SX, Whitehead S, Aylsworth A, Slinn J, Zurakowski B, Chan K et al (2010) Neuropilin 1 directly interacts with Fer kinase to mediate semaphorin 3A-induced death of cortical neurons. J Biol Chem 285(13):9908-9918. Epub 2010/02/06. https://doi.org/10.1074/jbc.M109.080689. PubMed PMID: 20133938; PubMed Central PMCID: PMCPMC2843238
31. Hou ST, Nilchi L, Li X, Gangaraju S, Jiang SX, Aylsworth A et al (2015) Semaphorin3A elevates vascular permeability and contributes to cerebral ischemia-induced brain damage. Sci Rep 5:7890. Epub 2015/01/21. https://doi.org/10.1038/srep07890. PubMed PMID: 25601765; PubMed Central PMCID: PMCPMC4298747
32. Dilly AK, Ekambaram P, Guo Y, Cai Y, Tucker SC, Fridman R et al (2013) Platelet-type 12-lipoxygenase induces MMP9 expression and cellular invasion via activation of PI3K/Akt/NF-kappaB. Int J Cancer 133(8):1784–1791. Epub 2013/03/26. https://doi.org/10.1002/ijc.28165. PubMed PMID: 23526143; PubMed Central PMCID: PMCPMC4269488
33. Armstrong MM, Freedman CJ, Jung JE, Zheng Y, Kalyanaraman C, Jacobson MP et al (2016) A potent and selective inhibitor targeting human and murine 12/15-LOX. Bioorg Med Chem 24(6):1183–1190. Epub 2016/02/24. https://doi.org/10.1016/j.bmc.2016.01.042. PubMed PMID: 26899595; PubMed Central PMCID: PMCPMC4778748
34. van Leyen K, Arai K, Jin G, Kenyon V, Gerstner B, Rosenberg PA et al (2008) Novel lipoxygenase inhibitors as neuroprotective reagents. J Neurosci Res 86(4):904–909. Epub 2007/10/27. https://doi.org/10.1002/jnr.21543. PubMed PMID: 17960827; PubMed Central PMCID: PMC2759176
35. Han H, Liang X, Ekberg M, Kritikou JS, Brunnstrom A, Pelcman B et al (2017) Human 15-lipoxygenase-1 is a regulator of dendritic-cell spreading and podosome formation. FASEB J 31(2):491–504. Epub 2016/11/09. https://doi.org/10.1096/fj.201600679RR. PubMed PMID: 27825104.
36. Lapchak PA, Boitano PD, Bombien R, Cook DJ, Doyan S, Lara JM et al (2018) CNB-001, a pleiotropic drug is efficacious in embolized agyrencephalic New Zealand white rabbits and ischemic gyrencephalic cynomolgus monkeys. Exp Neurol 313:98–108. Epub 2018/12/07. https://doi.org/10.1016/j.expneurol.2018.11.010. PubMed PMID: 30521790
37. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ et al (2015) A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 372(1):11–20. Epub 2014/12/18. https://doi.org/10.1056/NEJMoa1411587. PubMed PMID: 25517348
38. Gaberel T, Gakuba C, Zheng Y, Lepine M, Lo EH, van Leyen K (2019) Impact of 12/15-Lipoxygenase on Brain Injury After Subarachnoid Hemorrhage. Stroke 50(2): 520–523. STROKEAHA118022325. https://doi.org/10.1161/STROKEAHA.118.022325. Epub 2019/01/04. PubMed PMID: 30602353.

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13 Systematic Understanding of Bioactive Lipids in Neuro-Immune Interactions: Lessons from an Animal Model of Multiple Sclerosis

Yasuyuki Kihara

Abstract

Bioactive lipids, or lipid mediators, are utilized for intercellular communications. They are rapidly produced in response to various stimuli and exported to extracellular spaces followed by binding to cell surface G protein-coupled receptors (GPCRs) or nuclear receptors. Many drugs targeting lipid signaling such as non-steroidal anti-inflammatory drugs (NSAIDs), prostaglandins, and antagonists for lipid GPCRs are in use. For example, the sphingolipid analog, fingolimod (also known as FTY720), was the first oral disease-modifying therapy (DMT) for relapsing-remitting multiple sclerosis (MS), whose mechanisms of action (MOA) includes sequestration of pathogenic lymphocytes into secondary lymphoid organs, as well as astrocytic modulation, via down-regulation of the sphingosine 1-phosphate (S1P) receptor, $S1P_1$, by *in vivo*-phosphorylated fingolimod. Though the cause of MS is still under debate, MS is considered to be an autoimmune demyelinating and neurodegenerative disease. This review summarizes the involvement of bioactive lipids (prostaglandins, leukotrienes, platelet-activating factors, lysophosphatidic acid, and S1P) in MS and the animal model, experimental autoimmune encephalomyelitis (EAE). Genetic ablation, along with pharmacological inhibition, of lipid metabolic enzymes and lipid GPCRs revealed that each bioactive lipid has a unique role in regulating immune and neural functions, including helper T cell (T_H1 and T_H17) differentiation and proliferation, immune cell migration, astrocyte responses, endothelium function, and microglial phagocytosis. A systematic understanding of bioactive lipids in MS and EAE dredges up information about understudied lipid signaling pathways, which should be clarified in the near future to better understand MS pathology and to develop novel DMTs.

Keywords

Neuroimmunology · Autoimmunity · Neuroinflammation · Neurodegeneration · Demyelination · Inflammation · Microglia · Astrocyte · Blood-brain barrier · Eicosanoid · PLA_2 · PGE_2 · LTB_4 · PAF · LPA

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13.1 Neuro-immune Interactions and Their Failures

The central nervous system (CNS) is separated from the peripheral immune system by the blood-brain barrier (BBB) and it is also immunologically inactive which provides immune privilege [1] despite the existence of autoimmune demyelinating diseases such as multiple sclerosis (MS). Recent studies clearly show the communication pathways between the CNS vs. peripheral immune system through a lymphatic system in the meninges [2] and tunnels connecting the brain and the skull [3]. Furthermore, an animal model of MS proposed that pathogenic T cells penetrate into the CNS via the fifth lumber spinal cord [4]. Failure of homeostatic neuro-immune interactions exerts pernicious effects on the CNS, resulting in the development of several diseases including MS [4], Alzheimer's disease [5], and social behavior impairment [6]. In this section, MS and its animal model, experimental autoimmune encephalomyelitis (EAE), is introduced.

13.1.1 Multiple Sclerosis

Publications in the 1830s described a disease exhibiting multiple, irregular foci of discoloration and shrinkage throughout neuroaxis [7]. In 1868, a French neurologist, Jean-Martin Charcot, gathered prior clinical depictions and framed the disease as *sclérose en plaques* (multiple sclerosis in French) [8]. The pathologic hallmarks of MS are myelin loss (demyelination), accompanied by inflammation within the CNS, which are called plaques or lesions. Introduction of magnetic resonance imaging (MRI) in 1981 [9] conquered the chaos in diagnosing MS, understanding MS pathogenesis, and evaluation of treatment. The current standard of MS diagnosis, the McDonald criteria [10, 11], uses MRI to identify CNS damage as seen over time and location. In 1983, John F. Kurtzke developed the Expanded Disability Status Scale (EDSS) [12, 13] that quantify the disabilities in MS patients whose symptoms are variable and numerous including fatigue, walking difficulties, numbness/tingling, vision problems, and dizziness/vertigo. Surprisingly, many of these symptoms were recorded in documents about St. Lidwina of Schiedam (1380–1433), which is thought to be the oldest depiction of MS [14].

Based on the disease course, MS is classified into four types: (1) clinically isolated syndrome (CIS), (2) relapsing-remitting MS (RRMS), (3) secondary progressive MS (SPMS), and (4) primary progressive MS (PPMS) (nationalmssociety.org). Currently, about 2.3 million people live with MS around the world, with a higher prevalence in North America and Europe (>100/100,000 inhabitants) compared to Asia and Africa (2/100,000 population) [15]. MS affects more women than men (3~4:1 ratio) (nationalmssociety.org). Although involvement of genetic and environmental factors in MS pathogenesis have been proposed, the cause of MS is still under debate. By 2018, the Food and Drug Administration (FDA) has approved 16 medications as disease-modifying therapies (DMTs). However, daclizumab, an anti-CD25 monoclonal antibody (Ab), was withdrawn from the market worldwide in March 2018 [16]. Additional details and updated information about MS and its treatment can be found on the National Multiple Sclerosis Society's website (nationalmssociety.org). Since MS is traditionally considered to be an autoimmune disorder, immunomodulation is the mechanism of action (MOA) of most MS-drugs such as natalizumab (anti-VLA4 Ab) [17], alemtzumab (anti-CD52 Ab) [18], ocrelizumab (anti-CD20 Ab) [19], interferon β (IFN-β) [20], fingolimod [21, 22], and dimethyl fumarate (DMF) [23, 24]. However, a growing body of literature supports that some MS drugs, such as fingolimod [25], IFN-β [26], and DMF [27], exhibit direct effects in the CNS. Exploring and developing CNS-targeted MS-drugs is meaningful for finding a cure for progressive forms of MS that show more neurodegenerative features than RRMS. Neuro-immune interactions underlying MS are far more complex than previously understood, and thus further exploration of the communications between the immune and nervous systems is highly desirable.

13.1.2 Experimental Autoimmune Encephalomyelitis

A breakthrough in understanding MS pathogenesis and MS drug development was the establishment of an animal model of MS, now called experimental autoimmune (or allergic) encephalomyelitis (EAE), by Thomas Rivers in 1933 [28]. The study provided the first evidence of immune cell-mediated CNS damage. Recapitulation of EAE by transfer of lymph node cells [29] guided several important findings that include EAE induction with T cells reactive against myelin basic protein (MBP) [30] and definition of encephalitogenic epitopes of MBP [31]. Further investigation revealed $CD4^+$ T helper type 1 (T_H1) cells recognize oligodendrocyte-specific proteins or peptide fragments (such as MBP; proteolipid protein, PLP; myelin oligodendrocyte glycoprotein, MOG) [32, 33] that are responsible for EAE induction and progression. In 2005, interleukin-23 (IL-23)-derived IL-17 producing encephalitogenic T cells, which are now known as T_H17 cells, were found to play key roles in CNS autoimmunity [34].

EAE susceptibility varies in mouse strains and may be explained by the major histocompatibility complex H2 haplotypes [35]. The most commonly used mouse strains in the scientific field are T_H1-prone C57BL/6 (*H2b*) and $CD4^+$ T helper type 2 (T_H2)-prone Balb/c (*H2d*) mice. Both active immunization with MOG peptide (MOG_{35-55}) and passive immunization by adoptive transfer of encephalitogenic T cells in C57BL/6 mice display monophasic disease course [23, 36–39]. All the knockout studies introduced below used the C57BL/6 genetic background unless otherwise stated. Balb/c mice are not susceptible to EAE, but some controversial results are reported [40]. SJL mice harboring *H2s* present a relapsing-remitting disease course when immunized with PLP peptide ($PLP_{139-151}$) [35]. Non-obese diabetic (NOD) mice harboring $H2g^7$ immunized with MOG_{35-55} display progressive MS-like disease signs [35]. This needs careful consideration since NOD mice develop spontaneous diabetes at a higher rate.

Failure of neuro-immune communication results in T cells attacking CNS elements, particularly oligodendrocytes, which causes demyelination and neurodegeneration. Although immunological aspects of these diseases have been well-studied, it remained unknown whether CNS cells are actively involved in the disease. By using genetically engineered mice harboring a tetracycline-controlled c-Fos reporter (a green fluorescent protein-histone H2B fusion protein under a tetO promoter controlled by a c-Fos inducible tetracycline transactivator), we identified that astrocytes were activated by EAE insults at the initial phase of disease course [41]. We named the astrocytes that experienced c-Fos activation during EAE as immediate-early astrocytes (*ieAstrocytes*) [41]. The number of *ieAstrocytes* increased linearly with EAE progression, indicating that astrocytes are the key CNS cell type in EAE pathogenesis [41]. Although there are several differences between MS and EAE, both diseases share the pathological, clinical features, which justify EAE as an MS model to study neuro-immune interactions. In the following sections, the roles of bioactive lipids in neuro-immune interactions from EAE studies that used knockout (KO) mice or pharmacological tools are summarized.

13.2 Arachidonic Acid Cascade in EAE and MS

Arachidonic acid (AA) cascade is a pathway that produces eicosanoids from ω-6 polyunsaturated fatty acid (PUFA), AA (5Z,8Z,11Z,14Z-eicosatetraenoic acid) [42]. Eicosanoid is the general term used for potent bioactive lipids that have 20 (eicosa in Greek) carbon units in their structure, which include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), hydroxyl eicosatetraenoic acids (HETEs), and epoxyeicosatrienoic acids (EETs). Figure 13.1 summarizes the relationship between AA cascade and EAE/MS pathology.

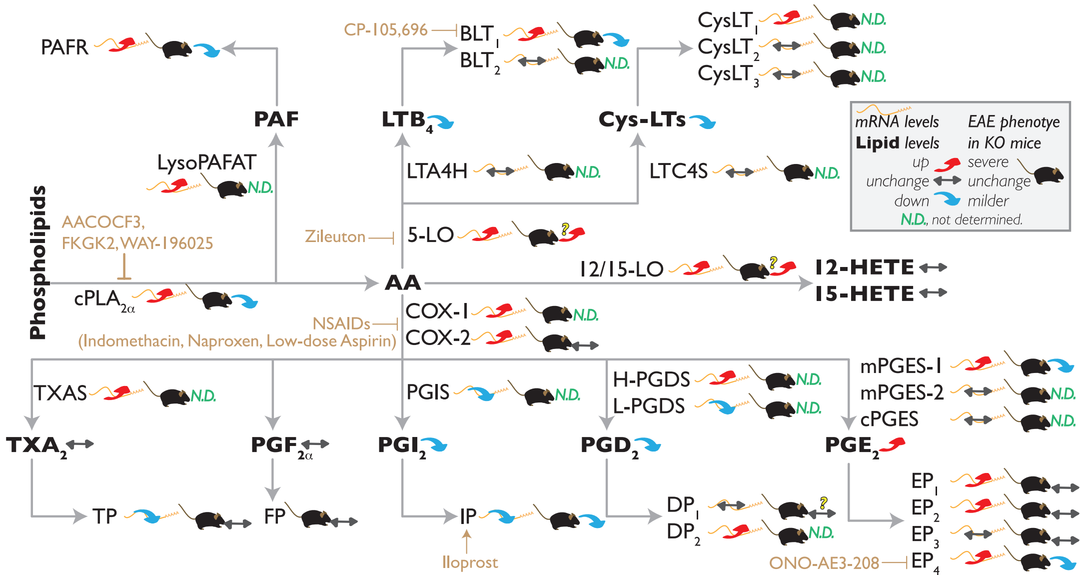

Fig. 13.1 EAE phenotypes in AA cascade-KO mice

13.2.1 Phospholipase A_2s (PLA_2s)

PLA_2s including cytosolic PLA_2s ($cPLA_2$), secreted PLA_2s ($sPLA_2$) and calcium-independent PLA_2s ($iPLA_2$), hydrolyze the *sn-2* position of membrane glycerophospholipids to liberate fatty acyls including AA [43]. Among them, group IVA $cPLA_2$ ($cPLA_2\alpha$; gene name, *Pla2g4a*) is a key enzyme to produce eicosanoids, since its deficiency lacks the ability of producing eicosanoids, resulting in resistance to various disease models [43]. Several lines of evidence, such as (1) up-regulation of Ca^{2+}-dependent PLA_2 activity and PLA_2 mRNAs ($cPLA_2\alpha$, $cPLA_2\beta$, $cPLA_2\zeta$, and $sPLA_2$-V) in EAE spinal cords (SCs) [38], (2) $cPLA_2\alpha$ expression in brain endothelium and infiltrated immune cells in EAE mice [44], and (3) increased $sPLA_2$ activity in both MS and EAE urine [45], suggest active involvement of these enzymes in EAE. Using $cPLA_2\alpha$-KO mice [46], Marusic et al. revealed the requirement of both peripheral and neuronal $cPLA_2\alpha$ in EAE development and progression [47]. No other PLA_2-KO mice have been tested for EAE. Pharmacological inhibition of PLA_2s (Pan inhibitors, AACOCF3 and FKGK2; $cPLA_2\alpha$ inhibitor, WAY-196025; $sPLA_2$ inhibitor, CHEC-9; and $iPLA_2$ inhibitor, FKGK11) also blocked EAE development and progression [44, 47–50]. These results suggest that eicosanoids, which are produced downstream of PLA_2, should have unique roles in EAE and MS.

13.2.2 Cyclooxygenases (COXs)

COXs (COX-1 and COX-2; gene names, *Ptgs1* and *Ptgs2*, respectively) insert two molecular oxygen into AA to produce an unstable endoperoxide intermediate, PGH_2, which in turn metabolizes to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 by isomerases and terminal synthases [51]. Acetylsalicylic acid (Aspirin®) is the first nonsteroidal anti-inflammatory drug (NSAID) synthesized by Bayer in 1897 [52]. The MOA of aspirin is suppression of prostaglandin synthesis by inhibiting COXs, which was revealed by John Vane in 1971 [53].

There were no beneficial effects of aspirin in guinea pig EAE [54, 55], while a high dose sodium salicylate, a major metabolite of aspirin, showed efficacy in guinea pig EAE [56]. Two clinical trials in the early 1960's examined aspirin's efficacy in MS failed [57, 58]. However, mouse EAE studies tested other NSAIDs including indomethacin and naproxen (non-selective

COX inhibitors) that clearly demonstrated the involvement of COXs in EAE [50, 59]. Miyamoto et al. reported that COX-2-KO mice showed equivalent disease course with controls [59]. The study also demonstrated that a COX-2 selective inhibitor, celecoxib, prevented EAE in mice, while COX-2-KO mice were not rescued by celecoxib. Thus, the MOA of celecoxib in EAE suppression is independent from COX-2 inhibition [59]. Recently, the efficacy of low-dose aspirin (1 ~ 3 mg/kg), which is commonly used for lowering cardiovascular risks [60], on the EAE phenotype was reported [61]. The study revealed that low-dose aspirin prevented loss of regulatory T cells (T_{reg}) by stimulating IL-11 production, and also suppressed T_H1/T_H17 differentiation, resulting in EAE amelioration [61]. However, since this MOA of low-dose aspirin is not tested in COX-1/2-KO mice and eicosanoid levels were not quantified, it remains unclear if the efficacy of low-dose aspirin is related to COX-1/2 inhibition. Considering the results from cPLA2α-KO and COX-2-KO studies, COX-1 may play some role in EAE, but EAE has not been tested in COX-1-KO mice to date.

13.2.3 Prostaglandins (PGs)

PGs are produced downstream of COX-1/2 and are functionally coupled with terminal PG synthases to produce each PG efficiently [62–64], followed by binding to their specific receptors (a member of the superfamily of G-protein coupled receptors (GPCRs)), to elicit a variety of biological responses [42].

We applied targeted lipidomics using liquid chromatography tandem mass spectrometry (LC-MS/MS) in combination with transcriptomics to identify a key eicosanoid pathway that is involved in EAE [37]. Lipidomics identified a metabolic shift from the PGD_2 pathway in naïve spinal cords (SCs) to the PGE_2 pathway in EAE SCs [37]. Among the three PGE_2 synthases, microsomal PGE synthase 1 (mPGES-1; gene name, *Ptges*) mRNA was uniquely up-regulated in EAE SCs. mPGES-1 protein was expressed in microglia/macrophages in both EAE and MS lesions [37]. mPGES-1 deficiency almost completely inhibited PGE_2 production in EAE SCs, while doubling PGI_2 production. mPEGS-1-KO mice showed EAE amelioration and impairment of T_H1/T_H17 differentiation [37]. Later, another group reported essentially the same results using the same mPGES-1-KO mice. They proposed that mPGES-1 in vascular endothelial cells controls IL-1β signaling in both $CD4^+$ T cells and endothelial cells, resulting in EAE exacerbation [65–67]. EAE phenomics using 8 PG receptor KO mice was reported [68], which identified a significant suppression of EAE only in PGE_2 receptor 4 (EP_4; gene name, *Ptger4*)-KO mice on the mixed background of C57BL/6 and 129/Ola. EAE phenotypes in other PGE_2 receptors (EP_1, EP_2, and EP_3) were equivalent to their matching controls [68]. Prophylactic administration of the EP_4 antagonist (ONO-AE3-208) prevented mice from developing EAE, while the therapeutic paradigm showed little effect on EAE development and severity [68]. The efficacy of ONO-AE3-208 was greater in EP_2-KO mice than wild-type (WT) mice, suggesting a redundancy of EP_4 and EP_2 [68]. Indeed, PGE_2 facilitated T_H1 differentiation and IL-23-induced T_H17 expansion through both EP_2 and EP_4 [69]. These results clearly demonstrate that the mPGES-1-PGE_2-$EP_{2/4}$ axis is an exacerbating pathway in EAE and MS.

Although the EAE phenomics study concluded no aggravation or facilitation of EAE in any PG receptor KO mice except for EP_4-KO mice, the clinical score of PGI_2 receptor (IP; gene name, *Ptgir*)-KO mice appeared to show a right shift (delayed onset and/or milder EAE) as compared to controls [68]. Indeed, another group reported a similar trend with significant differences between IP-KO mice vs. controls [70]. They also demonstrated that PGI_2-IP signaling induces IL-17A production in naïve $CD4^+$ T cells and promotes T_H17 differentiation [70]. The decreased PGI_2 in EAE SCs could be explained by down-regulation of PGI synthase (PGIS; gene name, *Ptgis*) mRNA that is abundant in endothelial cells [37], suggesting a direct neuroprotective role of PGI_2 in the CNS. Muramatsu et al. reported that knockdown of PGIS and IP by siRNA delivered directly into the CNS, delayed recovery

from motor dysfunction associated with EAE [71]. Furthermore, PGI_2 promoted neurite elongation *in vitro*. Systemic administration of an IP agonist, iloprost, improved motor deficits in EAE, indicating that vascular endothelial cell-derived PGI_2 promotes neuronal rewiring in EAE via the PGIS-PGI_2-IP pathway [71]. Collectively, PGI_2 shows anti-immune and neuroprotective effects.

PGD_2 is the most abundant eicosanoid in the CNS [72]. The decreased PGD_2 in EAE SCs might be associated with reductions in lipocalin-type PGD synthase (L-PGDS; gene name, *Ptgds*) mRNA that is highly expressed in oligodendrocytes (OLs) [37]. L-PGDS deficiency in a genetic demyelinating mouse model (twicher mice harboring a homozygote for a nonsense point mutation of the galactosylceramidase) showed increases of apoptotic neurons and OLs [73]. Moreover, increased L-PGDS expression was reported in remyelinated lesions of MS brains [76], indicating an anti-apoptotic and pro-remyelinating property of the L-PGDS-PGD_2 pathway in OLs. No difference in EAE signs between PGD_2 receptor (DP_1; gene name, *Ptgdr1*)-KO mice vs. controls was reported [68], while DP_1-KO mice appeared to show a slightly severer disease course than controls. This requires a more careful evaluation of the EAE phenotype in DP_1-KO mice. Moreover, little is known about the roles of L-PGDS, hematopoietic-type PGD synthase (H-PGDS; gene name, *Ptgds2*), and another PGD_2 receptor (DP_2/$CRTH_2$; gene name, *Ptgdr2*) in EAE.

Although PGD_2 levels decreased in EAE SCs, one PGD_2 metabolite, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), was substantially increased in EAE SCs [37]. 15d-PGJ_2 binds selectively to one of three peroxisome proliferator-activated receptors, PPARγ (gene name, *Pparg*), which inhibits pro-inflammatory cytokine production [75]. Both PPARγ-hetero [76, 77] and PPARγ antagonist treated mice developed severe EAE [77]. Furthermore, prophylactic and therapeutic administration of 15d-PGJ_2 [78], as well as prophylactic treatment of a PPARγ agonist (troglitazone) [79], ameliorated EAE [78], indicating that 15d-PGJ_2 production in EAE SCs might be a defensive reaction as an innate response.

13.2.4 Lipoxygenases (LOs)

LOs (5-LO, 8-LO, and 12/15-LO; gene names, *Alox5*, *Alox8*, and *Alox12*, respectively) insert one molecule of molecular oxygen into the 5, 8, 12, and 15 positions of AA, to produce hydroperoxy eicosatetraenoic acids (HpETE) [80, 81]. 5-LO catalyzes the two-step conversion of AA to LTA_4 that are metabolized to LTs and HETEs by enzymatic and non-enzymatic reactions, respectively [80]. LTs elicit their biological actions by binding to their specific GPCRs [42]. In addition to LTs, LOs also produce specialized pro-resolving mediators (SPMs) including AA-derived lipoxins, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)-drived resolvins (RvDs and RvEs, respectively), and DHA-derived neuroprotectin (NPD_1) [82–84].

Gene expression profiling in MS lesions and EAE brains identified an up-regulation of the 5-LO gene [85]. 5-LO-KO mice on a 129 genetic background showed severe disease as compared to controls on the 129S1/SvImJ background [86]. 12/15-LO-KO mice on a mixed B6:129S2 background also showed severe disease as compared to controls on a C57BL/6 J background [86]. These severe phenotypes may be accounted for by a metabolic shift towards the COX-PG pathway in 5-LO-KO mice, and/or by a reduction of SPMs in 12/15-LO-KO mice. However, the mismatched genetic backgrounds between KO vs. controls make it difficult to conclude roles of these LOs. On the other hand, the FDA-approved anti-asthma drug, zileuton, which is an 5-LO selective inhibitor, effectively delayed EAE onset and ameliorated EAE [49], suggesting that LTs produced by 5-LO may worsen EAE.

13.2.5 Leukotrienes (LTs)

LTs are produced downstream of 5-LO. LTA_4 hydrolase (LTA_4H; gene name, *Lta4h*) metabolizes LTA_4 to LTB_4 [87]. Cysteinyl LTs (cys-LTs) are produced by LTC_4 synthase (LTC_4S; gene name, *Ltc4s*) that conjugates reduced glutathione to LTA_4, resulting in LTC_4 formation that is

metabolized to LTD_4 followed by LTE_4 [88, 89]. Levels of these 5-LO metabolites in naïve SCs were very low as compared to COX metabolites, which were further declined in EAE SCs [37]. Therefore, LTs may not play pivotal roles in EAE. However, mRNAs of the LTB_4 receptor 1 (BLT_1; gene name, *Ltb4r1*) and the cys-LT receptor 1 ($CysLT_1$; gene name, *Cysltr1*) were highly up-regulated in EAE SCs from disease onset through the acute phase [37, 39], implying accumulation of immune cells expressing these receptors into the CNS. The BLT_1 antagonist, CP-105,696, prevented EAE and suppressed eosinophil infiltration into the CNS in a dose-dependent manner [90]. We reported that BLT_1-KO mice showed delayed onset, less severe EAE, and reduced T_H1/T_H17 responses than controls [39]. Moreover, BLT_1-KO SCs from asymptomatic EAE mice showed no T cell, neutrophil, or macrophage infiltrations, whereas those cells were found in asymptomatic WT EAE controls [39]. These results suggest that BLT_1 is responsible for immune cell recruitment into the CNS in the early phase of the disease. EAE studies using KO mice of LTA_4H, LTC_4S, BLT_2 (gene name, *Ltb4r2*), $CysLT_{1,2,3}$ (gene name, *Cysltr1, Cysltr2, Cyltr3/Oxgr1/Gpr99*) are not available at this time.

13.3 Platelet-Activating Factor (PAF) in EAE and MS

PAF (1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent lipid mediator that activates immune cells and induces vascular hyperpermeability, hypotension, and bronchoconstriction via its specific GPCR, PAF receptor (PAFR; gene name, *Ptafr*) [91, 92]. PAF production through a remodeling pathway requires membrane phospholipid hydrolysis by PLA_2s that supply lyso-PAF (1-O-alkyl-sn-glycero-3-phosphocholine), which in turn metabolizes to PAF by acetyl-CoA:lyso-PAF acetyltransferase (LysoPAFAT; gene name, *Lpcat2*) [93]. PAF is rapidly degraded to lyso-PAF by PAF acetylhydrolase (PAF-AH; gene name, *Pafah1b1, Pafah1b2, Pafah1b3*) [94]. Although a clinical trial using ginkgolide B, a PAFR antagonist extracted from *Ginkgo biloba*, showed no efficacy for the treatment of acute exacerbations of MS [95], several lines of evidence support an involvement of PAF in MS/EAE to certain extent, including (1) elevated PAF levels in cerebrospinal fluids (CSF) and plasma of RRMS patients [96], (2) up-regulation of PAFR mRNA in chronic MS plaques [97], (3) a relationship between PAFR gene missense mutation vs. the susceptibility for MS [98], and (4) an efficacy of PAFR antagonist (BN52021) on EAE [99]. We found increased activity of PAF producing enzymes (PLA_2 and LysoPAFAT) and unchanged PAFAH activity in EAE SCs as compared to controls, resulting in a significant elevation of PAF levels [36, 38]. We also tested EAE in PAFR-KO mice that showed a lower incidence and less severe disease than controls [36]. PAF-PAFR signaling promoted cytokine and chemokine production in SCs before disease onset [36]. In addition, PAF accelerated phagocytotic activity and its associated TNF-α production in macrophages via PAFR [36]. Another group independently confirmed these results using the same PAFR-KO mice [100]. These results suggest a dual role of PAF-PAFR signaling in EAE development and progression.

13.4 Lysophospholipid (LP) Mediators in EAE and MS

LP is a derivative of a phospholipid in which one acyl chain is missing from the glycerol backbone combined with a phosphate head group, such as lysophosphatidic acid (LPA, 1 or 2-acyl-sn-glycero-3-phosphate), lysophosphatidylserine (LysoPS), lysophosphatidylinositol (LPI), and lysophosphatidylcholine (LPC) [101]. Since LIPID MAPS® classifies sphingosine 1-phosphate (S1P) in the sphingolipid group [102], it is technically not a lysophospholipid. However, S1P is included here based on the IUPAR (International Union of Basic and Clinical Pharmacology) GPCR classification [103]. Figure 13.2 summarizes the relationship between LPs and EAE/MS pathology.

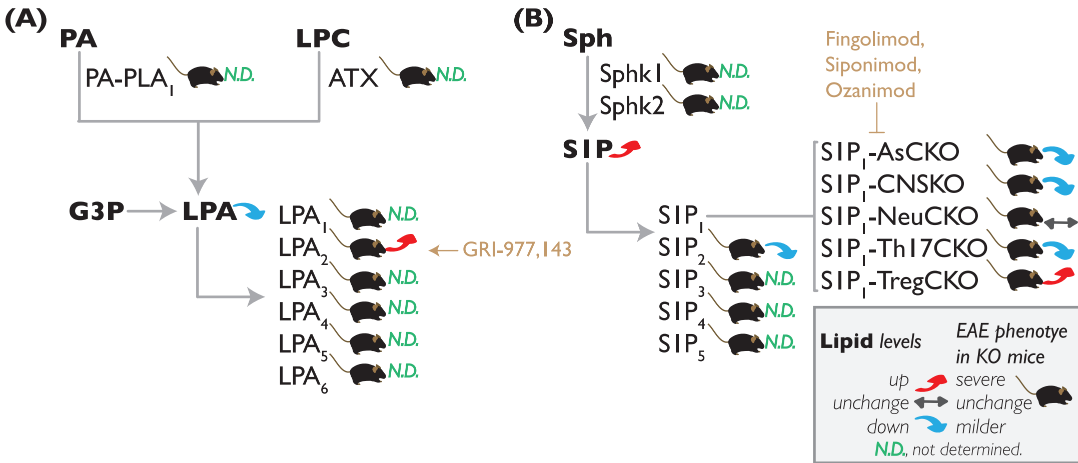

Fig. 13.2 EAE phenotypes in LP signaling-KO mice. (**a**) LPA pathway. (**b**) S1P pathway

13.4.1 Lysophosphatidic Acid (LPA)

LPA is produced by three distinct pathways: (1) *de novo* synthesis from glycerol-3-phosphate (G3P) by G3P acyltransferase (GPAT; gene names, *Gpam*, *Gpat2, Gpat3 and Gpat4*) [93, 104], (2) head group removal from LPs by autotaxin (ATX; gene name, *Enpp2*) [105, 106], and (3) hydrolysis of phosphatidic acid (PA) by PA-specific PLA_1 ($PA\text{-}PLA_1\alpha$; gene name, *Liph*) [105, 107]. LPA levels in blood (0.1 μM in plasma ~ 10 μM in serum) are of sufficiently high concentration to activate six cognate GPCRs ($LPA_{1\text{-}6}$; gene names, *Lpar1-6*) [101, 108–111], in which LPA_1 dissociation constants (K_d) for LPA approximated subnanomolar range [112]. Although LPA was isolated and identified from soy beans in 1978 [113], it has not been well-studied in MS and EAE until recently. In 2017, Schmitz et al. reported a meticulous investigation of the relationship between LPA and MS and EAE [114]. LPA concentrations quantified by LC-MS/MS were significantly reduced in the serum of MS patients, regardless of disease type, and EAE mice on an SJL/J background as compared to their controls [114]. Moreover, unsaturated LPA levels in serum were responsive to some DMTs (fingolimod and natalizumab), which were also observed in EAE mice on an SJL/J background [114]. In a spontaneous relapsing-remitting EAE model (T cell receptor (TCR) transgenic mice carrying TCR specific for $MOG_{92\text{-}106}$ in the context of $I\text{-}A^s$ in SJL/J genetic background; TCR^{1640} mice [115]), unsaturated LPAs in plasma and SCs were highly elevated during the first remission, followed by suppression in the second relapse [114]. Receptor expression profiles in spleen cells of EAE mice identified a suppression of LPA_2 protein expression on $CD4^+$ T cells and myeloid cells [114]. LPA_1 mRNA in spleen, LPA_2 and LPA_3 mRNA in white blood cells, and LPA_2, LPA_3, LPA_4 and LPA_5 mRNA in SCs were upregulated in EAE mice [114]. In LPA_2-KO mice on a C57BL6 x Sv129 mixed background, about 25% developed EAE with increased T cell and myeloid cell infiltration into the SCs, while their matching WT controls showed very mild disease course because Sv129 mice are resistant to EAE [114]. LPA_2 agonist, GRI-977,143, effectively ameliorated EAE in SJL mice [114]. Controversial, small cohort studies were also reported that LPA levels increased in serum of RRMS patients, while they were determined by ELISA or phosphate quantification [116, 117]. ATX activity was elevated in the CSF and serum of RRMS patients [118]. A naturally occurring LPA analog, cyclic phosphatidic acid (cPA, 1-acyl-sn-glycero-2,3-cyclic phosphate), is reported to activate LPA receptors [119]. Treatment using a metabolically stable analog of cPA (2ccPA) in both a prophylactic and therapeutic manner ameliorated EAE and reduced inflam-

mation in the SCs [120], which may be mediated via LPA_2. The EAE course of LPA_4-KO mice was equivalent to controls (unpublished observation). The EAE disease course in LPA_1, LPA_3, LPA_5, and LPA_6 deficiency, as well as LPA-producing and degrading enzyme deficiency, need to be studied to characterize their roles in EAE.

13.4.2 Sphingosine 1-phosphate (S1P)

S1P is produced from sphingosine (2-amino-4-octadecene-1,3-diol) by the action of sphingosine kinases (Sphks; gene name, *Sphk1*, *Sphk2*) [121]. S1P is enriched in blood plasma (low micromolar range), where S1P binds to albumin (gene name, *Alb*) and apolipoprotein M (ApoM; gene name, *Apom*) tethered to high-density lipoprotein (HDL) at a ratio of approximately three to seven [122]. S1P levels in lymph and tissues are lower than in blood (nanomolar range), which generates an S1P gradient between the circulatory system and tissues [122]. The biological significance of this gradient was recognized about 10 years after the discovery of fingolimod (trade name, Gilenya™), originally called FTY720.

In 1995, fingolimod was first synthesized from an immunosuppressive natural product, myriocin (ISP-I), which was isolated from culture broths of *Isaria sinclairii* [123–125]. Chiba et al. reported that fingolimod strikingly reduced the number of circulating lymphocytes [125], while the molecular basis of fingolimod activity remained unclear at that time. This immunosuppressive effect of fingolimod was clinically tested for preventing organ graft rejection, while phase III clinical trials did not support a superior efficacy of fingolimod in renal transplantation as compared to the prior standard treatment [126]. During 1998–2000, five S1P receptors ($S1P_{1-5}$: gene names, *S1pr1-5*) that belong to rhodopsin family GPCRs were identified [101, 108]. In 2002, the MOA of fingolimod was independently proposed by two pharmaceutical companies, Merck and Novartis [127, 128]. Both groups clearly demonstrated that phosphorylated fingolimod (fingolimod-P) was generated *in vivo* after administration of fingolimod. Moreover, fingolimod-P was identified as a high affinity agonist to four out of the five known S1P receptors ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$). Brinkman et al. also reported the efficacy of fingolimod in EAE by a prophylactic treatment paradigm [127]. In 2003, Sphk2 was reported to show a greater effect of fingolimod phosphorylation as compared to Sphk1 [129]. In 2004, Jason Cyster's group revised the MOA of fingolimod from agonism to functional antagonism based upon findings that (1) $S1P_1$ was required for lymphocyte egress from lymphoid organs to circulation, and (2) fingolimod treatment down-regulated $S1P_1$ cell surface expression [130, 131]. These studies provided a basis for the biological significance of the S1P gradient in lymphocyte trafficking between circulatory and lymph systems, which are supported by other studies using S1P-generating (Sphks) and -degrading (S1P lyase; gene name, *Sgpl1*) enzyme KO mice [132, 133]. Additional studies provided more evidence of $S1P_1$ involvement in MS and EAE. Proteomics analysis on MS brain tissue identified $S1P_1$ phosphorylation on a C-terminal serine residue that is crucial for receptor internalization [134]. A knock-in mouse harboring alanine mutations on $S1P_1$ C-terminal serine residues ($S1P_1$-S5A mice) [135] developed more severe EAE than controls with an enhanced T_H17 immunity. $S1P_1$ floxed mice ($S1P_1^{flox/flox}$) were used to study cell-specific roles of $S1P_1$ in EAE, since global $S1P_1$-KO mice were embryonically lethal due to impairment of vascular maturation [136]. T_H17-specific $S1P_1$-KO mice (IL-17A-Cre: $S1P_1^{flox/flox}$ mice) were completely resistant to EAE development [137]. On the other hand, T_{reg}-specific $S1P_1$-KO mice (Foxp3-Cre: $S1P_1^{flox/flox}$ mice) developed systemic autoimmunity, since T_{reg} cells were retained in lymphoid organs and reduced in peripheral tissues. Also, $S1P_1$ deletion, as well as fingolimod treatment, promoted to effector T_{reg} cell conversion from central T_{reg} cell. Tamoxifen-inducible T_{reg}-specific $S1P_1$-KO mice (Foxp3-CreER^{T2}: $S1P_1^{flox/flox}$ mice) developed severe EAE [137]. The immunological roles of ApoM-bound S1P were investigated using ApoM-KO mice, which revealed that ApoM-S1P suppresses

expansion of $Lin^{-}Sac\text{-}1^{+}cKit^{+}$ hematopoietic progenitor cells (LSKs) and common lymphoid progenitors (CLPs) through $S1P_1$ [138]. This immune stimulation led to exacerbation of EAE in mice lacking ApoM, while less severe EAE was observed in ApoM transgenic mice (driving with endogenous promoter). Although endothelium-specific $S1P_1$-KO mice (Cdh5-Cre-ER^{T2}: $S1P_1^{flox/flox}$ mice) showed pulmonary vascular leakage, EAE disease course in these mice were equivalent to their matching controls [138]. Collectively, $S1P_1$ signaling is responsible for homeostatic lymphocyte trafficking and lymphopoiesis, whose functional antagonism by fingolimod reduces circulating pathogenic T cells, resulting in EAE/MS amelioration.

Given the fact that fingolimod penetrated and accumulated in the CNS [139] where S1P receptors are abundant ($S1P_1$ in astrocytes and $S1P_5$ in oligodendrocytes) [36], direct CNS effects of fingolimod were expected. In 2011, Jerold Chun's group reported EAE amelioration and no fingolimod efficacy in CNS-specific $S1P_1$-KO mice (Nestin-Cre: $S1P_1^{flox/flox}$ mice) and astrocyte-specific $S1P_1$-KO mice (GFAP-Cre: $S1P_1^{flox/flox}$ mice), while neuron-specific $S1P_1$-KO mice (Synapsin-Cre: $S1P_1^{flox/flox}$ mice) did not show such phenotypes [140]. Most recently, we identified a novel functional astrocyte, *ieAstrocyte*, in EAE SCs, whose formation was significantly blocked by fingolimod administration and $S1P_1$ deficiency [41]. These results suggest that $S1P_1$ inhibition on astrocytes appears to be another MOA of fingolimod, which seems to be desirable for the treatment of progressive type of MS (PPMS and SPMS). Development of S1P receptor modulators in next generation compounds are designed to have a receptor subtype selectivity, such as the $S1P_1$/$S1P_5$ specificity in siponimod, ponesimod, and ozanimod, and $S1P_1$ specificity in KRP-203 and GSK2018682 [108]. Among these, the phase III clinical trial (EXPAND) demonstrated that siponimod reduced the risk of disability progression in SPMS patients [141], which might be mediated through direct CNS action. Several reviews have been published that summarize direct CNS effect of fingolimod and S1P receptor modulators [21, 25, 108, 140].

Knockout and antagonist studies targeting $S1P_3$, $S1P_4$, and $S1P_5$ have not been reported, but the EAE phenotype in $S1P_2$-KO mice was reported [142, 143]. Microarray analysis searching for sexually dimorphic genes in the CNS of naïve SJL mice identified higher $S1P_2$ expression levels in female vs. male [142]. $S1P_2$ expression increased in brain endothelial cells of EAE-induced female mice and female MS patients, which enhanced BBB permeability to Na-Fluorescein [142]. Therapeutic treatment with the $S1P_2$ antagonist (JTE-013) ameliorated EAE in SJL mice [142]. Furthermore, $S1P_2$-KO mice on a C57BL/6 × 129S mixed background showed milder EAE course than controls [142]. Using the same $S1P_2$-KO mice, another group also reported that $S1P_2$ deficiency ameliorated EAE, increased the number of oligodendrocytes in EAE lesions, and decreased fibrinogen extravasation and macrophage infiltration [143].

13.5 Conclusions

Collectively, this review provides an overview of lipid signaling pathways in MS and EAE. Each bioactive lipid, lipid metabolic enzyme, and lipid GPCR play key roles in regulating immune and neural functions. One of the most important contributions to the lipid biology field in neurodegenerative diseases was the development of fingolimod for the treatment of RRMS patients. Although most DMTs are immunomodulatory, fingolimod shows dual regulatory roles in the immune and nervous systems. Considering the neurodegenerative features of progressive forms of MS, not only immunomodulation, but also neuroprotection and neuroregeneration, should be explored. Unfortunately, we still have a lot to learn about the roles of lipid mediators including (EETs, HETEs, SPMs, cannabinoids, and lysophospholipids), lipid metabolic enzymes (PLA_2s, COX-1, PGDS, LysoPAFAT and other acyltransferases, diacylglycerol lipase, Sphks, and ATX), lipid GPCRs (DP_2, BLT_2, $CysLT_{1,\,2,\,3}$, cannabinoid receptors, fatty acid receptors, $LPA_{1,\,3,\,5,\,6}$, $S1P_{3\text{-}5}$, and other LP receptors), and more in EAE and MS. Among them, several proteins (L-PGDS,

LPA_1, $S1P_5$, and GPR17) that are highly expressed in oligodendrocytes and anti-inflammatory/pro-resolving lipid mediators (EETs and SPMs), are attractive neuroprotective targets to study in EAE/MS.

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References

1. Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC (2006) CNS immune privilege: hiding in plain sight. Immunol Rev 213:48–65
2. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523:337–341
3. Herisson F, Frodermann V, Courties G, Rohde D, Sun Y, Vandoorne K, Wojtkiewicz GR, Masson GS, Vinegoni C, Kim J, Kim DE, Weissleder R, Swirski FK, Moskowitz MA, Nahrendorf M (2018) Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. Nat Neurosci 21:1209–1217
4. Arima Y, Harada M, Kamimura D, Park JH, Kawano F, Yull FE, Kawamoto T, Iwakura Y, Betz UA, Marquez G, Blackwell TS, Ohira Y, Hirano T, Murakami M (2012) Regional neural activation defines a gateway for autoreactive T cells to cross the blood-brain barrier. Cell 148:447–457
5. Da Mesquita S, Louveau A, Vaccari A, Smirnov I, Cornelison RC, Kingsmore KM, Contarino C, Onengut-Gumuscu S, Farber E, Raper D, Viar KE, Powell RD, Baker W, Dabhi N, Bai R, Cao R, Hu S, Rich SS, Munson JM, Lopes MB, Overall CC, Acton ST, Kipnis J (2018) Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. Nature 560:185–191
6. Filiano AJ, Xu Y, Tustison NJ, Marsh RL, Baker W, Smirnov I, Overall CC, Gadani SP, Turner SD, Weng Z, Peerzade SN, Chen H, Lee KS, Scott MM, Beenhakker MP, Litvak V, Kipnis J (2016) Unexpected role of interferon-gamma in regulating neuronal connectivity and social behaviour. Nature 535:425–429
7. Hickey WF (1999) The pathology of multiple sclerosis: a historical perspective. J Neuroimmunol 98:37–44
8. Murray TJ (2009) The history of multiple sclerosis: the changing frame of the disease over the centuries. J Neurol Sci 277(Suppl 1):S3–S8
9. Young IR, Hall AS, Pallis CA, Legg NJ, Bydder GM, Steiner RE (1981) Nuclear magnetic resonance imaging of the brain in multiple sclerosis. Lancet 2:1063–1066
10. Mcdonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, Mcfarland HF, Paty DW, Polman CH, Reingold SC, Sandberg-Wollheim M, Sibley W, Thompson A, Van Den Noort S, Weinshenker BY, Wolinsky JS (2001) Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol 50:121–127
11. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K, Galetta SL, Hartung HP, Kappos L, Lublin FD, Marrie RA, Miller AE, Miller DH, Montalban X, Mowry EM, Sorensen PS, Tintore M, Traboulsee AL, Trojano M, Uitdehaag BMJ, Vukusic S, Waubant E, Weinshenker BG, Reingold SC, Cohen JA (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162–173
12. Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33:1444–1452
13. Kurtzke JF (2015) On the origin of EDSS. Mult Scler Relat Disord 4:95–103
14. Medaer R (1979) Does the history of multiple sclerosis go back as far as the 14th century? Acta Neurol Scand 60:189–192
15. Leray E, Moreau T, Fromont A, Edan G (2016) Epidemiology of multiple sclerosis. Rev Neurol (Paris) 172:3–13
16. Lancet T (2018) End of the road for daclizumab in multiple sclerosis. Lancet 391:1000
17. Derfuss T, Kuhle J, Lindberg R, Kappos L (2013) Natalizumab therapy for multiple sclerosis. Semin Neurol 33:26–36
18. Willis MD, Robertson NP (2016) Alemtuzumab for multiple sclerosis. Curr Neurol Neurosci Rep 16:84
19. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, Lublin F, Montalban X, Rammohan KW, Selmaj K, Traboulsee A, Wolinsky JS, Arnold DL, Klingelschmitt G, Masterman D, Fontoura P, Belachew S, Chin P, Mairon N, Garren H, Kappos L, Opera I, Investigators OIC (2017) Ocrelizumab versus interferon Beta-1a in relapsing multiple sclerosis. N Engl J Med 376:221–234

20. Jakimovski D, Kolb C, Ramanathan M, Zivadinov R, Weinstock-Guttman B (2018) Interferon beta for multiple sclerosis. Cold Spring Harb Perspect Med 8:a032003
21. Chun J, Brinkmann V (2011) A mechanistically novel, first oral therapy for multiple sclerosis: the development of fingolimod (FTY720, Gilenya). Discov Med 12:213–228
22. Cohen JA, Chun J (2011) Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis. Ann Neurol 69:759–777
23. Kihara Y, Groves A, Rivera RR, Chun J (2015a) Dimethyl fumarate inhibits integrin alpha4 expression in multiple sclerosis models. Ann Clin Transl Neurol 2:978–983
24. Von Glehn F, Dias-Carneiro RPC, Moraes AS, Farias AS, Silva V, Oliveira FTM, Silva C, De Carvalho F, Rahal E, Baecher-Allan C, Santos LMB (2018) Dimethyl fumarate downregulates the immune response through the HCA2/GPR109A pathway: implications for the treatment of multiple sclerosis. Mult Scler Relat Disord 23:46–50
25. Groves A, Kihara Y, Chun J (2013) Fingolimod: direct CNS effects of sphingosine 1-phosphate (S1P) receptor modulation and implications in multiple sclerosis therapy. J Neurol Sci 328:9–18
26. Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, Chao CC, Patel B, Yan R, Blain M, Alvarez JI, Kebir H, Anandasabapathy N, Izquierdo G, Jung S, Obholzer N, Pochet N, Clish CB, Prinz M, Prat A, Antel J, Quintana FJ (2016) Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. Nat Med 22:586–597
27. Al-Jaderi Z, Maghazachi AA (2016) Utilization of Dimethyl Fumarate and related molecules for treatment of multiple sclerosis, cancer, and other diseases. Front Immunol 7:278
28. Rivers TM, Sprunt DH, Berry GP (1933) Observations on Attempts to Produce Acute Disseminated Encephalomyelitis in Monkeys. J Exp Med 58:39–53
29. Paterson PY (1960) Transfer of allergic encephalomyelitis in rats by means of lymph node cells. J Exp Med 111:119–136
30. Ben-Nun A, Wekerle H, Cohen IR (1981) Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein. Nature 292:60–61
31. Zamvil SS, Mitchell DJ, Moore AC, Kitamura K, Steinman L, Rothbard JB (1986) T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. Nature 324:258–260
32. Rangachari M, Kuchroo VK (2013) Using EAE to better understand principles of immune function and autoimmune pathology. J Autoimmun 45:31–39
33. Windhagen A, Nicholson LB, Weiner HL, Kuchroo VK, Hafler DA (1996) Role of Th1 and Th2 cells in neurologic disorders. Chem Immunol 63:171–186
34. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, Mcclanahan T, Kastelein RA, Cua DJ (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 201:233–240
35. Yu CY, Whitacre CC (2004) Sex, MHC and complement C4 in autoimmune diseases. Trends Immunol 25:694–699
36. Kihara Y, Ishii S, Kita Y, Toda A, Shimada A, Shimizu T (2005) Dual phase regulation of experimental allergic encephalomyelitis by platelet-activating factor. J Exp Med 202:853–863
37. Kihara Y, Matsushita T, Kita Y, Uematsu S, Akira S, Kira J, Ishii S, Shimizu T (2009) Targeted lipidomics reveals mPGES-1-PGE2 as a therapeutic target for multiple sclerosis. Proc Natl Acad Sci U S A 106:21807–21812
38. Kihara Y, Yanagida K, Masago K, Kita Y, Hishikawa D, Shindou H, Ishii S, Shimizu T (2008) Platelet-activating factor production in the spinal cord of experimental allergic encephalomyelitis mice via the group IVA cytosolic phospholipase A2-lyso-PAFAT axis. J Immunol 181:5008–5014
39. Kihara Y, Yokomizo T, Kunita A, Morishita Y, Fukayama M, Ishii S, Shimizu T (2010) The leukotriene B4 receptor, BLT1, is required for the induction of experimental autoimmune encephalomyelitis. Biochem Biophys Res Commun 394:673–678
40. Terry RL, Ifergan I, Miller SD (2016) Experimental autoimmune encephalomyelitis in Mice. Methods Mol Biol 1304:145–160
41. Groves A, Kihara Y, Jonnalagadda D, Rivera R, Kennedy G, Mayford M, Chun J (2018) A functionally defined in vivo astrocyte population identified by c-Fos activation in a mouse model of multiple sclerosis modulated by S1P signaling: immediate-early Astrocytes (ieAstrocytes). eNeuro 5
42. Shimizu T (2009) Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. Annu Rev Pharmacol Toxicol 49:123–150
43. Kita Y, Ohto T, Uozumi N, Shimizu T (2006) Biochemical properties and pathophysiological roles of cytosolic phospholipase A2s. Biochim Biophys Acta 1761:1317–1322
44. Kalyvas A, David S (2004) Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. Neuron 41:323–335
45. Cunningham TJ, Yao L, Oetinger M, Cort L, Blankenhorn EP, Greenstein JI (2006) Secreted phospholipase A2 activity in experimental autoimmune encephalomyelitis and multiple sclerosis. J Neuroinflammation 3:26
46. Uozumi N, Kume K, Nagase T, Nakatani N, Ishii S, Tashiro F, Komagata Y, Maki K, Ikuta K, Ouchi Y, Miyazaki J, Shimizu T (1997) Role of cytosolic phospholipase A2 in allergic response and parturition. Nature 390:618–622
47. Marusic S, Leach MW, Pelker JW, Azoitei ML, Uozumi N, Cui J, Shen MW, Declercq CM,

Miyashiro JS, Carito BA, Thakker P, Simmons DL, Leonard JP, Shimizu T, Clark JD (2005) Cytosolic phospholipase A2 alpha-deficient mice are resistant to experimental autoimmune encephalomyelitis. J Exp Med 202:841–851
48. Kalyvas A, Baskakis C, Magrioti V, Constantinou-Kokotou V, Stephens D, Lopez-Vales R, Lu JQ, Yong VW, Dennis EA, Kokotos G, David S (2009) Differing roles for members of the phospholipase A2 superfamily in experimental autoimmune encephalomyelitis. Brain 132:1221–1235
49. Marusic S, Thakker P, Pelker JW, Stedman NL, Lee KL, Mckew JC, Han L, Xu X, Wolf SF, Borey AJ, Cui J, Shen MW, Donahue F, Hassan-Zahraee M, Leach MW, Shimizu T, Clark JD (2008) Blockade of cytosolic phospholipase A2 alpha prevents experimental autoimmune encephalomyelitis and diminishes development of Th1 and Th17 responses. J Neuroimmunol 204:29–37
50. Thakker P, Marusic S, Stedman NL, Lee KL, Mckew JC, Wood A, Goldman SJ, Leach MW, Collins M, Kuchroo VK, Wolf SF, Clark JD, Hassan-Zahraee M (2011) Cytosolic phospholipase A2alpha blockade abrogates disease during the tissue-damage effector phase of experimental autoimmune encephalomyelitis by its action on APCs. J Immunol 187:1986–1997
51. Smith WL, Urade Y, Jakobsson PJ (2011) Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chem Rev 111:5821–5865
52. Vane JR, Botting RM (2003) The mechanism of action of aspirin. Thromb Res 110:255–258
53. Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231:232–235
54. Kolb LC, Karlson AG, Sayre GP (1952) Prevention of experimental allergic encephalomyelitis by various agents. Trans Am Neurol Assoc 56:117–121
55. Tsau S, Emerson MR, Lynch SG, Levine SM (2015) Aspirin and multiple sclerosis. BMC Med 13:153
56. Good RA, Campbell B, Good TA (1949) Prophylactic and therapeutic effect of para-aminobenzoic acid and sodium salicylate on experimental allergic encephalomyelitis. Proc Soc Exp Biol Med 72:341–347
57. Miller H, Newell DJ, Ridley A (1961) Multiple sclerosis. Trials of maintenance treatment with prednisolone and soluble aspirin. Lancet 1:127–129
58. Miller HG, Foster JB, Newell DJ, Barwick DD, Brewis RA (1963) Multiple sclerosis: therapeutic trials of chloroquine, soluble aspirin, and gamma-globulin. Br Med J 2:1436–1439
59. Miyamoto K, Miyake S, Mizuno M, Oka N, Kusunoki S, Yamamura T (2006) Selective COX-2 inhibitor celecoxib prevents experimental autoimmune encephalomyelitis through COX-2-independent pathway. Brain 129:1984–1992
60. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C (2005) Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med 353:2373–2383
61. Mondal S, Jana M, Dasarathi S, Roy A, Pahan K (2018) Aspirin ameliorates experimental autoimmune encephalomyelitis through interleukin-11-mediated protection of regulatory T cells. Sci Signal 11:eaar8278
62. Kihara Y, Gupta S, Maurya MR, Armando A, Shah I, Quehenberger O, Glass CK, Dennis EA, Subramaniam S (2014a) Modeling of eicosanoid fluxes reveals functional coupling between cyclooxygenases and terminal synthases. Biophys J 106:966–975
63. Murakami M, Kambe-Ohkura T, Kudo I (2002) Functional coupling between phospholipase A2S and cyclooxygenases in immediate and delayed prostanoid biosynthetic pathways. Adv Exp Med Biol 507:15–19
64. Ueno N, Takegoshi Y, Kamei D, Kudo I, Murakami M (2005) Coupling between cyclooxygenases and terminal prostanoid synthases. Biochem Biophys Res Commun 338:70–76
65. Takemiya T, Kawakami M, Takeuchi C (2018) Endothelial microsomal prostaglandin E Synthetase-1 Upregulates vascularity and endothelial interleukin-1beta in deteriorative progression of experimental autoimmune encephalomyelitis. Int J Mol Sci 19:3647
66. Takemiya T, Takeuchi C, Kawakami M (2017) Microsomal prostaglandin E Synthase-1 facilitates an intercellular interaction between CD4(+) T cells through IL-1beta Autocrine function in experimental autoimmune encephalomyelitis. Int J Mol Sci 18:2758
67. Takeuchi C, Matsumoto Y, Kohyama K, Uematsu S, Akira S, Yamagata K, Takemiya T (2013) Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis. Neurochem Int 62:271–280
68. Esaki Y, Li Y, Sakata D, Yao C, Segi-Nishida E, Matsuoka T, Fukuda K, Narumiya S (2010) Dual roles of PGE2-EP4 signaling in mouse experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 107:12233–12238
69. Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, Sugimoto Y, Narumiya S (2009) Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. Nat Med 15:633–640
70. Zhou W, Dowell DR, Huckabee MM, Newcomb DC, Boswell MG, Goleniewska K, Lotz MT, Toki S, Yin H, Yao S, Natarajan C, Wu P, Sriram S, Breyer RM, Fitzgerald GA, Peebles RS Jr (2012) Prostaglandin I2 signaling drives Th17 differentiation and exacerbates experimental autoimmune encephalomyelitis. PLoS One 7:e33518
71. Muramatsu R, Takahashi C, Miyake S, Fujimura H, Mochizuki H, Yamashita T (2012) Angiogenesis induced by CNS inflammation promotes neuronal remodeling through vessel-derived prostacyclin. Nat Med 18:1658–1664
72. Narumiya S, Ogorochi T, Nakao K, Hayaishi O (1982) Prostaglandin D2 in rat brain, spinal cord and

pituitary: basal level and regional distribution. Life Sci 31:2093–2103
73. Huang YC, Lyu RK, Tseng MY, Chang HS, Hsu WC, Kuo HC, Chu CC, Wu YR, Ro LS, Huang CC, Chen CM (2009) Decreased intrathecal synthesis of prostaglandin D2 synthase in the cerebrospinal fluid of patients with acute inflammatory demyelinating polyneuropathy. J Neuroimmunol 206:100–105
74. Kagitani-Shimono K, Mohri I, Oda H, Ozono K, Suzuki K, Urade Y, Taniike M (2006) Lipocalin-type prostaglandin D synthase (beta-trace) is upregulated in the alphaB-crystallin-positive oligodendrocytes and astrocytes in the chronic multiple sclerosis. Neuropathol Appl Neurobiol 32:64–73
75. Cai W, Yang T, Liu H, Han L, Zhang K, Hu X, Zhang X, Yin KJ, Gao Y, Bennett MVL, Leak RK, Chen J (2018) Peroxisome proliferator-activated receptor gamma (PPARgamma): a master gatekeeper in CNS injury and repair. Prog Neurobiol 163–164:27–58
76. Natarajan C, Muthian G, Barak Y, Evans RM, Bright JJ (2003) Peroxisome proliferator-activated receptor-gamma-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis. J Immunol 171:5743–5750
77. Raikwar HP, Muthian G, Rajasingh J, Johnson C, Bright JJ (2005) PPARgamma antagonists exacerbate neural antigen-specific Th1 response and experimental allergic encephalomyelitis. J Neuroimmunol 167:99–107
78. Diab A, Deng C, Smith JD, Hussain RZ, Phanavanh B, Lovett-Racke AE, Drew PD, Racke MK (2002) Peroxisome proliferator-activated receptor-gamma agonist 15-deoxy-Delta(12,14)-prostaglandin J(2) ameliorates experimental autoimmune encephalomyelitis. J Immunol 168:2508–2515
79. Niino M, Iwabuchi K, Kikuchi S, Ato M, Morohashi T, Ogata A, Tashiro K, Onoe K (2001) Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-gamma. J Neuroimmunol 116:40–48
80. Radmark O, Werz O, Steinhilber D, Samuelsson B (2015) 5-Lipoxygenase, a key enzyme for leukotriene biosynthesis in health and disease. Biochim Biophys Acta 1851:331–339
81. Singh NK, Rao GN (2018) Emerging role of 12/15-Lipoxygenase (ALOX15) in human pathologies. Prog Lipid Res 73:28–45
82. Serhan CN (2017) Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. FASEB J 31:1273–1288
83. Serhan CN, Chiang N, Dalli J (2018) New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. Mol Asp Med 64:1–17
84. Serhan CN, Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J Clin Invest 128:2657–2669
85. Whitney LW, Ludwin SK, Mcfarland HF, Biddison WE (2001) Microarray analysis of gene expression in multiple sclerosis and EAE identifies 5-lipoxygenase as a component of inflammatory lesions. J Neuroimmunol 121:40–48
86. Emerson MR, Levine SM (2004) Experimental allergic encephalomyelitis is exacerbated in mice deficient for 12/15-lipoxygenase or 5-lipoxygenase. Brain Res 1021:140–145
87. Yokomizo T, Nakamura M, Shimizu T (2018) Leukotriene receptors as potential therapeutic targets. J Clin Invest 128:2691–2701
88. Kanaoka Y, Boyce JA (2014) Cysteinyl leukotrienes and their receptors; emerging concepts. Allergy Asthma Immunol Res 6:288–295
89. NationalMSSociety.org (2018) National Multiple Sclerosis Society [Online]. Available: https://www.nationalmssociety.org/. Accessed 2018
90. Gladue RP, Carroll LA, Milici AJ, Scampoli DN, Stukenbrok HA, Pettipher ER, Salter ED, Contillo L, Showell HJ (1996) Inhibition of leukotriene B4-receptor interaction suppresses eosinophil infiltration and disease pathology in a murine model of experimental allergic encephalomyelitis. J Exp Med 183:1893–1898
91. Honda Z, Ishii S, Shimizu T (2002) Platelet-activating factor receptor. J Biochem 131:773–779
92. Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, Okado H, Toh H, Ito K, Miyamoto T, Et AL (1991) Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349:342–346
93. Shindou H, Shimizu T (2009) Acyl-CoA:lysophospholipid acyltransferases. J Biol Chem 284:1–5
94. Kono N, Arai H (2019) Platelet-activating factor acetylhydrolases: an overview and update. Biochim Biophys Acta Mol Cell Biol Lipids 1864(6):922–931
95. Brochet B, Guinot P, Orgogozo JM, Confavreux C, Rumbach L, Lavergne V (1995) Double blind placebo controlled multicentre study of ginkgolide B in treatment of acute exacerbations of multiple sclerosis. The Ginkgolide Study Group in multiple sclerosis. J Neurol Neurosurg Psychiatry 58:360–362
96. Callea L, Arese M, Orlandini A, Bargnani C, Priori A, Bussolino F (1999) Platelet activating factor is elevated in cerebral spinal fluid and plasma of patients with relapsing-remitting multiple sclerosis. J Neuroimmunol 94:212–221
97. Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L (2002) Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med 8:500–508
98. Osoegawa M, Miyagishi R, Ochi H, Nakamura I, Niino M, Kikuchi S, Murai H, Fukazawa T, Minohara M, Tashiro K, Kira J (2005) Platelet-activating

factor receptor gene polymorphism in Japanese patients with multiple sclerosis. J Neuroimmunol 161:195–198
99. Bellizzi MJ, Geathers JS, Allan KC, Gelbard HA (2016) Platelet-activating factor receptors mediate excitatory postsynaptic hippocampal injury in experimental autoimmune Encephalomyelitis. J Neurosci 36:1336–1346
100. Rodrigues DH, Lacerda-Queiroz N, De Miranda AS, Fagundes CT, Campos RD, Arantes RE, Vilela Mde C, Rachid MA, Teixeira MM, Teixeira AL (2011) Absence of PAF receptor alters cellular infiltrate but not rolling and adhesion of leukocytes in experimental autoimmune encephalomyelitis. Brain Res 1385:298–306
101. Kihara Y, Maceyka M, Spiegel S, Chun J (2014b) Lysophospholipid receptor nomenclature review: IUPHAR review 8. Br J Pharmacol 171:3575–3594
102. Fahy E, Sud M, Cotter D, Subramaniam S (2007) LIPID MAPS online tools for lipid research. Nucleic Acids Res 35:W606–W612
103. Alexander SP, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD, Pawson AJ, Sharman JL, Southan C, Buneman OP, Cidlowski JA, Christopoulos A, Davenport AP, Fabbro D, Spedding M, Striessnig J, Davies JA, Collaborators C (2017) The concise guide to pharmacology 2017/18: overview. Br J Pharmacol 174(Suppl 1):S1–S16
104. Shindou H, Hishikawa D, Harayama T, Yuki K, Shimizu T (2009) Recent progress on acyl CoA: lysophospholipid acyltransferase research. J Lipid Res 50(Suppl):S46–S51
105. Aikawa S, Hashimoto T, Kano K, Aoki J (2015) Lysophosphatidic acid as a lipid mediator with multiple biological actions. J Biochem 157:81–89
106. Nishimasu H, Ishitani R, Aoki J, Nureki O (2012) A 3D view of autotaxin. Trends Pharmacol Sci 33:138–145
107. Aoki J, Nagai Y, Hosono H, Inoue K, Arai H (2002) Structure and function of phosphatidylserine-specific phospholipase A1. Biochim Biophys Acta 1582:26–32
108. Kihara Y, Mizuno H, Chun J (2015b) Lysophospholipid receptors in drug discovery. Exp Cell Res 333:171–177
109. Sheng X, Yung YC, Chen A, Chun J (2015) Lysophosphatidic acid signalling in development. Development 142:1390–1395
110. Yung YC, Stoddard NC, Chun J (2014) LPA receptor signaling: pharmacology, physiology, and pathophysiology. J Lipid Res 55:1192–1214
111. Yung YC, Stoddard NC, Mirendil H, Chun J (2015) Lysophosphatidic acid signaling in the nervous system. Neuron 85:669–682
112. Mizuno H, Kihara Y, Kussrow A, Chen A, Ray M, Rivera R, Bornhop D, Chun J (2018) Lysophospholipid G protein-coupled receptor binding parameters as determined by backscattering interferometry (BSI). J Lipid Res 60:212–217
113. Tokumura A, Fukuzawa K, Akamatsu Y, Yamada S, Suzuki T, Tsukatani H (1978) Identification of vasopressor phospholipid in crude soybean lecithin. Lipids 13:468–472
114. Schmitz K, Brunkhorst R, De Bruin N, Mayer CA, Haussler A, Ferreiros N, Schiffmann S, Parnham MJ, Tunaru S, Chun J, Offermanns S, Foerch C, Scholich K, Vogt J, Wicker S, Lotsch J, Geisslinger G, Tegeder I (2017) Dysregulation of lysophosphatidic acids in multiple sclerosis and autoimmune encephalomyelitis. Acta Neuropathol Commun 5:42
115. Pollinger B, Krishnamoorthy G, Berer K, Lassmann H, Bosl MR, Dunn R, Domingues HS, Holz A, Kurschus FC, Wekerle H (2009) Spontaneous relapsing-remitting EAE in the SJL/J mouse: MOG-reactive transgenic T cells recruit endogenous MOG-specific B cells. J Exp Med 206:1303–1316
116. Balood M, Zahednasab H, Siroos B, Mesbah-Namin SA, Torbati S, Harirchian MH (2014) Elevated serum levels of lysophosphatidic acid in patients with multiple sclerosis. Hum Immunol 75:411–413
117. Jiang D, Ju W, Wu X, Zhan X (2018) Elevated lysophosphatidic acid levels in the serum and cerebrospinal fluid in patients with multiple sclerosis: therapeutic response and clinical implication. Neurol Res 40:335–339
118. Zahednasab H, Balood M, Harirchian MH, Mesbah-Namin SA, Rahimian N, Siroos B (2014) Increased autotaxin activity in multiple sclerosis. J Neuroimmunol 273:120–123
119. Fujiwara Y (2008) Cyclic phosphatidic acid - a unique bioactive phospholipid. Biochim Biophys Acta 1781:519–524
120. Yamamoto S, Yamashina K, Ishikawa M, Gotoh M, Yagishita S, Iwasa K, Maruyama K, Murakami-Murofushi K, Yoshikawa K (2017) Protective and therapeutic role of 2-carba-cyclic phosphatidic acid in demyelinating disease. J Neuroinflammation 14:142
121. Maceyka M, Harikumar KB, Milstien S, Spiegel S (2012) Sphingosine-1-phosphate signaling and its role in disease. Trends Cell Biol 22:50–60
122. Proia RL, Hla T (2015) Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. J Clin Invest 125:1379–1387
123. Chiba K (2005) FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 108:308–319
124. Chiba K, Adachi K (2012) Discovery of fingolimod, the sphingosine 1-phosphate receptor modulator and its application for the therapy of multiple sclerosis. Future Med Chem 4:771–781
125. Chiba K, Hoshino Y, Suzuki C, Masubuchi Y, Yanagawa Y, Ohtsuki M, Sasaki S, Fujita T (1996) FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rats. Transplant Proc 28:1056–1059

126. Mansoor M, Melendez AJ (2008) Recent trials for FTY720 (fingolimod): a new generation of immunomodulators structurally similar to sphingosine. Rev Recent Clin Trials 3:62–69
127. Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457
128. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 296:346–349
129. Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel S (2003) The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 554:189–193
130. Lo CG, Xu Y, Proia RL, Cyster JG (2005) Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. J Exp Med 201:291–301
131. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 427:355–360
132. Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW, Huang Y, Cyster JG, Coughlin SR (2007) Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. Science 316:295–298
133. Schwab SR, Pereira JP, Matloubian M, Xu Y, Huang Y, Cyster JG (2005) Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. Science 309:1735–1739
134. Garris CS, Wu L, Acharya S, Arac A, Blaho VA, Huang Y, Moon BS, Axtell RC, Ho PP, Steinberg GK, Lewis DB, Sobel RA, Han DK, Steinman L, Snyder MP, Hla T, Han MH (2013) Defective sphingosine 1-phosphate receptor 1 (S1P1) phosphorylation exacerbates TH17-mediated autoimmune neuroinflammation. Nat Immunol 14:1166–1172
135. Thangada S, Khanna KM, Blaho VA, Oo ML, Im DS, Guo C, Lefrancois L, Hla T (2010) Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines lymphocyte egress kinetics. J Exp Med 207:1475–1483
136. Liu Y, Wada R, Yamashita T, Mi Y, Deng CX, Hobson JP, Rosenfeldt HM, Nava VE, Chae SS, Lee MJ, Liu CH, Hla T, Spiegel S, Proia RL (2000) Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. J Clin Invest 106:951–961
137. Eken A, Duhen R, Singh AK, Fry M, Buckner JH, Kita M, Bettelli E, Oukka M (2017) S1P1 deletion differentially affects TH17 and Regulatory T cells. Sci Rep 7:12905
138. Blaho VA, Galvani S, Engelbrecht E, Liu C, Swendeman SL, Kono M, Proia RL, Steinman L, Han MH, Hla T (2015) HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. Nature 523:342–346
139. Foster CA, Howard LM, Schweitzer A, Persohn E, Hiestand PC, Balatoni B, Reuschel R, Beerli C, Schwartz M, Billich A (2007) Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: consequences for mode of action in multiple sclerosis. J Pharmacol Exp Ther 323:469–475
140. Choi JW, Gardell SE, Herr DR, Rivera R, Lee CW, Noguchi K, Teo ST, Yung YC, Lu M, Kennedy G, Chun J (2011) FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. Proc Natl Acad Sci U S A 108:751–756
141. Kappos L, Bar-Or A, Cree BAC, Fox RJ, Giovannoni G, Gold R, Vermersch P, Arnold DL, Arnould S, Scherz T, Wolf C, Wallstrom E, Dahlke F, Investigators EC (2018) Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. Lancet 391:1263–1273
142. Cruz-Orengo L, Daniels BP, Dorsey D, Basak SA, Grajales-Reyes JG, Mccandless EE, Piccio L, Schmidt RE, Cross AH, Crosby SD, Klein RS (2014) Enhanced sphingosine-1-phosphate receptor 2 expression underlies female CNS autoimmunity susceptibility. J Clin Invest 124:2571–2584
143. Seyedsadr MS, Weinmann O, Amorim A, Ineichen BV, Egger M, Mirnajafi-Zadeh J, Becher B, Javan M, Schwab ME (2018) Inactivation of sphingosine-1-phosphate receptor 2 (S1PR2) decreases demyelination and enhances remyelination in animal models of multiple sclerosis. Neurobiol Dis 124:189–201

14 Role of Bioactive Sphingolipids in Inflammation and Eye Diseases

Koushik Mondal and Nawajes Mandal

Abstract

Inflammation is a common underlying factor in a diversity of ocular diseases, ranging from macular degeneration, autoimmune uveitis, glaucoma, diabetic retinopathy and microbial infection. In addition to the variety of known cellular mediators of inflammation, such as cytokines, chemokines and lipid mediators, there is now considerable evidence that sphingolipid metabolites also play a central role in the regulation of inflammatory pathways. Various sphingolipid metabolites, such as ceramide (Cer), ceramide-1-phosphate (C1P), sphingosine-1-phosphate (S1P), and lactosylceramide (LacCer) can contribute to ocular inflammatory diseases through multiple pathways. For example, inflammation generates Cer from sphingomyelins (SM) in the plasma membrane, which induces death receptor ligand formation and leads to apoptosis of retinal pigment epithelial (RPE) and photoreceptor cells. Inflammatory stress by reactive oxygen species leads to LacCer accumulation and S1P secretion and induces proliferation of retinal endothelial cells and eventual formation of new vessels. In sphingolipid/lysosomal storage disorders, sphingolipid metabolites accumulate in lysosomes and can cause ocular disorders that have an inflammatory etiology. Sphingolipid metabolites activate complement factors in the immune-response mediated pathogenesis of macular degeneration. These examples highlight the integral association between sphingolipids and inflammation in ocular diseases.

Keywords

Inflammation · Sphingolipid · Ceramide · Ceramide-1-phosphate · Sphingosine-1-phosphate · Lactosylceramide · AMD · Uveitis · Diabetic retinopathy · Glaucoma

14.1 Introduction

Inflammation is a defensive mechanism of a host organism against infectious agents and injury. Inflammatory mechanisms represent a network of complex processes requiring the involvement of different metabolic and signaling pathways to

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resolve damage to tissue or to fight against infection. However, inflammation may also be detrimental if it progresses out of control. Host organisms have evolved different signaling mechanisms to respond appropriately against a range of threats by utilizing specialized immune cells such as neutrophils, resident and recruited macrophages, dendritic cells, and lymphocytes [57]. Host immune machinery is activated against microbial pathogens and recognizes molecular structures found in pathogens, known as Pathogen-Associated Molecular Patterns (PAMPs) [80], whereas signals released from stressed and damaged host cells are known as Damage Associated Molecular Patterns (DAMPs) [175]. Both PAMPs and DAMPs are recognized by molecular structures on immune cells, known as Pattern recognition receptors (PRR). The Toll-like receptors (TLR) are important class of PRR [82] that recognize bacterial (pathogen) membrane lipopolysaccharides and viral RNA as well as endogenous molecules that are secreted from damaged or dying cells [126]. After activation, TLRs recruit downstream signal adaptor proteins, including Myeloid differentiation primary response 88 (MyD88) and TIR-domain containing adapter inducing interferon β (TRIF), which leads to activation of kinases, such as Inhibitor of kappa B (IkB) and Mitogen activated protein kinase (MAPK), downstream transcription factors, such as Nuclear factor kappa B (NF-kB), Activator protein-1 (AP-1), and interferon regulatory factor family proteins. These factors can stimulate transcription of several amplifiers and effectors [155]. Different types of cytokines, such as Tumor necrosis factor (TNF)-α, Interleukin (IL)-1β and IL-6, and chemokines, e.g., C-C motif chemokine ligand 2 (CCL2), C-X-C motif chemokine ligand 1 (CXCL1), and C-X-C motif chemokine ligand 10 (CXCL10) act as amplifiers and effector molecules in this mechanism. Another member of the innate immune sensor is the NOD-like receptor (NLR), which is a component of the inflammasome multiprotein complex [125]. The third type of pattern recognition receptor is Retinoic acid inducible gene 1 (RIG1) [87]. Cooperative interactions of inflammasomes and complement cascades play significant roles in immune surveillance and inflammatory processes [10]. During complement-mediated targeted cell lysis, there is an initiation of strong opsonization of the foreign pathogen or apoptotic cell/cellular compartment, followed by induction of proinflammatory signaling by anaphylatoxins, which lead to recruitment of macrophages and eventually phagocytosis of the pathogen through the formation of the membrane attack complex (MAC) [129]. Thus, the inflammasome-complement pathway eliminates the pathogen and clears and eliminates potential mediators of damage and injury. Acute and chronic inflammation can influence vascular permeability of the cell. Activation of proinflammatory cytokines up-regulates selectins (e.g., P-selectin) and integrin ligands, e.g., Vascular cell adhesion molecule 1 (VCAM-1) and Intercellular adhesion molecule 1 (ICAM-1), on the lumen of endothelial cells. These are sensed by selectin ligands, e.g., P-selectin glycoprotein ligand 1 (PSGL1) and integrins, e.g., Lymphocyte function-associated antigen 1 (LFA1) on the surface of leukocytes, and promote loosening of tight junctions between endothelial cells while permitting transfer of solutes to peripheral tissues and leukocyte infiltration through the blood-brain barrier [127]. Inflammasome activation can also regulate synthesis of various eicosanoids, such as prostaglandins (PGs), thromboxane, hydroxyeicosatetraenoic acid (HETEs) and leukotrienes [128]. In addition to inflammasome-mediated canonical activation of caspase-1-dependent maturation of proinflammatory cytokines IL-1β and IL-18, caspase-1 independently activates cytosolic phospholipase A2 (cPLA2) that stimulates eicosanoid synthesis. In this mechanism, there is formation of a membrane pore, which drives rapid Ca^{2+} influx. The influx of Ca^{2+} then activates cPLA2 and generates arachidonic acid (AA) from membrane phospholipids. This arachidonic acid is further converted to prostaglandins and thromboxanes by cyclooxygenases-1 (COX-1) and COX-2, and leukotrienes and HETEs are converted by lipoxygenases [163]. The generation of eicosanoids is responsible for increasing vascular permeability and leucocyte recruitment during diverse homeostatic and path-

ological processes [30]. Thus, during injury or disease, the immune cells become reactive and their PRRs are activated, which leads to generation of innate inflammatory mediators including complement pathway, chemokines and cytokines, and inflammatory enzymes. These proinflammatory mediators stimulate immune cells to proliferate, migrate and induce expression of adhesion molecules on endothelial cells, which promote loosening of tight junctions and eventually infiltration of immune cells leading to recovery from injury or infection from pathogens or otherwise pathological changes in diseased state.

14.2 Sphingolipid Metabolites and Inflammation

Sphingolipids serve both structural and regulatory roles in eukaryotic cells [31, 56]. The sphingolipid metabolites Ceramide (Cer), Sphingosine-1-phosphate (S1P), Ceramide-1 phosphate (C1P), and Lactosylceramide (LacCer) are the major signaling molecules regulating key physiological functions and a variety of pathological processes, mainly related to inflammatory responses or inflammation-associated diseases [55]. Cer acts as a potent pro-inflammatory agent, whereas C1P and S1P can regulate either inflammation or participate in anti-inflammatory functions. LacCer plays a role as a signaling molecule in inflammation-induced proliferation or angiogenesis. Immune cell mediated secretion of pro-inflammatory cytokines stimulate inflammatory sphingolipid metabolic enzymes to convert sphingomyelin (SM) into Cer. Cer is then converted into either S1P or C1P or glycosphingolipid. The schematic diagram of sphingolipid metabolism and the biosynthetic enzymes involved in this process has been presented in Fig. 14.1. The conversion of different sphingolipid metabolites varies with cell type. These inflammatory sphingolipid mediators then induce different types of inflammatory transcription factors (e.g., NFkB) or they may activate cyclooxygenase -2, leading to production of pro-inflammatory prostaglandins. In this review, we provide an overview of the significant body of research that reveals involvement of sphingolipid metabolites in inflammation and their role in ocular diseases.

14.2.1 Ceramides

Sphingolipid metabolism is regulated by cascades of enzyme activation within different cellular compartments wherein Cer occupies a central position. [56, 109] Cer plays a structural role by regulating membrane properties and its permeability [161] leading to promotion of raft fusion [46]. Cer- enriched platforms facilitate clustering of receptor molecules and their ligands [65]. This in turn helps the induction of apoptosis by clustering of CD95/Fas death receptor ligand [47]. In addition to its structural role, Cer acts as a second messenger by activating a diverse set of kinases and phosphatases [132]. The *de novo* Cer biosynthesis pathway is an anabolic pathway, which begins with condensation of serine and palmitoyl-CoA catalyzed by Serine palmitoyltransferase (SPT) in the endoplasmic reticulum (ER). The catabolic pathway of Cer generation occurs in the plasma membrane and lysosome via degradation of sphingomyelin (SM) to Cer and phosphorylcholine by sphingomyelinase. The third pathway is the lysosomal salvage pathway involving complex sphingolipids. Ceramide synthases play a significant role in the salvage pathway, thereby bypassing the formation of dihydroceramide. The fourth pathway of Cer biosynthesis occurs in liver mitochondria. The first report of the involvement of Cer in the inflammatory process demonstrated intracellular induction of a proinflammatory cytokine, TNF-α, which induced sphingomyelinase and, in turn, elevated Cer [75, 94]. Sphingolipidomics and transcriptomics studies revealed that lipopolysaccharide (LPS) induces inflammation through TLR4 in macrophage cell lines by inducing an increase in Cer [29]. In macrophages, the LPS-induced TLR4-mediated increase in *de novo* Cer biosynthesis is necessary for autophagosome formation, which could play a role in innate immunity [140]. Production of Cer subsequently activates a proinflammatory transcription factor, NFkB, the

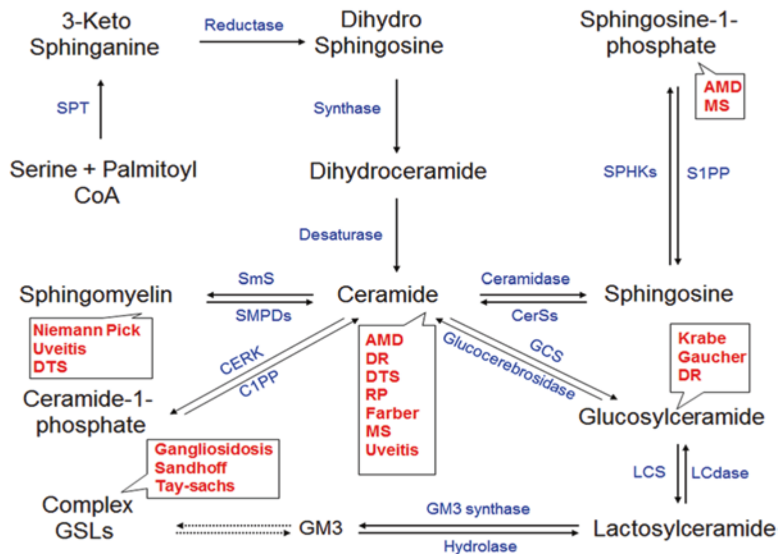

Fig. 14.1 Sphingolipid Metabolites in Ocular Diseases. The name of the disorders are presented in red and enzymes responsible for these metabolites are presented in blue. The *de-novo* Ceramide (Cer) biosynthesis is mediated by Serine palmitoyltransferase (SPT). In salvage pathway Ceramide synthase isoenzymes (CerSs) plays role in formation of Cer from Sphingosine. Sphingomyelin phosphodiesterase isoenzymes (SMPDs) converts Sphingomyelin (SM) to Cer. Sphingomyelin synthase (SmS) is the enzyme that converts Cer to SM. Ceramide kinase (CerK) and Ceramide-1-phosphate phosphatase (C1PP), respectively converts Cer to Ceramide-1-phosphate or vice versa. Conversion of Sphingosine-1-phosphate (S1P) is mediated by Sphingosine kinase isoenzymes (SPHKs) and for reverse reaction Sphingosine-1-phosphate phosphatase (S1PP) plays its role. Addition of glucose moiety with Cer is mediated by Glucosylceramide synthase (GCS). Lactosylceramide synthase (LCS) converts Glucosylceramide (GlcCer) to Lactosylceramide (LacCer) and Lactosylceramidase (LCdase) converts LacCer to GlcCer.GM3 ganglioside formation is mediated by GM3 synthase. Complex Gangliosides are generated by other carbohydrate moiety adding enzymes, which we discussed in our earlier review. *AMD* Age-related macular degeneration, *DR* Diabetic Retinopathy, *DTS* Dysfunctional Tear Syndrome, *MS* Multiple Sclerosis, *RP* Retinitis Pigmentosa

ubiquitously expressed transcription factor in mammalian cells [139]. The induction of NFkB encodes different cytokines, such as IL-1β, IL-6, IL-8, as well as chemokines, Monocyte chemoattractant protein-1 (MCP-1) including proinflammatory enzymes Cyclooxygenase -2 (COX-2), which is involved in the production of prostaglandins [169]. All these factors play important roles in inflammation. Another family of transcription factors, CCAAT/enhancer binding proteins (cEBP), is also activated by Cer in hepatocytes and macrophages. In hepatocytes, proinflammatory cytokine IL-1β induced CCAAT activation through Cer-mediated Extracellular signal regulated kinase 1/2 (ERK-1/2) pathway [42]. Cer can lead to induction of COX-2 via cEBP activation in macrophages stimulated with LPS [25]. Cer also plays a role in obesity by modulating inflammatory pathways. The Nlrp3 dependent inflammasome pathway increased caspase-1 activity through lipotoxicity-associated ceramide production in macrophage and adipose tissue of obese mice [162]. In mouse models, it has been reported that increases in Cer production leads to TLR-4 dependent insulin resistance by inhibiting Akt [60]. Stimulation of protein kinase C ζ (PKCζ) by Cer is another pathway which inhibits Akt activity [39]. In summary, these studies support a strong link between Cer and inflammation-related disorders.

14.2.2 Ceramide-1-P

Ceramide-1-phosphate (C1P) is mitogenic and anti-apoptotic for different cell types [44].

Interestingly, C1P can have a dual regulatory role by serving as an intracellular second messenger to regulate cell survival and/or as an extracellular receptor ligand to stimulate chemotaxis [43]. In inflammation, CIP also behaves in a promiscuous manner, either as a pro-inflammatory or anti-inflammatory agent. The biosynthesis of C1P takes place in the trans-Golgi network through phosphorylation of Cer by Ceramide kinase (CerK). The role of C1P in inflammation was first reported in A549 lung adenocarcinoma cells, where it stimulated release of arachidonic acid (AA) and subsequent production of proinflammatory eicosanoids [120]. C1P-mediated inflammation is directly regulated by activation of cytosolic phospholipase-A2α (cPLA2α), an enzyme that releases AA from membrane phospholipids [121]. Further studies demonstrated that C1P-mediated activation of group IV cPLA2 proinflammatory enzyme is chain length-specific; C1P bearing an acyl chain of 6 carbon or higher, activated cPLA2α in vitro, whereas CIP with a shorter acyl chain length (C_2-C1P) was unable to activate this proinflammatory enzyme [150, 166]. Interestingly, prostaglandin production is a coordinated function of C1P and S1P, where S1P stimulates COX-2 activity and then cPLA2α functions in synthesis of AA to produce prostaglandins [122]. The interaction of C1P in proinflammation has also been proposed in some studies. It has been shown that, compared to wild type mice, CerK knock-out mice generate decreased level of proinflammatory cytokines, IL-6 and TNF-α in high fat diets and show normal insulin signaling [98]. These knock out animals show higher expression of insulin receptors and glucose transporter GLUT4 and decreased signaling of MCP-1 in infiltrating macrophages of adipose tissue. However, the role of C1P in proinflammation is complex, as other studies have demonstrated that C1P may also reduce inflammation. C1P was shown to inhibit tumor necrosis factor (TNF)-converting enzyme or TACE, which is the major metalloprotease for cleaving pro-TNF to its active form that plays a role in inflammation [79]. Also, exogenous use of C1P was shown to suppress production of IL-6, IL-8, TNF, and IL-1β in LPS treated human peripheral blood mononuclear cells [54]. The bimodal behavior of C1P in pro- and anti-inflammation could provide a novel therapeutic strategy for modulating inflammation associated with different diseases.

14.2.3 Sphingosine-1-P

To date, Sphingosine-1-phosphate (S1P) is the best-described mediator within the sphingolipid pathway. Unlike Cer, which promotes apoptosis, S1P is responsible for suppressing apoptosis [146]. As an extracellular and intracellular messenger, S1P plays diversified roles in inflammation, cancer, atherosclerosis, autoimmunity, and angiogenesis [153]. The diversified actions of S1P are mainly regulated by five widely expressed S1P G-protein-coupled receptors, S1PR(1–5) [147]. S1P is produced by phosphorylation of free sphingosine by Sphingosine kinase isoenzymes 1 and 2 (SphK1 and SphK2). Topologically, SphK1 is a cytosolic protein and may also be localized at the plasma membrane and endocytic membrane trafficking network, whereas SphK2 mainly resides in the nucleus, mitochondria or in the ER [53]. It has been well documented that S1P gradient in circulation plays major role in lymphocyte migration and trafficking [12]. Usually, in the blood and lymph S1P level is higher than in tissues, which is important for maintaining vascular integrity. The maintenance of circulating lymphocytes is balanced through S1P; lymphocytes are attached through S1P with their receptors in the lymphoid organ to prevent their egression into blood. In inflamed tissues, there is production of S1P by SphK1 in endothelial cells. Simultaneously, S1PR1 is also activated by inflammatory signals, which in turn lead to disbalance in circulating lymphocytes [133]. The synthetic S1P analog FTY720 (Fingolimod) prevents egression of lymphocytes in circulation and accumulation in the lymph nodes [131]. Thus, FTY720 acts as an immunosuppressive drug in inflammation and autoimmune diseases. The role of S1P in inflammation varies with cell type. It plays an important role during activation of mast cells and the subsequent development of

inflammatory responses [111]. The involvement of S1P in inflammation is supported by the fact that loss of generation of S1P leads to decreased levels of proinflammatory cytokines (TNF-α, IL-6) and proinflammatory factor, arachidonic acid, in SphK2 deficient fetal liver mast cells [112]. TNF-α induced proinflammatory enzyme, COX2 and production of prostaglandin E2 are regulated by activation of the Sphk1/S1P axis [120]. In addition, in an animal model of inflammation, S1P levels were decreased in dextran-sulfate induced colitis of Sphk1-lacking mice [143]. TNF-α mediated activation of Sphk1 is responsible for activation of inflammatory transcription factor NFkB. S1P binds and stimulates ubiquitin E3 ligase TRAF2 activity, resulting in Lys-63 linked polyubiquitination of Receptor interacting protein-1 (RIP-1), leading to phosphorylation of IKK complex and activation of NFkB [6]. Similarly, in Rheumatoid arthritis, S1P/S1P receptor 1 signaling upregulates pro-inflammatory receptor activator of NFkB ligand (RANKL) [154]. Contrary to its role in inflammation, S1P has also been reported to have anti-inflammatory effects by causing a switch from pro-inflammatory macrophage subtype M1 to anti-inflammatory subtype M2. [130]. In mice, it has been reported that lymphopoiesis and neuro-inflammation has been restrained by HDL-bound S1P [18]. Although lots of information supports the role of the S1P/Sphk1 axis in activation of NFkB, nevertheless it has also been reported that downregulation of SphK1 enhances chemokine CCL5, which plays an active role in recruiting leukocytes in inflammatory sites. Downregulation of Sphk2 reduced CCL5 expression. In this mechanism p38MAPK may play a significant role without affecting NFkB [2]. Another complexity in S1P biology is that not all tissues respond in a similar fashion to regulation of SIP production. For example, in a mouse model, deletion of *Sphk1* decreases S1P in blood, whereas deletion of *Sphk2* increases it [86, 143]. Although we have progressed significantly in various aspects of S1P research, we are still lacking in a comprehensive understanding of the involvement of SIP mechanistic pathways in inflammation related diseases.

14.2.4 Lactosylceramide

Lactosylceramide (LacCer) acts as a common precursor of all types of the lactose series of complex glycosphingolipids (GSLs) (e.g. the gangliosides and globotriosylceramide). It acts as a bioactive lipid in various physiological processes ranging from inflammation, proliferation, expression of adhesion molecules, angiogenesis and endocytosis [17]. LacCer is synthesized from ceramide generated by the *de novo* pathway and from other sphingolipids. Glucosylceramide (GlcCer) is the first glycosylation product of ceramide generated at the cytosolic surface of the Golgi. GlcCer is then translocated to the lumen of the Golgi and LacCer synthase [UDP-Galactose: glucosylceramide β1,4 galactosyl transferase (β4GalT)] converts GlcCer to LacCer. LacCer is an important signaling component in astrogliosis and induction of inflammatory mediators in neuroinflammatory diseases. The proinflammatory factor arachidonic acid is generated by LacCer-mediated activation of cytosolic phospholipase A2α [103]. The inflammatory mediators TNF α [116] and LPS/IFN-γ [117] activate LacCer, which in turn induces inflammatory factors, namely inducible nitric oxide synthase enzyme (iNOS) that eventually generates nitric oxide(NO) and causes neuroinflammation. In this LacCer-mediated inflammatory mechanism, Ras-NFkB-MAPK [117] and Ras-ERK1/2 [116] pathways play significant roles. LacCer also induces inflammatory mediator, NADPH oxidase and generates reactive oxygen species (ROS) from endothelial cells [16] and from neutrophils [9]. Inflammation stimulates proliferation and migration of immune cells and induces expression of adhesion molecules on endothelial cells. LacCer induces expression of Platelet endothelial cell adhesion molecule-1 (PECAM1) on endothelial cells in the microenvironment of monocyte accumulated cells at the site of inflammation [45]. This LacCer-mediated expression of PECAM1 is regulated by cross-talk between activation of PKC-α/ζ and stimulation of phospholipase A2. The inflammation induced LacCer activation plays a role in PECAM1 expression

and induces angiogenesis. LacCer mediated induction of angiogenesis has been shown in Human umbilical vein endothelial cells (HUVEC). Loss of the LacCer synthase gene in HUVEC cells leads to reduction of angiogenesis by reduction of PECAM1 expression when induced with Vascular endothelial growth factor (VEGF) [124]. LacCer serves as a lipid second messenger in VEGF induced angiogenesis, separate from S1P mediated pathway [77]. LacCer also contributes to mitochondrial dysfunction and generation of ROS in murine model of diabetes [107]. This information suggests that LacCer acts as a connecting modulator between inflammation and angiogenesis by expression of cell adhesion molecules and eventually, angiogenesis.

14.3 Sphingolipids in Ocular Disease

14.3.1 Ocular Inflammation

Interestingly, ocular tissues prevent and resolve inflammation by different mechanisms. The barrier between circulating blood and the retina, lack of a direct lymphatic drainage pathway, and the intraocular microenvironment limit local immune and inflammatory responses and make the eye an immune-privileged tissue [148]. A major population of innate inflammatory cells involved in ocular inflammation are macrophages [27], whereas adaptive immune elements, CD4+ and CD8+ T cells, express effector function in the eye [119]. In the most common ocular inflammatory disease, uveitis, inflammation is associated with Th1 and Th17 cells [21] along with different innate effectors, such as monocytes and neutrophils. In Age-related macular degeneration (AMD), innate/complement inflammatory responses play a significant role [32] along with adaptive immune responses [108]. Diabetic retinopathy (DR) is another inflammatory and a classical microvascular disease, where presence of microglia has been reported in the outer nuclear layer and photoreceptor layer [173].

14.3.2 Sphingolipidoses and Ocular Inflammation

There are similar reports on ocular inflammation from metabolic diseases, which often cause neurodegeneration and visual impairment. Many of these diseases are lysosomal storage disorders resulting in functional impairment of lysosomal enzymes or co-factors responsible for accumulation of sphingolipid metabolites in the cell [23]. Sphingolipids are fatty amino alcohols, which regulate cell survival, growth, inflammation, senescence and apoptosis [89]. In the mouse model of GM1 and GM2 gangliosidoses, there is microglial activation leading to elevation of pro-inflammatory cytokines TNF-α, IL-1β [68]. The GM1 and GM2 Gangliosides are sialic acid-containing glycosphingolipids, which plays important role in cell-cell recognition, adhesion and signal transduction. The loss of beta hexosaminidase A and hexosaminidase B causes accumulation of gangliosides in the brain and nerve cells during Tay Sachs and Sandhoff disease, the lysosomal storage disorders [171]. The accumulation of gangliosides in patients' retinas has been reported in Tay Sachs [104] and Sandhoff disease [135]. Accumulation of glucocerebroside has been observed in Gaucher patients leading to pathological abnormalities in the eye ranging from ocular motor apraxia [35] to corneal opacity [51] followed by infiltration of monocytes/macrophages [40]. In Krabbe's disease (globoid cell leukodystrophy or galactosyl ceramide lipidosis), neuroinflammation is the major component of pathogenesis. A reduction of retinal ganglion cells and nerve fiber layers of the retina are observed in Krabbe's disease [34]. In murine models, there is a generation of numerous proinflammatory molecules, including Major histocompatibility complexes (MHC) [95], TNF-α, IL-6 [84], and Monocyte chemoattractant protein-1 (MCP 1), and IL-10 [167]. Activated microglia produce an inflammatory signaling mediator, Prostaglandin D_2 (PGD_2), which activates astrocytes in mouse models of Krabbe's disease [101]. Monoglycosylsphingolipid (Psychosine) plays a significant role as an inflammatory component in the pathogenesis of

Krabbe's disease [152]. Niemann-Pick is a lysosomal storage disease caused by a mutation in the acid sphingomyelinase gene leading to dysfunction of sphingolipid signaling [81]. In Neimann-Pick Type A, ocular abnormalities range from corneal opacification to retinal opacification with a macular cherry red spot [164]. Whereas in the case of Neimann-Pick Type B, the ocular manifestation is mainly retinal with pathological features including macular halos and cherry-red maculae [96]. Optic nerve pallor and perimacular gray discoloration in Neimann-Pick Type C have been observed, both clinically and histologically. In knock-out mouse models of Neimann-Pick disease, there is enhancement of microglial activity and upregulation of IL-1β from astrocytes [15]. In Farber disease, there is accumulation of ceramide in the joints, liver, throat, CNS and retina due to deficiency of ceramidase [41]. The pathological changes are observed in retinal ganglion cells with gross distention and inclusions [172]. Fabry disease is a deficiency of α-galactosidase A which leads to accumulation of globotriosylceramide. The most common ocular condition arising from this is cornea verticillate [144]. Overall, these observations suggest a role for sphingolipids in ocular inflammatory diseases.

14.3.3 Sphingolipids and Autoimmune Eye Diseases

Uveitis is an autoimmune eye disease where the uvea is pathologically affected. There is inflammation of the uvea, which composes the middle layer of eye including the iris, ciliary body and choroid. There are different types of uveitis, which is classified according to International Uveitis Study Group (IUSG) Classification, which includes Anterior Uveitis, Intermediate Uveitis, Posterior Uveitis and Panuveitis [66]. Anterior Uveitis is acute type and it is most common kind of uveitis where anterior chamber is inflamed. It mainly affects the iris and is often called as Iritis. Iridocyclitis and Anterior cyclitis are also included in this category of Anterior Uveitis. In Intermediate Uveitis there is chronic inflammation, which affects the vitreous. It includes Pars planitis, Posterior cyclitis and Hyalitis. During Posterior Uveitis inflammation affects retina, choroid and optic nerve. It could be chronic and recurrent in nature. The disease named Chorioretinitis, Retinochoroiditis, Retinitis and Neuroretinitis are under the category of Posterior Uveitis. Pathologically, Posterior Uveitis involves breakdown of blood-retinal barrier (BRB), whereas in other forms of uveitis do not [37]. Like Posterior uveitis pathology, in Experimental Autoimmune Uveitis (EAU), there is an extensive breakdown of BRB and release of retinal autoantigen [49]. The EAU follows classical example of organ-specific autoimmune disease that resembles Posterior Uveitis in humans [22]. In case of Panuveitis, the inflammation affects entire uvea. The inflammation associated with uveitis is due to infiltration of both innate and adaptive immune cells [61]. Using a murine model of uveitis, it has been confirmed that T cells are involved and that Th17 and Th1 play a significant role in the inflammatory mechanism. Th17 and Th1 recruit different innate effector molecules: Th17 recruits neutrophils and Th1 recruits monocytes; both cause tissue destruction with independent mechanisms of pathology, with proinflammatory cytokines playing a major role [119]. Interestingly, FTY720, a structural analog of Sphingosine (Sph) and an FDA-approved therapeutic drug for Multiple sclerosis (MS), has been found to be effective in a rat model of experimental autoimmune uveitis [26]. The exact mechanism of FTY720 is still unknown, but it acts in a complex way on sphingolipid metabolism. Sphk2 phosphorylates FTY720 to FTY720-P, which is a mimetic of S1P and inactivates S1P receptor mediated signaling [91]. It also inhibits *de novo* ceramide synthesis and also acts to inhibit ceramide synthase enzymes [23]. This same drug was earlier used in experimental treatment of Vogt-Koyanagi-Harada (VKH) uveitis patients to suppress production of granulocyte monocyte colony stimulating factor by T cells [134]. The T cell clones (TCC) from aqueous humor (AH) or peripheral blood mononuclear cells (PBMCs) from VKH patients pro-

duced significantly higher level of proinflammatory cytokines IL-6, IL-8 and IFN-γ in comparison with healthy donors. This finding suggests a role for sphingolipid in inflammation and lymphocyte migration in uveitis. Recently, in a Wister rat model of endotoxin-induced uveitis (EIV), increased levels of proinflammatory cytokines IL-6 and TNF-α were noted in the aqueous humor [165]. Increased levels of ceramides C24:0 and C24:1, and sphingomyelin C24:0 were also reported in the aqueous humor. In the retina, similar length carbon chain species of ceramide also have been noticed in EIV rats. Increased levels of proinflammatory transcription factor NFkB were also observed in the retina of EIV rats. These observations suggest that infiltration of innate and adaptive immune cells induces inflammation, which could be mediated through modulation of sphingolipid metabolites. Thus, sphingolipids may play a major role in uveitis pathology.

Multiple Sclerosis is an autoimmune disease. Inflammation-related retinal atrophy is one of the pathological features related to MS. Significant loss of retinal ganglion cells and the presence of human leukocyte antigen-DR positive cells in the retina, with activation of microglia are characteristic abnormalities associated with MS [48, 160]. In the central nervous system (CNS), oligodendrocytes are the myelin forming cells, which are affected during MS by activation of glial cells and infiltration of lymphocytes and macrophages, leading to apoptosis of oligodendrocytes. Sphingolipids are the major component of myelin sheath and there are multiple pathophysiological roles of sphingolipids in MS. In MS patients, increased levels of ceramide have been reported in oligodendrocytes [141] in association with an increase in sphingosine in white matter [102]. In addition to NSMase- Ceramide upregulation in MS, sphingosine kinase 1- S1P receptor signaling regulates astroglial proliferation and gliosis [168]. As S1P- S1P receptor1 is a main pathway of lymphocyte egression in MS, application of Fingolimod or FTY720, an immunosuppressive S1P receptor agonist drug reduces lesion formation in MS patients [73]. Neutral Sphingomyelinase (NSMase) activation and production of ceramide has been linked with types of neuroinflammation other than MS, including those that are connected to NFkB regulated pathways that cause blood brain barrier disruption, vascular leakage, and lymphocyte migration with upregulation of ICAM1, VCAM1 and selectin [67]. Although Fingolimod is currently used in the treatment of MS, one of the common side effects of this drug is Fingolimod-associated macular edema (FAME) [90]. Retinal hemorrhages and retinal vein occlusion can also occur in Fingolimod treated patients. Although information pertaining to molecular mechanisms associated with FAME is still lacking, a possible mechanism could be disruption of cell-to-cell and cell-to-matrix adhesion complexes in retinal vessels resulting in stress in vascular permeability and subsequent macular edema [97, 113]. While our current understanding of MS is incomplete, there appears to be a strong correlation between MS related retinal degeneration and ceramide-related inflammatory pathways.

14.3.4 Sphingolipids and Degenerative Retinal Diseases

Progressive damage to the retina and death of photoreceptors is a hallmark for degenerative retinal diseases, including Age-related macular degeneration (AMD) and Retinitis Pigmentosa (RP). AMD is associated with several pathological disorders, ranging from inflammation, malfunctioning of autophagy and chronic oxidative stress leading to degeneration of retinal pigment epithelium (RPE) and ultimately photoreceptor death with vision loss [99, 123]. RPE is the pigmented cell layer, which is attached to underlying choroid and provides nourishment to overlying retinal visual cells. It also functions in phagocytosis, secretion and immune modulation. Photoreceptor cells function in visual phototransduction and visual signal generation. There are two types of photoreceptor cells in mammalian retinas, rods and cones, along with second and third order neurons, bipolar and ganglion cells, respectively. During early stage of AMD there is

accumulation of extracellular deposits called drusen in the retina, between RPE and Bruch's membrane. Drusen formation is linked to chronic low-level inflammation and complement activation during initial stages in the pathogenesis of AMD [7, 69]. Activation and secretion of various cytokines and chemokines, e.g., IL-1β, IL-6, TNF-α, CXCL8 play a significant role in initiation of inflammation [83]. The later stage of disease progression is classified as either 'Dry AMD' or 'Wet AMD'. Dry AMD is limited to damage of the macula region of the retina caused by atrophy whereas wet AMD also includes both macular atrophy as well as choroidal neovascularization (CNV). Inflammation mediated by complement factor plays an important role in AMD. In addition to this, genetic mutations associated with complement factor gene is among the major risk factors in AMD pathogenesis. One such factor in AMD is the inheritable genetic mutation, *Y402H* in complement factor H (*CFH*) [52, 76]. Other variants are present in *C3*, *CFB, C2* genes, associated with susceptibility to AMD [8]. These mutations are associated with a reduction of anti-inflammatory iC3b component and an increase of proinflammatory cytokines TNF-α and IFN-γ [24]. RPE also plays role in autophagy by autophagic degradation of photoreceptor outer segments (POS) in the process called heterophagy [70]. In aging, the function of RPE declines and results in accumulation of POS, which eventually forms lipofuscin in lysosomes leading to malfunctioning of lysosomes, generation of oxidative stress and retinal inflammation [36]. Oxidative stress-induced Cer biosynthesis genes are involved in photoreceptor cell death [13]. Increased Cer levels in RPE cells raises the level of inflammatory factor and ROS, which leads to mitochondrial permeabilization and activation of caspase-3, followed by apoptosis [72]. Use of desipramine protects photoreceptor death by reducing inflammatory factors and oxidative stress augmented by Cer, as desipramine inhibits sphingomyelinase's ability to convert sphingomyelin to Cer [136]. Similarly, overexpression of Acid ceramidase (ASAH1) in ARPE19 cells (Human retinal pigment epithelial cell line) protects from oxidative stress by reducing Cer level [151]. On the other hand, overexpression of Sphingomyelin phosphodiesterase 3 (SMPD3) enhances Cer production, which in turn leads to enhancement of RPE cell death by increasing inflammatory factors and stress [174]. In mouse models, it has been reported that cholesterol mediates activation of acid sphingomyelinase, which disrupts autophagy in RPE and leads to early onset macular degeneration [159]. Increases in Cer eventually promote inflammatory factors and oxidative stress, which prevent proper endosomal recycling of complement regulatory proteins after complement attack and disrupt endosomal biogenesis [156]. Aberrant endosomal biogenesis mediates complement activation in the RPE cells in murine model of macular degeneration [74]. In Rd10 mouse models, inhibition of *de novo* Cer biosynthesis by myriocin lowers retinal Cer levels and restricts photoreceptor death in RP [149]. Accumulation of POS increases oxidative stress and activates CFB, leading to AMD associated neovascularization [157]. The inflammatory factor also activates complement factor C3 and aggravates AMD pathogenesis [105]. Ceramide induces retinal degeneration, whereas choroidal neovascularization (CNV) is promoted by administration of alpha-galactosylceramide into the vitreous cavity of C57BL/6 mice [58]. Similarly, S1P2 receptor deficient mice do not develop neovascularization in the murine model of ischemia driven retinopathy [142]. The blockage of S1P by sonepcizumab, a humanized monoclonal antibody, also significantly reduces CNV in mouse models [170]. In summary, sphingolipids appear to play a significant role in retinal degenerative diseases by increasing inflammation, generating oxidative stress and deregulating lysosomal function in RPE and triggering photoreceptor cell death and/or neovascularization.

14.3.5 Sphingolipids and Diabetic Retinopathy

Diabetic retinopathy is a microvascular disease affecting retinal vascular degeneration and defective repair of retinal endothelial cells with persis-

tent low-grade inflammation. It has been reported that activated retinal glial cells and pigment epithelial cells express proinflammatory cytokines and VEGF in diabetes, which contributes to damage of retinal vasculature [1, 20, 100]. In addition to this, activation of circulating myelomonocytic cells from bone marrow increases leukocyte adhesion and contributes to retinal inflammation [85, 138]. There is also myeloid derived monocyte infiltration in diabetic retinas and exacerbation of inflammation by secreting proinflammatory cytokines, which further activates resident microglia, astrocytes and Muller glia in the retina [1, 59, 145]. The proinflammatory cytokines secreted from these cells cause endothelial cells to produce Acid sphingomyelinase (ASMase). Endothelial cells produce up to 20-fold more secretory sphingomyelinase than macrophages in response to cytokine stimulation [92]. The increase in ASMase regulates cytokine-mediated inflammation by generation of Cer in diabetic human and animal models [115]. Using different inhibitors, it has been observed that ASMase plays a major role in diabetic retinopathy. Increases in ASMase by TNF-α and IL-1β induce VEGF and ICAM-1 in Human retinal endothelial cells (HREC) and regulate retinal microangiopathy [114]. Retinal vascular permeability is mediated by very long chain ceramide, which is decreased in diabetic conditions due to decreases of biosynthetic enzyme, an Elongation of very long-chain fatty acids protein 4 (ELOVL4) [71, 158]. The streptozocin-induced rat models exhibit decreased levels of Cer and a concomitant increase of Glucosylceramide (GlcCer). The inhibition of glucosylceramide synthase increases the viability of retinal neuronal cells and insulin sensitivity in retinal neurons [38]. In addition to this it has also been observed that the pharmacological inhibition of glucosylceramide synthase increases insulin sensitivity in Zucker diabetic fatty (ZDF) rat [3]. Thus sphingolipid, more specifically Cer and GlcCer, play significant role in inflammation and retinal neovascularization in diabetic retinopathy.

14.3.6 Sphingolipids and Glaucoma

Glaucoma is a neurodegenerative disease where retinal ganglion cells (RGC) and their axons in the optic nerve are affected. The major risk factor of glaucoma is elevation of intraocular pressure (IOP). The neuroinflammatory responses during early stages of glaucoma are mediated by astrocytes, resident microglia, and other monocyte-derived cells in the optic nerve head (ONH). Proteomic analysis of human glaucomatous retinas revealed upregulation of TLR signaling, where TLR2, TLR3 and TLR4 was observed in microglia and astrocytes from glaucomatous retinas [88]. In DBA/2J (Dilute Brown Non Agouti, which develops progressive eye abnormalities that closely mimic hereditary glaucoma) mice in early stages of glaucoma, 11 of the 13 TLRs were upregulated in the optic nerve head (ONH) [62, 64]. As there was upregulation of TLR, the downstream factors such as MyD88, MAPK and NFkB all were activated, which in turn lead to activation of proinflammatory cytokines. In RGC degeneration, Fas ligand also is a major effector in DBA/2J mice models [50]. In disease pathology apart from monocytes derived cells [63], the complement cascade system plays a significant part in inflammation in DBA/2J animal models. Upregulation of C1 complex was observed in microglial cells in ONH in DBA/2J glaucomatous mice [62]. The second component of the complement cascade that plays a damaging role is complement component C5, a necessary component for MAC generation. Although there is incomplete information on role of sphingolipids in glaucoma development, it has been reported that in the aqueous humor there are native sphingolipid species. The levels of sphingomyelin and sphingoid base were reduced in hypertensive state from normotensive conditions, whereas S1P and ceramide levels increased in a hypertensive state [33] in DBA/2J mouse models. Data pertaining to sphingolipid composition of human aqueous humor [5] and trabecular meshwork [4] have also been generated. There is still a significant lack of information regarding the sphingolipid biology in glaucoma.

14.3.7 Sphingolipids and Dry Eye Syndrome

Dysfunctional tear syndrome (DTS), commonly known as dry eye disease, is caused by tear deficiency or excessive evaporation [19]. In addition to this, ocular surface inflammation due to increase in tear osmolarity plays a major role in DTS [78]. The tear film performs diversified functions, ranging from maintaining light refraction, supplying the cornea with nutrients and oxygen, lubrication of the cornea and conjunctiva, and ocular surface protection against foreign materials [110]. There are three different layers in tear film composition: the carbohydrate-rich glycocalyx layer, the intermediate aqueous layer, and the superficial tear film lipid layer (TFLL) [28]. Meibomian glands are the major source of TFLL lipids. Meibomian Gland Dysfunction (MGD) is a complex multifactorial disorder arise from combination of five different pathophysiological mechanisms; these are eyelid inflammation, conjunctival inflammation, corneal damage, microbiological changes and dry eye disease resulting from tear film instability [14]. Mass spectrometry (MS) data from dry eye disease patient reveals the role of sphingolipid in maintaining TFLL integrity [78]. In patient meibomian samples, significant increases of SM levels were observed compared to normal subjects. The individual short chain GlcCer species were significantly increased in patient meibomian samples. Whereas in case of meibomian keratoconjunctivitis (MKC), increased levels of Cer were reported due to abnormal hyperkeratinization [93]. Similarly, increased Cer levels disrupt stability and elasticity of TFLL [11]. However, patients with chronic blepharitis had been reported with decreased amount of cerebrosides in their meibomian gland [106]. In animal models, very long chain ceramides (acylceramide) in TFLL prevent dry eye disease, as transgene ELOVL1 mice developed corneal opacity with vascular invasion, accompanied by epidermalization of the cornea due to lower level of acylceramide in epidermis and in the meibomian gland [137]. Recently, our lab has reported increase in Cer/S1P ratio from poor quality meibomian gland tear film as compare to the good quality individuals [118]. The sphingolipid metabolites in meibomian gland tear film could serve as clinical signature of different types of eye diseases.

14.4 Summary and Conclusion

This chapter summarizes our current understanding of inflammation and its correlation with sphingolipid metabolites in eye diseases. Although ocular immune privilege protects the eye and retina from inflammation, the modulation and accumulation of different sphingolipid metabolites can perturb the ocular anti-inflammatory environment and lead to ocular pathology in different lysosomal storage disorders, autoimmune diseases, age related macular degeneration and diabetic retinopathy, suggesting an involvement of sphingolipid metabolites in maintaining homeostasis of the eye. Fig. 14.1 shows a schematic diagram of sphingolipid metabolism and involvement of different sphingolipid metabolites in ocular diseases. The bioactive sphingolipid ceramide acts as a proinflammatory lipid, whereas C1P and S1P have both pro- and anti-inflammatory functions. LacCer, on the other hand, acts as an angiogenic sphingolipid and induces neovascularization. In ocular diseases, ceramides are found to be inflammatory factors for stimulating proinflammatory cytokines and in some cases, proinflammatory cytokines induce the formation of ceramide that may be ultimately responsible for retinal cell death. GlcCer, LacCer, and S1P have been found to be associated with the cross talk between immune cells and endothelial cells that eventually develop neovascularization in ‘wet AMD’ and diabetic retinopathy. The loss of homeostasis in diseased conditions leads to stress in the endoplasmic reticulum, mitochondria, lysosomes and ultimately to activation of different proinflammatory factors. A more complete understanding of sphingolipid metabolites and their role in inflammation will help in our understanding of the etiology and pathobiology of various eye diseases that have inflammatory links.

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References

1. Abcouwer SF (2013) Angiogenic factors and cytokines in diabetic retinopathy. J Clin Cell Immunol Suppl 1. https://doi.org/10.4172/2155-9899
2. Adada MM, Orr-Gandy KA, Snider AJ, Canals D, Hannun YA, Obeid LM, Clarke CJ (2013) Sphingosine kinase 1 regulates tumor necrosis factor-mediated RANTES induction through p38 mitogen-activated protein kinase but independently of nuclear factor kappaB activation. J Biol Chem 288:27667–27679. https://doi.org/10.1074/jbc.M113.489443
3. Aerts JM et al (2007) Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. Diabetes 56:1341–1349. https://doi.org/10.2337/db06-1619
4. Aljohani AJ, Edwards G, Guerra Y, Dubovy S, Miller D, Lee RK, Bhattacharya SK (2014) Human trabecular meshwork sphingolipid and ceramide profiles and potential latent fungal commensalism. Invest Ophthalmol Vis Sci 55:3413–3422. https://doi.org/10.1167/iovs.13-13570
5. Aljohani AJ, Munguba GC, Guerra Y, Lee RK, Bhattacharya SK (2013) Sphingolipids and ceramides in human aqueous humor. Mol Vis 19:1966–1984
6. Alvarez SE et al (2010) Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. Nature 465:1084–1088. https://doi.org/10.1038/nature09128
7. Anderson DH, Mullins RF, Hageman GS, Johnson LV (2002) A role for local inflammation in the formation of drusen in the aging eye. Am J Ophthalmol 134:411–431
8. Anderson DH et al (2010) The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res 29:95–112. https://doi.org/10.1016/j.preteyeres.2009.11.003
9. Arai T, Bhunia AK, Chatterjee S, Bulkley GB (1998) Lactosylceramide stimulates human neutrophils to upregulate Mac-1, adhere to endothelium, and generate reactive oxygen metabolites in vitro. Circ Res 82:540–547
10. Arbore G, Kemper C (2016) A novel "complement-metabolism-inflammasome axis" as a key regulator of immune cell effector function. Eur J Immunol 46:1563–1573. https://doi.org/10.1002/eji.201546131
11. Arciniega JC, Uchiyama E, Butovich IA (2013) Disruption and destabilization of meibomian lipid films caused by increasing amounts of ceramides and cholesterol. Invest Ophthalmol Vis Sci 54:1352–1360. https://doi.org/10.1167/iovs.12-10662
12. Baeyens A, Fang V, Chen C, Schwab SR (2015) Exit Strategies: S1P Signaling and T Cell Migration. Trends Immunol 36:778–787. https://doi.org/10.1016/j.it.2015.10.005
13. Barak A, Morse LS, Goldkorn T (2001) Ceramide: a potential mediator of apoptosis in human retinal pigment epithelial cells. Invest Ophthalmol Vis Sci 42:247–254
14. Baudouin C et al (2016) Revisiting the vicious circle of dry eye disease: a focus on the pathophysiology of meibomian gland dysfunction. Br J Ophthalmol 100:300–306. https://doi.org/10.1136/bjophthalmol-2015-307415
15. Baudry M, Yao Y, Simmons D, Liu J, Bi X (2003) Postnatal development of inflammation in a murine model of Niemann-Pick type C disease: immunohistochemical observations of microglia and astroglia. Exp Neurol 184:887–903. https://doi.org/10.1016/S0014-4886(03)00345-5
16. Bhunia AK, Han H, Snowden A, Chatterjee S (1996) Lactosylceramide stimulates Ras-GTP loading, kinases (MEK, Raf), p44 mitogen-activated protein kinase, and c-fos expression in human aortic smooth muscle cells. J Biol Chem 271:10660–10666
17. Bhunia AK, Han H, Snowden A, Chatterjee S (1997) Redox-regulated signaling by lactosylceramide in the proliferation of human aortic smooth muscle cells. J Biol Chem 272:15642–15649
18. Blaho VA et al (2015) HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. Nature 523:342–346. https://doi.org/10.1038/nature14462
19. Bron AJ et al (2014) Rethinking dry eye disease: a perspective on clinical implications. Ocul Surf 12:S1–S31. https://doi.org/10.1016/j.jtos.2014.02.002
20. Busik JV, Mohr S, Grant MB (2008) Hyperglycemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. Diabetes 57:1952–1965. https://doi.org/10.2337/db07-1520
21. Caspi RR (2010) A look at autoimmunity and inflammation in the eye. J Clin Invest 120:3073–3083. https://doi.org/10.1172/JCI42440
22. Caspi RR (2011) Understanding autoimmune uveitis through animal models. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 52:1872–1879. https://doi.org/10.1167/iovs.10-6909
23. Chen H, Chan AY, Stone DU, Mandal NA (2014) Beyond the cherry-red spot: Ocular manifestations of sphingolipid-mediated neurodegenerative and inflammatory disorders. Surv Ophthalmol 59:64–76. https://doi.org/10.1016/j.survophthal.2013.02.005
24. Chen M, Muckersie E, Robertson M, Forrester JV, Xu H (2008) Up-regulation of complement factor

B in retinal pigment epithelial cells is accompanied by complement activation in the aged retina. Exp Eye Res 87:543–550. https://doi.org/10.1016/j.exer.2008.09.005
25. Cho YH, Lee CH, Kim SG (2003) Potentiation of lipopolysaccharide-inducible cyclooxygenase 2 expression by C2-ceramide via c-Jun N-terminal kinase-mediated activation of CCAAT/enhancer binding protein beta in macrophages. Mol Pharmacol 63:512–523
26. Commodaro AG, Peron JP, Lopes CT, Arslanian C, Belfort R Jr, Rizzo LV, Bueno V (2010) Evaluation of experimental autoimmune uveitis in mice treated with FTY720. Invest Ophthalmol Vis Sci 51:2568–2574. https://doi.org/10.1167/iovs.09-4769
27. Cousins SW, Espinosa-Heidmann DG, Csaky KG (2004) Monocyte activation in patients with age-related macular degeneration: a biomarker of risk for choroidal neovascularization? Arch Ophthalmol 122:1013–1018. https://doi.org/10.1001/archopht.122.7.1013
28. Cwiklik L (2016) Tear film lipid layer: a molecular level view. Biochim Biophys Acta 1858:2421–2430. https://doi.org/10.1016/j.bbamem.2016.02.020
29. Dennis EA et al (2010) A mouse macrophage lipidome. J Biol Chem 285:39976–39985. https://doi.org/10.1074/jbc.M110.182915
30. Dennis EA, Norris PC (2015) Eicosanoid storm in infection and inflammation. Nat Rev Immunol 15:511–523. https://doi.org/10.1038/nri3859
31. Dressler KA, Mathias S, Kolesnick RN (1992) Tumor necrosis factor-alpha activates the sphingomyelin signal transduction pathway in a cell-free system. Science 255:1715–1718
32. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA (2005) Complement factor H polymorphism and age-related macular degeneration. Science 308:421–424. https://doi.org/10.1126/science.1110189
33. Edwards G, Aribindi K, Guerra Y, Bhattacharya SK (2014) Sphingolipids and ceramides of mouse aqueous humor: comparative profiles from normotensive and hypertensive DBA/2J mice. Biochimie 105:99–109. https://doi.org/10.1016/j.biochi.2014.06.019
34. Emery JM, Green WR, Huff DS (1972) Krabbe's disease. Histopathology and ultrastructure of the eye. Am J Ophthalmol 74:400–406
35. Erikson A, Wahlberg I (1985) Gaucher disease–Norrbottnian type. Ocular abnormalities. Acta Ophthalmol (Copenh) 63:221–225
36. Feeney-Burns L, Berman ER, Rothman H (1980) Lipofuscin of human retinal pigment epithelium. Am J Ophthalmol 90:783–791
37. Forrester JV, Kuffova L, Dick AD (2018) Autoimmunity, autoinflammation, and infection in uveitis. Am J Ophthalmol 189:77–85. https://doi.org/10.1016/j.ajo.2018.02.019
38. Fox TE et al (2006) Diabetes alters sphingolipid metabolism in the retina: a potential mechanism of cell death in diabetic retinopathy. Diabetes 55:3573–3580. https://doi.org/10.2337/db06-0539
39. Fox TE et al (2007) Ceramide recruits and activates protein kinase C zeta (PKC zeta) within structured membrane microdomains. J Biol Chem 282:12450–12457. https://doi.org/10.1074/jbc.M700082200
40. Fujiwaki T, Yamaguchi S, Tasaka M, Takayanagi M, Isobe M, Taketomi T (2004) Evaluation of sphingolipids in vitreous bodies from a patient with Gaucher disease, using delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 806:47–51. https://doi.org/10.1016/j.jchromb.2004.02.027
41. Gangoiti P et al (2010) Control of metabolism and signaling of simple bioactive sphingolipids: implications in disease. Prog Lipid Res 49:316–334. https://doi.org/10.1016/j.plipres.2010.02.004
42. Giltiay NV, Karakashian AA, Alimov AP, Ligthle S, Nikolova-Karakashian MN (2005) Ceramide- and ERK-dependent pathway for the activation of CCAAT/enhancer binding protein by interleukin-1beta in hepatocytes. J Lipid Res 46:2497–2505. https://doi.org/10.1194/jlr.M500337-JLR200
43. Gomez-Munoz A, Gangoiti P, Arana L, Ouro A, Rivera IG, Ordonez M, Trueba M (2013) New insights on the role of ceramide 1-phosphate in inflammation. Biochim Biophys Acta 1831:1060–1066. https://doi.org/10.1016/j.bbalip.2013.02.001
44. Gomez-Munoz A, Presa N, Gomez-Larrauri A, Rivera IG, Trueba M, Ordonez M (2016) Control of inflammatory responses by ceramide, sphingosine 1-phosphate and ceramide 1-phosphate. Prog Lipid Res 61:51–62. https://doi.org/10.1016/j.plipres.2015.09.002
45. Gong N, Wei H, Chowdhury SH, Chatterjee S (2004) Lactosylceramide recruits PKCalpha/epsilon and phospholipase A2 to stimulate PECAM-1 expression in human monocytes and adhesion to endothelial cells. Proc Natl Acad Sci USA 101:6490–6495. https://doi.org/10.1073/pnas.0308684101
46. Goni FM, Alonso A (2009) Effects of ceramide and other simple sphingolipids on membrane lateral structure. Biochim Biophys Acta 1788:169–177. https://doi.org/10.1016/j.bbamem.2008.09.002
47. Grassme H et al (2001) CD95 signaling via ceramide-rich membrane rafts. J Biol Chem 276:20589–20596. https://doi.org/10.1074/jbc.M101207200
48. Green AJ, McQuaid S, Hauser SL, Allen IV, Lyness R (2010) Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration. Brain 133:1591–1601. https://doi.org/10.1093/brain/awq080
49. Greenwood J (1992) The blood-retinal barrier in experimental autoimmune uveoretinitis (EAU): a review. Curr Eye Res 11(Suppl):25–32
50. Gregory MS et al (2011) Opposing roles for membrane bound and soluble Fas ligand in glaucoma-associated retinal ganglion cell death. PLoS

One 6:e17659. https://doi.org/10.1371/journal.pone.0017659

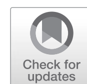
51. Guemes A, Kosmorsky GS, Moodie DS, Clark B, Meisler D, Traboulsi EI (1998) Corneal opacities in Gaucher disease. Am J Ophthalmol 126:833–835
52. Hageman GS et al (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 102:7227–7232. https://doi.org/10.1073/pnas.0501536102
53. Hait NC, Maiti A (2017) The role of sphingosine-1-phosphate and ceramide-1-phosphate in inflammation and cancer. Mediat Inflamm 2017:4806541. https://doi.org/10.1155/2017/4806541
54. Hankins JL, Fox TE, Barth BM, Unrath KA, Kester M (2011) Exogenous ceramide-1-phosphate reduces lipopolysaccharide (LPS)-mediated cytokine expression. J Biol Chem 286:44357–44366. https://doi.org/10.1074/jbc.M111.264010
55. Hannun YA (1996) Functions of ceramide in coordinating cellular responses to stress. Science 274:1855–1859
56. Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol 9:139–150. https://doi.org/10.1038/nrm2329
57. Heneka MT, McManus RM, Latz E (2018) Inflammasome signalling in brain function and neurodegenerative disease. Nat Rev Neurosci 19:610–621. https://doi.org/10.1038/s41583-018-0055-7
58. Hijioka K, Sonoda KH, Tsutsumi-Miyahara C, Fujimoto T, Oshima Y, Taniguchi M, Ishibashi T (2008) Investigation of the role of CD1d-restricted invariant NKT cells in experimental choroidal neovascularization. Biochem Biophys Res Commun 374:38–43. https://doi.org/10.1016/j.bbrc.2008.06.080
59. Hinze A, Stolzing A (2011) Differentiation of mouse bone marrow derived stem cells toward microglia-like cells. BMC Cell Biol 12:35. https://doi.org/10.1186/1471-2121-12-35
60. Holland WL et al (2011) Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest 121:1858–1870. https://doi.org/10.1172/JCI43378
61. Horai R et al (2013) Breakdown of immune privilege and spontaneous autoimmunity in mice expressing a transgenic T cell receptor specific for a retinal autoantigen. J Autoimmun 44:21–33. https://doi.org/10.1016/j.jaut.2013.06.003
62. Howell GR et al (2011a) Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. J Clin Invest 121:1429–1444. https://doi.org/10.1172/JCI44646
63. Howell GR et al (2012) Radiation treatment inhibits monocyte entry into the optic nerve head and prevents neuronal damage in a mouse model of glaucoma. J Clin Invest 122:1246–1261. https://doi.org/10.1172/JCI61135
64. Howell GR, Walton DO, King BL, Libby RT, John SW (2011b) Datgan, a reusable software system for facile interrogation and visualization of complex transcription profiling data. BMC Genomics 12:429. https://doi.org/10.1186/1471-2164-12-429
65. Hueber AO, Bernard AM, Herincs Z, Couzinet A, He HT (2002) An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes. EMBO Rep 3:190–196. https://doi.org/10.1093/embo-reports/kvf022
66. Jabs DA, Nussenblatt RB, Rosenbaum JT, Standardization of Uveitis Nomenclature Working G (2005) Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol 140:509–516
67. Jana A, Pahan K (2010) Sphingolipids in multiple sclerosis. NeuroMolecular Med 12:351–361. https://doi.org/10.1007/s12017-010-8128-4
68. Jeyakumar M et al (2003) Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidosis. Brain 126:974–987
69. Johnson LV, Leitner WP, Staples MK, Anderson DH (2001) Complement activation and inflammatory processes in Drusen formation and age related macular degeneration. Exp Eye Res 73:887–896. https://doi.org/10.1006/exer.2001.1094
70. Kaarniranta K et al (2013) Autophagy and heterophagy dysregulation leads to retinal pigment epithelium dysfunction and development of age-related macular degeneration. Autophagy 9:973–984. https://doi.org/10.4161/auto.24546
71. Kady NM et al (2018) ELOVL4-mediated production of very long-chain ceramides stabilizes tight junctions and prevents diabetes-induced retinal vascular permeability. Diabetes 67:769–781. https://doi.org/10.2337/db17-1034
72. Kannan R, Jin M, Gamulescu MA, Hinton DR (2004) Ceramide-induced apoptosis: role of catalase and hepatocyte growth factor. Free Radic Biol Med 37:166–175. https://doi.org/10.1016/j.freeradbiomed.2004.04.011
73. Kappos L et al (2006) Oral fingolimod (FTY720) for relapsing multiple sclerosis. N Engl J Med 355:1124–1140. https://doi.org/10.1056/NEJMoa052643
74. Kaur G et al (2018) Aberrant early endosome biogenesis mediates complement activation in the retinal pigment epithelium in models of macular degeneration. Proc Natl Acad Sci USA 115:9014–9019. https://doi.org/10.1073/pnas.1805039115
75. Kim MY, Linardic C, Obeid L, Hannun Y (1991) Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. J Biol Chem 266:484–489
76. Klein R, Peto T, Bird A, Vannewkirk MR (2004) The epidemiology of age-related macular degeneration. Am J Ophthalmol 137:486–495. https://doi.org/10.1016/j.ajo.2003.11.069

77. Kolmakova A, Rajesh M, Zang D, Pili R, Chatterjee S (2009) VEGF recruits lactosylceramide to induce endothelial cell adhesion molecule expression and angiogenesis in vitro and in vivo. Glycoconj J 26:547–558. https://doi.org/10.1007/s10719-008-9206-9
78. Lam SM, Tong L, Yong SS, Li B, Chaurasia SS, Shui G, Wenk MR (2011) Meibum lipid composition in Asians with dry eye disease. PLoS One 6:e24339. https://doi.org/10.1371/journal.pone.0024339
79. Lamour NF, Wijesinghe DS, Mietla JA, Ward KE, Stahelin RV, Chalfant CE (2011) Ceramide kinase regulates the production of tumor necrosis factor alpha (TNFalpha) via inhibition of TNFalpha-converting enzyme. J Biol Chem 286:42808–42817. https://doi.org/10.1074/jbc.M111.310169
80. Lampron A, Elali A, Rivest S (2013) Innate immunity in the CNS: redefining the relationship between the CNS and Its environment. Neuron 78:214–232. https://doi.org/10.1016/j.neuron.2013.04.005
81. Ledesma MD, Prinetti A, Sonnino S, Schuchman EH (2011) Brain pathology in Niemann Pick disease type A: insights from the acid sphingomyelinase knockout mice. J Neurochem 116:779–788. https://doi.org/10.1111/j.1471-4159.2010.07034.x
82. Lehnardt S (2010) Innate immunity and neuroinflammation in the CNS: the role of microglia in Toll-like receptor-mediated neuronal injury. Glia 58:253–263. https://doi.org/10.1002/glia.20928
83. Lentsch AB, Ward PA (2000) Regulation of inflammatory vascular damage. J Pathol 190:343–348. https://doi.org/10.1002/(SICI)1096-9896(200002)190:3<343::AID-PATH522>3.0.CO;2-M
84. LeVine SM, Brown DC (1997) IL-6 and TNFalpha expression in brains of twitcher, quaking and normal mice. J Neuroimmunol 73:47–56
85. Li G, Veenstra AA, Talahalli RR, Wang X, Gubitosi-Klug RA, Sheibani N, Kern TS (2012) Marrow-derived cells regulate the development of early diabetic retinopathy and tactile allodynia in mice. Diabetes 61:3294–3303. https://doi.org/10.2337/db11-1249
86. Liang J et al (2013) Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. Cancer Cell 23:107–120. https://doi.org/10.1016/j.ccr.2012.11.013
87. Loo YM, Gale M Jr (2011) Immune signaling by RIG-I-like receptors. Immunity 34:680–692. https://doi.org/10.1016/j.immuni.2011.05.003
88. Luo C, Yang X, Kain AD, Powell DW, Kuehn MH, Tezel G (2010) Glaucomatous tissue stress and the regulation of immune response through glial Toll-like receptor signaling. Invest Ophthalmol Vis Sci 51:5697–5707. https://doi.org/10.1167/iovs.10-5407
89. Maceyka M, Spiegel S (2014) Sphingolipid metabolites in inflammatory disease. Nature 510:58–67. https://doi.org/10.1038/nature13475
90. Mandal P, Gupta A, Fusi-Rubiano W, Keane PA, Yang Y (2017) Fingolimod: therapeutic mechanisms and ocular adverse effects. Eye (Lond) 31:232–240. https://doi.org/10.1038/eye.2016.258
91. Mann H (2010) Oral cladribine and fingolimod for relapsing multiple sclerosis. N Engl J Med 362:1738.; author reply 1739–1740. https://doi.org/10.1056/NEJMc1002550
92. Marathe S, Schissel SL, Yellin MJ, Beatini N, Mintzer R, Williams KJ, Tabas I (1998) Human vascular endothelial cells are a rich and regulatable source of secretory sphingomyelinase. Implications for early atherogenesis and ceramide-mediated cell signaling. J Biol Chem 273:4081–4088
93. Mathers WD, Lane JA (1998) Meibomian gland lipids, evaporation, and tear film stability. Adv Exp Med Biol 438:349–360
94. Mathias S, Dressler KA, Kolesnick RN (1991) Characterization of a ceramide-activated protein kinase: stimulation by tumor necrosis factor alpha. Proc Natl Acad Sci USA 88:10009–10013
95. Matsushima GK, Taniike M, Glimcher LH, Grusby MJ, Frelinger JA, Suzuki K, Ting JP (1994) Absence of MHC class II molecules reduces CNS demyelination, microglial/macrophage infiltration, and twitching in murine globoid cell leukodystrophy. Cell 78:645–656
96. McGovern MM, Wasserstein MP, Aron A, Desnick RJ, Schuchman EH, Brodie SE (2004) Ocular manifestations of Niemann-Pick disease type B. Ophthalmology 111:1424–1427. https://doi.org/10.1016/j.ophtha.2003.10.034
97. McVerry BJ, Garcia JG (2004) Endothelial cell barrier regulation by sphingosine 1-phosphate. J Cell Biochem 92:1075–1085. https://doi.org/10.1002/jcb.20088
98. Mitsutake S, Date T, Yokota H, Sugiura M, Kohama T, Igarashi Y (2012) Ceramide kinase deficiency improves diet-induced obesity and insulin resistance. FEBS Lett 586:1300–1305. https://doi.org/10.1016/j.febslet.2012.03.032
99. Mitter SK et al (2014) Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. Autophagy 10:1989–2005. https://doi.org/10.4161/auto.36184
100. Mohr S (2004) Potential new strategies to prevent the development of diabetic retinopathy. Expert Opin Investig Drugs 13:189–198. https://doi.org/10.1517/13543784.13.3.189
101. Mohri I et al (2006) Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. J Neurosci 26:4383–4393. https://doi.org/10.1523/JNEUROSCI.4531-05.2006
102. Moscatelli EA, Isaacson E (1969) Gas liquid chromatographic analysis of sphingosine bases in sphingolipids of human normal and multiple sclerosis cerebral white matter. Lipids 4:550–555
103. Nakamura H, Moriyama Y, Makiyama T, Emori S, Yamashita H, Yamazaki R, Murayama T (2013)

Lactosylceramide interacts with and activates cytosolic phospholipase A2alpha. J Biol Chem 288:23264–23272. https://doi.org/10.1074/jbc.M113.491431
104. Nakaya-Onishi M, Suzuki A, Okamoto N, Fukada M (2000) Observations on time course changes of the cherry red spot in a patient with Tay-Sachs disease. Br J Ophthalmol 84:1320–1321
105. Natoli R et al (2017) Retinal macrophages synthesize C3 and activate complement in AMD and in models of focal retinal degeneration. Invest Ophthalmol Vis Sci 58:2977–2990. https://doi.org/10.1167/iovs.17-21672
106. Nicolaides N, Kaitaranta JK, Rawdah TN, Macy JI, Boswell FM 3rd, Smith RE (1981) Meibomian gland studies: comparison of steer and human lipids. Invest Ophthalmol Vis Sci 20:522–536
107. Novgorodov SA et al (2016) Lactosylceramide contributes to mitochondrial dysfunction in diabetes. J Lipid Res 57:546–562. https://doi.org/10.1194/jlr.M060061
108. Nussenblatt RB, Liu B, Wei L, Sen HN (2013) The immunological basis of degenerative diseases of the eye. Int Rev Immunol 32:97–112. https://doi.org/10.3109/08830185.2012.740536
109. Ogretmen B (2018) Sphingolipid metabolism in cancer signalling and therapy. Nat Rev Cancer 18:33–50. https://doi.org/10.1038/nrc.2017.96
110. Ohashi Y, Dogru M, Tsubota K (2006) Laboratory findings in tear fluid analysis. Clin Chim Acta 369:17–28. https://doi.org/10.1016/j.cca.2005.12.035
111. Olivera A (2008) Unraveling the complexities of sphingosine-1-phosphate function: the mast cell model. Prostaglandins Other Lipid Mediat 86:1–11. https://doi.org/10.1016/j.prostaglandins.2008.02.005
112. Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, Rivera J (2007) The sphingosine kinase-sphingosine-1-phosphate axis is a determinant of mast cell function and anaphylaxis. Immunity 26:287–297. https://doi.org/10.1016/j.immuni.2007.02.008
113. Oo ML et al (2011) Engagement of S1P(1)-degradative mechanisms leads to vascular leak in mice. J Clin Invest 121:2290–2300. https://doi.org/10.1172/JCI45403
114. Opreanu M, Lydic TA, Reid GE, McSorley KM, Esselman WJ, Busik JV (2010) Inhibition of cytokine signaling in human retinal endothelial cells through downregulation of sphingomyelinases by docosahexaenoic acid. Invest Ophthalmol Vis Sci 51:3253–3263. https://doi.org/10.1167/iovs.09-4731
115. Opreanu M et al (2011) The unconventional role of acid sphingomyelinase in regulation of retinal microangiopathy in diabetic human and animal models. Diabetes 60:2370–2378. https://doi.org/10.2337/db10-0550
116. Pannu R, Singh AK, Singh I (2005) A novel role of lactosylceramide in the regulation of tumor necrosis factor alpha-mediated proliferation of rat primary astrocytes. Implications for astrogliosis following neurotrauma. J Biol Chem 280:13742–13751. https://doi.org/10.1074/jbc.M411959200
117. Pannu R, Won JS, Khan M, Singh AK, Singh I (2004) A novel role of lactosylceramide in the regulation of lipopolysaccharide/interferon-gamma-mediated inducible nitric oxide synthase gene expression: implications for neuroinflammatory diseases. J Neurosci 24:5942–5954. https://doi.org/10.1523/JNEUROSCI.1271-04.2004
118. Paranjpe V, Tan J, Nguyen J, Lee J, Allegood J, Galor A, Mandal N (2018) Clinical signs of meibomian gland dysfunction (MGD) are associated with changes in meibum sphingolipid composition. Ocul Surf. https://doi.org/10.1016/j.jtos.2018.12.006
119. Perez VL, Caspi RR (2015) Immune mechanisms in inflammatory and degenerative eye disease. Trends Immunol 36:354–363. https://doi.org/10.1016/j.it.2015.04.003
120. Pettus BJ, Bielawska A, Spiegel S, Roddy P, Hannun YA, Chalfant CE (2003) Ceramide kinase mediates cytokine- and calcium ionophore-induced arachidonic acid release. J Biol Chem 278:38206–38213. https://doi.org/10.1074/jbc.M304816200
121. Pettus BJ et al (2004) Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. J Biol Chem 279:11320–11326. https://doi.org/10.1074/jbc.M309262200
122. Pettus BJ et al (2005) The coordination of prostaglandin E2 production by sphingosine-1-phosphate and ceramide-1-phosphate. Mol Pharmacol 68:330–335. https://doi.org/10.1124/mol.104.008722
123. Piippo N et al (2014) Decline in cellular clearance systems induces inflammasome signaling in human ARPE-19 cells. Biochim Biophys Acta 1843:3038–3046. https://doi.org/10.1016/j.bbamcr.2014.09.015
124. Rajesh M, Kolmakova A, Chatterjee S (2005) Novel role of lactosylceramide in vascular endothelial growth factor-mediated angiogenesis in human endothelial cells. Circ Res 97:796–804. https://doi.org/10.1161/01.RES.0000185327.45463.A8
125. Ransohoff RM, Brown MA (2012) Innate immunity in the central nervous system. J Clin Invest 122:1164–1171. https://doi.org/10.1172/JCI58644
126. Ransohoff RM, Engelhardt B (2012) The anatomical and cellular basis of immune surveillance in the central nervous system. Nat Rev Immunol 12:623–635. https://doi.org/10.1038/nri3265
127. Ransohoff RM, Kivisakk P, Kidd G (2003) Three or more routes for leukocyte migration into the central nervous system. Nat Rev Immunol 3:569–581. https://doi.org/10.1038/nri1130
128. Rathinam VA, Fitzgerald KA (2016) Inflammasome complexes: emerging mechanisms and effector functions. Cell 165:792–800. https://doi.org/10.1016/j.cell.2016.03.046
129. Ricklin D, Hajishengallis G, Yang K, Lambris JD (2010) Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11:785–797. https://doi.org/10.1038/ni.1923

130. Rivera J, Proia RL, Olivera A (2008) The alliance of sphingosine-1-phosphate and its receptors in immunity. Nat Rev Immunol 8:753–763. https://doi.org/10.1038/nri2400
131. Rosen H, Sanna G, Alfonso C (2003) Egress: a receptor-regulated step in lymphocyte trafficking. Immunol Rev 195:160–177
132. Ruvolo PP (2003) Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol Res 47:383–392
133. Saba JD (2015) A B cell-dependent mechanism restrains T cell transendothelial migration. Nat Med 21:424–426. https://doi.org/10.1038/nm.3858
134. Sakaguchi M, Sugita S, Sagawa K, Itoh K, Mochizuki M (1998) Cytokine production by T cells infiltrating in the eye of uveitis patients. Jpn J Ophthalmol 42:262–268
135. Sandhoff K, Harzer K (2013) Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. J Neurosci 33:10195–10208. https://doi.org/10.1523/JNEUROSCI.0822-13.2013
136. Sanvicens N, Cotter TG (2006) Ceramide is the key mediator of oxidative stress-induced apoptosis in retinal photoreceptor cells. J Neurochem 98:1432–1444. https://doi.org/10.1111/j.1471-4159.2006.03977.x
137. Sassa T, Tadaki M, Kiyonari H, Kihara A (2018) Very long-chain tear film lipids produced by fatty acid elongase ELOVL1 prevent dry eye disease in mice. FASEB J 32:2966–2978. https://doi.org/10.1096/fj.201700947R
138. Schroder S, Palinski W, Schmid-Schonbein GW (1991) Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. Am J Pathol 139:81–100
139. Schutze S, Potthoff K, Machleidt T, Berkovic D, Wiegmann K, Kronke M (1992) TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced “acidic” sphingomyelin breakdown. Cell 71:765–776
140. Sims K et al (2010) Kdo2-lipid A, a TLR4-specific agonist, induces de novo sphingolipid biosynthesis in RAW264.7 macrophages, which is essential for induction of autophagy. J Biol Chem 285:38568–38579. https://doi.org/10.1074/jbc.M110.170621
141. Singh I, Pahan K, Khan M, Singh AK (1998) Cytokine-mediated induction of ceramide production is redox-sensitive. Implications to proinflammatory cytokine-mediated apoptosis in demyelinating diseases. J Biol Chem 273:20354–20362
142. Skoura A, Sanchez T, Claffey K, Mandala SM, Proia RL, Hla T (2007) Essential role of sphingosine 1-phosphate receptor 2 in pathological angiogenesis of the mouse retina. J Clin Invest 117:2506–2516. https://doi.org/10.1172/JCI31123
143. Snider AJ, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Hannun YA, Obeid LM (2009) A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. FASEB J 23:143–152. https://doi.org/10.1096/fj.08-118109
144. Sodi A, Ioannidis AS, Mehta A, Davey C, Beck M, Pitz S (2007) Ocular manifestations of Fabry’s disease: data from the Fabry Outcome Survey. Br J Ophthalmol 91:210–214. https://doi.org/10.1136/bjo.2006.100602
145. Soulet D, Rivest S (2008) Bone-marrow-derived microglia: myth or reality? Curr Opin Pharmacol 8:508–518. https://doi.org/10.1016/j.coph.2008.04.002
146. Spiegel S, Milstien S (2002) Sphingosine 1-phosphate, a key cell signaling molecule. J Biol Chem 277:25851–25854. https://doi.org/10.1074/jbc.R200007200
147. Spiegel S, Milstien S (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol 4:397–407. https://doi.org/10.1038/nrm1103
148. Streilein JW (2003) Ocular immune privilege: the eye takes a dim but practical view of immunity and inflammation. J Leukoc Biol 74:179–185
149. Strettoi E, Gargini C, Novelli E, Sala G, Piano I, Gasco P, Ghidoni R (2010) Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of retinitis pigmentosa. Proc Natl Acad Sci USA 107:18706–18711. https://doi.org/10.1073/pnas.1007644107
150. Subramanian P, Stahelin RV, Szulc Z, Bielawska A, Cho W, Chalfant CE (2005) Ceramide 1-phosphate acts as a positive allosteric activator of group IVA cytosolic phospholipase A2 alpha and enhances the interaction of the enzyme with phosphatidylcholine. J Biol Chem 280:17601–17607. https://doi.org/10.1074/jbc.M414173200
151. Sugano E et al (2018) Overexpression of acid-ceramidase (ASAH1) protects retinal cells (ARPE19) from oxidative stress. J Lipid Res. https://doi.org/10.1194/jlr.M082198
152. Suzuki K (2003) Globoid cell leukodystrophy (Krabbe’s disease): update. J Child Neurol 18:595–603. https://doi.org/10.1177/08830738030180090201
153. Takabe K, Paugh SW, Milstien S, Spiegel S (2008) “Inside-out” signaling of sphingosine-1-phosphate: therapeutic targets. Pharmacol Rev 60:181–195. https://doi.org/10.1124/pr.107.07113
154. Takeshita H et al (2012) Sphingosine 1-phosphate (S1P)/S1P receptor 1 signaling regulates receptor activator of NF-kappaB ligand (RANKL) expression in rheumatoid arthritis. Biochem Biophys Res Commun 419:154–159. https://doi.org/10.1016/j.bbrc.2012.01.103
155. Takeuchi O, Akira S (2010) Pattern recognition receptors and inflammation. Cell 140:805–820. https://doi.org/10.1016/j.cell.2010.01.022
156. Tan LX, Toops KA, Lakkaraju A (2016) Protective responses to sublytic complement in the retinal pigment epithelium. Proc Natl Acad Sci USA 113:8789–8794. https://doi.org/10.1073/pnas.1523061113

157. Tanaka K, Nakayama T, Mori R, Sato N, Kawamura A, Yuzawa M (2014) Associations of complement factor B and complement component 2 genotypes with subtypes of polypoidal choroidal vasculopathy. BMC Ophthalmol 14:83. https://doi.org/10.1186/1471-2415-14-83
158. Tikhonenko M et al (2010) Remodeling of retinal Fatty acids in an animal model of diabetes: a decrease in long-chain polyunsaturated fatty acids is associated with a decrease in fatty acid elongases Elovl2 and Elovl4. Diabetes 59:219–227. https://doi.org/10.2337/db09-0728
159. Toops KA, Tan LX, Jiang Z, Radu RA, Lakkaraju A (2015) Cholesterol-mediated activation of acid sphingomyelinase disrupts autophagy in the retinal pigment epithelium. Mol Biol Cell 26:1–14. https://doi.org/10.1091/mbc.E14-05-1028
160. Trip SA et al (2005) Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. Ann Neurol 58:383–391. https://doi.org/10.1002/ana.20575
161. van Blitterswijk WJ, van der Luit AH, Veldman RJ, Verheij M, Borst J (2003) Ceramide: second messenger or modulator of membrane structure and dynamics? Biochem J 369:199–211. https://doi.org/10.1042/BJ20021528
162. Vandanmagsar B et al (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–188. https://doi.org/10.1038/nm.2279
163. von Moltke J et al (2012) Rapid induction of inflammatory lipid mediators by the inflammasome in vivo. Nature 490:107–111. https://doi.org/10.1038/nature11351
164. Walton DS, Robb RM, Crocker AC (1978) Ocular manifestations of group A Niemann-Pick disease. Am J Ophthalmol 85:174–180
165. Wang HY, Wang Y, Zhang Y, Wang J, Xiong SY, Sun Q (2018) Crosslink between lipids and acute uveitis: a lipidomic analysis. Int J Ophthalmol 11:736–746. https://doi.org/10.18240/ijo.2018.05.05
166. Wijesinghe DS et al (2009) Chain length specificity for activation of cPLA2alpha by C1P: use of the dodecane delivery system to determine lipid-specific effects. J Lipid Res 50:1986–1995. https://doi.org/10.1194/jlr.M800367-JLR200
167. Wu YP, McMahon EJ, Matsuda J, Suzuki K, Matsushima GK, Suzuki K (2001) Expression of immune-related molecules is downregulated in twitcher mice following bone marrow transplantation. J Neuropathol Exp Neurol 60:1062–1074
168. Wu YP, Mizugishi K, Bektas M, Sandhoff R, Proia RL (2008) Sphingosine kinase 1/S1P receptor signaling axis controls glial proliferation in mice with Sandhoff disease. Hum Mol Genet 17:2257–2264. https://doi.org/10.1093/hmg/ddn126
169. Xiao Y, Zhong Y, Su H, Zhou Z, Chiao P, Zhong G (2005) NF-kappa B activation is not required for Chlamydia trachomatis inhibition of host epithelial cell apoptosis. J Immunol 174:1701–1708
170. Xie B, Shen J, Dong A, Rashid A, Stoller G, Campochiaro PA (2009) Blockade of sphingosine-1-phosphate reduces macrophage influx and retinal and choroidal neovascularization. J Cell Physiol 218:192–198. https://doi.org/10.1002/jcp.21588
171. Yu RK, Tsai YT, Ariga T, Yanagisawa M (2011) Structures, biosynthesis, and functions of gangliosides–an overview. J Oleo Sci 60:537–544
172. Zarbin MA, Green WR, Moser HW, Morton SJ (1985) Farber's disease. Light and electron microscopic study of the eye. Arch Ophthalmol 103:73–80
173. Zeng XX, Ng YK, Ling EA (2000) Neuronal and microglial response in the retina of streptozotocin-induced diabetic rats. Vis Neurosci 17:463–471
174. Zhu D, Sreekumar PG, Hinton DR, Kannan R (2010) Expression and regulation of enzymes in the ceramide metabolic pathway in human retinal pigment epithelial cells and their relevance to retinal degeneration. Vis Res 50:643–651. https://doi.org/10.1016/j.visres.2009.09.002
175. Zhu H et al (2011) An efficient delivery of DAMPs on the cell surface by the unconventional secretion pathway. Biochem Biophys Res Commun 404:790–795. https://doi.org/10.1016/j.bbrc.2010.12.061



15 Roles of Ceramides and Other Sphingolipids in Immune Cell Function and Inflammation

Sabrin Albeituni and Johnny Stiban

Abstract

Ceramides are bioactive sphingolipids that support the structure of the plasma membrane and mediate numerous cell-signaling events in eukaryotic cells. The finding that ceramides act as second messengers transducing cellular signals has attracted substantial attention in several fields of Biology. Since all cells contain lipid plasma membranes, the impact of various ceramides, ceramide synthases, ceramide metabolites, and other sphingolipids has been implicated in a vast range of cellular functions including, migration, proliferation, response to external stimuli, and death. The roles of lipids in these functions widely differ among the diverse cell types. Herein, we discuss the roles of ceramides and other sphingolipids in mediating the function of various immune cells; particularly dendritic cells, neutrophils, and macrophages. In addition, we highlight the main studies describing effects of ceramides in inflammation, specifically in various inflammatory settings including insulin resistance, graft-versus-host disease, immune suppression in cancer, multiple sclerosis, and inflammatory bowel disease.

Keywords

Ceramides · Immune cells · Sphingolipids · Inflammation · Disease

Abbreviations

3KS	3-keto-sphinganine
acyl-CoA	fatty acyl-coenzyme A
aSMase	acid sphingomyelinase
ATM	adipose tissue macrophages
C1P	ceramide 1-phosphate
CerS	ceramide synthases
CNS	central nervous system
COX-2	cyclooxygenase-2
CTL	cytotoxic T lymphocytes
CXCR2	C-X-C motif chemokine receptor type 2
DAG	diacylglycerol
DC	dendritic cells
DSS	dextran sulfate sodium
EAE	autoimmune encephalomyelitis
ERK	extracellular signal-regulated kinases

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fMLP	N-formylmethionine-leucyl-phenylalanine
GalCer	galactosylceramides
G-CSF	granulocyte-colony stimulating factor
GI	gastrointestinal
GVHD	Graft-Versus-Host Disease
HIV	human immunodeficiency virus
IBD	Inflammatory Bowel Disease
IFNγ	interferon gamma
IL-	interleukin
iNOS	inducible nitric oxide synthase
IRS-1	insulin receptor substrate-1
JNK	c-Jun N-terminal kinase
LacCer	lactosylceramide
LipC6	nanoliposome-loaded C_6-ceramide
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
M-CSF	macrophage-colony stimulating factor
MDSC	myeloid-derived suppressor cells
MS	Multiple Sclerosis
NETs	neutrophil extracellular traps
Nlrp3	Nod-like receptor pyrin domain-containing-3
NO	nitric oxide
PI3K	phosphatidylinositol 3 kinase
PKB	protein kinase B
PKCζ	protein kinase C zeta
PRR	pattern recognition receptors
ROS	reactive oxygen species
S1P	sphingosine 1-phosphate
Sa	sphinganine
SK	sphingosine kinase
SLs	sphingolipids
SMase	sphingomyelinase
So	sphingosine
SPT	serine palmitoyltransferase
TAM	tumor-associated macrophages
TCR	T cell receptor
TEM	tumor microenvironment
TGFβ	transforming growth factor beta
TLRs	toll-like receptor
TNFα	tumor necrosis factor alpha
T_{reg}	regulatory CD4+ T cells

15.1 Introduction

Ceramides are sphingolipids (SLs) that along with sterols and glycerolipids constitute the "fluid" part of the plasma membrane of eukaryotic cells. Ceramides are biologically active metabolites of the SL family, composed of a sphingoid base that mainly consists of the 18-carbon amino alcohols sphinganine (Sa) or sphingosine (So) covalently bound to a long fatty acyl side-chain [1, 2]. Conjugation of various headgroups to ceramide leads to the production of sphingomyelin (choline phosphate group), ceramide 1-phosphate (C1P) (phosphate group), glucosylceramide (glucose), galactosylceramides (GalCer) (galactose), or diverse glycolipids of the ganglioside and globoside families (addition of various saccharides) [1, 3, 4]. Apart from the differences in the headgroups, the variations in the number of carbons of the sphingoid base, the length of the fatty acyl side-chain, and location of double bonds lead to the diversification of ceramide structure and biological function [5].

In eukaryotic cells, ceramide generation occurs via three main pathways: *de novo* synthesis, sphingomyelin hydrolysis, and the salvage pathway [6, 7]. In the *de novo* biosynthetic pathway, ceramide synthesis is first mediated by serine palmitoyltransferase (SPT) that transfers serine to fatty acyl-coenzyme A (acyl-CoA), leading to the generation of 3-keto-sphinganine (3KS), which is then reduced by 3KS reductase producing the saturated amino alcohol Sa. Sa is later *N*-acylated through the action of any of the 6 identified ceramide synthases (CerS) forming dihydroceramide which is finally converted to ceramide via the action of dihydroceramide desaturases [8–10]. The second major reaction that results in ceramide production is sphingomyelin hydrolysis. In this reaction ceramides are generated via hydrolysis of the phosphocholine head group from sphingomyelin by sphingomyelinase (SMase) enzymes [11, 12]. The third major pathway by which ceramide is produced is

the SL recycling or salvage pathway. In this pathway, complex SLs are catabolized to So which is subsequently *N*-acylated to ceramide. A more complex network of enzymes involving SMases, ceramidases, and CerS are implicated in the pathway [13].

In mammals CerS family consists of 6 enzyme isoforms, named CerS1-6 [9, 14, 15]. Different CerS are distributed differentially in cells and tissues in the body (Fig. 15.1) [14, 16]. All CerS transfer acyl-CoA of variable lengths to the amine group of a sphingoid base [2]. Each CerS has a higher specificity towards the transfer of acyl-CoA of a certain length [9]. CerS1 shows specificity towards the transfer of C_{18}-CoA [17]; CerS2 is specific to C_{20-26} [16]; CerS3 acylates sphingoid bases with very long-chain fatty acyl CoAs (>26) [18]. CerS4 is specific for the transfer of C_{18-22} acyl CoAs [19], whereas the preferred substrates for CerS5 and CerS6 are C_{14-18} CoAs [20], and C_{14-16} CoAs [16], respectively. Even though the specificity of CerS towards different CoAs has been well established, little is known about the CerS regions that determine such specificity, because the crystal structure of different CerS has not been solved. However, it has been recently reported by Tidhar et al., that 11 key amino acid residues might be critical in determining the acyl chain length specificity of CerS2, CerS4, and CerS5 [21].

Functional CerS are important players for the wellbeing of cells. Loss of CerS has led to abnormalities in mouse models (Table 15.1): CerS1 knockout mice suffered from reduced ganglioside levels and Purkinje cell loss leading to impaired behavioral and motor development [17, 22]. CerS2 knockout mice developed hepatocarcinoma and cerebral degeneration [28–30]. In addition, CerS3 loss resulted in disruption of the skin barrier and spermatogenic arrest [18, 31]. While CerS4 deficient mice developed alopecia [32] due to destabilization in epidermal stem/progenitor cell homeostasis [35]. Interestingly, CerS5 loss led to improved adipose tissue health and function after consumption of high fat diet [20]. Deficiency of CerS6 led to behavioral abnormalities and abnormal clasping of the hind limbs in mice [34].

Ceramides are the simplest SLs composed of two hydrophobic tails and a simple rather than complex hydrophilic head, consisting of a hydroxyl group [36]. Because ceramides are

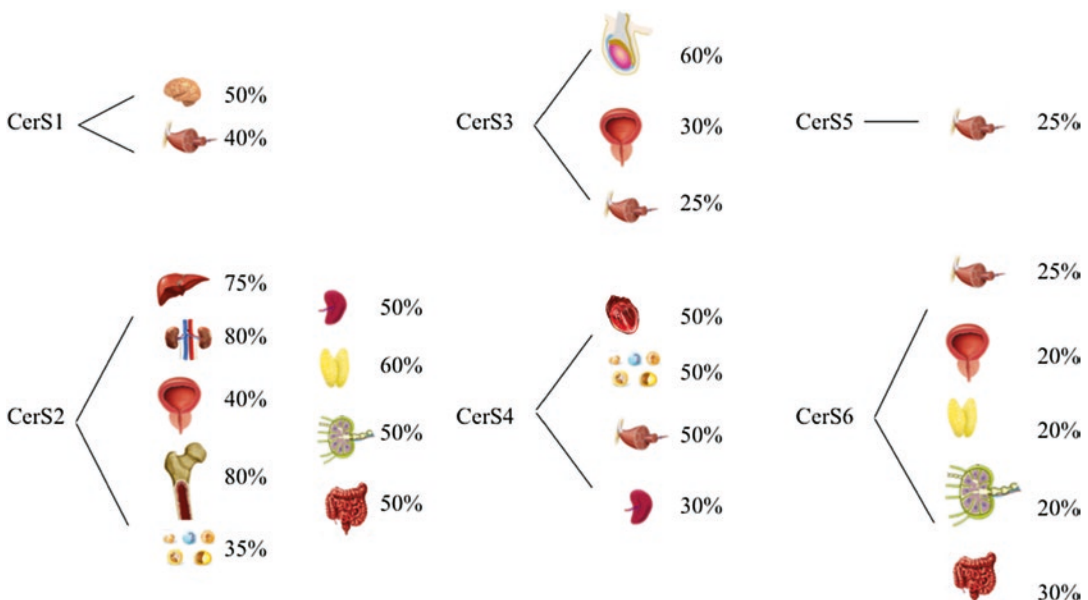

Fig. 15.1 Tissue distribution of CerS isoforms in mice. Tissue distribution of all CerS isoforms based on mRNA levels [14, 16]. The percentage next to organ pictures represents the relative amount of each CerS isoform in that organ

Table 15.1 Cellular abnormalities resulting from CerS deficiency

Deficient CerS isoforom	Abnormality[a]	Reference(s)
CerS1	Purkinje cell loss; motor development impairment	Ginkel et al. [17] and Zhao et al. [22]
CerS2	Delayed liver regeneration after partial hepatectomy	Jin et al. [23]
	Increased intestinal permeability	Chen et al. [24]
	Pheochromocytoma	Park et al. [25]
	Impaired neutrophil migration	Barthelmes et al. [26]
	Enhanced liver tumorigenesis	Chen et al. [27]
	Cerebral degeneration, myelin sheath defects	Imgrund et al. [28] and Pewzner-Jung et al. [29, 30]
CerS3	Skin barrier deformation	Jennemann et al. [18]
	Blocked spermatogenesis	Rabionet et al. [31]
CerS4	Altered lipid composition in skin; alopecia	Ebel et al. [32]
CerS5	Improved glucose homeostasis and adipose tissue health following high fat diet[a]	Gosejacob et al. [20]
CerS6	Protection against the development of colitis[a]	Scheffel et al. [33]
	Behavioral problems	Ebel et al. [34]

[a]Abnormality may not necessarily be detrimental to cells, it may be beneficial, but still it is a change from normal conditions

hydrophobic and usually composed of a C_{18} sphingoid base and a long $C_{14–26}$ N-linked-fatty acyl group, these lipids are embedded in the membrane making it harder to study their physiological functions. For this reason, more permeable ceramide analogs with short chains have been used in the laboratory (C_2, C_6, and C_8) to better understand ceramide biological function [37]. Nevertheless, numerous studies have been performed on the biophysical effects of ceramides in membranes ([30, 38–40]; Stiban et al. [41]). In all, ceramides are not mere structural components in membranes. Along with So, sphingosine 1-phosphate (S1P), C1P and lyso-sphingomyelin, ceramides have been implicated as bioactive lipids [42] that act as second messengers and regulate cellular functions including apoptosis and stress responses [43], tumor cell death and metabolism [44], and cytokine signaling and inflammation [45, 46]. Interestingly, numerous evidences point to the ability of ceramides to self-assemble into protein-permeable channels [47] in artificial membranes [48, 49], mitochondria [50–53] and lysosomes [54] that are upstream to caspase-dependent apoptotic cell death.

Despite the enormous progress in understanding the effects of ceramides in regulating key events in cellular biology, their role in regulating immune cell function and inflammatory diseases has only gained momentum in the last two decades. Herein, we discuss the main research findings describing the roles of ceramides in regulating the function of various immune cells, including dendritic cells (DC) and neutrophils, and the modulation of T cell function and macrophages in different disease settings. In addition, we will address the main findings highlighting ceramide function in various inflammatory diseases.

15.2 Ceramides and Other SLs in Immune Cell Function

15.2.1 Dendritic Cells

Unraveling the mechanisms of DC development, differentiation, maturation, antigen uptake, processing, and presentation have lied in the core of immunology research since their discovery by Steinman more than four decades ago [55, 56]. The main function associated with DC is linking the innate and adaptive immune responses through the uptake of foreign antigens and subsequent presentation to T cells in order to mount effective inflammatory and immune responses. In early studies, the role of ceramides

in regulating DC function was initially associated with induction of apoptosis by C_2-ceramide [57]. Similarly, induction of DC apoptosis was linked to increased accumulation of ceramides in DC cultured with tumor supernatants, subsequently leading to down regulation of the following survival signaling pathways, phosphatidylinositol 3 kinase (PI3K), Akt kinase, Bcl-x_L, and NF-κB [58]. However, induction of ceramide accumulation in DC by factors that cause DC maturation including, CD40L, interleukin 1β (IL-1β), tumor necrosis factor alpha (TNFα), and the gram-negative bacterial endotoxin lipopolysaccharide (LPS) did not induce DC apoptosis [59, 60]. These controversial findings were later reconciled by Franchi and colleagues demonstrating that ceramide-induced cell death in DC is only exacerbated in the absence of serum, while in the presence of serum and LPS, DC survival was achieved by the action of cellular ceramidases that deacylate ceramides to So, thus, preventing ceramide accumulation and DC apoptosis [61].

The study of ceramides in DC gained further attention due to the structural resemblance between the toxic component of LPS, lipid A, and ceramide (Fig. 15.2) [62]. It was suggested that LPS mediates its function by mimicking ceramides since, similar to LPS inducing DC maturation, C_2-ceramide reduces micropinocytosis and antigen presentation to T cells by DC [60]. However, while both LPS and C_2-ceramide induce c-Jun N-terminal kinase (JNK), only LPS activates extracellular signal-regulated kinases (ERKs) and NF-κB. In addition, LPS stimulates the production of ceramides regardless of whether macrophages are genetically responsive or unresponsive to LPS [63]. Therefore, this further supported that LPS exerts its function by inducing ceramide accumulation and not by interacting with ceramide-producing enzymes or as a structural mimic of ceramide.

Moreover, ceramides play important roles in DC during viral infections. GalCer on epithelial cells binds the human immunodeficiency virus (HIV)-1 envelope glycoproteins gp120 [64, 65]

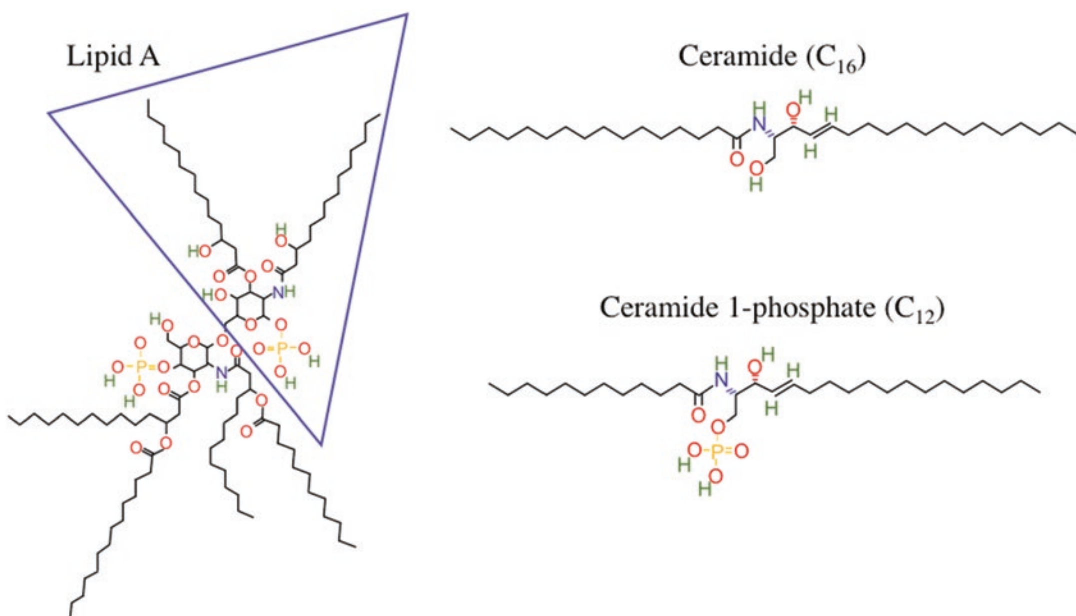

Fig. 15.2 Structural similarity between Lipid A of LPS and ceramides. Two representative ceramides are included for comparison, the most abundant C_{16}-ceramide and the phosphorylated C_{12}-ceramide 1-phosphate. The blue triangle identifies the comparable structure to ceramides. The 2D structures were obtained from PubChem and were redrawn to increase resolution. *PubChem CID: 9877306 (Lipid A), 71314646 (C_{16}-ceramide) and 5283580 (C_{12}-ceramide 1-phosphate).*

and gp41 [66, 67]. In addition, GalCer is essential in the formation of membrane lipid rafts that allow for the internalization of HIV-1 through endocytosis and transcytosis [68]. Interestingly, GalCer is present in monocyte-derived immature DC and human primary DC isolated from mucosa, suggesting that DC mediates HIV-1 transfer to its target cells through GalCer [69]. Ceramides also stabilized the membrane for measles virus entry in DC, as Dendritic Cell-specific intercellular adhesion molecule-3-grabbing non-integrin ligation on DC results in sphingomyelinase activation and subsequent ceramide accumulation in DC exposed to either mannan or measles virus [70].

The role of ceramides was further extended beyond viral entry in DC. It has been demonstrated that local administration of a ceramide analog C_8-ceramide causes the induction of DC maturation, secretion of the pro-inflammatory cytokines IL-12p70 and TNFα, and enhanced virus-specific T cell responses in murine models of chronic lymphocytic choriomeningitis virus clone 13 and influenza virus [71].

Collectively, these findings demonstrated that DC apoptosis, maturation, and antigen presenting capacity, are finely tuned by the action of endogenous ceramides or treatment with exogenous ceramide analogs. In addition, ceramides are key structural components of lipid rafts in DC required for binding, uptake, and internalization of viruses. These steps are key for initiating DC response allowing for viral internalization, virus peptide processing, and presentation to T cells that subsequently kill virus-infected cells. Since these findings strongly suggest that ceramides are key effectors of DC function, it is important to note that future studies elucidating the role of ceramides on the various DC subsets (e.g. plasmacytoid DC, migratory classical DC, tissue resident classical DC) could be of great therapeutic relevance in numerous immunologic diseases.

15.2.2 Neutrophils

Neutrophil extravasation, migration, production of cytokines and superoxide, and formation of neutrophil extracellular traps (NETs) in response to inflammatory stimuli are crucial for mounting an effective frontline immune response against invading microorganisms, especially extracellular pathogens [72]. Since the isolation of the free long-chain base So from human neutrophils [73], the role of ceramides in mediating and regulating neutrophil function has gained considerable traction. This was particularly due to early studies linking TNFα signaling, a potent inducer of superoxide and apoptosis in neutrophils [74], to ceramide function [75]. In HL-60 human promyelocytic leukemia cells, TNFα causes early sphingomyelin hydrolysis and ceramide production [76]. In addition, in a cell-free system both sphingomyelin content and ceramide concentration were reported to be increased in response to TNFα [77]. It was therefore hypothesized that ceramides might regulate neutrophil function in response to TNFα. However, later studies have proposed that ceramides activated a negative feedback loop to inhibit superoxide production in human neutrophils. It has been shown that ceramides not only did not mediate TNFα-induced superoxide production in human neutrophils [78] but also C_2-ceramide inhibited the 20:4 (n-6)-mediated superoxide formation in human neutrophils [79]. C_2-ceramide also inhibited respiratory burst of N-formylmethionine-leucyl-phenylalanine (fMLP)-stimulated adherent neutrophils [80–82]. Modulation of neutrophil response to polarity in response to a chemotactic agent such as fMLP has been shown to be dependent on neutral sphingomyelinase activity that converts sphingomyelin to ceramide through the modulation of Rac1/2/RhoA GTPases [83]. These combined results suggested that induction of ceramides might delay the response of neutrophils to TNFα to allow for neutrophil extravasation and migration prior to superoxide production.

Ceramides have also been implicated in the modulation of phagocytosis and migration in neutrophils. C_2-ceramides reduced phagocytosis of IgG-opsonized erythrocytes by fMLP-activated neutrophils through the inhibition of MAP kinase activation and tyrosine phosphorylation of ERK1 and ERK2 [84]; and by inhibiting

phospholipase D (PLD) function required for phagocytosis [85, 86]. Similarly, in COS-1 monkey kidney immortalized cells transfected with FcγIIA receptor, inhibition of ceramide synthesis led to enhanced phagocytosis [87]. On the other hand, chemotaxis, phagocytosis and NETs formation in fMLP-stimulated neutrophils were enhanced by the selective estrogen receptor antagonist, tamoxifen, through induction of ceramide accumulation, and subsequently protein kinase C zeta (PKCζ) activation [88]. These conflicting findings might suggest the opposing roles of various ceramides on neutrophil function.

A more direct role of ceramides is also prevalent in neutrophils. Ceramides are pro-apoptotic metabolites whose concentration in cells rises prior to the execution of the apoptotic pathway. Neutrophil apoptosis is mediated by C_{16}- and C_{24}-ceramides via caspase activation. This is correlated with the ability of granulocyte-macrophage colony-stimulating factor, a neutrophil survival factor, to reduce the accumulation of ceramides in neutrophils [89]. During early neutrophil apoptosis ceramide is generated by acid SMase (aSMase). In aSMase −/− mice, neutrophil apoptosis is delayed compared to WT mice [90]. In an anti-microbial setting, *Pseudomonas auroginosa* release pyocyanin that induce reactive oxygen species (ROS), which subsequently activates mitochondrial SMase, therefore, increasing mitochondrial ceramide levels and inducing cytochrome *c* release from mitochondria. This initiates cell death in neutrophils [91]. In addition, the enzyme sphingomyelin synthase, which mediates the transfer of choline phosphate to ceramide from phosphatidylcholine, leads to the production of sphingomyelin and diacylglycerol (DAG) [92, 93] and mediates neutrophil killing of fungus *Cryptococcus neoformans* [94].

Nevertheless, the aforementioned negative regulation by ceramides was not reported upon stimulation with the glycosphingolipid, lactosylceramide (LacCer). In neutrophils, LacCer compose more than two thirds of the glycolipid molecules in the plasma membrane and is mainly associated with a pro-inflammatory phenotype [95]. This large composition was later demonstrated to be not only relevant in supporting the membrane structure but also as a transducer of cellular signals. *In vitro* studies have revealed that LacCer enhances the upregulation of the integrin, CD11b, on neutrophil surface, inducing neutrophil adherence to endothelium, and mediating the production of ROS through activation of NADPH oxidase [96]. NADPH oxidase activation in neutrophils results from the association of LacCer in lipid rafts with the Src family kinase Lyn, and subsequent activation of PI3K, mitogen-activated protein kinase (MAPK) and PKC [97]. In addition, LacCer with long-chain fatty acids (C_{24}) are required for the coupling of Lyn to LacCer lipid rafts resulting in the production of superoxides and induction of migration in neutrophils [98–100]. Association of Lyn with the LacCer containing C_{24}-fatty acid chain is necessary for the phagocytosis of mycobacteria by neutrophils. Interestingly, LacCer-enriched lipid rafts are modulated by pathogenic bacteria to evade neutrophil-mediated killing. For instance, *Mycobacterium tuberculosis* prevented phagolysosome formation in neutrophils through binding of bacterial mannose-capped lipoarabinomannan to LacCer rafts in neutrophils [101].

In addition, inhibition of inflammation in neutrophils has also been associated with SL biology. Ceramides inhibit immune cell responses by binding to CD300f inhibitory receptor in sepsis [102], LPS-induced skin inflammation [103], allergic responses [104], and experimental colitis [105]. Interestingly, disruption of ceramide-CD300f binding induces neutrophil infiltration in sepsis and skin inflammation [102]. Ceramide metabolites have also been implicated in the modulation of neutrophil function. For instance, chemotactic migration of neutrophils in response to IL-8 and fMLP is inhibited by S1P, an immediate metabolite of ceramide [102]. Moreover, IL-8 gene expression and secretion was shown to be induced by S1P in lung H441 epithelial cells [107]. Similarly, C1P dampens inflammation in a model of LPS-induced lung inflammation. Particularly, C_{16}-C1P and synthetic C_8-C1P analog inhibit NF-κB activation in neutrophils and LPS-mediated IL-8 production [108].

These observations make it difficult, at a first glance, to conclude whether ceramides induce or inhibit neutrophil function. It is important to note that neutrophils are short-lived innate immune cells that sense extracellular pathogens early on during infection. Therefore, early on during activation, it is possible that ceramide induction in neutrophils leads to a delay in the production of TNFα allowing for neutrophil extravasation. However, it is not yet clear whether, as neutrophil activation progresses, a certain threshold of ceramides is required to turn on the apoptotic program in neutrophils. Data using ceramide analogs must be interpreted with caution, since the use of ceramide analogs might not correspond to the physiologic concentration of ceramide during infection. Nevertheless, the importance of understanding ceramide biology in neutrophils has started to attract more attention, especially in studies describing ceramide inhibitory role in multiple models of inflammation including models of sepsis, skin inflammation, allergic responses, and experimental colitis. Thus, these results may possibly be opening new doors for the inclusion of novel ceramide inhibitors that dampen neutrophil activation in diseases in which neutrophils are the main cause of pathophysiology. The effects of different SLs on neutrophil function are summarized in Table 15.2.

15.2.3 Macrophages

As the name implies, macrophages or the 'big eaters', along with DC and monocytes, compose the mononuclear phagocytic system [109, 110]. Due to their phagocytic nature, macrophages have the capacity to engulf debris, dead cells, and foreign pathogens [110, 111]. The heterogeneity and plasticity of macrophages enable these cells to mount a spectrum of pro-inflammatory or anti-inflammatory responses to various stimuli [111, 112]. Due to their vast distribution throughout the body, understanding macrophage biology has been a high pursuit since their description by Metchnikoff in the late 1800s. It is therefore not surprising that such a cell type with high membrane activity has recently gained considerable attention from lipid biochemists and immunologists alike [113, 114].

Macrophages recognize pathogen-associated molecular patterns through pattern recognition receptors (PRR), including toll-like receptors (TLRs) [115, 116]. The role of ceramides in modulating the ability of macrophages to sense and respond to microbes has been documented. Global lipidome analysis revealed that ceramides accumulated in all cellular membranes of activated macrophages in response to TLR4 stimulation with Kdo2-lipid A [117–119]. Furthermore, ceramide production was induced in macro-

Table 15.2 SLs in Neutrophil Function

SL	Function	Modulation	Reference(s)
Ceramide	Superoxide production	Inhibition	Robinson et al. [79], Nakamura et al. [82], Ahmed and Berridge [80], and Fuortes et al. [81]
	Extravasation and migration	Activation	
	Phagocytosis	Inhibition	Suchard et al. [84], Hinkovska-Galcheva et al. [85], Suchard et al. [86]
	Chemotaxis	Activation	Corriden et al. [88]
	Apoptosis	Activation	Manago et al. [91]
	Inflammation	Inhibition	Izawa et al. [102], Shiba et al. [103], Izawa et al. [104], and Matsukawa et al. [105]
LacCer	Adherence to endothelium	Activation	Arai et al. [96]
	ROS production	Activation	Iwabuchi and Nagaoka [97], Chiricozzi et al. [98], Iwabuchi et al. [99], and Sonnino et al. [100]
	Migration	Activation	
S1P	Chemotaxis	Inhibition	Kawa et al. [106]
C1P	Inflammation	Inhibition	Baudiss et al. [108]

phages stimulated with TLR4 in combination with palmitate, suggesting that lipotoxic conditions induce *de novo* ceramide synthesis in macrophages. Increased ceramide production was also associated with induced TNFα and IL1β production by macrophages [150]. In addition, palmitate and LPS synergistic effect was also shown to induce NLRP3 inflammasome activation, resulting in induced IL-1β and IL-18 production [121] in a manner independent of the SPT subunit Sptlc2 [122]. Interestingly, ceramide accumulation has also been implicated in macrophage response to oxidative stress in a TLR2-dependent manner [123]. In the aforementioned study, ceramide accumulation led to suppression of mitochondrial respiration shifting the use of the citric acid cycle metabolites to the production of the antioxidant glutathione.

Ceramide accumulation has also been associated with induced cell death in macrophages [124]. It has been demonstrated that apoptosis in adipose triglyceride lipase-deficient macrophages is mediated by increased C_{16}-ceramide concentrations [125]. Interestingly, withdrawal of macrophage-colony stimulating factor (M-CSF) from bone marrow-derived macrophages resulted in enhanced SPT activation and ceramide production prior to apoptosis [126]. This finding was further supported by the partial rescue of aSMase-deficient macrophages following M-CSF withdrawal, suggesting that apoptosis was at least in part mediated by ceramides. Additionally, ceramide accumulation was shown to mediate apoptosis in macrophages exposed to a lipotoxic diet consisting of saturated fatty acids [127].

Altogether, these studies suggest a crucial role for ceramides in modulating macrophage function and metabolism early and late during infection. The ability of ceramides to induce downstream signaling for the production of pro-inflammatory cytokines following an infection, allows macrophages to persist and orchestrate activation of immune cells that subsequently produce survival factors such as M-CSF. Once the infection is cleared and macrophage survival factors are no longer high, ceramides mediate macrophage apoptosis winding down the cycle of immune cell activation.

15.3 Ceramides and Other SLs in Inflammatory Diseases

Most of what is known today about the roles of ceramides and other SLs in modulating immune cell responses arises from a plethora of studies performed by lipid biochemists around the world. In the current era of lipidomics and targeted lipid therapeutics, lipid biology and metabolism have also started to attract many immunologists worldwide. In this section we present the main studies highlighting the roles of SLs in some major inflammatory diseases.

15.3.1 Insulin Resistance

Insulin Resistance refers to the impaired response of cells to insulin, resulting in a reduced uptake of glucose and glucose accumulation in the bloodstream. Insulin resistance is the main hallmark of type 2 diabetes mellitus and is highly associated with induction of cardiovascular disease, obesity, and various types of cancer [128]. Early studies established a strong link between ceramide accumulation and insulin resistance by demonstrating that SLs inhibited glucose transport into 3T3-L1 mouse adipose fibroblasts [129] and that bacterial SMase and the short-chain ceramides, C_2 and C_6, reduced insulin-induced tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) downstream the glucose transporter, GLUT4, in insulin-sensitive rat hepatoma Fao cells [130]. Interestingly, inhibition of IRS-1 was later demonstrated to be mediated by TNFα via induction of tumor necrosis factor receptor and SMase activity [131]. However, inhibition of IRS-1 activity by ceramides seems to be a result of protein kinase B (PKB) activity rather than direct inhibition of IRS-1 function [132–136]. Further investigation of the mechanisms of PKB inhibition by ceramides demonstrated that C_2-ceramide inhibited nuclear translocation of Akt1 [137], reduced phosphorylation of serine 473 in PKB [138], inhibited translocation of Akt/PKB to the plasma membrane and promoted dephosphorylation of Akt/PKB by protein phosphatase 2A [139–141].

Recent studies have also demonstrated induction of PKR/JNK activation [142, 143].

Further investigations aimed to determine whether ceramides induce insulin resistance. Analysis of muscle biopsies from insulin intolerant obese individuals revealed an increased accumulation of ceramides [144–147]. In addition, exogenous C_2-ceramide induced apoptosis in skeletal muscle myotubes, reducing the cell capacity to uptake glucose, a process that can be reversed by the CerS inhibitor, fumonisin B_1 [148, 149]. In muscle cells, C_2-ceramide also mediated insulin resistance by inhibiting Rac activation, thus reducing GLUT4 translocation to the plasma membrane in response to insulin [150]. These studies suggested that targeting ceramide accumulation could protect muscles against insulin resistance. In line with this hypothesis, Bruce and coworkers demonstrated that overexpression of sphingosine kinase 1 (SK1) led to reduced ceramide accumulation and insulin resistance in mice given high-fat diet [151]. Inhibition of CerS and ceramide synthesis with the S1P analog, FTY720 [152], also reduced insulin tolerance in mice on high-fat diet [153]. Furthermore, in obese rodents, blockade of *de novo* ceramide synthesis with the SPT) inhibitor, myriocin, improved glucose tolerance [128, 154].

Since CerS are required for *de novo* ceramide generation, several studies have focused on the role of CerS in inducing insulin tolerance. Overexpression of CerS1, CerS2, CerS4, CerS5, and CerS6 in L6 myotubes induced ceramide production; however, none of the CerS was able to inhibit insulin signaling [139]. Given the opposing role of CerS2 and CerS6 in inflammation, current studies have been focusing on the role of these CerS in insulin resistance *in vivo*. CerS2 haploinsufficient mice have altered patterns of ceramide acylation, leading to reduced levels of very-long-chain ceramides and increased levels of long-chain C_{16}-ceramide as a compensatory mechanism leading to insulin resistance when fed with high-fat diet [155]. In agreement with these findings, CerS6 expression and levels of C_{16}-ceramide are induced in the adipose tissue of obese humans. Moreover, CerS6- and CerS5-deficient mice fed with high-fat diet are protected from obesity, glucose intolerance, and insulin resistance suggesting that targeting CerS6 could be beneficial for the treatment of obesity and type 2 diabetes [20, 156]. It is worth noting that the products of CerS5 (C_{16}-ceramide) and CerS2 (C_{24}-ceramide) antagonized each other's ability to form channels in mitochondrial outer membranes to induce apoptosis [157]. This may be another mechanism by which different CerS affect insulin resistance and tolerance differentially.

Ceramide accumulation has been linked to inflammatory pathways mainly involving signaling events downstream of TLR4. More specifically, Holland et al., was the first to provide a link between lipotoxicity and inflammation in the induction of insulin intolerance [158]. Particularly, saturated fatty acids induced *de novo* ceramide synthesis via TLR4 activation, which also altered the metabolic program of skeletal muscle, inducing insulin resistance. However, Galbo et al. showed that lipid-induced insulin resistance is a result of increased accumulation of DAG and induced DAG-PKCε signaling rather than induced TLR4-ceramide pathway [159]. These conclusions were based on the observation that knockdown of TLR4 or the adaptor protein MyD88 prevented hepatic steatosis in mice fed with a saturated fat diet through reduction of appetite but not hepatic insulin signaling. When mice were given saturated fat by oral gavage, loss of TLR4 or MyD88 did not protect mice from hepatic insulin resistance. Interestingly, another link between ceramides and inflammatory pathways in insulin tolerance involves stimulation of the Nod-like receptor pyrin domain-containing-3 (Nlrp3) inflammasome [160]. In obese mice, ceramides activated Nlrp3 inflammasome and IL-1β secretion through caspase-1 activation in adipose tissue macrophages in a Nlrp3-dependent manner. This induction subsequently led to T cell activation. However, this study does not exclude the possibility that inducers other than ceramides present in the diet induced caspase-1 activation. Further studies are required to establish whether ceramides are truly sensed by PRR in immune cells.

15.3.2 Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation. It is an inflammatory response mediated primarily by donor T cells, resulting in destruction of host tissues including skin, liver, and gastrointestinal (GI) tract [161]. The role of ceramides in mediating T cell cytotoxic function has only been recently explored in two studies of mouse models of GVHD. Rotolo et al. reported that aSMase in the host mediated GVHD [162]. Adoptive transfer of allogeneic T cells to aSMase-deficient hosts reduced morbidity and mortality. This induction in survival resulted from reduced inflammatory responses, cytokine storm, CD8+ T cell proliferation and activation, and apoptosis of host hepatocytes, skin and intestinal cells. Interestingly, aSMase is required for the formation of ceramide-rich platforms on target cells for cytotoxic T lymphocytes (CTL) efficient killing. Further investigation of requirement of ceramide bioactive function in donor T cells by Sofi and colleagues demonstrated that CerS6-deficient donor T cells reduced GVHD [163]. In addition, T cells lacking CerS6 had an aberrant T cell receptor (TCR) signal transduction due to the reduction of tyrosine phosphorylation and CD3-PKCθ colocalization required for T cell proliferation and response to pro-inflammatory cytokines, particularly interferon gamma (IFNγ). Furthermore, inhibition of Cer6S with its specific inhibitor, ST1072 [164], reduced T cell proliferation and IFNγ production. This could also be a result of reduced IL-2 secretion by aSMase-deficient T cells upon TCR stimulation as previously reported [165]. Moreover, aSMase has also been described to be required for proper TCR signaling downstream CD3/CD28 activation in CD4+ T cells, since blockade of aSMase bioactivity with imipramine impaired PLCγ1, JNK, ERK, Akt, and mTOR phosphorylation downstream TCR [166]. More studies are required to further elucidate the roles of the aSMase and lipid metabolism in driving T cell activation and its role in GVHD pathogenesis.

15.3.3 Immune Suppression in the Tumor Microenvironment

Immunotherapy has recently been employed as a novel strategy to eradicate tumors. During tumor development, immune cell responses include massive tumor infiltration and production of cytokines in an attempt to contain tumor growth and kill tumor cells. If not contained, tumor growth leads to the establishment of a tumor microenvironment (TEM) characterized by induced hypoxia [167] and lactic acid accumulation leading to suppression of effector CD8+ CTL [168]. CD8+ T cell responses are inhibited by suppressive immune cells of various types that initially infiltrated the tumor to contain growth but reversed its effector phenotype in the TEM. Among these suppressive immune cells are tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and regulatory CD4+ T (T_{reg}) cells [111]. Recently, it has been appreciated that tumor cells gain advantage over immune cells by altering their glucose and lipid metabolism. Despite many studies describing the effects of alterations in glucose metabolism in immune cell function, only recently has similar alterations in immune cell lipid metabolism been considered [169].

S1P has been implicated in the polarization of macrophages to an M2 anti-inflammatory phenotype in the TEM. During tumor progression, apoptotic cells are released in the TEM serving as a source for S1P [170]. Interestingly, S1P released from engulfed apoptotic cells by macrophages induces macrophage survival through the activation of PIRK, ERK/2, and Ca^{+2} [171] and polarization to an M2 phenotype through the inhibition of inducible nitric oxide synthase (iNOS) and reduction of TNFα and IL-8 production [172–175]. Knock down of the S1P-producing enzyme, sphingosine kinase 2 (SK2) in MCF-7 breast carcinoma cells delayed tumor growth in a xenograft model in nude mice by promoting macrophage polarization to an M1 pro-inflammatory phenotype characterized by increased expression of nitric oxide (NO), TNFα, IL-12, and major histocompatibility

complex class II and reduced expression of IL-10 and CD206,which are markers associated with macrophage suppression [176].

Modulation of TAM function by ceramides has been also achieved in a mouse model of hepatocellular cancer through the administration of nanoliposome-loaded C_6-ceramide (LipC6) [177]. LipC6 exerts its modulatory function by acting as a ROS scavenger, therefore inducing macrophage polarization from an M2 to an M1 phenotype and resulting in reversal of CD8+ T cell exhaustion and induction of CD8+ cytotoxic function against tumor cells. The effect of ceramides has also been explored in suppressive MDSC in mice bearing CMS4-metastic tumors. Treatment of tumor-bearing mice with acid ceramidase inhibitor, LCL521, reduced MDSC accumulation in tumors without reducing tumor growth. In addition, LCL521 treatment led to an increased accumulation of C_{16}-ceramide that resulted in cathepsin-mediated cell death of tumor and MDSC-like J774 cells [178]. Suppression of CD8+ T cell function in tumors also results from suppression mediated by T_{reg} cells. To our knowledge, there have been no studies reporting the effect of ceramides on T_{reg} cell function in tumor-bearing mice. However, recent studies have described the effect of ceramide accumulation on T_{reg} cells in healthy WT and aSMase-deficient mice. T_{reg} cell frequency and suppressive function are induced in aSMase-deficient mice [179]. In addition, this was associated with increased percentage of induced T_{reg} (iT_{reg}) cells from aSMase$^{-/-}$ CD4+ T cells treated with transforming growth factor beta (TGFβ) and IL-2. Moreover, aSMase product C_6-ceramide induced a Th17-associtated cytokine in CD4+ T cell hampering T_{reg} cell induction, suggesting that aSMase is a negative regulator of T_{reg} cell development [180]. The relevance of these findings remains to be further explored in models of inflammation for further development of lipid-targeted therapies.

15.3.4 Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease that results in demyelination due to autoimmune responses against myelin in the central nervous system (CNS) [181]. In experimental autoimmune encephalomyelitis (EAE), *de novo* synthesis of C_{16}-ceramide by CerS6 is required for the production of pro-inflammatory TNFα and iNOS in macrophages in response to IFNγ [182]. Upregulation of CerS6 in EAE led to further speculations on its role in driving MS. Interestingly, upregulation of CerS6 and TNFα mRNA expression was found to be higher in females compared to male littermates in spontaneous relapsing remitting EAE [183]. This increase was correlated with the ability of females to initiate anti-inflammatory responses during the course of the disease, suggesting that CerS6 could promote anti-inflammatory responses. This observation was later confirmed with experiments showing that EAE progression is worsened in CerS6 knockout mice [184]. In addition, EAE was enhanced in chimeric mice in which only leukocytes were CerS6-deficient, providing further evidence that leukocytes lacking CerS6 drive the exacerbated phenotype. Moreover, expression of genes driving leukocyte migration and CNS infiltration (e.g. CCL2, CCL5, CXCL2) was increased, especially in CerS6-deficient neutrophils due to increased C-X-C motif chemokine receptor type 2 (CXCR2) in response to granulocyte-colony stimulating factor. On the contrary, CerS2-deficient mice had delayed development of EAE. This delay in disease onset was correlated with the reduced expression of CXCR2 in CerS2-deficient neutrophils [26]. In addition, the induction of ceramides was also associated with disease development in EAE, since aSMase deficiency protected mice from disease [185]. Collectively, these findings suggest that CerS6 and CerS2 have opposing roles in driving disease progression in EAE.

Excitingly, the S1P receptor agonist, fingolimod FTY720, was FDA approved in 2010 as a first-line therapy for the treatment of MS [186, 187]. FTY720 is phosphorylated *in vivo* by SK to form FTY720-P, which binds with a high affinity to S1P receptors and competes with its natural ligand, S1P. Therefore, FTY720 functionally antagonizes S1P by strongly binding to S1PR leading to internalization and inhibition of the receptor [188–190]. By modulating the S1P receptor, fingolimod prevented autoreactive S1P

expressing inflammatory T cells from exiting the lymph nodes, therefore, inhibiting CNS infiltration by autoreactive T cells. Subsequently, this resulted in reduced destruction of myelin sheath surround the axons of nerve cells [188–191]. This is a perfect example of the potential outcome of modulating lipid metabolism in immune cells driving forward the era of lipid therapeutics.

15.3.5 Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a group of diseases, including ulcerative colitis and Crohn's disease, characterized by chronic inflammation of gastrointestinal tract. Pathology is mediated by leukocytes infiltration and massive production of pro-inflammatory cytokines leading to intestinal damage [192, 193]. The first link between SL metabolism and IBD originated from studies showing that TNFα, a cytokine that induces the generation of S1P and synthesis of cyclooxygenase-2 (COX-2) [194, 195], was induced in patients with IBD [192, 196]. In addition, TNFα blockade alleviated clinical symptoms in mouse models of disease [197, 198]. Since ceramides also induced the activation of MAPK, a key inducer of inflammatory responses [199], follow up studies demonstrated that inhibition of ceramide accumulation by targeting SMase or S1P receptor diminished clinical manifestations of disease in mouse models of colitis. For example, in a dextran sulfate sodium (DSS) model of colitis, inhibition of SMase with a sphingomyelin analogue-7 reduced the formation of ceramide, levels of cytokines, and intestinal injury [200]. In addition, administration of FTY720 reduced disease symptoms in a mouse model of colitis, associated with a reduced production of the pro-inflammatory cytokine IL-12p70 and Th1 cytokines, and upregulation of CD4+ Foxp3+ suppressive T_{reg} cells [201]. Furthermore, administration of a new S1P receptor agonist, KRP-203, resulted in reduced lymphocyte infiltration and production of pro-inflammatory Th1 cytokines in the colonic mucosa [202]. Similarly, chemical and genetic inhibition of SK1 that is responsible for the production of S1P reduced manifestations of colitis, COX-2 expression, and neutrophil infiltration [203–205]. In support of these findings, high SK1 expression has also been reported in intestinal mucosa of patients with ulcerative colitis [205]. Currently, modulators of S1P receptor, such as ozanimod (RPC1063) and etrasimod (APD334) are being tested in clinical trials for the treatment of IBD [206, 207], further emphasizing the importance of investigating the role of SL metabolism in this disease setting.

Along with inhibition of S1P receptor function, modulation of CerS has also been explored in IBD, particularly CerS2 and CerS6. In a mouse model of colitis, loss of CerS2 destabilized the epithelial barrier and tight junction in the intestinal membrane leading to increased intestinal permeability. CerS2-deficient mice have reduced expression of junctional adhesion molecule A [208] and tight junction protein ZO-1 [209]. This outcome is associated with reduced levels of very-long acyl chain ceramides (C_{24}) and increased levels of long-chain sphingoid bases and C_{16}-ceramides [208]. These findings further suggest that CerS2 is protective against colitis as opposed to EAE in which CerS2-deficient mice are protected [26]. Interestingly, further exploration of the role of CerS6 in colitis has led to opposing conclusions. Scheffel et al. reported that the transfer of CerS6-deficient CD4+ T cells is protective in colitis [33]. On the contrary, Helke et al. showed that in a model of DSS-induced colitis, loss of CerS6 exacerbates inflammation [210]. Interestingly, pathology in CerS6-deficient mice is not a result of reduced intestinal permeability but is associated with enhanced neutrophil infiltration. However, more studies are needed to further elucidate the role of CerS in various models of colitis and its implementation in the development of CerS targeted therapies in humans with IBD.

15.4 Conclusions and Future Directions

While it has been thoroughly reviewed in other cells, the roles of ceramides in immune cells have not been proportionally presented. In this chapter we attempted to summarize the significant effects

of ceramides in immune cells and immune diseases. Numerous studies have highlighted the importance of ceramides in a variety of pathways necessary for the development, function, and metabolism of immune cells. Ceramide accumulation has been described as a key step mediating and regulating various immune cell functions, including regulation of immune cell responses to viruses, bacteria, and other foreign pathogens, migration, phagocytosis, and cytokine production. For instance, ceramides have been implicated in the modulation of responses to LPS, cytokine production, antigen presentation, and viral entry in DC; control of migration, cytokine and superoxide production, and formation of NETs in neutrophils; and response to TLR stimulation, inducing apoptosis, and regulating oxidative stress in macrophages. Moreover, ceramide functions extend to T cells, stabilizing the T cell receptor complex and mediating T cell responses during inflammation. Ceramide metabolites and upstream regulators including CerS in immune cell function have also been thoroughly described in the literature, further emphasizing the important role that ceramides play during inflammation.

Investigating the roles of ceramides and other SLs as modulators of the biology and metabolism of immune cells has unraveled new avenues in targeting ceramides in life-threatening inflammatory diseases that negatively impact the life of millions around the globe. For instance, recently, the FDA approved the CerS inhibitor, FTY720, as the first-line therapy for MS patients. This certainly has raised an interest among immunologists and lipid biochemists alike in developing novel inhibitors to target ceramide metabolism in various models of inflammation in the laboratory to hopefully translate such findings in the clinic.

It is also strictly important to consider the multiple effects of a plethora of ceramide species on the function of a certain immune cell or different cells when targeting ceramides in disease. Despite the encouraging routes that ceramide research has taken, further studies and collaborations between scientists of multidisciplinary fields are still needed to elucidate the mechanisms of ceramides in immune cell function and other cell types. Since diversity of ceramides, similar to other membrane and bioactive lipids, arises from the different possible combinations of head groups and/or degree of saturation, the effect of each type of SL and ceramide on every immune cell opens endless possibilities in targeting various ceramides to retune immune cell function in cancer, transplantation, diabetes, MS, IBD and many other diseases.

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References

1. Merrill AH Jr (2011) Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chem Rev 111:6387–6422
2. Park JW, Park WJ, Futerman AH (2014) Ceramide synthases as potential targets for therapeutic intervention in human diseases. Biochim Biophys Acta 1841:671–681
3. Don AS, Lim XY, Couttas TA (2014) Re-configuration of sphingolipid metabolism by oncogenic transformation. Biomol Ther 4:315–353
4. Lopez PH, Schnaar RL (2009) Gangliosides in cell recognition and membrane protein regulation. Curr Opin Struct Biol 19:549–557
5. Hannun YA, Obeid LM (2011) Many ceramides. J Biol Chem 286:27855–27862
6. Abou-Ghali M, Stiban J (2015) Regulation of ceramide channel formation and disassembly: insights on the initiation of apoptosis. Saudi J Biol Sci 22:760–772
7. Rappocciolo E, Stiban J (2019) Prokaryotic and mitochondrial lipids: a survey of evolutionary origins. Adv Exp Med Biol 1159. https://doi.org/10.1007/978-3-030-21162-2. (in press)
8. Bikman BT, Summers SA (2011) Ceramides as modulators of cellular and whole-body metabolism. J Clin Invest 121:4222–4230
9. Stiban J, Tidhar R, Futerman AH (2010) Ceramide synthases: roles in cell physiology and signaling. Adv Exp Med Biol 688:60–71
10. Tidhar R, Futerman AH (2013) The complexity of sphingolipid biosynthesis in the endoplasmic reticulum. Biochim Biophys Acta 1833:2511–2518
11. Boulgaropoulos B, Amenitsch H, Laggner P, Pabst G (2010) Implication of sphingomyelin/ceramide

molar ratio on the biological activity of sphingomyelinase. Biophys J 99:499–506
12. Claus RA, Dorer MJ, Bunck AC, Deigner HP (2009) Inhibition of sphingomyelin hydrolysis: targeting the lipid mediator ceramide as a key regulator of cellular fate. Curr Med Chem 16:1978–2000
13. Kitatani K, Idkowiak-Baldys J, Hannun YA (2008) The sphingolipid salvage pathway in ceramide metabolism and signaling. Cell Signal 20:1010–1018
14. Mullen TD, Hannun YA, Obeid LM (2012) Ceramide synthases at the Centre of sphingolipid metabolism and biology. Biochem J 441:789–802
15. Zelnik ID, Rozman B, Rosenfeld-Gur E, Ben-Dor S, Futerman AH (2019) A stroll down the CerS lane. Adv Exp Med Biol 1159. https://doi.org/10.1007/978-3-030-21162-2. (in press)
16. Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH Jr, Futerman AH (2008) Characterization of ceramide synthase 2: tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. J Biol Chem 283:5677–5684
17. Ginkel C, Hartmann D, vom Dorp K, Zlomuzica A, Farwanah H, Eckhardt M, Sandhoff R, Degen J, Rabionet M, Dere E, Dormann P, Sandhoff K, Willecke K (2012) Ablation of neuronal ceramide synthase 1 in mice decreases ganglioside levels and expression of myelin-associated glycoprotein in oligodendrocytes. J Biol Chem 287:41888–41902
18. Jennemann R, Rabionet M, Gorgas K, Epstein S, Dalpke A, Rothermel U, Bayerle A, van der Hoeven F, Imgrund S, Kirsch J, Nickel W, Willecke K, Riezman H, Grone HJ, Sandhoff R (2012) Loss of ceramide synthase 3 causes lethal skin barrier disruption. Hum Mol Genet 21:586–608
19. Mizutani Y, Kihara A, Igarashi Y (2005) Mammalian Lass6 and its related family members regulate synthesis of specific ceramides. Biochem J 390:263–271
20. Gosejacob D, Jager PS, Vom Dorp K, Frejno M, Carstensen AC, Kohnke M, Degen J, Dormann P, Hoch M (2016) Ceramide synthase 5 is essential to maintain C16:0-ceramide pools and contributes to the development of diet-induced obesity. J Biol Chem 291:6989–7003
21. Tidhar R, Zelnik ID, Volpert G, Ben-Dor S, Kelly S, Merrill AH Jr, Futerman AH (2018) Eleven residues determine the acyl chain specificity of ceramide synthases. J Biol Chem 293:9912–9921
22. Zhao L, Spassieva SD, Jucius TJ, Shultz LD, Shick HE, Macklin WB, Hannun YA, Obeid LM, Ackerman SL (2011) A deficiency of ceramide biosynthesis causes cerebellar Purkinje cell neurodegeneration and lipofuscin accumulation. PLoS Genet 7:e1002063
23. Jin H, Wang C, Gu D, Zhang Y, Fan S, Xing S, Wang H, Ruan H, Yang C, Lv Y, Feng H, Yao M, Qin W (2017) Liver-specific deletion of LASS2 delayed regeneration of mouse liver after partial hepatectomy. Biochem Biophys Res Commun 493:1176–1183
24. Chen Z, Han Y, Gu Y, Liu Y, Jiang Z, Zhang M, Cao X (2013) CD11c(high)CD8+ regulatory T cell feedback inhibits CD4 T cell immune response via Fas ligand-Fas pathway. J Immunol (Baltimore, Md: 1950) 190:6145–6154
25. Park WJ, Brenner O, Kogot-Levin A, Saada A, Merrill AH Jr, Pewzner-Jung Y, Futerman AH (2015) Development of pheochromocytoma in ceramide synthase 2 null mice. Endocr Relat Cancer 22:623–632
26. Barthelmes J, de Bazo AM, Pewzner-Jung Y, Schmitz K, Mayer CA, Foerch C, Eberle M, Tafferner N, Ferreiros N, Henke M, Geisslinger G, Futerman AH, Grosch S, Schiffmann S (2015) Lack of ceramide synthase 2 suppresses the development of experimental autoimmune encephalomyelitis by impairing the migratory capacity of neutrophils. Brain Behav Immun 46:280–292
27. Chen L, Lu X, Zeng T, Chen Y, Chen Q, Wu W, Yan X, Cai H, Zhang Z, Shao Q, Qin W (2014) Enhancement of DEN-induced liver tumourigenesis in hepatocyte-specific Lass2-knockout mice coincident with upregulation of the TGF-beta1-Smad4-PAI-1 axis. Oncol Rep 31:885–893
28. Imgrund S, Hartmann D, Farwanah H, Eckhardt M, Sandhoff R, Degen J, Gieselmann V, Sandhoff K, Willecke K (2009) Adult ceramide synthase 2 (CERS2)-deficient mice exhibit myelin sheath defects, cerebellar degeneration, and hepatocarcinomas. J Biol Chem 284:33549–33560
29. Pewzner-Jung Y, Brenner O, Braun S, Laviad EL, Ben-Dor S, Feldmesser E, Horn-Saban S, Amann-Zalcenstein D, Raanan C, Berkutzki T, Erez-Roman R, Ben-David O, Levy M, Holzman D, Park H, Nyska A, Merrill AH Jr, Futerman AH (2010a) A critical role for ceramide synthase 2 in liver homeostasis: II. Insights into molecular changes leading to hepatopathy. J Biol Chem 285:10911–10923
30. Pewzner-Jung Y, Park H, Laviad EL, Silva LC, Lahiri S, Stiban J, Erez-Roman R, Brugger B, Sachsenheimer T, Wieland F, Prieto M, Merrill AH Jr, Futerman AH (2010b) A critical role for ceramide synthase 2 in liver homeostasis: I. alterations in lipid metabolic pathways. J Biol Chem 285:10902–10910
31. Rabionet M, Bayerle A, Jennemann R, Heid H, Fuchser J, Marsching C, Porubsky S, Bolenz C, Guillou F, Grone HJ, Gorgas K, Sandhoff R (2015) Male meiotic cytokinesis requires ceramide synthase 3-dependent sphingolipids with unique membrane anchors. Hum Mol Genet 24:4792–4808
32. Ebel P, Imgrund S, Vom Dorp K, Hofmann K, Maier H, Drake H, Degen J, Dormann P, Eckhardt M, Franz T, Willecke K (2014) Ceramide synthase 4 deficiency in mice causes lipid alterations in sebum and results in alopecia. Biochem J 461:147–158
33. Scheffel MJ, Helke K, Lu P, Bowers JS, Ogretmen B, Garrett-Mayer E, Paulos CM, Voelkel-Johnson

C (2017) Adoptive transfer of ceramide synthase 6 deficient Splenocytes reduces the development of colitis. Sci Rep 7:15552

34. Ebel P, Vom Dorp K, Petrasch-Parwez E, Zlomuzica A, Kinugawa K, Mariani J, Minich D, Ginkel C, Welcker J, Degen J, Eckhardt M, Dere E, Dormann P, Willecke K (2013) Inactivation of ceramide synthase 6 in mice results in an altered sphingolipid metabolism and behavioral abnormalities. J Biol Chem 288:21433–21447
35. Peters F, Vorhagen S, Brodesser S, Jakobshagen K, Bruning JC, Niessen CM, Kronke M (2015) Ceramide synthase 4 regulates stem cell homeostasis and hair follicle cycling. J Invest Dermatol 135:1501–1509
36. Futerman AH, Hannun YA (2004) The complex life of simple sphingolipids. EMBO Rep 5:777–782
37. Hannun YA, Luberto C (2000) Ceramide in the eukaryotic stress response. Trends Cell Biol 10:73–80
38. Pinto SN, Silva LC, Futerman AH, Prieto M (2011) Effect of ceramide structure on membrane biophysical properties: the role of acyl chain length and unsaturation. Biochim Biophys Acta 1808:2753–2760
39. Silva LC, Ben David O, Pewzner-Jung Y, Laviad EL, Stiban J, Bandyopadhyay S, Merrill AH Jr, Prieto M, Futerman AH (2012) Ablation of ceramide synthase 2 strongly affects biophysical properties of membranes. J Lipid Res 53:430–436
40. Silva LC, Futerman AH, Prieto M (2009) Lipid raft composition modulates sphingomyelinase activity and ceramide-induced membrane physical alterations. Biophys J 96:3210–3222
41. Stiban JS, Silva LC, Futerman AH (2008) Ceramide-containing membranes: the interface between biophysics and biology. Trends Glycosci Glycotechnol 20:297–313
42. Stiban J (2019) Introduction: enigmas of sphingolipids. Adv Exp Med Biol 1159. https://doi.org/10.1007/978-3-030-21162-2. (in press)
43. Hannun YA, Obeid LM (2002) The ceramide-centric universe of lipid-mediated cell regulation: stress encounters of the lipid kind. J Biol Chem 277:25847–25850
44. Nganga R, Oleinik N, Ogretmen B (2018) Mechanisms of ceramide-dependent cancer cell death. Adv Cancer Res 140:1–25
45. Kolesnick R, Golde DW (1994) The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77:325–328
46. Maceyka M, Spiegel S (2014) Sphingolipid metabolites in inflammatory disease. Nature 510:58–67
47. Colombini M (2019) Ceramide channels. Adv Exp Med Biol 1159. https://doi.org/10.1007/978-3-030-21162-2. (in press)
48. Siskind LJ, Colombini M (2000) The lipids C2- and C16-ceramide form large stable channels. Implications for apoptosis. J Biol Chem 275:38640–38644
49. Siskind LJ, Davoody A, Lewin N, Marshall S, Colombini M (2003) Enlargement and contracture of C2-ceramide channels. Biophys J 85:1560–1575
50. Samanta S, Stiban J, Maugel TK, Colombini M (2011) Visualization of ceramide channels by transmission electron microscopy. Biochim Biophys Acta 1808:1196–1201
51. Siskind LJ, Kolesnick RN, Colombini M (2002) Ceramide channels increase the permeability of the mitochondrial outer membrane to small proteins. J Biol Chem 277:26796–26803
52. Siskind LJ, Kolesnick RN, Colombini M (2006) Ceramide forms channels in mitochondrial outer membranes at physiologically relevant concentrations. Mitochondrion 6:118–125
53. Stiban J, Fistere D, Colombini M (2006) Dihydroceramide hinders ceramide channel formation: implications on apoptosis. Apoptosis 11:773–780
54. Yamane M, Moriya S, Kokuba H (2017) Visualization of ceramide channels in lysosomes following endogenous palmitoyl-ceramide accumulation as an initial step in the induction of necrosis. Biochem Biophys Rep 11:174–181
55. Steinman RM (2012) Decisions about dendritic cells: past, present, and future. Annu Rev Immunol 30:1–22
56. Steinman RM, Cohn ZA (2007) Pillars article: identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Immunol (Baltimore, Md: 1950) 178:5–25
57. Ashany D, Savir A, Bhardwaj N, Elkon KB (1999) Dendritic cells are resistant to apoptosis through the Fas (CD95/APO-1) pathway. J Immunol (Baltimore, Md: 1950) 163:5303–5311
58. Kanto T, Kalinski P, Hunter OC, Lotze MT, Amoscato AA (2001) Ceramide mediates tumor-induced dendritic cell apoptosis. J Immunol (Baltimore, Md: 1950) 167:3773–3784
59. Sallusto F, Cella M, Danieli C, Lanzavecchia A (1995) Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 182:389–400
60. Sallusto F, Nicolo C, De Maria R, Corinti S, Testi R (1996) Ceramide inhibits antigen uptake and presentation by dendritic cells. J Exp Med 184: 2411–2416
61. Franchi L, Malisan F, Tomassini B, Testi R (2006) Ceramide catabolism critically controls survival of human dendritic cells. J Leukoc Biol 79:166–172
62. Joseph CK, Wright SD, Bornmann WG, Randolph JT, Kumar ER, Bittman R, Liu J, Kolesnick RN (1994) Bacterial lipopolysaccharide has structural similarity to ceramide and stimulates ceramide-activated protein kinase in myeloid cells. J Biol Chem 269:17606–17610

63. MacKichan ML, DeFranco AL (1999) Role of ceramide in lipopolysaccharide (LPS)-induced signaling. LPS increases ceramide rather than acting as a structural homolog. J Biol Chem 274:1767–1775
64. Harouse JM, Bhat S, Spitalnik SL, Laughlin M, Stefano K, Silberberg DH, Gonzalez-Scarano F (1991) Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. Science (New York, NY) 253:320–323
65. Yahi N, Sabatier JM, Baghdiguian S, Gonzalez-Scarano F, Fantini J (1995) Synthetic multimeric peptides derived from the principal neutralization domain (V3 loop) of human immunodeficiency virus type 1 (HIV-1) gp120 bind to galactosylceramide and block HIV-1 infection in a human CD4-negative mucosal epithelial cell line. J Virol 69:320–325
66. Alfsen A, Bomsel M (2002) HIV-1 gp41 envelope residues 650-685 exposed on native virus act as a lectin to bind epithelial cell galactosyl ceramide. J Biol Chem 277:25649–25659
67. Alfsen A, Iniguez P, Bouguyon E, Bomsel M (2001) Secretory IgA specific for a conserved epitope on gp41 envelope glycoprotein inhibits epithelial transcytosis of HIV-1. J Immunol (Baltimore, Md: 1950) 166:6257–6265
68. Lingwood CA, Branch DR (2011) The role of glycosphingolipids in HIV/AIDS. Discov Med 11:303–313
69. Magerus-Chatinet A, Yu H, Garcia S, Ducloux E, Terris B, Bomsel M (2007) Galactosyl ceramide expressed on dendritic cells can mediate HIV-1 transfer from monocyte derived dendritic cells to autologous T cells. Virology 362:67–74
70. Avota E, Gulbins E, Schneider-Schaulies S (2011) DC-SIGN mediated sphingomyelinase-activation and ceramide generation is essential for enhancement of viral uptake in dendritic cells. PLoS Pathog 7:e1001290
71. Pritzl CJ, Seo YJ, Xia C, Vijayan M, Stokes ZD, Hahm B (2015) A ceramide analogue stimulates dendritic cells to promote T cell responses upon virus infections. J Immunol (Baltimore, Md: 1950) 194:4339–4349
72. Mantovani A, Cassatella MA, Costantini C, Jaillon S (2011) Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 11:519–531
73. Wilson E, Wang E, Mullins RE, Uhlinger DJ, Liotta DC, Lambeth JD, Merrill AH Jr (1988) Modulation of the free sphingosine levels in human neutrophils by phorbol esters and other factors. J Biol Chem 263:9304–9309
74. Utsumi T, Klostergaard J, Akimaru K, Edashige K, Sato EF, Utsumi K (1992) Modulation of TNF-alpha-priming and stimulation-dependent superoxide generation in human neutrophils by protein kinase inhibitors. Arch Biochem Biophys 294:271–278
75. Ohta H, Yatomi Y, Sweeney EA, Hakomori S, Igarashi Y (1994) A possible role of sphingosine in induction of apoptosis by tumor necrosis factor-alpha in human neutrophils. FEBS Lett 355:267–270
76. Kim MY, Linardic C, Obeid L, Hannun Y (1991) Identification of sphingomyelin turnover as an effector mechanism for the action of tumor necrosis factor alpha and gamma-interferon. Specific role in cell differentiation. J Biol Chem 266:484–489
77. Dressler KA, Mathias S, Kolesnick RN (1992) Tumor necrosis factor-alpha activates the sphingomyelin signal transduction pathway in a cell-free system. Science (New York, NY) 255:1715–1718
78. Fiore S, Nicolaou KC, Caulfield T, Kataoka H, Serhan CN (1990) Evaluation of synthetic sphingosine, lysosphingolipids and glycosphingolipids as inhibitors of functional responses of human neutrophils. Biochem J 266:25–31
79. Robinson BS, Hii CS, Poulos A, Ferrante A (1997) Activation of neutral sphingomyelinase in human neutrophils by polyunsaturated fatty acids. Immunology 91:274–280
80. Ahmed N, Berridge MV (2000) Ceramides that mediate apoptosis reduce glucose uptake and transporter affinity for glucose in human leukaemic cell lines but not in neutrophils. Pharmacol Toxicol 86:114–121
81. Fuortes M, Jin W, Nathan C (1996) Ceramide selectively inhibits early events in the response of human neutrophils to tumor necrosis factor. J Leukoc Biol 59:451–460
82. Nakamura T, Abe A, Balazovich KJ, Wu D, Suchard SJ, Boxer LA, Shayman JA (1994) Ceramide regulates oxidant release in adherent human neutrophils. J Biol Chem 269:18384–18389
83. Sitrin RG, Sassanella TM, Petty HR (2011) An obligate role for membrane-associated neutral sphingomyelinase activity in orienting chemotactic migration of human neutrophils. Am J Respir Cell Mol Biol 44:205–212
84. Suchard SJ, Mansfield PJ, Boxer LA, Shayman JA (1997b) Mitogen-activated protein kinase activation during IgG-dependent phagocytosis in human neutrophils: inhibition by ceramide. J Immunol (Baltimore, Md: 1950) 158:4961–4967
85. Hinkovska-Galcheva V, Kjeldsen L, Mansfield PJ, Boxer LA, Shayman JA, Suchard SJ (1998) Activation of a plasma membrane-associated neutral sphingomyelinase and concomitant ceramide accumulation during IgG-dependent phagocytosis in human polymorphonuclear leukocytes. Blood 91:4761–4769
86. Suchard SJ, Hinkovska-Galcheva V, Mansfield PJ, Boxer LA, Shayman JA (1997a) Ceramide inhibits IgG-dependent phagocytosis in human polymorphonuclear leukocytes. Blood 89:2139–2147
87. Hinkovska-Galcheva V, Boxer L, Mansfield PJ, Schreiber AD, Shayman JA (2003) Enhanced phagocytosis through inhibition of de novo ceramide synthesis. J Biol Chem 278:974–982
88. Corriden R, Hollands A, Olson J, Derieux J, Lopez J, Chang JT, Gonzalez DJ, Nizet V (2015) Tamoxifen

augments the innate immune function of neutrophils through modulation of intracellular ceramide. Nat Commun 6:8369
89. Seumois G, Fillet M, Gillet L, Faccinetto C, Desmet C, Francois C, Dewals B, Oury C, Vanderplasschen A, Lekeux P, Bureau F (2007) De novo C16- and C24-ceramide generation contributes to spontaneous neutrophil apoptosis. J Leukoc Biol 81:1477–1486
90. Scheel-Toellner D, Wang K, Assi LK, Webb PR, Craddock RM, Salmon M, Lord JM (2004) Clustering of death receptors in lipid rafts initiates neutrophil spontaneous apoptosis. Biochem Soc Trans 32:679–681
91. Manago A, Becker KA, Carpinteiro A, Wilker B, Soddemann M, Seitz AP, Edwards MJ, Grassme H, Szabo I, Gulbins E (2015) Pseudomonas aeruginosa pyocyanin induces neutrophil death via mitochondrial reactive oxygen species and mitochondrial acid sphingomyelinase. Antioxid Redox Signal 22:1097–1110
92. Tafesse FG, Huitema K, Hermansson M, van der Poel S, van den Dikkenberg J, Uphoff A, Somerharju P, Holthuis JC (2007) Both sphingomyelin synthases SMS1 and SMS2 are required for sphingomyelin homeostasis and growth in human HeLa cells. J Biol Chem 282:17537–17547
93. Tafesse FG, Ternes P, Holthuis JC (2006) The multigenic sphingomyelin synthase family. J Biol Chem 281:29421–29425
94. Qureshi A, Subathra M, Grey A, Schey K, Del Poeta M, Luberto C (2010) Role of sphingomyelin synthase in controlling the antimicrobial activity of neutrophils against Cryptococcus neoformans. PLoS One 5:e15587
95. Symington FW, Hedges DL, Hakomori S (1985) Glycolipid antigens of human polymorphonuclear neutrophils and the inducible HL-60 myeloid leukemia line. J Immunol (Baltimore, Md: 1950) 134:2498–2506
96. Arai T, Bhunia AK, Chatterjee S, Bulkley GB (1998) Lactosylceramide stimulates human neutrophils to upregulate Mac-1, adhere to endothelium, and generate reactive oxygen metabolites in vitro. Circ Res 82:540–547
97. Iwabuchi K, Nagaoka I (2002) Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils. Blood 100:1454–1464
98. Chiricozzi E, Ciampa MG, Brasile G, Compostella F, Prinetti A, Nakayama H, Ekyalongo RC, Iwabuchi K, Sonnino S, Mauri L (2015) Direct interaction, instrumental for signaling processes, between LacCer and Lyn in the lipid rafts of neutrophil-like cells. J Lipid Res 56:129–141
99. Iwabuchi K, Prinetti A, Sonnino S, Mauri L, Kobayashi T, Ishii K, Kaga N, Murayama K, Kurihara H, Nakayama H, Yoshizaki F, Takamori K, Ogawa H, Nagaoka I (2008) Involvement of very long fatty acid-containing lactosylceramide in lactosylceramide-mediated superoxide generation and migration in neutrophils. Glycoconj J 25:357–374
100. Sonnino S, Prinetti A, Nakayama H, Yangida M, Ogawa H, Iwabuchi K (2009) Role of very long fatty acid-containing glycosphingolipids in membrane organization and cell signaling: the model of lactosylceramide in neutrophils. Glycoconj J 26:615–621
101. Nakayama H, Kurihara H, Morita YS, Kinoshita T, Mauri L, Prinetti A, Sonnino S, Yokoyama N, Ogawa H, Takamori K, Iwabuchi K (2016) Lipoarabinomannan binding to lactosylceramide in lipid rafts is essential for the phagocytosis of mycobacteria by human neutrophils. Sci Signal 9:ra101
102. Izawa K, Maehara A, Isobe M, Yasuda Y, Urai M, Hoshino Y, Ueno K, Matsukawa T, Takahashi M, Kaitani A, Shiba E, Takamori A, Uchida S, Uchida K, Maeda K, Nakano N, Yamanishi Y, Oki T, Voehringer D, Roers A, Nakae S, Ishikawa J, Kinjo Y, Shimizu T, Ogawa H, Okumura K, Kitamura T, Kitaura J (2017) Disrupting ceramide-CD300f interaction prevents septic peritonitis by stimulating neutrophil recruitment. Sci Rep 7:4298
103. Shiba E, Izawa K, Kaitani A, Isobe M, Maehara A, Uchida K, Maeda K, Nakano N, Ogawa H, Okumura K, Kitamura T, Shimizu T, Kitaura J (2017) Ceramide-CD300f binding inhibits lipopolysaccharide-induced skin inflammation. J Biol Chem 292:2924–2932
104. Izawa K, Yamanishi Y, Maehara A, Takahashi M, Isobe M, Ito S, Kaitani A, Matsukawa T, Matsuoka T, Nakahara F, Oki T, Kiyonari H, Abe T, Okumura K, Kitamura T, Kitaura J (2012) The receptor LMIR3 negatively regulates mast cell activation and allergic responses by binding to extracellular ceramide. Immunity 37:827–839
105. Matsukawa T, Izawa K, Isobe M, Takahashi M, Maehara A, Yamanishi Y, Kaitani A, Okumura K, Teshima T, Kitamura T, Kitaura J (2016) Ceramide-CD300f binding suppresses experimental colitis by inhibiting ATP-mediated mast cell activation. Gut 65:777–787
106. Kawa S, Kimura S, Hakomori S, Igarashi Y (1997) Inhibition of chemotactic motility and trans-endothelial migration of human neutrophils by sphingosine 1-phosphate. FEBS Lett 420:196–200
107. Chandru H, Boggaram V (2007) The role of sphingosine 1-phosphate in the TNF-alpha induction of IL-8 gene expression in lung epithelial cells. Gene 391:150–160
108. Baudiss K, de Paula Vieira R, Cicko S, Ayata K, Hossfeld M, Ehrat N, Gomez-Munoz A, Eltzschig HK, Idzko M (2016) C1P attenuates lipopolysaccharide-induced acute lung injury by preventing NF-kappaB activation in neutrophils. J Immunol (Baltimore, Md: 1950) 196:2319–2326

109. Italiani P, Boraschi D (2015) New insights into tissue macrophages: from their origin to the development of memory. Immune Netw 15:167–176
110. Murray PJ, Wynn TA (2011) Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 11:723–737
111. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA (2014) Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 41:14–20
112. Stout RD, Suttles J (2004) Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol 76:509–513
113. Bhargava P, Lee CH (2012) Role and function of macrophages in the metabolic syndrome. Biochem J 442:253–262
114. Prieur X, Roszer T, Ricote M (2010) Lipotoxicity in macrophages: evidence from diseases associated with the metabolic syndrome. Biochim Biophys Acta 1801:327–337
115. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2:675–680
116. Beutler B, Hoebe K, Georgel P, Tabeta K, Du X (2005) Genetic analysis of innate immunity: identification and function of the TIR adapter proteins. Adv Exp Med Biol 560:29–39
117. Andreyev AY, Fahy E, Guan Z, Kelly S, Li X, McDonald JG, Milne S, Myers D, Park H, Ryan A, Thompson BM, Wang E, Zhao Y, Brown HA, Merrill AH, Raetz CR, Russell DW, Subramaniam S, Dennis EA (2010) Subcellular organelle lipidomics in TLR-4-activated macrophages. J Lipid Res 51:2785–2797
118. Dennis EA, Deems RA, Harkewicz R, Quehenberger O, Brown HA, Milne SB, Myers DS, Glass CK, Hardiman G, Reichart D, Merrill AH Jr, Sullards MC, Wang E, Murphy RC, Raetz CR, Garrett TA, Guan Z, Ryan AC, Russell DW, McDonald JG, Thompson BM, Shaw WA, Sud M, Zhao Y, Gupta S, Maurya MR, Fahy E, Subramaniam S (2010) A mouse macrophage lipidome. J Biol Chem 285:39976–39985
119. Sims K, Haynes CA, Kelly S, Allegood JC, Wang E, Momin A, Leipelt M, Reichart D, Glass CK, Sullards MC, Merrill AH Jr (2010) Kdo2-lipid A, a TLR4-specific agonist, induces de novo sphingolipid biosynthesis in RAW264.7 macrophages, which is essential for induction of autophagy. J Biol Chem 285:38568–38579
120. Schilling JD, Machkovech HM, He L, Sidhu R, Fujiwara H, Weber K, Ory DS, Schaffer JE (2013) Palmitate and lipopolysaccharide trigger synergistic ceramide production in primary macrophages. J Biol Chem 288:2923–2932
121. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP (2011) Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 12:408–415
122. Camell CD, Nguyen KY, Jurczak MJ, Christian BE, Shulman GI, Shadel GS, Dixit VD (2015) Macrophage-specific de novo synthesis of ceramide is dispensable for Inflammasome-driven inflammation and insulin resistance in obesity. J Biol Chem 290:29402–29413
123. Serbulea V, Upchurch CM, Ahern KW, Bories G, Voigt P, DeWeese DE, Meher AK, Harris TE, Leitinger N (2018) Macrophages sensing oxidized DAMPs reprogram their metabolism to support redox homeostasis and inflammation through a TLR2-Syk-ceramide dependent mechanism. Mol Metabol 7:23–34
124. Hundal RS, Gomez-Munoz A, Kong JY, Salh BS, Marotta A, Duronio V, Steinbrecher UP (2003) Oxidized low density lipoprotein inhibits macrophage apoptosis by blocking ceramide generation, thereby maintaining protein kinase B activation and Bcl-XL levels. J Biol Chem 278:24399–24408
125. Aflaki E, Doddapattar P, Radovic B, Povoden S, Kolb D, Vujic N, Wegscheider M, Koefeler H, Hornemann T, Graier WF, Malli R, Madeo F, Kratky D (2012) C16 ceramide is crucial for triacylglycerol-induced apoptosis in macrophages. Cell Death Dis 3:e280
126. Wang SW, Hojabrpour P, Zhang P, Kolesnick RN, Steinbrecher UP, Gomez-Munoz A, Duronio V (2015) Regulation of ceramide generation during macrophage apoptosis by ASMase and de novo synthesis. Biochim Biophys Acta 1851:1482–1489
127. Zhang Y, Rao E, Zeng J, Hao J, Sun Y, Liu S, Sauter ER, Bernlohr DA, Cleary MP, Suttles J, Li B (2017) Adipose fatty acid binding protein promotes saturated fatty acid-induced macrophage cell death through enhancing ceramide production. J Immunol (Baltimore, Md: 1950) 198:798–807
128. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA (2007) Inhibition of ceramide synthesis ameliorates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 5:167–179
129. Nelson DH, Murray DK (1986) Sphingolipids inhibit insulin and phorbol ester stimulated uptake of 2-deoxyglucose. Biochem Biophys Res Commun 138:463–467
130. Kanety H, Hemi R, Papa MZ, Karasik A (1996) Sphingomyelinase and ceramide suppress insulin-induced tyrosine phosphorylation of the insulin receptor substrate-1. J Biol Chem 271:9895–9897
131. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM (1996) Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. J Biol Chem 271:13018–13022

132. Schmitz-Peiffer C, Craig DL, Biden TJ (1999) Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. J Biol Chem 274:24202–24210
133. Summers SA, Garza LA, Zhou H, Birnbaum MJ (1998) Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. Mol Cell Biol 18:5457–5464
134. Wang CN, O'Brien L, Brindley DN (1998) Effects of cell-permeable ceramides and tumor necrosis factor-alpha on insulin signaling and glucose uptake in 3T3-L1 adipocytes. Diabetes 47:24–31
135. Zhou H, Summers SA, Birnbaum MJ, Pittman RN (1998) Inhibition of Akt kinase by cell-permeable ceramide and its implications for ceramide-induced apoptosis. J Biol Chem 273:16568–16575
136. Zinda MJ, Vlahos CJ, Lai MT (2001) Ceramide induces the dephosphorylation and inhibition of constitutively activated Akt in PTEN negative U87mg cells. Biochem Biophys Res Commun 280:1107–1115
137. Salinas M, Lopez-Valdaliso R, Martin D, Alvarez A, Cuadrado A (2000) Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide-activated protein phosphatase in PC12 cells. Mol Cell Neurosci 15:156–169
138. Schubert KM, Scheid MP, Duronio V (2000) Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J Biol Chem 275:13330–13335
139. Frangioudakis G, Diakanastasis B, Liao BQ, Saville JT, Hoffman NJ, Mitchell TW, Schmitz-Peiffer C (2013) Ceramide accumulation in L6 skeletal muscle cells due to increased activity of ceramide synthase isoforms has opposing effects on insulin action to those caused by palmitate treatment. Diabetologia 56:2697–2701
140. Stratford S, Hoehn KL, Liu F, Summers SA (2004) Regulation of insulin action by ceramide: dual mechanisms linking ceramide accumulation to the inhibition of Akt/protein kinase B. J Biol Chem 279:36608–36615
141. Teruel T, Hernandez R, Lorenzo M (2001) Ceramide mediates insulin resistance by tumor necrosis factor-alpha in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. Diabetes 50:2563–2571
142. Chen CL, Lin CF, Chang WT, Huang WC, Teng CF, Lin YS (2008) Ceramide induces p38 MAPK and JNK activation through a mechanism involving a thioredoxin-interacting protein-mediated pathway. Blood 111:4365–4374
143. Hage Hassan R, Pacheco de Sousa AC, Mahfouz R, Hainault I, Blachnio-Zabielska A, Bourron O, Koskas F, Gorski J, Ferre P, Foufelle F, Hajduch E (2016) Sustained action of ceramide on THE insulin signaling pathway in muscle cells: implication of the double-stranded RNA-activated protein kinase. J Biol Chem 291:3019–3029
144. Adams JM 2nd, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ (2004) Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. Diabetes 53:25–31
145. Blachnio-Zabielska AU, Chacinska M, Vendelbo MH, Zabielski P (2016) The crucial role of C18-Cer in fat-induced skeletal muscle insulin resistance. Cell Physiol Biochem 40:1207–1220
146. Coen PM, Dube JJ, Amati F, Stefanovic-Racic M, Ferrell RE, Toledo FG, Goodpaster BH (2010) Insulin resistance is associated with higher intramyocellular triglycerides in type I but not type II myocytes concomitant with higher ceramide content. Diabetes 59:80–88
147. Fillmore N, Keung W, Kelly SE, Proctor SD, Lopaschuk GD, Ussher JR (2015) Accumulation of ceramide in slow-twitch muscle contributes to the development of insulin resistance in the obese JCR:LA-cp rat. Exp Physiol 100:730–741
148. Pickersgill L, Litherland GJ, Greenberg AS, Walker M, Yeaman SJ (2007) Key role for ceramides in mediating insulin resistance in human muscle cells. J Biol Chem 282:12583–12589
149. Turpin SM, Lancaster GI, Darby I, Febbraio MA, Watt MJ (2006) Apoptosis in skeletal muscle myotubes is induced by ceramides and is positively related to insulin resistance. Am J Physiol Endocrinol Metab 291:E1341–E1350
150. JeBailey L, Wanono O, Niu W, Roessler J, Rudich A, Klip A (2007) Ceramide- and oxidant-induced insulin resistance involve loss of insulin-dependent Rac-activation and actin remodeling in muscle cells. Diabetes 56:394–403
151. Bruce CR, Risis S, Babb JR, Yang C, Kowalski GM, Selathurai A, Lee-Young RS, Weir JM, Yoshioka K, Takuwa Y, Meikle PJ, Pitson SM, Febbraio MA (2012) Overexpression of sphingosine kinase 1 prevents ceramide accumulation and ameliorates muscle insulin resistance in high-fat diet-fed mice. Diabetes 61:3148–3155
152. Lahiri S, Park H, Laviad EL, Lu X, Bittman R, Futerman AH (2009) Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an acyl-CoA chain length-dependent manner. J Biol Chem 284:16090–16098
153. Bruce CR, Risis S, Babb JR, Yang C, Lee-Young RS, Henstridge DC, Febbraio MA (2013) The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. Endocrinology 154:65–76
154. Ussher JR, Koves TR, Cadete VJ, Zhang L, Jaswal JS, Swyrd SJ, Lopaschuk DG, Proctor SD, Keung W, Muoio DM, Lopaschuk GD (2010) Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. Diabetes 59:2453–2464

155. Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, Dogra S, Ohman MK, Takeda K, Sugii S, Pewzner-Jung Y, Futerman AH, Summers SA (2014) CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. Cell Metab 20:687–695
156. Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, Mauer J, Xu E, Hammerschmidt P, Bronneke HS, Trifunovic A, LoSasso G, Wunderlich FT, Kornfeld JW, Bluher M, Kronke M, Bruning JC (2014) Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. Cell Metab 20:678–686
157. Stiban J, Perera M (2015) Very long chain ceramides interfere with C16-ceramide-induced channel formation: A plausible mechanism for regulating the initiation of intrinsic apoptosis. Biochim Biophys Acta 1848:561–567
158. Holland WL, Bikman BT, Wang LP, Yuguang G, Sargent KM, Bulchand S, Knotts TA, Shui G, Clegg DJ, Wenk MR, Pagliassotti MJ, Scherer PE, Summers SA (2011) Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest 121:1858–1870
159. Galbo T, Perry RJ, Jurczak MJ, Camporez JP, Alves TC, Kahn M, Guigni BA, Serr J, Zhang D, Bhanot S, Samuel VT, Shulman GI (2013) Saturated and unsaturated fat induce hepatic insulin resistance independently of TLR-4 signaling and ceramide synthesis in vivo. Proc Natl Acad Sci U S A 110:12780–12785
160. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, Ravussin E, Stephens JM, Dixit VD (2011) The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 17:179–188
161. Ferrara JLM, Chaudhry MS (2018) GVHD: biology matters. Blood advances 2:3411–3417
162. Rotolo JA, Stancevic B, Lu SX, Zhang J, Suh D, King CG, Kappel LW, Murphy GF, Liu C, Fuks Z, van den Brink MR, Kolesnick R (2009) Cytolytic T cells induce ceramide-rich platforms in target cell membranes to initiate graft-versus-host disease. Blood 114:3693–3706
163. Sofi MH, Heinrichs J, Dany M, Nguyen H, Dai M, Bastian D, Schutt S, Wu Y, Daenthanasanmak A, Gencer S, Zivkovic A, Szulc Z, Stark H, Liu C, Chang YJ, Ogretmen B, Yu XZ (2017) Ceramide synthesis regulates T cell activity and GVHD development. JCI Insight 2
164. Schiffmann S, Hartmann D, Fuchs S, Birod K, Ferreiros N, Schreiber Y, Zivkovic A, Geisslinger G, Grosch S, Stark H (2012b) Inhibitors of specific ceramide synthases. Biochimie 94:558–565
165. Stoffel B, Bauer P, Nix M, Deres K, Stoffel W (1998) Ceramide-independent CD28 and TCR signaling but reduced IL-2 secretion in T cells of acid sphingomyelinase-deficient mice. Eur J Immunol 28:874–880
166. Bai A, Kokkotou E, Zheng Y, Robson SC (2015) Role of acid sphingomyelinase bioactivity in human CD4+ T-cell activation and immune responses. Cell Death Dis 6:e1828
167. Manoochehri Khoshinani H, Afshar S, Najafi R (2016) Hypoxia: A double-edged sword in cancer therapy. Cancer Investig 34:536–545
168. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R (2014) Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature 513:559–563
169. Renner K, Singer K, Koehl GE, Geissler EK, Peter K, Siska PJ, Kreutz M (2017) Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. Front Immunol 8:248
170. Gude DR, Alvarez SE, Paugh SW, Mitra P, Yu J, Griffiths R, Barbour SE, Milstien S, Spiegel S (2008) Apoptosis induces expression of sphingosine kinase 1 to release sphingosine-1-phosphate as a "come-and-get-me" signal. FASEB J 22:2629–2638
171. Weigert A, Johann AM, von Knethen A, Schmidt H, Geisslinger G, Brune B (2006) Apoptotic cells promote macrophage survival by releasing the anti-apoptotic mediator sphingosine-1-phosphate. Blood 108:1635–1642
172. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 101:890–898
173. Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35
174. Hughes JE, Srinivasan S, Lynch KR, Proia RL, Ferdek P, Hedrick CC (2008) Sphingosine-1-phosphate induces an antiinflammatory phenotype in macrophages. Circ Res 102:950–958
175. Weigert A, Tzieply N, von Knethen A, Johann AM, Schmidt H, Geisslinger G, Brune B (2007) Tumor cell apoptosis polarizes macrophages role of sphingosine-1-phosphate. Mol Biol Cell 18:3810–3819
176. Weigert A, Schiffmann S, Sekar D, Ley S, Menrad H, Werno C, Grosch S, Geisslinger G, Brune B (2009) Sphingosine kinase 2 deficient tumor xenografts show impaired growth and fail to polarize macrophages towards an anti-inflammatory phenotype. Int J Cancer 125:2114–2121
177. Li G, Liu D, Kimchi ET, Kaifi JT, Qi X, Manjunath Y, Liu X, Deering T, Avella DM, Fox T, Rockey DC, Schell TD, Kester M, Staveley-O'Carroll KF (2018) Nanoliposome C6-ceramide increases the anti-tumor immune response and slows growth of liver tumors in mice. Gastroenterology 154:1024–1036.e1029
178. Liu F, Li X, Lu C, Bai A, Bielawski J, Bielawska A, Marshall B, Schoenlein PV, Lebedyeva IO, Liu

K (2016) Ceramide activates lysosomal cathepsin B and cathepsin D to attenuate autophagy and induces ER stress to suppress myeloid-derived suppressor cells. Oncotarget 7:83907–83925
179. Hollmann C, Werner S, Avota E, Reuter D, Japtok L, Kleuser B, Gulbins E, Becker KA, Schneider-Schaulies J, Beyersdorf N (2016) Inhibition of acid Sphingomyelinase allows for selective targeting of CD4+ conventional versus Foxp3+ regulatory T cells. J Immunol (Baltimore, Md: 1950) 197:3130–3141
180. Zhou Y, Salker MS, Walker B, Munzer P, Borst O, Gawaz M, Gulbins E, Singh Y, Lang F (2016) Acid Sphingomyelinase (ASM) is a negative regulator of regulatory T cell (Treg) development. Cell Physiol Biochem 39:985–995
181. Reich DS, Lucchinetti CF, Calabresi PA (2018) Multiple Sclerosis. N Engl J Med 378:169–180
182. Schiffmann S, Ferreiros N, Birod K, Eberle M, Schreiber Y, Pfeilschifter W, Ziemann U, Pierre S, Scholich K, Grosch S, Geisslinger G (2012a) Ceramide synthase 6 plays a critical role in the development of experimental autoimmune encephalomyelitis. J Immunol (Baltimore, Md: 1950) 188:5723–5733
183. Eberle M, Ebel P, Wegner MS, Mannich J, Tafferner N, Ferreiros N, Birod K, Schreiber Y, Krishnamoorthy G, Willecke K, Geisslinger G, Grosch S, Schiffmann S (2014) Regulation of ceramide synthase 6 in a spontaneous experimental autoimmune encephalomyelitis model is sex dependent. Biochem Pharmacol 92:326–335
184. Eberle M, Ebel P, Mayer CA, Barthelmes J, Tafferner N, Ferreiros N, Ulshofer T, Henke M, Foerch C, de Bazo AM, Grosch S, Geisslinger G, Willecke K, Schiffmann S (2015) Exacerbation of experimental autoimmune encephalomyelitis in ceramide synthase 6 knockout mice is associated with enhanced activation/migration of neutrophils. Immunol Cell Biol 93:825–836
185. Becker KA, Halmer R, Davies L, Henry BD, Ziobro-Henry R, Decker Y, Liu Y, Gulbins E, Fassbender K, Walter S (2017) Blockade of experimental multiple sclerosis by inhibition of the acid Sphingomyelinase/ceramide system. Neurosignals 25:88–97
186. Sharma S, Mathur AG, Pradhan S, Singh DB, Gupta S (2011) Fingolimod (FTY720): first approved oral therapy for multiple sclerosis. J Pharmacol Pharmacother 2:49–51
187. Singh M, Cugati G, Singh P, Singh AK (2011) Fingolimod: the first oral drug approved by food and drug administration; A breakthrough in treatment of multiple sclerosis. J Pharm Bioallied Sci 3:460–461
188. Brinkmann V, Cyster JG, Hla T (2004) FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg 4:1019–1025
189. Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457
190. Matloubian M, Lo CG, Cinamon G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 427:355–360
191. Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thornton R, Shei GJ, Card D, Keohane C, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science (New York, NY) 296:346–349
192. Baumgart DC, Sandborn WJ (2012) Crohn's disease. Lancet (London, England) 380:1590–1605
193. Danese S, Fiocchi C (2011) Ulcerative colitis. N Engl J Med 365:1713–1725
194. De Palma C, Meacci E, Perrotta C, Bruni P, Clementi E (2006) Endothelial nitric oxide synthase activation by tumor necrosis factor alpha through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 phosphate receptors: a novel pathway relevant to the pathophysiology of endothelium. Arterioscler Thromb Vasc Biol 26:99–105
195. Pettus BJ, Bielawski J, Porcelli AM, Reames DL, Johnson KR, Morrow J, Chalfant CE, Obeid LM, Hannun YA (2003) The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-alpha. FASEB J 17:1411–1421
196. Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P, Walker-Smith JA, MacDonald TT (1994) Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 106:1455–1466
197. Ngo B, Farrell CP, Barr M, Wolov K, Bailey R, Mullin JM, Thornton JJ (2010) Tumor necrosis factor blockade for treatment of inflammatory bowel disease: efficacy and safety. Curr Mol Pharmacol 3:145–152
198. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N (2008) Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest 118:560–570
199. Spiegel S, Foster D, Kolesnick R (1996) Signal transduction through lipid second messengers. Curr Opin Cell Biol 8:159–167
200. Sakata A, Ochiai T, Shimeno H, Hikishima S, Yokomatsu T, Shibuya S, Toda A, Eyanagi R, Soeda S (2007) Acid sphingomyelinase inhibition suppresses lipopolysaccharide-mediated release of inflammatory cytokines from macrophages and

protects against disease pathology in dextran sulphate sodium-induced colitis in mice. Immunology 122:54–64

201. Daniel C, Sartory N, Zahn N, Geisslinger G, Radeke HH, Stein JM (2007) FTY720 ameliorates Th1-mediated colitis in mice by directly affecting the functional activity of CD4+CD25+ regulatory T cells. J Immunol (Baltimore, Md: 1950) 178:2458–2468
202. Song J, Matsuda C, Kai Y, Nishida T, Nakajima K, Mizushima T, Kinoshita M, Yasue T, Sawa Y, Ito T (2008) A novel sphingosine 1-phosphate receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukin-10 gene-deficient mice. J Pharmacol Exp Ther 324:276–283
203. Maines LW, Fitzpatrick LR, French KJ, Zhuang Y, Xia Z, Keller SN, Upson JJ, Smith CD (2008) Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. Dig Dis Sci 53:997–1012
204. Pulkoski-Gross MJ, Uys JD, Orr-Gandy KA, Coant N, Bialkowska AB, Szulc ZM, Bai A, Bielawska A, Townsend DM, Hannun YA, Obeid LM, Snider AJ (2017) Novel sphingosine kinase-1 inhibitor, LCL351, reduces immune responses in murine DSS-induced colitis. Prostaglandins Other Lipid Mediat 130:47–56
205. Snider AJ, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Hannun YA, Obeid LM (2009) A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. FASEB J 23:143–152
206. Danese S, Furfaro F, Vetrano S (2018) Targeting S1P in inflammatory bowel disease: new avenues for modulating intestinal leukocyte migration. J Crohns Colitis 12:S678–s686
207. Peyrin-Biroulet L, Christopher R, Behan D, Lassen C (2017) Modulation of sphingosine-1-phosphate in inflammatory bowel disease. Autoimmun Rev 16:495–503
208. Kim YR, Volpert G, Shin KO, Kim SY, Shin SH, Lee Y, Sung SH, Lee YM, Ahn JH, Pewzner-Jung Y, Park WJ, Futerman AH, Park JW (2017) Ablation of ceramide synthase 2 exacerbates dextran sodium sulphate-induced colitis in mice due to increased intestinal permeability. J Cell Mol Med 21:3565–3578
209. Oertel S, Scholich K, Weigert A, Thomas D, Schmetzer J, Trautmann S, Wegner MS, Radeke HH, Filmann N, Brune B, Geisslinger G, Tegeder I, Grosch S (2017) Ceramide synthase 2 deficiency aggravates AOM-DSS-induced colitis in mice: role of colon barrier integrity. Cell Mol Life Sci CMLS 74:3039–3055
210. Helke K, Angel P, Lu P, Garrett-Mayer E, Ogretmen B, Drake R, Voelkel-Johnson C (2018) Ceramide synthase 6 deficiency enhances inflammation in the DSS model of colitis. Sci Rep 8:1627

16

Bioactive Lipids in Cancer, Inflammation and Related Diseases

Acute and Chronic Mild Traumatic Brain Injury Differentially Changes Levels of Bioactive Lipids in the CNS Associated with Headache

Emma Leishman, Phillip E. Kunkler, Joyce H. Hurley, Sally Miller, and Heather B. Bradshaw

Abstract

Headache is a common complaint after mild traumatic brain injury (mTBI). Changes in the CNS lipidome were previously associated with acrolein-induced headache in rodents. mTBI caused similar headache-like symptoms in rats; therefore, we tested the hypothesis that mTBI might likewise alter the lipidome. Using a stereotaxic impactor, rats were given either a single mTBI or a series of 4 mTBIs 48 h apart. 72 h later for single mTBI and 7 days later for repeated mTBI, the trigeminal ganglia (TG), trigeminal nucleus (TNC), and cerebellum (CER) were isolated. Using HPLC/MS/MS, ~80 lipids were measured in each tissue and compared to sham controls. mTBI drove widespread alterations in lipid levels. Single mTBI increased arachidonic acid and repeated mTBI increased prostaglandins in all 3 tissue types. mTBI affected multiple TRPV agonists, including N-arachidonoyl ethanolamine (AEA), which increased in the TNC and CER after single mTBI. After repeated mTBI, AEA increased in the TG, but decreased in the TNC. Common to all tissue types in single and repeated mTBI was an increase the AEA metabolite, N-arachidonoyl glycine, a potent activator of microglial migration. Changes in the CNS lipidome associated with mTBI likely play a role in headache and in long-term neurodegenerative effects of repeated mTBI.

Keywords

mTBI · Endocannabinoids · TRP · Lipidomics · Lipoamine · Acyl amino acid · Acyl amide · Docosahexaenoyl ethanolamine · N-arachidonoyl glycine · BV-2

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16.1 Traumatic Brain Injury, Headache, and Endogenous Cannabinoids

~40 million people worldwide endure a mild traumatic brain injury (mTBI; also known as concussion) each year [1], accounting for over 75% of brain injuries [2]. Headache is a common complaint after mTBI [2–6]. In studies of TBI patient populations in the United States, around 70% of participants reported having headaches during the first-year post-injury [3, 5]. Demonstrating that post-mTBI headache is a global phenomenon, a study of TBI patients in China found that, regardless of TBI severity, 58% of patients had headaches at 3 months post-injury, 54% at 6 months, and 49% at 1 year [6]. In contrast, only 12% of the patients reported pre-injury headaches [6]. Often, post-mTBI headaches are migraine-like [4, 6–9]. For example, in the study by Xu and colleagues, around 70% of the headaches described by TBI patients were classified as migraine-type [6]. Activation of the trigeminovascular system (TVS), the trigeminal innervation of cranial vasculature [10], is hypothesized to underlie migraine pain [11]. Sensitization can occur after repeated TVS activation [11]. After the initial mTBI, there is a secondary inflammatory biochemical insult that occurs days later [12, 13]. These biochemical changes may promote headaches through the activity of inflammatory mediators, many of which are bioactive lipids, that are produced during chronic immune responses [4, 8].

Bioactive lipids such as endogenous cannabinoids (eCBs), the endogenous activators of CB_1 and CB_2 cannabinoid receptors [14–17], help regulate inflammation [17] and have emerging roles in the TVS [18]. Brain levels of the eCB 2-arachidonoyl glycerol (2-AG) [15, 16] significantly increased in a mouse model of TBI, starting at 4 h post-injury [19]. As 2-AG was neuroprotective after TBI [12, 19, 20], it was hypothesized that the increase in 2-AG was a compensatory mechanism to mitigate the damage of pro-inflammatory mediators released after injury [19]. The effects of 2-AG were mostly mediated by CB_1 [19]. However, CB_2 agonists were also neuroprotective in rodent TBI models by reducing blood-brain barrier disruption, as measured by sodium fluorescein uptake, which prevented the entry of pro-inflammatory immune cells, and by attenuating microglial activation, as measured by immunohistochemistry [21]. Cannabinoid receptor activation is hypothesized to reduce headache pain [4, 18], suggesting convergent biochemistry between headache and TBI.

16.2 Lipoamines as Endogenous Activators of Transient Receptor Potential (TRP) Channels

TRP channels are ubiquitous ligand-gated ion channels that serve a variety of functions throughout the body [22–31]. There are 28 known TRP channels in mammalian systems; however, they are arguably understudied to date as their endogenous ligands, distributions, and signaling properties are still in question [26, 29, 31]. In the context of disease, and more specifically the symptom of pain, TRP vanilloid type 1 (TRPV1) has been the focus of intense study and may represent the TRP channel being the most studied overall [23, 27, 28, 31–34]. More than a dozen bioactive lipids have been shown to activate TRPV1 [31, 35–40]. Our group recently identified 8 of these [41]. Given the ubiquity of TRPs, it is not surprising that they appear to have interconnected responses with other TRPs and GPCRs. A combination of TRPV1-TRP ankyrin 1 (TRPA1) activity has been observed in an animal model for headache wherein ablation of TRPV1 in the trigeminal ganglia (TG) inhibited TVS activation driven by TRPA1 agonists [42]. We recently showed that TRPA1 activation by acrolein drives increases in TRPV1 endogenous bioactive lipids in the CNS [43].

Many endogenous TRPV ligands are lipoamines, which are conjugations of fatty acids and amines [22, 44–46]. The eCB *N*-arachidonoyl ethanolamine (AEA) [14] is a lipoamine that activates TRPV1, as well as cannabinoid receptors [35]. Additional lipoamines activate TRPV1 such as the *N*-acyl ethanolamines (NAEs) *N*-oleoyl

ethanolamine (OEA) [37, 38], *N*-linoleoyl ethanolamine (LEA), and *N*-docosahexaenoyl ethanolamine (DEA) [41], and certain lipoamines derived from arachidonic acid (AA) such as *N*-arachidonoyl GABA (A-GABA) [41] and *N*-arachidonoyl taurine (A-Taur) [39]. *N*-arachidonoyl serine (A-Ser) was neuroprotective in a mouse model of TBI, and these effects were attenuated with a TRPV1 antagonist, as well as a CB_2 antagonist [47, 48].

16.3 Bioactive Lipid Levels Change in a Model of Headache

Showing cross-talk with TRPV1, TRPA1 is expressed in sensory neurons [49]. The TRPA1 agonist acrolein is an irritant and a major component of air pollution [50, 51]. Molecular mechanisms of inflammatory pain and hypersensitization of the TVS caused by irritants like acrolein partially depend on TRPA1 activity [52, 53]. In a rat model of headache driven by exposure to acrolein, TVS sensitization [53] and headache-like behaviors [54] were correlated with altered levels of bioactive lipids in the TVS and the cerebellum (CER), such as eCBs and endogenous lipoamine TPRV1 ligands [43]. TVS tissues analyzed were the TG, where cell bodies of trigeminal nerve fibers are located [10], and the trigeminal nucleus caudalis (TNC), part of the brainstem which receives sensory signals from the TG and projects them onto thalamo-cortical pathways [55]. These experiments provided evidence that acrolein exposure drives changes in CNS lipids, supporting a working hypothesis that both TRPA1 and TRPV1 activity play a role in headache.

Exposure to a single mTBI caused headache-like symptoms in rats [56, 57]. Specifically, mTBI caused periorbital allodynia, meaning that stimuli that were non-nociceptive before the injury became painful after the injury [56, 57]. Providing more evidence of alterations in the TVS that could support headache post-TBI, levels of pro-nociceptive peptides were upregulated in the TNC after TBI [56, 57]. Periorbital allodynia was a marker of headache-like symptoms measured in the acrolein model [54], showing these very different models have similar effects on measures of headache-like pain in rodents. Given that headache driven by acrolein exposure was linked to alterations in bioactive lipids [43], we wanted to know whether headache caused by a very different model, mTBI, would drive similar alterations in lipid levels and whether the effects of repeated mTBIs would differ from the effects of a single mTBI. To test the hypothesis that acute and repeated mTBI alters the CNS lipidome, we performed targeted lipidomics screens of the TG, TNC and the CER, from rats exposed to single or repeated mTBI. Analyzing 74 lipoamine structural analogs, 2-acyl glycerol structural analogs, prostaglandins (PGs), and free fatty acids through targeted high-pressure liquid chromatography-coupled tandem mass spectrometry (HPLC/MS/MS) we were able to determine how this lipidome is modified. Both single and repeated mTBI modified many bioactive lipids including increased levels of the AEA metabolite [58] and GPR18 ligand [59], *N*-arachidonoyl glycine (NAGly), and alterations of TRPV1 and CB ligands. Increased levels of PGs were associated with repeated mTBI. These findings may shed new light on how bioactive lipids in the CNS play a role in headache associated with TBI.

16.4 mTBI Protocols, Tissue Collection, and HPLC/MS/MS Analysis

All animal procedures were approved by the Institutional Animal Care and Use Committee of Indiana University School of Medicine and were in accordance with the ethics guidelines set by the International Association for the Study of Pain [60]. In total, 24 adult male (170–250 g) Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were used: 12 were assigned to the single mTBI paradigm and 12 were assigned to the repeated mTBI paradigm. Rats were housed in pairs with food and water available ad libitum, and were kept on a standard light/dark cycle. Using an Impact One stereotaxic impactor (Leica Microsystems, Wetzlar, Germany) with a 3 mm

diameter tip, mTBI was induced by 4.0 m/s impact to 2.0 mm depth on the right hemisphere adjacent to bregma and sagittal sutures. This was performed once on single mTBI rats and performed 4 times, with 48 h between each impact, on repeated mTBI rats. 6 of the 12 rats in each paradigm (single or repeated) received mTBI and the other 6 received a sham treatment.

To better model concussion, the injury was a closed-head injury and the rats did not undergo craniotomy [61]. Confirming a mild injury, the impact did not damage the skull. As reviewed by Fehily and Fitzgerald, other rodent models of repeated mTBI use similar parameters [62]. For example, a study by David Brody's group described modeling repeated mTBI in mice by striking the exposed, intact skull with a rubber tip at 5.0 m/s to a depth of 3.3 mm [63]. Although this injury caused cognitive deficits, lasting microglial activation and disruption to white-matter axonal cytoskeletal integrity, there was no damage to the skull or any readily observable damage to the underlying brain tissue [63]. Bolton and Saatman tested various impact depths in a closed-head mouse model of repeated mTBI and found that 2.0 mm impact depth did not damage the skull but caused gliosis which was worsened when mTBI was repeated every 24 h [64]. Thus, our parameters are consistent with models employed by other groups studying mTBI in rodents. Rats were sacrificed via decapitation, either 72 h after the single mTBI or 1 week after the last session in repeated mTBI. Brains were immediately removed. Following removal of the brain, the left TG was removed from the skull and frozen on dry ice. The brains were dissected into targeted regions: TNC and CER, and frozen on dry ice. TG, TNC and CER samples were stored at −80 °C before being processed for lipid extraction.

Tissue extracts were performed as previously described [41, 43, 46, 65–72]. Lipids were analyzed using an Applied Biosystems API 3000 triple quadrupole mass spectrometer with electrospray ionization (Foster City, CA, USA) as previously described [43]. Levels of each compound were determined by running each sample using a multiple-reactions monitoring method (MRM) tailored for each group of structurally similar compounds (Fig. 16.1). HPLC/MS/MS data was analyzed using Analyst software (Applied Biosystems) as previously described [41, 43, 46, 65–72]. Chromatograms were generated by determining the retention time of analytes from the C18 analytical column with a [M−1] or [M + 1] parent peak and a fragmentation peak corresponding to the programmed values. Extraction efficiency was calculated with the d_8NAGly or d_8AEA-spiked recovery vial as a standard as done previously [41, 43, 46, 65–72]. For each individual lipid in the TG, TNC and CER, concentrations in moles per gram of wet tissue weight adjusted for percent recovery from mTBI animals were compared to sham using a one-way ANOVA. Statistical tests were carried out using SPSS Statistics (IBM, Armonk, NY, USA) and significance was defined as $p < 0.05$ and trending significance was defined as $p < 0.10$. Using ANOVAs instead of more flexible t-tests provides more constraints on this type of larger data sets.

As a first level of analysis presented here, the percentage of the detected lipids affected by single and repeated mTBI in each tissue type was calculated. To calculate the percent of the lipids detected that were unaffected by mTBI, the number of changes ($p < 0.10$) in a tissue type for a specific condition (single or repeated) was first subtracted from the number of lipids detected in that tissue (which represents the number of possible changes). This number was then divided by the number of lipids detected and multiplied by 100. For example, in the TNC in the repeated mTBI condition, there were 73 lipids detected and 29 of them changed with repeated mTBI, meaning that 44 did not change. 44 divided by 73 times 100 is 60.27%, so 60.27% of the detected lipidome did not change with repeated mTBI in the TNC. This percentage can be viewed in the left, blue columns of Fig. 16.2. The next portion of Fig. 16.2 (middle, green) gives the percentage of the lipids detected in each tissue that increased in mTBI. Sticking with the repeated mTBI TNC, of the 29 changes that were detected, 14 of them were increases. 14 is then divided by the number of lipids detected (73 in this case) and multiplied

***N*-acyl alanine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl alanine	**326.5**	**88.09**
N-stearoyl alanine	**354.55**	**88.09**
N-oleoyl alanine	**352.53**	**88.09**
N-linoleoyl alanine	**350.52**	**88.09**
N-arachidonoyl alanine	**374.5**	**88.09**
N-docosahexaenoyl alanine	**398.56**	**88.09**
***N*-acyl dopamine**	**$[M - H]^-$**	**Fragment**
N-oleoyl dopamine	**416.3**	**123.2**
N-arachidonoyl dopamine	**438.4**	**123.2**
***N*-acyl ethanolamine**	**$[M + H]^+$**	**Fragment**
N-palmitoyl ethanolamine	**300.29**	**62.1**
N-stearoyl ethanolamine	**328.3**	**62.1**
N-oleoyl ethanolamine	**326.3**	**62.1**
N-linoleoyl ethanolamine	**324.3**	**62.1**
N-arachidonoyl ethanolamine	**348.3**	**62.1**
N-docosahexaenoyl ethanolamine	**372.6**	**62.1**
***N*-acyl GABA**	**$[M - H]^-$**	**Fragment**
N-palmitoyl GABA	**340.54**	**102.1**
N-stearoyl GABA	**368.58**	**102.1**
N-oleoyl GABA	**366.57**	**102.1**
N-linoleoyl GABA	**364.54**	**102.1**
N-arachidonoyl GABA	**388.57**	**102.1**
N-docosahexaenoyl GABA	**412.59**	**102.1**
***N*-acyl glycine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl glycine	**312.26**	**74.2**
N-stearoyl glycine	**340.3**	**74.2**
N-oleoyl glycine	**338.3**	**74.2**
N-linoleoyl glycine	**336.3**	**74.2**
N-arachidonoyl glycine	**360.3**	**74.2**
N-docosahexaenoyl glycine	**384.3**	**74.2**
***N*-acyl leucine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl leucine	**368.58**	**130.1**
N-stearoyl leucine	**396.63**	**130.1**
N-oleoyl leucine	**394.61**	**130.1**
N-linoleoyl leucine	**392.6**	**130.1**
N-docosahexaenoyl leucine	**440.64**	**130.1**
***N*-acyl methionine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl methionine	**386.62**	**148.2**
N-stearoyl methionine	**414.64**	**148.2**
N-oleoyl methionine	**412.65**	**148.2**
N-linoleoyl methionine	**410.64**	**148.2**
N-arachidonoyl methionine	**434.66**	**148.2**
N-docosahexaenoyl methionine	**458.68**	**148.2**
***N*-acyl phenylalanine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl phenylalanine	**402.59**	**164.1**
N-stearoyl phenylalanine	**430.65**	**164.1**
N-oleoyl phenylalanine	**428.63**	**164.1**
N-linoleoyl phenylalanine	**426.61**	**164.1**
N-arachidonoyl phenylalanine	**450.64**	**164.1**
N-docosahexaenoyl phenylalanine	**474.66**	**164.1**

***N*-acyl proline**	**$[M - H]^-$**	**Fragment**
N-palmitoyl proline	**352.53**	**114.12**
N-stearoyl proline	**380.59**	**114.12**
N-oleoyl proline	**378.31**	**114.12**
N-linoleoyl proline	**376.56**	**114.12**
N-arachidonoyl proline	**400.58**	**114.12**
N-docosahexaenoyl proline	**424.6**	**114.12**
***N*-acyl serine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl serine	**342.3**	**74**
N-stearoyl serine	**370.3**	**74**
N-oleoyl serine	**368.3**	**74**
N-linoleoyl serine	**366.27**	**74**
N-arachidonoyl serine	**390.3**	**74**
N-docosahexaenoyl serine	**414.3**	**74**
***N*-acyl taurine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl taurine	**362.6**	**124**
N-stearoyl taurine	**390.6**	**124**
N-oleoyl taurine	**388.6**	**124**
N-arachidonoyl taurine	**410.6**	**124**
***N*-acyl tryptophan**	**$[M - H]^-$**	**Fragment**
N-palmitoyl tryptophan	**441.63**	**203.1**
N-stearoyl tryptophan	**469.68**	**203.1**
N-oleoyl tryptophan	**467.67**	**203.1**
N-linoleoyl tryptophan	**465.65**	**203.1**
N-arachidonoyl tryptophan	**489.67**	**203.1**
N-docosahexaenoyl tryptophan	**513.69**	**203.1**
***N*-acyl tyrosine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl tyrosine	**418.59**	**180.18**
N-stearoyl tyrosine	**446.65**	**180.18**
N-oleoyl tyrosine	**444.63**	**180.18**
N-linoleoyl tyrosine	**442.61**	**180.18**
N-arachidonoyl tyrosine	**466**	**180.18**
N-docosahexaenoyl tyrosine	**490.66**	**180.18**
***N*-acyl valine**	**$[M - H]^-$**	**Fragment**
N-palmitoyl valine	**354.31**	**116.31**
N- stearoyl valine	**382.6**	**116.14**
N-oleoyl valine	**380.59**	**116.14**
N-linoleoyl valine	**378.58**	**116.14**
N-docosahexaenoyl valine	**426.62**	**116.14**
2-acyl glycerol	**$[M + H]^+$**	**Fragment**
2-palmitoyl glycerol	**331.5**	**239.5**
2-oleoyl glycerol	**357.5**	**265.2**
2-linoleoyl glycerol	**355.5**	**245**
2-arachidonoyl glycerol	**379.3**	**287.5**
Free Fatty Acids	**$[M - H]^-$**	**Fragment**
Oleic acid	**281.5**	**263**
Linoleic acid	**279.5**	**261**
Arachidonic acid	**303.5**	**285**
PhosphoNAEs	**$[M - H]^-$**	**Fragment**
PhosphoLEA	**403.5**	**58.5**
Prostaglandins	**$[M - H]^-$**	**Fragment**
PGE_2	**351.2**	**315**
$PGF_{2\alpha}$	**353.3**	**309.2**
6-keto-$PGF_{1\alpha}$	**369.3**	**206.9**

Fig. 16.1 Lipids in HPLC/MS/MS screening library with parent ion and fragment ion masses. Lipids are grouped by amide family and all members of that lipid family are screened in a multiple reactions monitoring (MRM) method. The parent ion mass is also listed: negative ionization mode, resulting in a $[M – H]^-$ parent ion, is used for all methods except the *N*-acyl ethanolamine and 2-acyl glycerol methods, which uses positive ionization and generates a parent ion with a mass of $[M + H]^+$. The parent ion is the only ion allowed to pass through the first quadrupole of the API3000. The parent ion is then fragmented into the collision chamber and an abundant fragment can be selected as the fragment ion. Only the selected fragment ion can pass from the collision chamber into the third quadrupole. Therefore, unknown lipids are matched to known standards according to retention time from the analytical column and according to their mass fingerprint

		% Lipids detected unchanged by mTBI	% Lipids detected increased by mTBI	% Lipids detected decreased by mTBI
Cerebellum	Single	53.85%	46.15%	0.00%
	Repeated	75.64%	21.80%	2.56%
Trigeminal Ganglia	Single	77.50%	18.75%	3.75%
	Repeated	63.64%	35.06%	1.30%
Trigeminal Nucleus	Single	63.16%	36.84%	0.00%
	Repeated	60.27%	19.18%	20.55%

Fig. 16.2 Summary of effects of single and repeated mild traumatic brain injury (mTBI) on lipid levels in the rat cerebellum, trigeminal ganglia, and trigeminal nucleus caudalis. The left, blue column represents the percentage of the lipids detected within each of tissue type (row) with concentrations that were unaffected by single or repeated mTBI. The middle, green column represents the percentage of lipids detected that increased with single and repeated mTBI and the right, orange column represents the percentage of lipids detected that decreased with single and repeated mTBI relative to sham. See Methods for more details on how percentages were calculated

by 100 to yield the percentage increased (19.18%). The right portion of Fig. 16.2 shows the percentage of the lipids detected that decreased with single or repeated mTBI. The percentage was calculated by taking the number of lipids with decreased concentrations in each tissue and dividing it by the number of lipids detected in that tissue. For the TNC, 15 lipids decreased with repeated mTBI, giving a percentage of 20.55% for the proportion of the lipidome that decreased. When the 3 different percentages for a tissue are added, they should equal 100. For the repeated mTBI TNC, 60.27 plus 19.18 plus 20.55 equals 100.

Analyzed lipidomics data from the TG, TNC, and CER are represented in tabular format illustrating both the direction and magnitude of change. For example, the mean level of AEA was 9.20×10^{-11} moles per gram in the CER of the rats subjected to a single mTBI and 7.86×10^{-11} moles per gram in the corresponding sham CER. 9.20×10^{-11} divided by 7.86×10^{-11} is 1.17, assigning it 1 up arrow in the figures because the magnitude of change was between 1 and 1.5 times higher than sham. Providing an example of a decrease, the average level of AEA in the TNC of rats exposed to repeated mTBI was 5.22×10^{-12} moles per gram and the average level of AEA in the TNC of the sham rats was 7.32×10^{-12} moles per gram; 5.22×10^{-12} divided by 7.32×10^{-12} equals 0.71, and the reciprocal of 0.71 is 1.41, meaning that the decrease is between 1 and 1.5 times sham levels and giving it 1 down arrow on our scale.

Immortalized BV2 microglia cells were a gift from Dr. N. Stella at University of Washington, Seattle. Boyden migration chamber assays and analyses were performed identically to those previously described in our laboratory [59, 73].

16.4.1 mTBI Has Broad Effects on the CER, TG, and TNC Lipidome

Of the 74 lipoamines in our screening library (Fig. 16.1), over 50 were detected in the TG, TNC, and CER. If a lipid was detected in the samples from injured rats it was also found in the corresponding sham samples. In both single and repeated mTBI, 78 lipids were detected in the CER (92% of the 85 lipids in the screening library). For the TG and TNC, different numbers of lipids were detected after single and repeated mTBI. In the TG, 80 lipids were detected in the single condition (94%) and 77 in the repeated condition (91%). Fewer lipids were detected in the TNC (single: 76 lipids – 89%; repeated: 73 lipids – 86%). Tables listing the lipids detected and their mean levels in each tissue type in sham and mTBI tissues are available upon request from the authors.

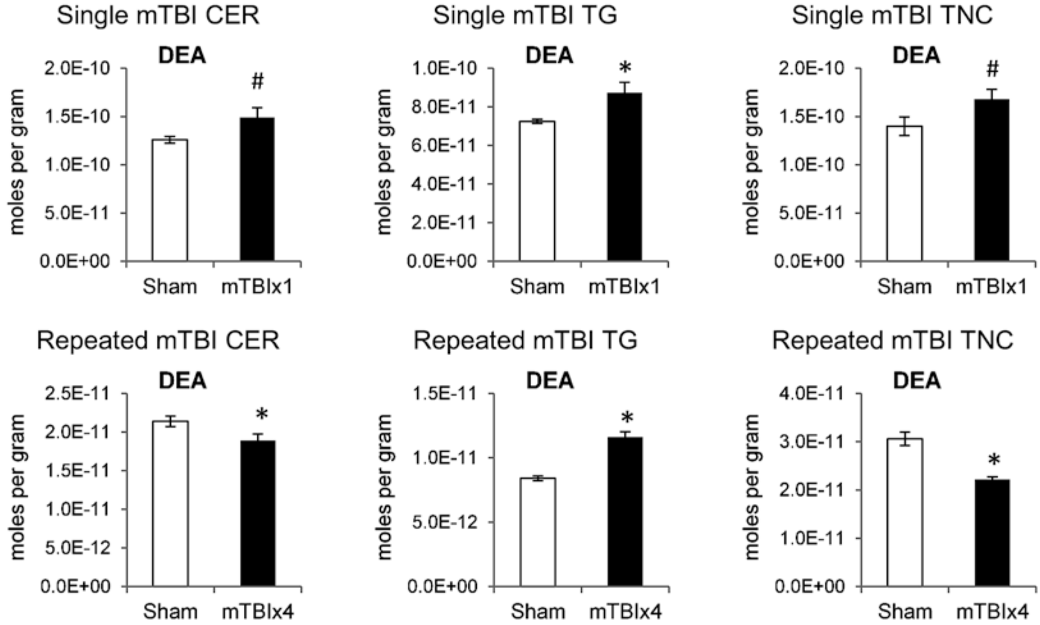
The first level of analysis examined the proportion of the lipids detected that were unchanged, upregulated, or downregulated with mTBI relative to sham. Effects of single mTBI on the lipidome were mostly tissue-dependent

(Figs. 16.2, 16.3, 16.4, and 16.5). The highest percentage of changes in lipid levels occurred in the CER (36 lipids – 46% of the lipids detected), followed by the TNC (28 lipids – 37%). The area with the lowest percentages of changes in lipid levels was the TG (18 lipids – 22.5%). All of the changes driven in the TNC and CER were increases in a lipid's concentration. In contrast, changes measured in the TG included decreases (15 increases, 3 decreases).

Repeated mTBI affected a similar number of the detected lipids (Figs. 16.2, 16.3, 16.4, and 16.5). However, the specific tissue most affected shifted in repeated mTBI. The highest percent-

	Cerebellum		Trigeminal Ganglia		Trigeminal Nucleus	
Lipid Species	**mTBI X 1**	**mTBI X 4**	**mTBI X 1**	**mTBI X 4**	**mTBI X 1**	**mTBI X 4**
***N*-acyl ethanolamine**						
N-palmitoyl ethanolamine						↓
N-stearoyl ethanolamine						
N-oleoyl ethanolamine				↓		
N-linoleoyl ethanolamine	↑			↑	↑	↓
N-arachidonoyl ethanolamine	↑			↑	↑	↓
N-docosahexaenoyl ethanolamine	↑	↓	↑	↑	↑	↓
***N*-acyl glycine**						
N-palmitoyl glycine					↑	
N-stearoyl glycine		↑	↑		↑	↓
N-oleoyl glycine	↑		↑		↑	
N-linoleoyl glycine					↑	
N-arachidonoyl glycine	↑	↑	↑	↑	↑	↑
N-docosahexaenoyl glycine	↑			↑	↑	
***N*-acyl taurine**						
N-palmitoyl taurine	↑				↑	
N-stearoyl taurine	↑	↓			↑	
N-oleoyl taurine	↑				↑	↑
N-arachidonoyl taurine	↑			↑	↑	↑
2-acyl glycerol						
2-palmitoyl glycerol		↑↑		↑	↑↑	
2-oleoyl glycerol				↑		↓
2-linoleoyl glycerol		↑		↑	↑	
2-arachidonoyl glycerol				↑	↑	
Free Fatty Acids						
Oleic acid	↑			↑		
Linoleic acid	↑	↑	↑	↑	↑	↓
Arachidonic acid	↑	↑	↑	↑	↑	
Prostaglandins						
PGE_2		↑		↑	↑↑	
$PGF_{2\alpha}$	↑	↑↑	↑	↑↑		↑
6-keto$PGF_{1\alpha}$	↑	↑	↓	↑↑	↑	↑

Fig. 16.3 Comparison of effects of single or repeated mild traumatic brain injury (mTBI) on levels of *N*-acyl ethanolamines, *N*-acyl glycines, *N*-acyl taurines, 2-acyl glycerols, free fatty acids, and prostaglandins. Levels of targeted bioactive lipids in the cerebellum, trigeminal ganglia, and trigeminal nucleus caudalis of rats given a single mTBI (mTBI × 1) or 4 repeated mTBIs (mTBI × 4) were compared to respective sham rats. Cells with shaded arrows indicate a significant or trending change in that lipid with mTBI. The arrow color indicates the direction of change relative to sham. Green colors represent increases, whereas orange colors represent decreases. Darker colors represent significant changes of $p < 0.05$ and lighter colors represent trending changes of $p < 0.10$, as determined by one-way ANOVA. The number of arrows indicates the magnitude of the difference between mTBI and sham. One arrow indicates a magnitude difference of less than 1.5 fold and 2 arrows indicate a 1.5–1.99 fold change. A blank cell indicates that there was no change in the lipid's level due to mTBI. See Methods for more detailed description of analysis



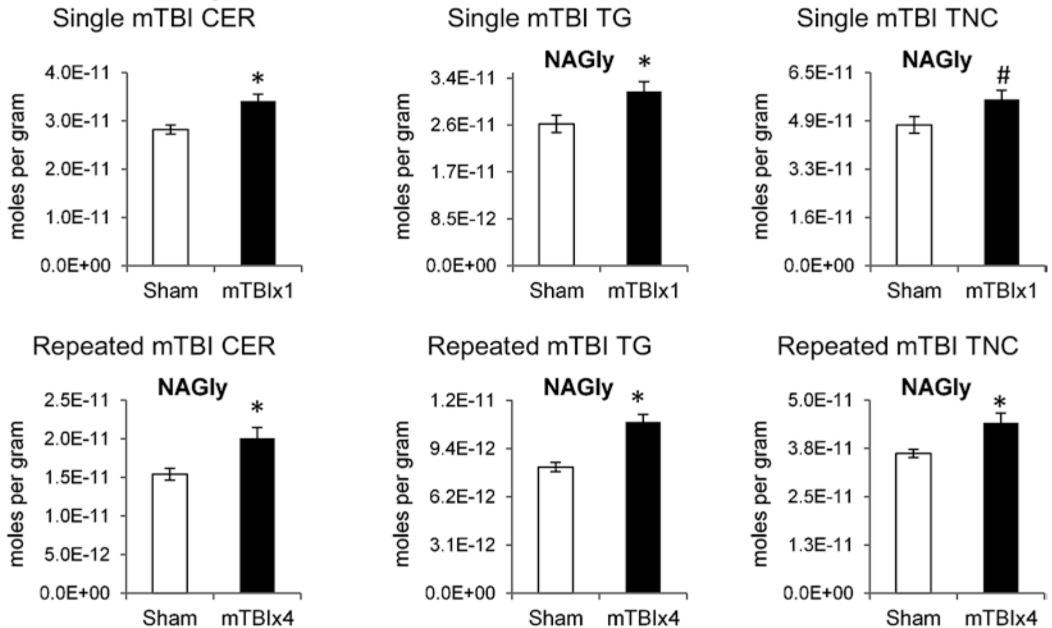


Fig. 16.4 Comparison of effects of single or repeated mild traumatic brain injury (mTBI) on levels of *N*-docosahexaenoyl ethanolamine (DEA) and *N*-arachidonoyl glycine (NAGly). Panel A shows bar graphs for mean levels in moles per gram tissue of DEA in the cerebellum (CER), trigeminal ganglia (TG), and trigeminal nucleus caudalis (TNC) of rats exposed to a single mild traumatic brain injury (single mTBI – black bars) or sham (open bars) and of rats exposed to a series of 4 mTBIs (repeated mTBI – black bars) or sham (open bars). Error bars are ± standard error of the mean. Asterisk (∗) indicates a difference between mTBI and sham of $p < 0.05$

age of changes occurred in the TNC (29 lipids – 40% of the lipids detected), followed by the TG (28 lipids – 36%). Unlike in single mTBI, where the most changes were detected in the CER, in repeated mTBI the CER had the fewest changes detected (19 lipids – 24%). The ratio of increases and decreases also shifted, with decreases occurring in every tissue type. In the TG and CER, most of the changes were increases (27 increases of 28 changes for TG and 17/19 for CER). The TNC stands out, because it was the only tissue type where most of the changes were decreases, suggesting functional specialization in this area that can potentially underlie a more dynamic response to injuries (15/29; Figs. 16.2, 16.3, 16.4, and 16.5). Given that the CER was the most affected area after a single mTBI, a single mTBI may therefore have a stronger effect on bioactive lipid production in sites closer to the injury, such as the CER, whereas the effects of repeated mTBI on bioactive lipid levels may spread to more distant areas.

16.4.2 Effects of mTBI on Bioactive Lipids with Relatively More Well-Studied Biosynthetic Pathways and Protein Targets

16.4.2.1 N-Acyl Glycine Lipid Species Are Upregulated After mTBI

Levels of NAGly increased in all 6 groups in the mTBI experiments (Figs. 16.3 and 16.4). Increases in NAGly were also found after repeated exposure to low levels of the environmental toxin, acrolein [43]. This means that increases in NAGly may serve as a marker of headache caused by very different stimuli, mTBI and TRPA1 activation. There are multiple pathways that regulate NAGly levels. NAGly is a metabolite of AEA and can be formed from AEA via 2 enzymatic pathways, one mediated by alcohol dehydrogenase and the other mediated by fatty acid amide hydrolase (FAAH) [58]. Indeed, in FAAH knockout (KO) mice, brain levels of NAGly are significantly reduced, whereas AEA levels are significantly higher compared to wild-type (WT) [69]. The concurrent reduction in AEA and other NAEs and increase in NAGly in the TNC after repeated mTBI could potentially be driven by an increase in AEA metabolism via FAAH. NAGly production can also be mediated by glycine *N*-acyl transferase enzymes [75] and by the newly-characterized enzyme peptidase M20 domain containing 1 (PM20D1) [76, 77]. PM20D1 regulates mitochondrial uncoupling via regulation of endogenous lipoamines. As mitochondrial dysfunction is a hallmark of neuroinflammation after TBI [13], the relationship between PM20D1, TBI, and lipoamine regulation should be further investigated.

All other *N*-acyl glycine lipids that changed with a single mTBI showed an increase (Fig. 16.3). After a single mTBI, *N*-oleoyl glycine (O-Gly) increased in all 3 tissue types, along with the increase in NAGly, which was still present after repeated mTBI. Alterations in O-Gly have previously been associated with TBI in rodent models, as O-Gly was upregulated in the mouse insular cortex 24 h after TBI [78]. Levels

Fig. 16.4 (continued) whereas the hash sign (#) indicates a difference between mTBI and sham of $p < 0.10$, as determined by one-way ANOVA. After single mTBI, DEA increased in all 3 tissue types, corresponding to green cells with 1 up arrow in Fig. 16.4. After repeated mTBI, DEA decreased in the CER and TNC, corresponding to orange cells with 1 down arrow in Fig. 16.4, but increased in the TG, corresponding to a green cell with 1 up arrow in Fig. 16.4. **Panel B** shows bar graphs for mean levels in moles per gram tissue of NAGly in the cerebellum (CER), trigeminal ganglia (TG), and trigeminal nucleus caudalis (TNC) of rats exposed to a single mild traumatic brain injury (single mTBI – black bars) or sham (open bars) and of rats exposed to a series of 4 mTBIs (repeated mTBI – black bars) or sham (open bars). Error bars are ± standard error of the mean. Asterisk (*) indicates a difference between mTBI and sham of $p < 0.05$ whereas the hash sign (#) indicates a difference between mTBI and sham of $p < 0.10$, as determined by one-way ANOVA. In all 3 tissue types, levels of NAGly increased after single and repeated mTBI, corresponding to green cells with 1 up arrow in Fig. 16.4

	Cerebellum		Trigeminal Ganglia		Trigeminal Nucleus	
Lipid Species	**mTBI X 1**	**mTBI X 4**	**mTBI X 1**	**mTBI X 4**	**mTBI X 1**	**mTBI X 4**
***N*-acyl alanine**						
N-palmitoyl alanine	↑		↑			↑
N-stearoyl alanine	↑		↑↑			↓
N-oleoyl alanine	↑	↑	↑			↑
N-arachidonoyl alanine	↑↑↑		↑↑↑			↑
N-docosahexaenoyl alanine		↑↑				↑↑
***N*-acyl GABA**						
N-palmitoyl GABA			↓	↑↑	↑	
N-stearoyl GABA	↑			↑		↓
N-oleoyl GABA	↑		↓			↓
N-linoleoyl GABA	↑		BAL	BAL		
N-arachidonoyl GABA	↑	↑			↑	↑
N-docosahexaenoyl GABA						↓
***N*-acyl leucine**						
N-palmitoyl leucine				↑		
N-stearoyl leucine				↑		↓
N-oleoyl leucine						↓
N-linoleoyl leucine		↑				
N-docosahexaenoyl leucine		↑				
***N*-acyl methionine**						
N-palmitoyl methionine	↑					
N-stearoyl methionine	↑		↑			
N-oleoyl methionine			↑			
N-arachidonoyl methionine						↑
N-docosahexaenoyl methionine		BAL		↑↑↑	BAL	BAL
***N*-acyl phenylalanine**						
N-stearoyl phenylalanine						↓
N-oleoyl phenylalanine					↑	
N-arachidonoyl phenylalanine	↑		↑	↑	↑	↑
N-docosahexaenoyl phenylalanine						↓↓
***N*-acyl proline**						
N-oleoyl proline		↑				↑
N-arachidonoyl proline	BAL	BAL	BAL	↑↑	BAL	BAL
***N*-acyl serine**						
N-palmitoyl serine	↑				↑	
N-stearoyl serine	↑					
N-oleoyl serine	↑					
N-linoleoyl serine				↑		
N-arachidonoyl serine	↑	↑		↑		
***N*-acyl tryptophan**						
N-stearoyl tryptophan				↑		
***N*-acyl tyrosine**						
N-palmitoyl tyrosine					↑	
N-oleoyl tyrosine	↑					
N-linoleoyl tyrosine	↑↑↑					BAL
N-arachidonoyl tyrosine	↑				↑	↑
***N*-acyl valine**						
N-palmitoyl valine	↑					
N- stearoyl valine	↑		↑		↑	
N-docosahexaenoyl valine	↑↑				BAL	BAL
phosphoLEA						
phosphoLEA	BAL	↑↑	BAL	↑↑		

Fig. 16.5 Effects of single and repeated mild traumatic brain injury (mTBI) on additional bioactive lipids in the cerebellum, trigeminal ganglia, and trigeminal nucleus. Specifically, this heatmap shows effects of mTBI on members of the *N*-acyl alanine, *N*-acyl GABA, *N*-acyl leucine, *N*-acyl methionine, *N*-acyl phenylalanine, *N*-acyl proline, *N*-acyl serine, *N*-acyl tryptophan, *N*-acyl tyrosine, and *N*-acyl valine families of bioactive lipids, and effects on phosphoLEA. Levels of targeted bioactive lipids in the cerebellum, trigeminal ganglia, and trigeminal nucleus caudalis of rats given a single mTBI (mTBI × 1) or 4 repeated mTBIs (mTBI × 4) were compared to respective sham rats. Only lipids that had significant or trending changes with either single or

of the TRPV1 agonist *N*-docosahexaenoyl glycine (D-Gly) [41] also increased in the CER and *N*-stearoyl glycine (S-Gly) also increased in the TG. The TNC had the most changes in *N*-acyl glycine levels after a single mTBI, with levels of all 6 *N*-acyl glycines increasing. After repeated mTBI, the increase in NAGly was the only increase in an *N*-acyl glycine in the TNC. In the TNC, S-Gly decreased after repeated mTBI, which was the only mTBI-driven decrease measured in *N*-acyl glycines. S-Gly changed in the opposite direction in the CER, increasing after repeated mTBI. D-Gly increased in the TG only (Figs. 16.3 and 16.4).

16.4.2.2 NAGly and Additional N-Acyl Glycine Lipids Drive Microglial Migration

NAGly drives dose-dependent migration in BV2 microglia via activation of GPR18, which can be inhibited by A-Ser [59]. A-Ser's effects on microglia could underlie some of its reported neuroprotective actions after mTBI [47, 48] because microglial migration is understood to be a marker of neuroinflammation [47]. Other studies have shown an increase in microglial activation after mTBI, with microglia rapidly migrating towards damaged tissue [79]. However, the utility of the microglial response is complex [13]. Initially, microglia may be useful at clearing away damaged axonal tissue, which can promote regeneration of damaged axons [13]. However, sustained microglial activation does not seem to be as beneficial and appears to promote inflammation rather than regeneration [13, 79]. The fact that the increase in NAGly was widespread, occurring in sites distant from the injury, and sustained up to 1 week after repeated mTBI suggests broad increases in neuroinflammation [79]. Microglia can release pro-inflammatory lipid signaling molecules such as PGs [4]. It is possible that the increases in PGs observed after repeated mTBI are a consequence of increased microglial activation. Experiments are planned to see if microglia release PGs when incubated with NAGly.

Novel data shown here demonstrates that NAGly is not the only *N*-acyl glycine that can induce microglial migration. At 10 μM, *N*-palmitoyl glycine, S-Gly, O-Gly, *N*-linoleoyl glycine, NAGly, and D-Gly induced migration in BV-2 microglia (Fig. 16.6a, b). This demonstrates how BV2 microglia might not be responding to the identity of the acyl chain but are instead sensitive to glycine-containing lipoamines. Follow-up studies should examine how combining these compounds affects migration, as levels of multiple *N*-acyl glycines change with mTBI. Unpublished data from the Hurley lab confirmed that there were increased numbers of microglia in the corpus callosum of mTBI rats, suggesting some effects on migration (*personal communication*). Highlighting potential cross-talk between TRPA1 and microglia, endogenous TRPA1 ligands released by microglia in the spinal cord are hypothesized to contribute to central sensitization after a peripheral nerve injury. Both TRPA1 antagonists and blockers of microglia can reduce central pain hypersensitivity in animal models [80]. Therefore, it is worth investigating whether TRPA1 antagonists or microglial blockers can similarly reduce headache pain secondary to TBI.

Fig. 16.5 (continued) repeated mTBI in at least 1 tissue type are shown here. Cells with shaded arrows indicate a significant or trending change in that lipid with mTBI. The arrow color indicates the direction of change relative to sham. Green colors represent increases, whereas orange colors represent decreases. Darker colors represent significant changes of $p < 0.05$ and lighter colors represent trending changes of $p < 0.10$, as determined by one-way ANOVA. The number of arrows indicates the magnitude of the difference between mTBI and sham. One arrow indicates a magnitude difference of less than 1.5 fold, 2 arrows indicate a 1.5–1.99 fold change, and 3 arrows indicate a 2–2.99 fold change. A blank cell indicates that there was no change in the lipid's level due to mTBI. BAL stands for below analytical limits and means that lipid was not detected in all samples in that tissue type. See Methods for more detailed description of analysis

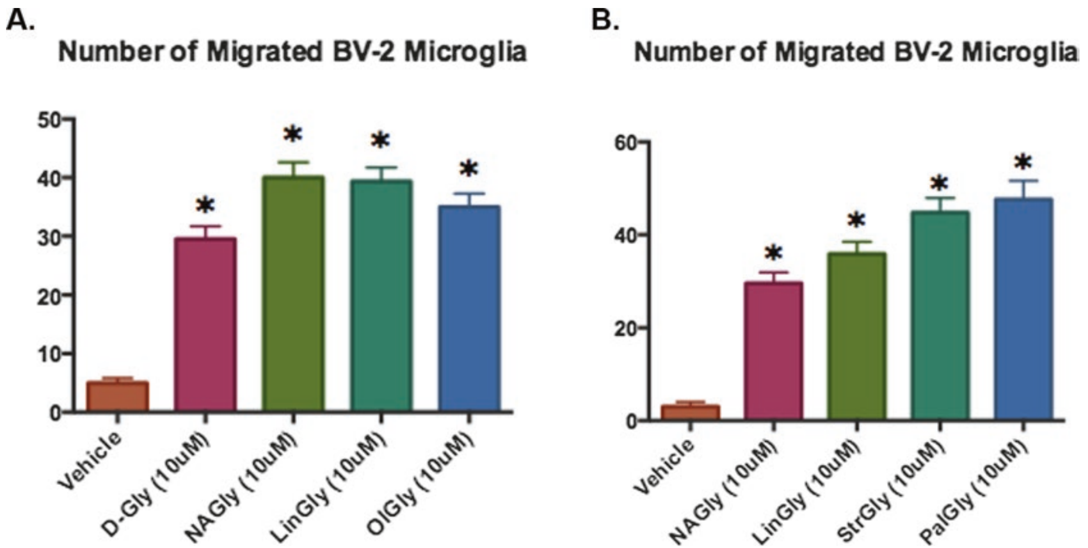


Fig. 16.6 Cellular migration activity of *N*-acyl glycines in BV2 microglial cells. Panel A: At 10 μM, *N*-docosahexaenoyl glycine (DocGly), *N*-arachidonoyl glycine (NAGly), *N*-linoleoyl glycine (LinGly), and *N*-oleoyl glycine (OlGly) caused cellular migration that was significantly greater than the vehicle control. 7 plates were analyzed with 20 repeats for each condition per plate. * indicates a mean difference significant at the 0.05 level when compared to vehicle. **Panel B:** At 10 μM, *N*-arachidonoyl glycine (NAGly), *N*-linoleoyl glycine (LinGly), *N*-stearoyl glycine (StrGly), and *N*-palmitoyl glycine (PalGly) caused cellular migration that was significantly greater than the vehicle control. 4 plates were analyzed with 20 repeats for each condition per plate. * indicates a mean difference significant at the 0.05 level when compared to vehicle

16.4.3 N-Docosahexaenoyl Ethanolamine (DEA) May Play a Key Role in the Effects of mTBI with a Potential Implication in Resolving Pathways

As the most affected NAE, the TRPV1 agonist DEA [41] increased in all single mTBI groups but decreased in the CER and the TNC with repeated mTBI. The eCB AEA increased in the CER and TNC after a single mTBI, but not in the TG. LEA also increased in the CER and TNC, meaning that multiple TRPV1 agonist NAEs [41] were elevated in these tissues after a single mTBI. Repeated mTBI drove a tissue-specific mixture of increases and decreases in NAEs, with increases restricted to the TG. The only change in NAEs in the CER was a decrease in DEA. In contrast, DEA increased in the TG, along with LEA and AEA. OEA decreased slightly in the TG. In contrast to single mTBI where LEA, AEA, and DEA increased, these NAEs decreased in the TNC after repeated mTBI, along with *N*-palmitoyl ethanolamine (Figs. 16.3 and 16.4). Reductions in NAEs in the TNC after repeated mTBI could be driven by a decrease in biosynthesis via *N*-acyl phosphatidyl ethanolamine-specific phospholipase D (NAPE-PLD) [70]. In NAPE-PLD KO mice, brain levels of NAGly either stayed the same or increased while AEA decreased [70], consistent with what was seen in the TNC after repeated mTBI. Increased levels of PGs in the TNC after repeated mTBI (Fig. 16.3) are also consistent with NAPE-PLD blockade, as NAPE-PLD KO mice have higher levels of PGs in the CNS compared to WT [70].

The omega-3 fatty acid, docosahexaenoic acid (DHA) is a precursor for DEA and can be neuroprotective after TBI [81–85]. For example, rats were subjected to an impact acceleration injury, which causes diffuse axonal injury [81]. For the next 30 days, injured rats were given vehicle or DHA [81]. In rats supplemented with DHA, plasma levels of DHA increased and the ratio of AA to eicosapentaenoic acid, a general

marker of inflammation, decreased [81]. In rats given vehicle, plasma levels of DHA were significantly reduced [81]. DHA had a protective effect and decreased the number of amyloid precursor protein-positive and caspase-3 positive (apoptotic) neurons in the brainstem [81]. The same group performed a similar follow-up study, but here they gave rats DHA for 30 days before the injury instead of after the injury [83]. Similar results were uncovered, with DHA having a neuroprotective effect against TBI, suggesting that DHA can also be used as a prophylactic to protect the brain against the consequences of injury [83].

In another study, rats were given a DHA-supplemented diet or a control diet for 30 days before receiving a mild fluid percussion injury [82]. Rats on the control diet experienced learning difficulties after the injury, as measured by the Morris water maze, whereas rats that received DHA did not show these deficits [82]. Furthermore, injury-related decreases in BDNF, CREB, and synapsin I, were not measured in the DHA-supplemented rats and lower levels of oxidized proteins were measured in the hippocampus of the DHA-rats, suggesting a reduction in oxidative stress [82]. DHA also appears to protect against inflammation after TBI, as rats that were given daily injections of DHA for 21 days showed a reduction in markers of activated microglia, which were elevated after TBI [85]. Of the activated microglia that were still present in the CNS of the DHA-treated rats, more of these expressed the anti-inflammatory marker CD206 and fewer expressed the pro-inflammatory marker CD16/32 [85]. On the other hand, when rats are raised on a diet low in DHA and other omega-3 fatty acids, they show worsened recovery from TBI-related motor deficits and increased neuronal death compared to rats on a control diet, as well as a 70% loss of DHA in the brain [84]. This data is an important reminder that diet can influence levels of bioactive lipids and that a diet rich in omega-3 polyunsaturated fatty acids can have measurable health benefits. Although metabolites of DHA were not quantified, the authors hypothesized that some of DHA's neuroprotective effects are due to conversion into pro-resolving mediators such as D-series resolvins and neuroprotectin [81].

The primary biosynthetic pathways for these DHA-derived lipids, 15-lipoxygenase (15-LOX) can convert DHA into 17S-hydroperoxydocosahexaenoic acid (17S-H(p)-DHA), whereas cyclooxygenase 2 can convert DHA into the enantiomer, 17R-hydroperoxydocosahexaenoic acid (17R-H(p)-DHA) [86]. 5-lipoxygenase (5-LOX) converts 17S-H(p)-DHA to resolvin D1, whereas the same enzyme converts 17R-H(p)-DHA into aspirin-triggered resolvin D1 [86]. Alternatively, 17S-H(p)-DHA and 17R-H(p)-DHA can first be converted into epoxide intermediates which are then hydrolyzed to produce neuroprotection D1 [86]. Resolvin D1 can reduce immune cell infiltration and reduces inflammatory pain [87]. Likewise, neuroprotectin D1 reduces inflammatory pain and can also inhibit pain signals via TRPV1 at intrathecal doses of just 0.1–10 ng [88]. Given that TRPV1 ligands are elevated after a single mTBI, it is possible that neuroprotectin D1 could reduce TVS sensitization and therefore decreases headache pain.

Pro-resolving mediators are currently being investigated as neuroprotective therapies for brain injuries [89], as they are highly potent anti-inflammatory bioactive lipids [90]. Treating mice with diffuse axonal injury with aspirin-triggered resolvin D1 for 3 days pre-injury and 7 days post-injury partially rescued the cognitive deficits and motor deficits, as measured by the novel object recognition task and the rotorod task [90]. Neuroprotectin D1 formation increased in the mouse hippocampus after cerebral artery occlusion, where it inhibited immune cell infiltration and pro-inflammatory gene induction [91]. Neuroprotectin D1 also inhibited cytokine production in neuronal cell cultures [91]. Highlighting the neuroprotective effect of this bioactive lipid, the infarct volume in the hippocampus was significantly reduced 48 h after the cerebral artery occlusion injury in mice given intracerebroventricular infusions of neuroprotectin D1 [91].

Decreases in DEA after repeated mTBI could potentially be driven by conversion to oxygenated metabolites. DEA has been identified as a precursor for several bioactive lipids, which are formed via similar pathways as the biosynthesis of resolvins and neuroprotectin from DHA [92]. For example, incubating mouse brain homogenates with DEA generated 17-hydroxydocosahexaenoyl ethanolamide (17-HDHEA), 4,17-dihydroxydocosahexaenoyl ethanolamide, as well as smaller amounts of 7,17-dihydroxydocosahexaenoyl ethanolamide, 10,17-dihydroxydocosahexaenoyl ethanolamide (10,17-diHDHEA), and 15-hydroxy-16(17)-epoxy-docosapentaenoyl ethanolamide (15-HEDPEA) [92]. 10,17-diHDHEA and 15-HEDPEA were agonists at CB_2 with EC_{50}'s in the low nanomolar range and blocked platelet-leukocyte aggregation formation in human whole blood at concentrations of just 10pM [92]. As CB_2 ligands were neuroprotective after TBI [21], it is possible that these DEA-derived lipids have neuroprotective effects via that receptor. Highlighting the potency of these resolving lipids, injections of 15-HEDPEA reduced lung injury after reperfusion at doses as low as 1 μg/mouse [92]. Given that these lipids are so potent, they might be bioactive at concentrations below the detection limits of our HPLC/MS/MS system. It might instead be a more viable strategy to measure the activity of 5-LOX after mTBI and acrolein exposure, as this is a rate-limiting enzyme for the formation of resolvins from DHA and might also be important for the formation of oxygenated metabolites of DEA [93]. Indeed, blocking 5-LOX with siRNA in head and neck squamous cell carcinoma cell lines attenuated the formation of reactive oxygen species and blocked the ability of DEA to inhibit cell proliferation [93]. 15-LOX is also an important enzyme to monitor, because it mediates the conversion of DEA to 17-hydroperoxydocosahexaenoyl ethanolamide, a precursor for the CB_2 agonists 10,17-diHDHEA and 15-HEDPEA, as well as 17-HDHEA [92].

The fact that multiple NAEs were altered with mTBI is important because there are emergent signaling properties when multiple TRPV1-ligand NAEs are combined in that the potency is increased when multiple agonists are present [43]. Multiple NAEs also changed in brain tissues of rats in a model of peripheral inflammation [41] and in a rodent model of headache driven by acrolein exposure [43], suggesting that these molecules are sensitive to inflammatory insults. The actions of multiple NAEs at TRP channels may override the analgesic effects of AEA via CB_1 [18], and prolonged exposure to elevated levels of NAEs has been associated with a nociceptive phenotype in response to capsaicin [66].

16.4.4 Additional Bioactive Lipids Effected by mTBI

16.4.4.1 N-Acyl Taurines

All 4 *N*-acyl taurines, including the TRPV1 and TRPV4 agonist [39] A-Taur, increased in the CER and TNC after a single mTBI. However, none of the targeted *N*-acyl taurines changed in the TG. Fewer changes in *N*-acyl taurines were measured after repeated mTBI and there were no longer increases in all 4 *N*-acyl taurines in the CER and TNC. Instead, *N*-stearoyl taurine decreased in the CER and A-Taur increased in the TG, which was the only change in *N*-acyl taurines associated with mTBI in the TG. A-Taur also increased in the TNC, as did *N*-oleoyl taurine (Fig. 16.3) [43].

16.4.4.2 2-Acyl Glycerols

After a single mTBI, 2-acyl glycerol levels were only affected in the TNC. Here, there were increases in the eCB 2-AG, as well as 2-palmitoyl glycerol (2-PG) and 2-linoleoyl glycerol (2-LG). This makes the TNC the only tissue where levels of both of the eCBs, AEA and 2-AG, were affected by a single mTBI. Whether levels of 2-acyl glycerols increased or decreased after repeated mTBI was dependent on tissue type. Changes in 2-acyl glycerols were increases in the CER and the TG, but decreases in the TNC. In the CER, 2-PG and 2-LG increased. In contrast, all 4 2-acyl glycerols in the screening library significantly increased in the TG. Only 1 change in 2-acyl glycerol levels was measured in the TNC – a reduction in 2-oleoyl glycerol (Fig. 16.3).

As 2-AG has been shown to be neuroprotective in multiple TBI studies [12, 19, 20], increases in 2-AG in the TG after repeated mTBI and in the

TNC after single mTBI may be part of a neuroprotective and regenerative response that mediates some of the moderate spontaneous recovery that occurs after TBI [74]. mTBI also impacted structurally analogous lipids to the eCB 2-AG, especially in the in the TG, where repeated mTBI increased 4 different 2-acyl glycerols. This implicates eCB system enzymes as potential mediators of mTBI's effects on lipid levels because these enzymes regulate lipid levels beyond eCB substrates [69, 70, 94]. A decrease in 2-acyl glycerol hydrolysis via monoacylglycerol lipase (MAGL) could increase 2-acyl glycerol levels, and we have shown that MAGL deletion causes widespread increases in 2-acyl glycerols in the mouse brain [69]. However, these increases in 2-acyl glycerols likely came at the expense of levels of AA and PGs [69]. Increases in AA and PGs were measured in the TG after repeated mTBI, making MAGL inhibition an unlikely mechanism.

Diacylglycerol lipases (DAGL) synthesize 2-acyl glycerols from their diacylglycerol (DAG) precursors, so an increase in DAGL activity could upregulate 2-acyl glycerols [95]. The DAGLα isoform appears more important for biosynthesis of 2-AG in the brain, whereas the DAGLβ isoform appears more important in the periphery [96]. There is evidence that levels of AA and AEA can change in the same direction as 2-AG upon a change in DAGL activity. Along with 2-AG levels, AEA levels were downregulated in the cerebellum, striatum, and hippocampus of a line of DAGLα KO mice [97]. Another line of DAGLα KO had an 80% reduction in whole brain 2-AG and AA levels and a 40% reduction in AEA [98]. A third line of DAGLα KO had reduced levels of 2-AG and AA in the forebrain, but did not detect a significant reduction in AEA in this area, hinting at some regional specificity for the contribution of DAGLα activity to AEA levels [99]. Reductions in whole-brain 2-AG, AA, and AEA also occur when DAGLα is pharmacologically inhibited [100]. It isn't yet known whether increasing DAGL activity causes concurrent increases in AA and AEA, which occurred in the TG after repeated mTBI.

Follow-up studies can determine if the effects of mTBI are abolished when DAGL is inhibited or deleted. If effects on the lipidome require DAGL, then more direct measurements of DAGL activity can be performed after mTBI or acrolein exposure. Recently, a fluorescent probe was developed to measure DAGL activity [101]. This probe can be used in activity based protein profiling (ABPP) assays in mouse brain proteasomes [102]. By performing ABPP after acute and repeated mTBI and after acute and chronic acrolein exposure, the impact on DAGL activity can be quantified. ABPP can also be employed to detect off-target serine hydrolase activity [103], which may reveal a more widespread impact of mTBI on enzymes important for maintaining levels of eCBs and other bioactive lipids.

16.4.4.3 The Potential Link Between TRPV1 and 2-Acyl Glycerol Lipids

What upstream changes can affect DAG production? Phospholipase C (PLC) intracellular pathways stimulate the release of DAGs from membrane phospholipids [104]. An earlier study demonstrated that TBI increased PLC activity in the cat brain [105]. Activation of TRP receptors can drive PLC pathways [36]. Indeed, we have shown that capsaicin activation of TRPV1 increases 2-acyl glycerol production [43]. Increases in levels of multiple TRPV1 ligands like LEA, AEA, and DEA in the TG after repeated mTBI could potentially increase PLC signaling, leading to an increase in DAG substrates for 2-acyl glycerol production. AA itself can also activate PLC pathways [106] and levels of AA were upregulated after repeated mTBI in the TG.

16.4.4.4 Free Fatty Acids and PGs

After a single mTBI, increases in AA and linoleic acid (LA) were common to all 3 tissues. Oleic acid (OA) also increased in the CER, meaning that all 3 free fatty acids screened for increased after single mTBI in this tissue type. After repeated mTBI, it was the TG that had increases in all 3 free fatty acids. AA and LA increased in the TG, but OA did not significantly change in this tissue. The only decrease in free fatty acid levels was restricted to the TNC, where LA decreased after repeated mTBI (Fig. 16.3).

PGs were also affected by mTBI. A single mTBI increased levels of 2 different PGs, $PGF_{2\alpha}$ and 6-keto$PGF_{1\alpha}$ in the CER. In the TG, $PGF_{2\alpha}$ increased, whereas 6-keto$PGF_{1\alpha}$ decreased. This was the only measured decrease in PG levels. In contrast, 2 PGs increased in the TNC, PGE_2 and 6-keto$PGF_{1\alpha}$. After repeated mTBI, $PGF_{2\alpha}$ and 6-keto$PGF_{1\alpha}$ increased in all 3 tissue types. In the CER and TG, PGE_2 also increased, such that all 3 PGs in the screening library increased in the CER and TG (Fig. 16.3).

Increases in AA in CNS tissues were measured after mTBI. AA can be directly released from phospholipids via phospholipase A_2, or indirectly via PLC [107]. Levels of AA are elevated in the CSF of TBI patients and higher levels of AA 1 week post-injury were correlated with worse outcomes [108]. Increased levels of AA are hypothesized to underlie increases in PGs in the brain after TBI [105] and here there were increases in levels of all 3 PGs in the CER and TG after repeated mTBI, where levels of AA also increased.

Levels of bioactive lipids are differentially affected by mTBI, mostly increasing after single and repeated mTBI. However, decreases in bioactive lipid levels were more common after repeated mTBI compared to single mTBI, especially in the TNC. After a single mTBI, certain changes were measured in all 3 tissue types and all of these were increases relative to sham. Specifically, DEA, O-Gly, NAGly, LA, and AA increased. After repeated mTBI, fewer changes were common to all 3 tissue types, although levels of PGs increased in all 3 tissues, along with NAGly. Thus, DEA levels changed in every tissue type and NAGly was upregulated in the CER, TG, and TNC, after a single or repeated mTBI (Figs. 16.3 and 16.4).

16.4.5 Effects of mTBI on Lipoamine Families That Include a TRPV Ligand

The following section summarizes some of the effects on the lipids that were measured in these models but are lipids that have members that have some activity at TRP channels. Many of these lipids have little data about them beyond those generated by our laboratory from which to compare; therefore, we will have to rely on those few reports. The lipids are subdivided into lipoamine "families" as characterized by the amine moiety and each section is labeled with this family for ease of referencing those that might be of particular interest to a reader.

N-Acyl GABAs *N*-linoleoyl GABA (L-GABA), A-GABA, and *N*-docosahexaenoyl GABA (D-GABA) are ligands at TRPV1 [41]. However, D-GABA did not change after single mTBI. After single mTBI, change in L-GABA was restricted to the CER, where it increased along with A-GABA and 2 additional *N*-acyl GABAs. Changes in *N*-acyl GABAs were decreases in the TG after single TBI and were restricted to *N*-acyl GABAs that lack TRPV1 activity. *N*-palmitoyl GABA changed in the opposite direction in the TNC, as it increased after a single mTBI, along with A-GABA. Fewer *N*-acyl GABAs changed in the CER after repeated mTBI, as an increase in A-GABA was the only detected change. Decreases in *N*-acyl GABAs were no longer found in the TG after repeated mTBI and instead 2 *N*-acyl GABAs with saturated acyl moieties increased. On the other hand, 3 *N*-acyl GABAs decreased in the TNC, including D-GABA. This was the only change in D-GABA associated with mTBI. However, A-GABA increased in the TNC. This means that increases in A-GABA were present in the CER and TNC after both single and repeated mTBI, but other changes in *N*-acyl GABAs were sensitive to the number of mTBIs (Fig. 16.5).

N-Acyl Prolines Although *N*-docosahexaenoyl proline is a TRPV1 antagonist [41], this lipid was not detected in any tissue type. This is likely to do with a poor signal to noise characteristic of our mass spectrometric analysis than due to a lack of endogenous production given that all other combinations are measurable. Even with the poor signal to noise of this lipoamine family in general, we are still able to measure many of the members

and showed that those *N*-acyl prolines only changed after repeated mTBI. *N*-oleoyl proline increased in the CER and TNC, whereas *N*-arachidonoyl proline (A-Pro) increased in the TG, which was the only tissue where it was detected in all samples (Fig. 16.5).

***N*-Acyl Serines** A single mTBI increased 4 different *N*-acyl serines in the CER, including A-Ser. The only other change in an *N*-acyl serine after a single mTBI was an increase in *N*-palmitoyl serine in the TNC. Repeated mTBI increased A-Ser in the CER and TG. *N*-linoleoyl serine also increased in the TG but no changes in *N*-acyl serines were measured in the TNC in the repeated mTBI paradigm. Levels of the TRPV1 agonist *N*-docosahexaenoyl serine [41] did not change with mTBI (Fig. 16.5).

***N*-Acyl Tryptophans** *N*-acyl tryptophans containing polyunsaturated acyl chains are agonists at TRPV4 [41]. However, these particular lipids were either not detected or unchanged by mTBI. The only mTBI-driven change in an *N*-acyl tryptophan was an increase in *N*-stearoyl tryptophan measured in the TG after repeated mTBI (Fig. 16.5).

***N*-Acyl Tyrosines** Multiple *N*-acyl tyrosines increased in the CER and TNC after single mTBI. The CER had the most changes, with increases in *N*-oleoyl tyrosine (O-Tyr), and the TRPV4 agonists [41] *N*-linoleoyl tyrosine (L-Tyr) and *N*-arachidonoyl tyrosine (A-Tyr). Although O-Tyr does not directly activate or inhibit a TRPV channel, a mixture of *N*-acyl tyrosines including O-Tyr activated TRPV2, TRPV3, and TRPV4 in transfected HEK cells [41]. Thus, the elevated levels in multiple N-acyl tyrosines might affect signaling via TRPV2-4. The increase in L-Tyr was a larger magnitude change than the other increases, with levels of L-Tyr being twice as high in the mTBI CER relative to the sham CER. On the other hand, no changes in *N*-acyl tyrosines were measured in the TG. In the TNC, single mTBI increased levels of the TRPV2/4 agonist *N*-palmitoyl tyrosine [41] and A-Tyr. An increase in A-Tyr in the TNC was the only change in *N*-acyl tyrosines measured after repeated mTBI (Fig. 16.5).

***N*-Acyl Valines** In all 3 tissue types, exposure to a single mTBI increased levels of the TRPV3 antagonist [41] *N*-stearoyl valine (S-Val). In the CER, levels of *N*-palmitoyl valine and *N*-docosahexaenoyl valine, which is also a TRPV3 antagonist [41], additionally increased. Repeated mTBI did not affect *N*-acyl valine levels (Fig. 16.5).

To summarize the effects mTBI on endogenous TRPV ligands, more endogenous TRPV ligands were affected after a single mTBI compared to after repeated mTBI,. A single mTBI elevated levels of TRPV ligands, whereas repeated mTBI produced a mixture of increases and decreases in levels of TRPV ligands. In the mTBI model, changes in TRPV ligands common to all 3 tissue types were only uncovered after a single mTBI, specifically, the TRPV1 agonist DEA [41] and the TRPV3 antagonist S-Val [41] increased.

16.4.6 Effects of mTBI on Orphan Bioactive Lipids

Those lipids for which we have no known molecular targets and are collectively described as "orphan lipids" are discussed here. Some of these lipoamine families have multiple members that demonstrate consistent changes across models and represent potentially important targets to focus future research efforts.

***N*-Acyl Alanines** *N*-acyl alanine species were responsive to mTBI. *N*-acyl alanines did not change in the TNC after a single TBI, whereas 4 different *N*-acyl alanines increased in the CER and TG. The increase in *N*-arachidonoyl alanine (A-Ala) was of a larger magnitude than the other increases, with levels of A-Ala being over twice

as high in the mTBI CER and TG compared to sham. In contrast, none of the *N*-acyl alanines changed in the TG after repeated mTBI and only 2 changed in the CER. In the repeated condition, the TNC had the most changes, with increases in 4 *N*-acyl alanines, including A-Ala, and with a decrease in *N*-stearoyl alanine (Fig. 16.5).

N-Acyl Leucines *N*-acyl leucines only changed after exposure to 4 mTBIs and did not change after a single mTBI. *N*-linoleoyl leucine and *N*-docosahexaenoyl leucine increased in the CER, whereas *N*-palmitoyl leucine and *N*-stearoyl leucine (S-Leu) increased in the TG. In contrast, S-Leu decreased in the TNC, as did *N*-oleoyl leucine (Fig. 16.5).

N-Acyl Methionines Alterations in *N*-acyl methionines were restricted to the CER and TG after a single mTBI. 2 *N*-acyl methionines increased in each of these tissue types and *N*-stearoyl methionine increased in both tissue types. These changes were no longer present after repeated mTBI. Only the TG and TNC had changes in *N*-acyl methionines in this condition. There was a large increase in *N*-docosahexaenoyl methionine in the TG and a more modest increase in *N*-arachidonoyl methionine (A-Met) in the TNC (Fig. 16.5).

N-Acyl Phenylalanines In all 3 tissue types, a single exposure to mTBI increased *N*-arachidonoyl phenylalanine (A-Phe). Single mTBI also increased *N*-oleoyl phenylalanine in the TNC only. Changes in *N*-acyl phenylalanines were not measured in the CER after 4 mTBIs and increases in A-Phe were restricted to the TG and TNC. 2 additional *N*-acyl phenylalanines decreased in the TNC (Fig. 16.5).

PhosphoLEA PhosphoNAEs are intermediates for NAE production [109]. PhosphoLEA is the only phosphoNAE that is available in the screening library. This lipid only changed after repeated mTBI. Specifically, repeated mTBI increased phosphoLEA in the CER and TG (Fig. 16.5) [43]. Interestingly, these increases did not always accompany an increase in LEA.

Multiple orphan lipids, which lack molecular targets, change with mTBI suggesting that a wider variety of signaling systems may be affected. Those orphan lipids that stand out include A-Phe, which increased in all 3 tissue types after a single mTBI. mTBI affected certain lipoamines derived from AA, both orphan and with known molecular targets. For example, A-Ala robustly increased in the CER and TG following a single mTBI, A-Met increased in the TNC after repeated mTBI, and A-Pro increased in the TG after repeated mTBI. In all 3 tissue types, a single exposure to mTBI increased levels of A-Phe. Increases in A-Phe were also found in the TG and TNC after repeated mTBI. mTBI might therefore have more of an effect on enzymes that preferentially act on substrates containing AA.

16.5 Effects on Bioactive Lipids Change Over Time But Are More Similar When Headache-Like Symptoms Are Present

Effects of mTBI and acrolein exposure were quite different when comparing acute acrolein exposure effects to mTBI effects. Specifically, levels of bioactive lipids were more likely to decrease after the acute acrolein exposure [43]. These lipids tended to increase after mTBI. One explanation for these differences is that the tissue was collected immediately after the single 4 h acrolein exposure, whereas tissue was collected at least 72 h later in the case of mTBI. It wasn't until after repeated acrolein exposure that headache-like behaviors appeared [54], whereas after the single mTBI exposure, there were measurable headache-like behaviors. Similar alterations in lipid levels were observed between the models at time points when headache behavior was measurable (4 acrolein exposures or 1 mTBI

exposure). One key finding is that chronic acrolein exposure increased levels of multiple TRPV ligands and increased levels of NAGly [43], as did mTBI. Thus, bioactive lipids may serve as a marker for the physiological changes in headache, regardless of the original stimulus that disrupted the TVS. Follow-up studies should examine whether there are similar changes in lipid levels immediately after the mTBI, as these could give insight into the lipid milieu that eventually gives rise to inflammation and pain.

Providing further evidence that the effects of painful insults on bioactive lipids are dynamic and dependent on time post-insult, in another rodent model of inflammatory pain, we also observed decreases in bioactive lipids in CNS tissues 1 h after intraplantar carrageenan application [41]. For example, A-GABA decreased in the cerebellum and brainstem and OEA decreased in the thalamus. At 3 h post-carrageenan, increases were observed in multiple NAEs in all 6 brain areas tested (striatum, hippocampus, cerebellum, thalamus, midbrain, and brainstem) [41]. These effects at 3 h included increases in NAGly in the striatum, hippocampus, and cerebellum [41]. Therefore, the effects of peripheral insults on bioactive lipids take some time to centralize, whereas the effects of central insults like mTBI may take some time to fully permeate the periphery. Overall, the effects of peripheral inflammation, acrolein exposure, and mTBI on bioactive lipids look more similar when pain-related behaviors emerge.

16.5.1 Implications for Therapeutics

Current therapeutics for headache are not particularly effective, underscoring the need to better understand the mechanisms that cause headache and develop new drugs that target these mechanisms [110]. TRPA1 may represent a novel target for treating headache [111]. Studies in the TRPA1 KO mouse [52] suggest that TRPA1 is required for pain and hypersensitivity associated with chronic inflammation, as TRPA1 functions as a gatekeeper of the release of endogenous inflammatory mediators from sensory neurons [24]. Systemic pretreatment with the TRPA1 antagonist AP-18 prevented the sensitization of the TVS due to chronic acrolein [11]. In acrolein-exposed rats treated with AP-18, changes in dural blood flow in response to either TRPA1 or TRPV1 agonist challenges did not sensitize the responses, implicating TRPA1 in the process of sensitization regardless of the exact receptor action of the agent [11]. The biochemical similarities between the 2 models (acrolein and mTBI), such as increases in NAGly [43], as well as the similarities in central and peripheral sensitization [11, 54], suggest that the same therapy may work in both models. A follow up study will test the hypothesis that pretreatment with AP-18 prevents headache driven by mTBI.

Novel headache therapeutics that block microglial activation are currently being developed [13, 112]. It is possible that attenuating microglial activation could be a therapy for headache due to mTBI, as both share changes in neuroinflammatory mediators that drive microglial activation [4, 13]. Strengthening the hypothesis that blocking microglial migration represents a strategy for treating mTBI, A-Ser, which attenuated NAGly-driven microglial migration [59], was also neuroprotective after TBI [47, 48]. A-Ser levels were elevated in the CER and TG post-repeated mTBI, potentially representing a compensatory strategy to reduce the microglial activation and migration that occurs after TBI. Because GPR18 activation drives microglial migration [59], it should be tested whether GPR18 antagonists are effective at preventing microglial migration after mTBI, as this might represent a novel strategy to reduce inflammation. FAAH inhibition also represents a strategy to reduce NAGly levels [69, 113]. Lipidomics studies have revealed that genetic [69] and pharmacological [113] inhibition of FAAH increases levels of AEA and other NAEs but at the expense of other AA-derived lipoamines such as NAGly. Treatment with the FAAH inhibitor URB597 reduced microglial and astrocytic activation after TBI in rats and was associated with improvement in behavioral measures [20]. The contribution of NAGly to these findings has not yet been investigated.

Cannabis-based medicines also represent a strategy to reduce inflammation and increase neuroprotection after mTBI, as well as reduce headache [4]. 85.1% of medical cannabis patients in Colorado who were recommended cannabis due to migraine experienced a reduction in migraine frequency [114]. Cannabidiol (CBD) might be a suitable candidate plant cannabinoid to treat headache, as it lacks abuse liability or intoxicating effects [4, 18]. Potentially reducing the inflammation associated with headache, CBD has known anti-inflammatory properties [4, 115]. Interestingly, CBD can block microglial migration driven by GPR18 [59], so it might be able to reduce neuroinflammation by blocking a target for NAGly and additional *N*-acyl glycines. As CBD has anti-epileptic properties [116], it might also protect against excitotoxicity that occurs after TBI [13].

16.6 Conclusions

In a rodent model of mTBI, there were widespread changes in levels of many bioactive lipids in the TVS and in the CER. Most of the mTBI-driven changes in lipids were increases. Some of these lipids are active at cannabinoid receptors or at TRPV1 receptors, with potential implications for the development of pain post TBI. Other lipids that were affected by mTBI act on microglia, which could play a role in the microglial activation and inflammation seen after mTBI. The most robust example being the AEA metabolite NAGly, which was upregulated in every tissue type screened after mTBI. NAGly is a potent inducer of migration in microglia, and global changes in NAGly levels could contribute to neuroinflammation and headache. Finally, the effects on DEA may provide an insight into the regulation of DHA-derived lipids such as the resolvins and further indicates that no lipid is an island. The interconnectedness that is a continuous theme in our understanding of bioactive lipids is particularly highlighted in the novel data presented in this chapter and also provides a new platform for thinking about how the long-term effects of mTBI has on lipid signaling.

References

1. Gardner RC, Yaffe K (2015) Epidemiology of mild traumatic brain injury and neurodegenerative disease. Mol Cell Neurosci 66(Pt B):75–80. https://doi.org/10.1016/j.mcn.2015.03.001
2. Evans RW (2004) Post-traumatic headaches. Neurol Clin 22(1):237–249
3. Lucas S, Smith BM, Temkin N, Bell KR, Dikmen S, Hoffman JM (2016) Comorbidity of headache and depression after mild traumatic brain injury. Headache 56(2):323–330. https://doi.org/10.1111/head.12762
4. Elliott MB, Ward SJ, Abood ME, Tuma RF, Jallo JI (2017) Understanding the endocannabinoid system as a modulator of the trigeminal pain response to concussion. Concussion 2(4):CNC49
5. Hoffman JM, Lucas S, Dikmen S, Braden CA, Brown AW, Brunner R, Diaz-Arrastia R, Walker WC, Watanabe TK, Bell KR (2011) Natural history of headache after traumatic brain injury. J Neurotrauma 28(9):1719–1725
6. Xu H, Pi H, Ma L, Su X, Wang J (2016) Incidence of headache after traumatic brain injury in China: a large prospective study. World Neurosurg 88:289–296
7. Mihalik JP, Stump JE, Collins MW, Lovell MR, Field M, Maroon JC (2005) Posttraumatic migraine characteristics in athletes following sports-related concussion. J Neurosurg 102(5):850–855
8. Ruff RL, Blake K (2016) Pathophysiological links between traumatic brain injury and post-traumatic headaches. F1000 Res 5:2116
9. Eckner JT, Seifert T, Pescovitz A, Zeiger M, Kutcher JS (2017) Is migraine headache associated with concussion in athletes? A case-control study. Clin J Sport Med 27(3):266–270
10. Goadsby PJ, Edvinsson L (1993) The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann Neurol 33(1):48–56. https://doi.org/10.1002/ana.410330109
11. Kunkler PE, Zhang L, Pellman JJ, Oxford GS, Hurley JH (2015) Sensitization of the trigeminovascular system following environmental irritant exposure. Cephalalgia 35(13):1192–1201. https://doi.org/10.1177/0333102415574845
12. Mayeux J, Katz P, Edwards S, Middleton JW, Molina PE (2017) Inhibition of endocannabinoid degradation improves outcomes from mild traumatic brain injury: a mechanistic role for synaptic hyperexcitability. J Neurotrauma 34(2):436–443
13. Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, Borlongan CV (2015) Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr Dis Treat 11:97
14. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum

A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258(5090):1946–1949
15. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215(1):89–97
16. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR et al (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50(1):83–90
17. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48(3):443–450
18. Leimuranta P, Khiroug L, Giniatullin R (2018) Emerging role of (Endo) cannabinoids in migraine. Front Pharmacol 9:420
19. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanuš L, Breuer A, Mechoulam R, Shohami E (2001) An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 413(6855):527–531
20. Katz PS, Sulzer JK, Impastato RA, Teng SX, Rogers EK, Molina PE (2015) Endocannabinoid degradation inhibition improves neurobehavioral function, blood–brain barrier integrity, and neuroinflammation following mild traumatic brain injury. J Neurotrauma 32(5):297–306
21. Amenta PS, Jallo JI, Tuma RF, Elliott MB (2012) A cannabinoid type 2 receptor agonist attenuates blood–brain barrier damage and neurodegeneration in a murine model of traumatic brain injury. J Neurosci Res 90(12):2293–2305
22. Bradshaw HB, Raboune S, Hollis JL (2013) Opportunistic activation of TRP receptors by endogenous lipids: exploiting lipidomics to understand TRP receptor cellular communication. Life Sci 92(8–9):404–409. https://doi.org/10.1016/j.lfs.2012.11.008
23. Marrone MC, Morabito A, Giustizieri M, Chiurchiu V, Leuti A, Mattioli M, Marinelli S, Riganti L, Lombardi M, Murana E, Totaro A, Piomelli D, Ragozzino D, Oddi S, Maccarrone M, Verderio C, Marinelli S (2017) TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. Nat Commun 8:15292. https://doi.org/10.1038/ncomms15292
24. Bautista DM, Pellegrino M, Tsunozaki M (2013) TRPA1: a gatekeeper for inflammation. Annu Rev Physiol 75:181–200. https://doi.org/10.1146/annurev-physiol-030212-183811
25. Oxford GS, Hurley JH (2013) The role of TRP channels in migraine. Open Pain J 6(Suppl 1):37–49
26. Clapham DE (2003) TRP channels as cellular sensors. Nature 426(6966):517–524
27. Julius D (2013) TRP channels and pain. Annu Rev Cell Dev Biol 29:355–384
28. Moran MM (2018) TRP channels as potential drug targets. Annu Rev Pharmacol Toxicol 58:309–330
29. Voets T, Talavera K, Owsianik G, Nilius B (2005) Sensing with TRP channels. Nat Chem Biol 1(2):85–92
30. Venkatachalam K, Luo J, Montell C (2014) Evolutionarily conserved, multitasking TRP channels: lessons from worms and flies. In: Mammalian transient receptor potential (TRP) cation channels. Springer, Berlin, pp 937–962
31. Nilius B, Szallasi A (2014) Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. Pharmacol Rev 66(3):676–814
32. Ho KW, Ward NJ, Calkins DJ (2012) TRPV1: a stress response protein in the central nervous system. Am J Neurodegener Dis 1(1):1–14
33. Starowicz K, Makuch W, Korostynski M, Malek N, Slezak M, Zychowska M, Petrosino S, De Petrocellis L, Cristino L, Przewlocka B, Di Marzo V (2013) Full inhibition of spinal FAAH leads to TRPV1-mediated analgesic effects in neuropathic rats and possible lipoxygenase-mediated remodeling of anandamide metabolism. PLoS One 8(4):e60040. https://doi.org/10.1371/journal.pone.0060040
34. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389(6653):816–824
35. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400(6743):452–457. https://doi.org/10.1038/22761
36. Zygmunt PM, Ermund A, Movahed P, Andersson DA, Simonsen C, Jonsson BA, Blomgren A, Birnir B, Bevan S, Eschalier A, Mallet C, Gomis A, Hogestatt ED (2013) Monoacylglycerols activate TRPV1--a link between phospholipase C and TRPV1. PLoS One 8(12):e81618. https://doi.org/10.1371/journal.pone.0081618
37. Ahern GP (2003) Activation of TRPV1 by the satiety factor oleoylethanolamide. J Biol Chem 278(33):30429–30434. https://doi.org/10.1074/jbc.M305051200
38. Movahed P, Jonsson BA, Birnir B, Wingstrand JA, Jorgensen TD, Ermund A, Sterner O, Zygmunt PM, Hogestatt ED (2005) Endogenous unsaturated C18 N-acylethanolamines are vanilloid receptor (TRPV1) agonists. J Biol Chem 280(46):38496–38504. https://doi.org/10.1074/jbc.M507429200
39. Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF (2006) A FAAH-regulated class of N-acyl taurines that activates TRP ion channels. Biochemistry 45(30):9007–9015. https://doi.org/10.1021/bi0608008
40. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ,

Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A 99(12):8400–8405. https://doi.org/10.1073/pnas.122196999

41. Raboune S, Stuart JM, Leishman E, Takacs SM, Rhodes B, Basnet A, Jameyfield E, McHugh D, Widlanski T, Bradshaw HB (2014) Novel endogenous N-acyl amides activate TRPV1-4 receptors, BV-2 microglia, and are regulated in brain in an acute model of inflammation. Front Cell Neurosci 8:195. https://doi.org/10.3389/fncel.2014.00195
42. Kunkler PE, Ballard CJ, Pellman JJ, Zhang L, Oxford GS, Hurley JH (2014) Intraganglionic signaling as a novel nasal-meningeal pathway for TRPA1-dependent trigeminovascular activation by inhaled environmental irritants. PLoS One 9(7):e103086. https://doi.org/10.1371/journal.pone.0103086
43. Leishman E, Kunkler PE, Manchanda M, Sangani K, Stuart JM, Oxford GS, Hurley JH, Bradshaw HB (2017) Environmental toxin acrolein alters levels of endogenous lipids, including TRP agonists: a potential mechanism for headache driven by TRPA1 activation. Neurobiol Pain 1:28–36. https://doi.org/10.1016/j.ynpai.2017.03.001
44. Milman G, Maor Y, Abu-Lafi S, Horowitz M, Gallily R, Batkai S, Mo F-M, Offertaler L, Pacher P, Kunos G (2006) N-arachidonoyl L-serine, an endocannabinoid-like brain constituent with vasodilatory properties. Proc Natl Acad Sci U S A 103(7):2428–2433
45. Smoum R, Bar A, Tan B, Milman G, Attar-Namdar M, Ofek O, Stuart JM, Bajayo A, Tam J, Kram V (2010) Oleoyl serine, an endogenous N-acyl amide, modulates bone remodeling and mass. Proc Natl Acad Sci 107(41):17710–17715
46. Tortoriello G, Rhodes BP, Takacs SM, Stuart JM, Basnet A, Raboune S, Widlanski TS, Doherty P, Harkany T, Bradshaw HB (2013) Targeted lipidomics in Drosophila melanogaster identifies novel 2-monoacylglycerols and N-acyl amides. PLoS One 8(7):e67865. https://doi.org/10.1371/journal.pone.0067865
47. Mann A, Cohen-Yeshurun A, Trembovler V, Mechoulam R, Shohami E (2016) Are the endocannabinoid-like compounds N-acyl aminoacids neuroprotective after traumatic brain injury? J Basic Clin Physiol Pharmacol 27(3):209–216
48. Cohen-Yeshurun A, Trembovler V, Alexandrovich A, Ryberg E, Greasley PJ, Mechoulam R, Shohami E, Leker RR (2011) N-arachidonoyl-L-serine is neuroprotective after traumatic brain injury by reducing apoptosis. J Cereb Blood Flow Metab 31(8):1768–1777. https://doi.org/10.1038/jcbfm.2011.53
49. Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Hogestatt ED, Meng ID, Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 427(6971):260–265. https://doi.org/10.1038/nature02282
50. Feng Z, Hu W, Hu Y, Tang MS (2006) Acrolein is a major cigarette-related lung cancer agent: preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci U S A 103(42):15404–15409. https://doi.org/10.1073/pnas.0607031103
51. Brone B, Peeters PJ, Marrannes R, Mercken M, Nuydens R, Meert T, Gijsen HJ (2008) Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor. Toxicol Appl Pharmacol 231(2):150–156. https://doi.org/10.1016/j.taap.2008.04.005
52. Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D (2006) TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124(6):1269–1282. https://doi.org/10.1016/j.cell.2006.02.023
53. Kunkler PE, Ballard CJ, Oxford GS, Hurley JH (2011) TRPA1 receptors mediate environmental irritant-induced meningeal vasodilatation. Pain 152(1):38–44. https://doi.org/10.1016/j.pain.2010.08.021
54. Kunkler PE, Zhang L, Johnson PL, Oxford GS, Hurley JH (2018) Induction of chronic migraine phenotypes in a rat model after environmental irritant exposure. Pain 159(3):540–549. https://doi.org/10.1097/j.pain.0000000000001124
55. Eftekhari S, Edvinsson L (2011) Calcitonin gene-related peptide (CGRP) and its receptor components in human and rat spinal trigeminal nucleus and spinal cord at C1-level. BMC Neurosci 12(1):1
56. Daiutolo BV, Tyburski A, Clark SW, Elliott MB (2016) Trigeminal pain molecules, allodynia, and photosensitivity are pharmacologically and genetically modulated in a model of traumatic brain injury. J Neurotrauma 33(8):748–760
57. Elliott MB, Oshinsky ML, Amenta PS, Awe OO, Jallo JI (2012) Nociceptive neuropeptide increases and periorbital allodynia in a model of traumatic brain injury. Headache J Head Face Pain 52(6):966–984
58. Bradshaw HB, Rimmerman N, Hu SS, Benton VM, Stuart JM, Masuda K, Cravatt BF, O'Dell DK, Walker JM (2009) The endocannabinoid anandamide is a precursor for the signaling lipid N-arachidonoyl glycine by two distinct pathways. BMC Biochem 10:14. https://doi.org/10.1186/1471-2091-10-14
59. McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, Bradshaw HB (2010) N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11:44. https://doi.org/10.1186/1471-2202-11-44
60. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16(2):109–110

61. Velosky AG, Tucker LB, Fu AH, Liu J, McCabe JT (2017) Cognitive performance of male and female C57BL/6J mice after repetitive concussive brain injuries. Behav Brain Res 324:115–124
62. Fehily B, Fitzgerald M (2017) Repeated mild traumatic brain injury: potential mechanisms of damage. Cell Transplant 26(7):1131–1155
63. Shitaka Y, Tran HT, Bennett RE, Sanchez L, Levy MA, Dikranian K, Brody DL (2011) Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. J Neuropathol Exp Neurol 70(7):551–567
64. Bolton AN, Saatman KE (2014) Regional neurodegeneration and gliosis are amplified by mild traumatic brain injury repeated at 24-hour intervals. J Neuropathol Exp Neurol 73(10):933–947
65. Tan B, Bradshaw HB, Rimmerman N, Srinivasan H, Yu YW, Krey JF, Monn MF, Chen JS, Hu SS, Pickens SR, Walker JM (2006) Targeted lipidomics: discovery of new fatty acyl amides. AAPS J 8(3):E461–E465. https://doi.org/10.1208/aapsj080354
66. Carey LM, Slivicki RA, Leishman E, Cornett B, Mackie K, Bradshaw H, Hohmann AG (2016) A pro-nociceptive phenotype unmasked in mice lacking fatty-acid amide hydrolase. Mol Pain 12:174480691664919. https://doi.org/10.1177/1744806916649192
67. Stuart JM, Paris JJ, Frye C, Bradshaw HB (2013) Brain levels of prostaglandins, endocannabinoids, and related lipids are affected by mating strategies. Int J Endocrinol 2013:436252. https://doi.org/10.1155/2013/436252
68. Sun X, Deng W, Li Y, Tang S, Leishman E, Bradshaw HB, Dey SK (2016) Sustained endocannabinoid signaling compromises decidual function and promotes inflammation-induced preterm birth. J Biol Chem 291(15):8231–8240. https://doi.org/10.1074/jbc.M115.707836
69. Leishman E, Cornett B, Spork K, Straiker A, Mackie K, Bradshaw HB (2016) Broad impact of deleting endogenous cannabinoid hydrolyzing enzymes and the CB1 cannabinoid receptor on the endogenous cannabinoid-related lipidome in eight regions of the mouse brain. Pharmacol Res 110:159–172. https://doi.org/10.1016/j.phrs.2016.04.020
70. Leishman E, Mackie K, Luquet S, Bradshaw HB (2016) Lipidomics profile of a NAPE-PLD KO mouse provides evidence of a broader role of this enzyme in lipid metabolism in the brain. Biochim Biophys Acta 1861(6):491–500. https://doi.org/10.1016/j.bbalip.2016.03.003
71. Leishman E, Murphy M, Mackie K, Bradshaw HB (2018) Delta(9)-tetrahydrocannabinol changes the brain lipidome and transcriptome differentially in the adolescent and the adult. Biochim Biophys Acta 1863(5):479–492. https://doi.org/10.1016/j.bbalip.2018.02.001
72. Leishman E, Murphy MN, Murphy MI, Mackie K, Bradshaw HB (2018) Broad and region-specific impacts of the synthetic cannabinoid CP 55,940 in adolescent and adult female mouse brains. Front Mol Neurosci 11:436
73. McHugh D, Wager-Miller J, Page J, Bradshaw HB (2012) siRNA knockdown of GPR18 receptors in BV-2 microglia attenuates N-arachidonoyl glycine-induced cell migration. J Mol Signal 7(1):10. https://doi.org/10.1186/1750-2187-7-10
74. Shohami E, Cohen-Yeshurun A, Magid L, Algali M, Mechoulam R (2011) Endocannabinoids and traumatic brain injury. Br J Pharmacol 163(7):1402–1410. https://doi.org/10.1111/j.1476-5381.2011.01343.x
75. Jeffries KA, Dempsey DR, Farrell EK, Anderson RL, Garbade GJ, Gurina TS, Gruhonjic I, Gunderson CA, Merkler DJ (2016) Glycine N-acyltransferase like 3 is responsible for long-chain N-acylglycine formation in N18TG2 cells. J Lipid Res 57(5):781–790. https://doi.org/10.1194/jlr.M062042
76. Long JZ, Roche AM, Berdan CA, Louie SM, Roberts AJ, Svensson KJ, Dou FY, Bateman LA, Mina AI, Deng Z, Jedrychowski MP, Lin H, Kamenecka TM, Asara JM, Griffin PR, Banks AS, Nomura DK, Spiegelman BM (2018) Ablation of PM20D1 reveals N-acyl amino acid control of metabolism and nociception. Proc Natl Acad Sci U S A 115:E6937–E6945. https://doi.org/10.1073/pnas.1803389115
77. Long JZ, Svensson KJ, Bateman LA, Lin H, Kamenecka T, Lokurkar IA, Lou J, Rao RR, Chang MR, Jedrychowski MP, Paulo JA, Gygi SP, Griffin PR, Nomura DK, Spiegelman BM (2016) The secreted enzyme PM20D1 regulates lipidated amino acid uncouplers of mitochondria. Cell 166(2):424–435. https://doi.org/10.1016/j.cell.2016.05.071
78. Donvito G, Piscitelli F, Muldoon P, Jackson A, Vitale RM, D'Aniello E, Giordano C, Ignatowska-Jankowska BM, Mustafa MA, Guida F (2018) N-Oleoyl-glycine reduces nicotine reward and withdrawal in mice. Neuropharmacology 148:320–331
79. Blennow K, Hardy J, Zetterberg H (2012) The neuropathology and neurobiology of traumatic brain injury. Neuron 76(5):886–899
80. Koivisto A (2012) Sustained TRPA1 activation in vivo. Acta Physiol 204(2):248–254
81. Bailes JE, Mills JD (2010) Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model. J Neurotrauma 27(9):1617–1624. https://doi.org/10.1089/neu.2009.1239
82. Wu A, Ying Z, Gomez-Pinilla F (2004) Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. J Neurotrauma 21(10):1457–1467
83. Mills JD, Hadley K, Bailes JE (2011) Dietary supplementation with the omega-3 fatty acid docosahexaenoic acid in traumatic brain injury. Neurosurgery 68(2):474–481
84. Desai A, Kevala K, Kim H-Y (2014) Depletion of brain docosahexaenoic acid impairs recovery from traumatic brain injury. PLoS One 9(1):e86472

85. Harvey LD, Yin Y, Attarwala IY, Begum G, Deng J, Yan HQ, Dixon CE, Sun D (2015) Administration of DHA reduces endoplasmic reticulum stress-associated inflammation and alters microglial or macrophage activation in traumatic brain injury. ASN Neuro 7(6):1759091415618969
86. Serhan CN, Recchiuti A (2012) Pro-resolving lipid mediators (SPMs) and their actions in regulating miRNA in novel resolution circuits in inflammation. Front Immunol 3:298
87. Xu Z-Z, Zhang L, Liu T, Park JY, Berta T, Yang R, Serhan CN, Ji R-R (2010) Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat Med 16(5):592–597
88. Park C-K, Lü N, Xu Z-Z, Liu T, Serhan CN, Ji R-R (2011) Resolving TRPV1-and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. J Neurosci 31(42):15072–15085
89. Harrison JL, Rowe RK, Lifshitz J (2016) Lipid mediators of inflammation in neurological injury: shifting the balance toward resolution. Neural Regen Res 11(1):77–78
90. Harrison JL, Rowe RK, Ellis TW, Yee NS, O'Hara BF, Adelson PD, Lifshitz J (2015) Resolvins AT-D1 and E1 differentially impact functional outcome, post-traumatic sleep, and microglial activation following diffuse brain injury in the mouse. Brain Behav Immun 47:131–140
91. Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN (2003) Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem 278(44):43807–43817
92. Yang R, Fredman G, Krishnamoorthy S, Agrawal N, Irimia D, Piomelli D, Serhan CN (2011) Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel bioactive signals. J Biol Chem 286(36):31532–31541. https://doi.org/10.1074/jbc.M111.237990
93. Park SW, Hah JH, Oh SM, Jeong WJ, Sung MW (2016) 5-lipoxygenase mediates docosahexaenoyl ethanolamide and N-arachidonoyl-L-alanine-induced reactive oxygen species production and inhibition of proliferation of head and neck squamous cell carcinoma cells. BMC Cancer 16:458. https://doi.org/10.1186/s12885-016-2499-3
94. Bradshaw HB, Leishman E (2017) Lipidomics: a corrective lens for enzyme myopia. In: Methods in enzymology, vol 593. Elsevier, Amsterdam, pp 123–141
95. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams E-J (2003) Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. J Cell Biol 163(3):463–468
96. Reisenberg M, Singh PK, Williams G, Doherty P (2012) The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signalling. Philos Trans R Soc Lond Ser B Biol Sci 367(1607):3264–3275. https://doi.org/10.1098/rstb.2011.0387
97. Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakimura K, Kano M (2010) The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. Neuron 65(3):320–327. https://doi.org/10.1016/j.neuron.2010.01.021
98. Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, Reisenberg M, Shen R, Zhang MY, Strassle BW, Lu P, Mark L, Piesla MJ, Deng K, Kouranova EV, Ring RH, Whiteside GT, Bates B, Walsh FS, Williams G, Pangalos MN, Samad TA, Doherty P (2010) Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci 30(6):2017–2024. https://doi.org/10.1523/JNEUROSCI.5693-09.2010
99. Shonesy BC, Bluett RJ, Ramikie TS, Báldi R, Hermanson DJ, Kingsley PJ, Marnett LJ, Winder DG, Colbran RJ, Patel S (2014) Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep 9(5):1644–1653
100. Ogasawara D, Deng H, Viader A, Baggelaar MP, Breman A, den Dulk H, van den Nieuwendijk AM, Soethoudt M, van der Wel T, Zhou J, Overkleeft HS, Sanchez-Alavez M, Mori S, Nguyen W, Conti B, Liu X, Chen Y, Liu QS, Cravatt BF, van der Stelt M (2016) Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. Proc Natl Acad Sci U S A 113(1):26–33. https://doi.org/10.1073/pnas.1522364112
101. van der Wel T, Janssen FJ, Baggelaar MP, Deng H, den Dulk H, Overkleeft HS, van der Stelt M (2015) A natural substrate-based fluorescence assay for inhibitor screening on diacylglycerol lipase α. J Lipid Res 56(4):927–935
102. Baggelaar MP, Janssen FJ, van Esbroeck A, den Dulk H, Allarà M, Hoogendoorn S, McGuire R, Florea BI, Meeuwenoord N, van den Elst H (2013) Development of an activity-based probe and in silico design reveal highly selective inhibitors for diacylglycerol lipase-α in brain. Angew Chem Int Ed 52(46):12081–12085
103. Liu Y, Patricelli MP, Cravatt BF (1999) Activity-based protein profiling: the serine hydrolases. Proc Natl Acad Sci 96(26):14694–14699
104. Sugiura T, Kishimoto S, Oka S, Gokoh M (2006) Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. Prog Lipid Res 45(5):405–446. https://doi.org/10.1016/j.plipres.2006.03.003

105. Wei EP, Lamb RG, Kontos HA (1982) Increased phospholipase C activity after experimental brain injury. J Neurosurg 56(5):695–698
106. Hwang SC, Jhon D-Y, Bae YS, Kim JH, Rhee SG (1996) Activation of phospholipase C-γ by the concerted action of tau proteins and arachidonic acid. J Biol Chem 271(31):18342–18349
107. Axelrod J, Burch RM, Jelsema CL (1988) Receptor-mediated activation of phospholipase A2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. Trends Neurosci 11(3):117–123
108. Pilitsis JG, Coplin WM, O'Regan MH, Wellwood JM, Diaz FG, Fairfax MR, Michael DB, Phillis JW (2003) Free fatty acids in cerebrospinal fluids from patients with traumatic brain injury. Neurosci Lett 349(2):136–138
109. Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G (2006) A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A 103(36):13345–13350. https://doi.org/10.1073/pnas.0601832103
110. Dussor G, Yan J, Xie JY, Ossipov MH, Dodick DW, Porreca F (2014) Targeting TRP channels for novel migraine therapeutics. ACS Chem Neurosci 5(11):1085–1096. https://doi.org/10.1021/cn500083e
111. Koivisto A, Chapman H, Jalava N, Korjamo T, Saarnilehto M, Lindstedt K, Pertovaara A (2014) TRPA1: a transducer and amplifier of pain and inflammation. Basic Clin Pharmacol Toxicol 114(1):50–55. https://doi.org/10.1111/bcpt.12138
112. Liu Q, Liu C, Jiang L, Li M, Long T, He W, Qin G, Chen L, Zhou J (2018) α7 nicotinic acetylcholine receptor-mediated anti-inflammatory effect in a chronic migraine rat model via the attenuation of glial cell activation. J Pain Res 11:1129–1140
113. Han B, Wright R, Kirchhoff AM, Chester JA, Cooper BR, Davisson VJ, Barker E (2013) Quantitative LC-MS/MS analysis of arachidonoyl amino acids in mouse brain with treatment of FAAH inhibitor. Anal Biochem 432(2):74–81. https://doi.org/10.1016/j.ab.2012.09.031
114. Russo EB (2016) Clinical endocannabinoid deficiency reconsidered: current research supports the theory in migraine, fibromyalgia, irritable bowel, and other treatment-resistant syndromes. Cannabis Cannabinoid Res 1(1):154–165
115. Russo EB, Hohmann AG (2013) Role of cannabinoids in pain management. In: Comprehensive treatment of chronic pain by medical, interventional, and integrative approaches. Springer, New York, pp 181–197
116. Reddy D (2017) The utility of cannabidiol in the treatment of refractory epilepsy. Clin Pharmacol Ther 101(2):182–184

17

Novel Anti-inflammatory and Vasodilatory ω-3 Endocannabinoid Epoxide Regioisomers

Lauren N. Carnevale and Aditi Das

Abstract

Accumulating evidence suggests that diets rich in ω-3 polyunsaturated fatty acids (PUFAs) offer protection against vascular inflammation, neuroinflammation, hypertension, and thrombosis. Recently, biochemical studies have demonstrated that these benefits are partially mediated by their conversion to ω-3 endocannabinoid epoxide metabolites. These lipid metabolites originate from the epoxidation of ω-3 endocannabinoids, docosahexanoyl ethanolamide (DHEA) and eicosapentaenoyl ethanolamide (EPEA) by cytochrome P450 (CYP) epoxygenases to form epoxydocosapentaenoic acid-ethanolamides (EDP-EAs) and epoxyeicosatetraenoic acid-ethanolamides (EEQ-EAs), respectively. The EDP-EAs and EEQ-EAs are endogenously produced in rat brain and peripheral organs. Additionally, EDP-EAs and EEQ-EAs dose-dependently decrease pro-inflammatory IL-6 cytokine and increased anti-inflammatory IL-10 cytokine. Furthermore, the EEQ-EAs and EDP-EAs attenuate angiogenesis and cell migration in cancer cells, induce vasodilation in bovine coronary arteries, and reciprocally regulate platelet aggregation in washed human platelets. Taken together, the ω-3 endocannabinoid epoxides represent a new class of dual acting molecules that display unique pharmacological properties.

Keywords

Cytochrome p450 · Epoxygenase · Neuroinflammation · Endocannabinoid · Epoxyeicosatrienoic acids · Cannabinoid receptors 1 and 2 · Omega-3 fatty acids

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Abbreviations

2-AG	2-arachidonoyl-glycerol
2-DHG	2-docosahexaenoyl-glycerol
2-EPG	2-eicosapentaenoyl-glycerol
AA	arachidonic acid
AEA	anandamide
cAMP	cyclic adenosine monophosphate
CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
CYP	cytochrome P450
DHA	docosahexaenoic acid
DHEA	docosahexaenoyl ethanolamide
eCB	endocannabinoid
EDP-EA	docosapentaenoic acid ethanolamide
EEQ-EA	epoxyeicosatetraenoic acid ethanolamide
EPA	eicosapentaenoic acid
EPEA	eicosapentaenoyl ethanolamide
FAAH	fatty amide hydrolase
GPCR	G-Protein coupled receptor
MAGL	monoacylglycerol lipase
sEH	soluble epoxide hydrolase
Δ^9-THC	Δ^9-tetrahydrocannabinol

17.1 Introduction

17.1.1 The Endocannabinoid System

The main psychoactive component of *Cannabis sativa,* Δ^9-tetrahydrocannabinol (Δ^9-THC), was identified in 1987. Howlett's group showed that the neurobehavioral effects of Δ^9-THC were mediated through a G-protein ($G_{i/o}$) coupled receptor (GPCR). Shortly thereafter, Pfizer developed the synthetic cannabinoid, CP 55,940. Later, CP 55,940 and Δ^9-THC helped identify a cannabinoid (CB) receptor [1]. The first identified cannabinoid receptor, termed cannabinoid receptor 1 (CB1), is the most abundant GPCR located within the central nervous system (CNS) [1–3]. More specifically, it is found at high levels in the hippocampus, pre-frontal cortex, dorsal root ganglion, cerebellum, and substantia nigra. Although CB1 is most abundant in the CNS, it is found to a lesser extent in the cardiovascular, [4, 5] pulmonary, intestinal, and reproductive tissues [6]. Three years after CB1 was cloned, a second cannabinoid receptor, termed CB2, was identified. While both CB1 and CB2 are GPCRs ($G_{i/o}$) activated by phytocannabinoids, their tissue distribution and resulting physiological effects differ. For example, CB2 is primarily expressed in the spleen and immune cells with the highest levels occurring in B lymphocytes, macrophages, and natural killer cells [6–8]. Furthermore, during times of injury or stress, the resident immune cells, microglial cells, express CB2 thereby suggesting a role in abating neuroinflammation. These results prompted the design of CB2 agonists to modulate neuroinflammation [9].

The discovery of CB1 and CB2 prompted the researchers to identify the endogenous ligands of these receptors. The first endocannabinoid identified was arachidonoyl ethanolamide (AEA), which was extracted from pig brain and could readily displace the potent cannabinoid receptor ligand, [^{3}H]-HU243 ($K_i = 52$ nM). AEA was identified to be an agonist at the CB1 receptor and was later named anandamide. The name anandamide was derived from 'ananda,' the Sanskrit word for 'bliss' due to the compound's ability of modulate neurobehavioral activity [10]. Accordingly, AEA and all future ligands for CB1 and CB2 were collectively termed the *endo*cannabinoids (eCBs) since they are endogenously produced molecules that bind and activate cannabinoid receptors. The second eCB, 2-arachidonoyl glycerol (2-AG), was discovered just shortly after AEA. It was shown to activate the CB2 with greater potency than at the CB1 [11]. Since the discovery of AEA and 2-AG, several other eCBs have been discovered, including, but are not limited to, 2-arachidonoylglycerylether (noladin ether), N-arachidonoyl dopamine (NADA), N-oleoylethanolamide (OEA), 2-docosahexaenoyl glycerol, (2-DHG), virodhamine (O-AEA), eicosapentaenoic acid ethanolamide (EPEA) and docosahexaenoyl ethanolamide (DHEA) [10, 12–15]. Several other eCB congeners have been identified that may not directly bind cannabinoid receptors but can bind to other receptors such as TRPPV1, GRP119, GPR55, and GPR18. In addition, many of these receptors interact with phytocannabinoids [5, 16, 17].

Cellular eCB levels are carefully regulated through multiple biosynthetic and degradative enzymes. For instance, the eCBs originate from dietary ω-3 and ω-6 polyunsaturated fatty acids (PUFAs). PUFAs are class of lipids that contain two or more *cis* carbon-carbon double bonds (points of unsaturation) and are identified as ω-3 or ω-6 based on the location of the first carbon-carbon double bond from the terminal methyl group. ω-6 eCBs derived from arachidonic acid (AA), such as AEA and 2-AG, are the most well studied eCBs. With the recent interest in the dietary effects of ω-3 PUFAs, there has been an increased interest in ω-3 eCBs. The ω-3 eCBs are derived from the ω-3 PUFA docosahexaenoic acid (DHA), eicosapentanoic acid (EPA, timnodonic acid), or α-linoleic acid (ALA).

Following their uptake, PUFAs are mobilized via transport proteins to tissues where they esterified and are temporarily stored in the cell membrane (Fig. 17.1). For example, AEA is stored as its membrane precursor *N*-arachidonoyl phosphatidylethanolamine (NAPE) [18] and is released from the membrane by phospholipase D. This catabolic process yields a free eCB [19]. After biosynthesis, the eCBs are tightly regulated by degradative enzymes through hydrolytic inactivation [20]. For example, fatty acid amide hydrolase (FAAH) catalyzes the hydrolytic cleavage of AEA to form AA and ethanolamine [21]. FAAH is the principal AEA and DHEA-hydrolyzing enzyme distributed throughout the nervous system [22] On the other hand, monoacylglycerol lipase (MAGL) is the degradative

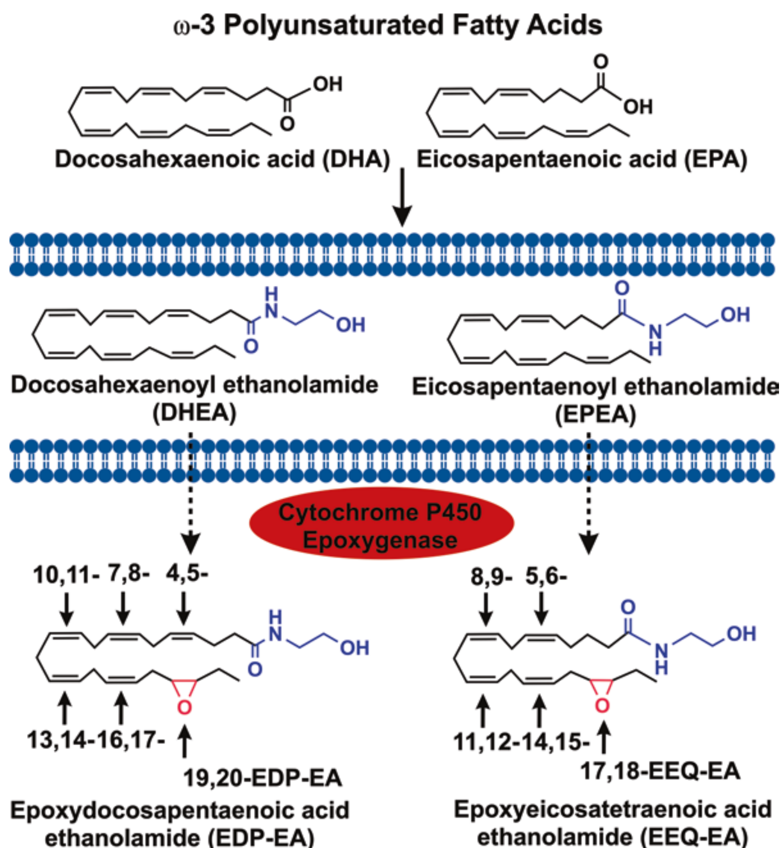

Fig. 17.1 Overview of the ω-3 Endocannabinoid Epoxide Biosynthetic Pathway. Dietary consumption of ω-3 polyunsaturated fatty acids results in the storage of DHA and EPA esters within the plasma membrane. Upon cleavage by a phospholipase, DHA and EPA are cleaved from their membrane precursor and undergo biosynthesis to the eCBs DHEA and EPEA, respectively. The metabolism of DHEA and EPEA by cytochrome P450 epoxygenases produces six (DHEA) or five (EPEA) constitutional isomers at each carbon-carbon double bond. DHEA epoxide metabolites are termed EDP-EAs while the EPEA epoxide metabolites are termed EEQ-EAs

enzymes for 2-AG and 2-DHG [20, 23]. Overall, these enzymes convert the eCBs back into their free fatty acid form, which can be esterified and stored in the phospholipid membrane bilayer or used for cellular functions [24].

Based on the widespread tissue distribution of the cannabinoid receptors there is a plethora of biochemical processes that utilize cannabinoid signaling [25–27]. The main role of CB1 is to mediate neurobehavioral activities, such as appetite regulation and executive functions, while the prominent function of CB2 is to mediate an anti-inflammatory immune response. More specifically, CB2 is involved in neuroinflammation through its immunoregulatory activity in murine brain microglia [5, 28]. Overall, these activities suggest that CB1 and CB2 are neuroprotective [29–32]. Given the prevalence of neuroinflammatory-based diseases such as Alzheimer's disease (AD), multiple sclerosis (MS), and Parkinson's disease (PD), selective agonists for CB2 activation or modulation of eCB levels have promising therapeutic potential for the control of neuroinflammation [29, 33–37]. Additionally, accumulating evidence suggests that eCBs target alternative receptors such as the nuclear peroxisome proliferator-activated receptors (PPAR) and the heat sensing receptor transient vanilloid type-1 (TRPV1), [38–40] prompting studies regarding their role in nociceptive pain.

While the eCBs directly bind to CB1 and CB2 receptors, they also serve as substrates for the eicosanoid synthesizing enzymes. Cyclooxygenases, lipoxygenases, and epoxygenases oxidize fatty acids to bioactive metabolites that have novel physiological and cannabinoid receptor activities that are unique compared to their parent molecule [5, 41]. Previously, the Serhan group reported the metabolism of DHEA by the lipoxygenase pathway. DHEA is metabolized to 10,12-diHDHA-EA and 15-HEDPEA, which reduce leukocyte infiltration (chemotaxis) during inflammation. They can activate the CB2 receptor with EC_{50} values at 3.9 nM and 1 nM, respectively [42]. In this chapter, we will discuss the monooxygenation of eCBs by epoxygenases to produce eCB epoxide metabolites with novel anti-inflammatory and potential anti-pain properties as well as vasoactive activities.

17.1.2 Endocannabinoid Metabolism by Cytochrome P450 (CYP) Epoxygenases

In humans, there are 57 CYPs which are involved in xenobiotic metabolism, steroid synthesis, and fatty acid metabolism [43]. These enzymes are distributed throughout the human body, with the highest levels reported in the liver. In 2008, the Hollenberg group was the first to demonstrate the metabolism of AEA by CYPs [44]. This represented a novel metabolic pathway for AEA that led to the formation of the emerging class of ω-6 derived eCB epoxides. Importantly, it was demonstrated that the AEA epoxide, 5,6-EET-EA, had 300-fold selectivity for the CB2 compared to CB1 and 1000-fold greater affinity for CB2 compared to AEA, indicating unique biological activity of the epoxides compared to their parent molecule [44]. To identify CYPs involved in the conversion of both AEA and 2-AG, the Hollenberg lab utilized LC-MS/MS to determine that CYP isoforms, CYP3A4, CYP4F2, and CYP2D6, metabolize AEA in human liver and kidney microsomes as well as in brain microsomal and mitochondrial preparations [45]. Furthermore, the Das group discovered the role of cardiac CYP, CYP2J2, in the metabolism of 2-AG and AEA. This finding inspired future studies on CYP2J2-mediated ω-3 and ω-6 eCB metabolism [12, 13, 46]. CYP2J2 converts PUFAs to bioactive fatty acid epoxides which display anti-inflammatory activities and enhance wound repair [47–49]. CYP epoxygenases mediate the metabolism of eCBs to eCB epoxides [13, 50, 51]. More recent studies have shown that CYP2J2 and other CYPs metabolize ω-3 eCBs such as DHEA and EPEA to more potent anti-inflammatory epoxide metabolites, as illustrated in Figs. 17.1 and 17.2. This metabolic pathway consists of CYP epoxygenases, which belong to the cysteinato-hemoprotein

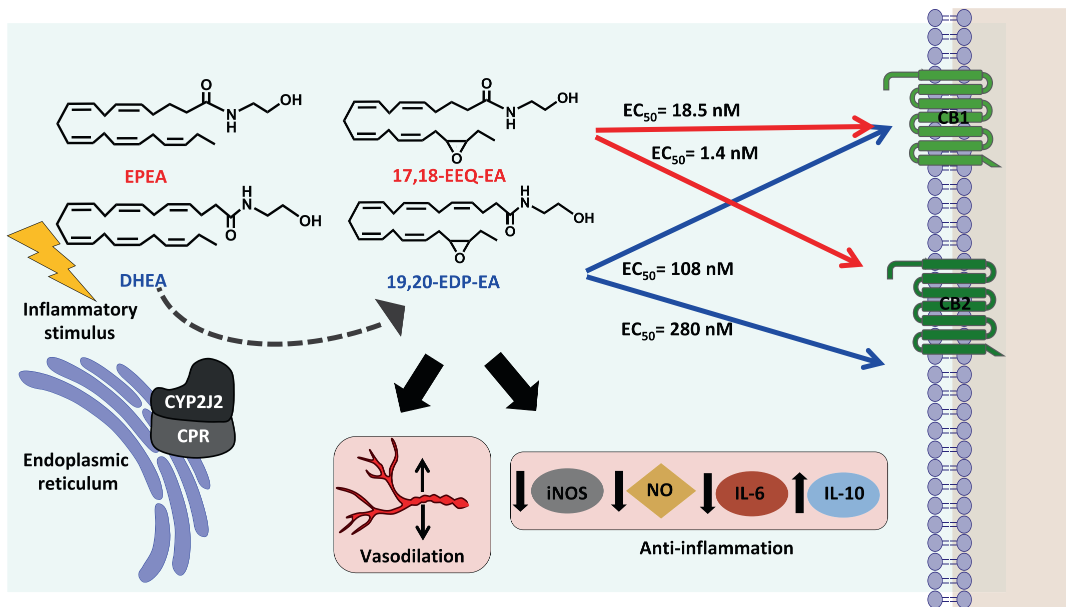

Fig. 17.2 Endocannabinoid Signaling Regulates Inflammation and Arterial Tone. Inflammatory stimuli such as LPS up-regulates cytochrome P450 expression which metabolize eCBs DHEA and EPEA to epoxide metabolites (19,20-EDP-EA and 17,18-EEQ-EA, respectively). These metabolites are more potent at CB2 than their eCB parent molecule and display CB2 selectivity. 19,20-EDP-EA and 17,18-EEQ-EA diminish pro-inflammatory NO and IL-6 while they elevate anti-inflammatory marker IL-10. In addition, DHEA, EPEA as well as their epoxide metabolites are vasoactive

superfamily and diflavin enzyme, CYP reductase (CPR), which shuttles electrons from NADPH to the CYP [52].

17.2 Endocannabinoid Epoxides

17.2.1 ω-3 Endocannabinoid DHA Ethanolamide (DHEA)

Encouraged by studies that identified AEA-like fatty acid ethanolamides across several tissues, the Di Marzo group sought to identify their ω-3 eCB counterparts. This group demonstrated that ω-3 eCB, DHEA is located in bovine retina. Furthermore, DHEA was found at twofold higher levels compared to AEA in the brain (3.77 ± 0.66 ng/g and 1.88 ± 0.46 ng/g, respectively) [15, 53]. Additionally, DHEA has been detected under peripheral inflammation in rat contralateral spinal tissue [54]. More recently, a targeted lipidomics method using liquid chromatography tandem mass spectrometry (LC-MS/MS) detected DHEA in rat brain (128.9 ± 27.9 pmol/g), heart (57.7 ± 24.4 pmol/g), kidney (49.6 ± 22.2 pmol/g), spleen (22.9 ± 1.3 pmol/g), liver (195.1 ± 48.7 pmol/g), pig brain (265.5 ± 100 pmol/g), and human plasma (13.3 ± 0.4 pmol/mL). Interestingly, dietary supplementation with DHA increased the levels of DHEA at the expense of AEA, thereby providing a method for elevating the levels of ω-3 eCBs *in vivo* [13, 15]. Although an exact biosynthetic pathway for DHEA has yet to be determined, it is believed that DHEA is produced in a similar manner to AEA. It has been suggested that a membrane precursor, *N*-docosahexaenoyl phosphatidylethanolamine (NDHPE) is hydrolyzed by phospholipase D2 to produce free DHEA, however, more studies are required to confirm this hypothesis [55]. Since DHEA is structurally similar to AEA and showed a high potential for physiological relevance, there was significant interest in investigating its physiological activities *in vitro* and *in vivo*. Additionally, DHEA displays immune-

modulatory activity in RAW264.7 cells. DHEA dose-dependently reduced monocyte chemoattractant protein 1 (MCP-1) mRNA, prostaglandin and thromboxane B_2 levels produced by COX-2 [56].

17.2.2 ω-3 Endocannabinoid DHEA Epoxides (Epoxydocosapentaenoic Acid-Ethanolamide, EDP-EA)

Recently, the Das group showed that DHEA is a substrate for enzymes in the CYP epoxygenase pathway. They designed a targeted lipidomics LC-MS/MS method in the multiple reaction monitoring mode (MRM) to simultaneously detect and quantify five DHEA epoxide (EDP-EA) regioisomers in rat brain, heart, kidney, spleen, liver tissues as well as in mouse microglial cells. These isomers share the same molecular weight but differ in the order in which their atoms are connected. Due to the reported upregulation of CYP epoxygenases in LPS-stimulated microglia, the Das lab sought to investigate these molecules under inflammatory conditions [44, 57]. By monitoring the production of EDP-EAs in LPS-stimulated microglia treated with DHEA, they confirmed that EDP-EAs are produced by CYPs as inhibitors of CYPs reduce their levels. It was further demonstrated that the CYP isozyme, CYP2J2, is responsible for this action. Current research is going on to identify other brain CYPs that are involved in eCB metabolism. Interestingly, the production of the terminal epoxide, 19,20-EDP-EA is favored over the other regioisomers in a reconstituted CYP2J2-CYP Reductase (CPR) Nanodisc system and by brain microsomes [13]. More research is needed to further understand why certain regioisomers are preferred by CYPs.

As previous studies consistently showed DHEA as a weak ligand at both CB1 and CB2, the Das lab tested to see if DHEA and its epoxide metabolites activated or bound to CB receptors [56]. Using the PRESTO-Tango assay, they showed that epoxygenation of DHEA by CYP epoxygenases led to an altered CB1 and CB2 activity profile compared to the DHEA parent molecule. For example, 19,20-EDP-EA was observed to be more potent at CB2 compared to DHEA and had a ten-fold lower activity at CB1 (EC_{50} at CB1: 108 nM and CB2: 280 nM) (Fig. 17.2) [13]. As 19,20-EDP-EA, was observed to display an altered cannabinoid receptor profile compared to DHEA, it would be interesting to characterize the other five DHEA epoxide regioisomers produced by the cross-talk of CYP metabolism pathways. From similar studies on other regioisomers, it is expected that DHEA epoxide regioisomers and enantiomers will display different activities at the CB1 and CB2 receptors.

17.2.3 Anti-Inflammatory Activity of DHA Ethanolamide Epoxides (EDP-EAs)

Multiple studies indicate that eCBs combat microglial-promoted neuroinflammation via CB2 activation [58]. In fact, there is a current drive towards the development of CB2-selective agonists to combat the inflammatory pathology associated with progression of neurodegenerative disease. As the DHEA epoxides (EDP-EAs) are naturally produced by microglia and the microglia express CYPs, the Das group examined the anti-inflammatory effects of these molecules in an *in vitro* model of neuroinflammation. As mentioned briefly above, microglial cells are the innate immune cells of the brain, which continuously survey the brain parenchyma for harmful stimuli. When harmful stimuli are encountered, microglia exhibit a pro-inflammatory phenotype to promote defense. As microglia clear the harmful stimuli, a phenotype change occurs that promotes an inflammation-resolution phase. This phenotype switching mechanism is classically known as macrophage polarization. Out of the EDP-EAs tested, 19,20-EDP-EA showed the most potent dose-dependent reduction of pro-inflammatory markers. (Fig. 17.2). Correspondingly, it decreased

pro-inflammatory cytokine IL-6 and increased the production of IL-10 [13]. Additionally, the anti-inflammatory effects of 19,20-EDP-EA were confirmed using freshly isolated primary piglet microglial cells stimulated with LPS, which suppressed IL-6 production, and thus corroborated the findings in immortalized BV-2 cells. Based on these findings, it is possible that the DHA-derived eCB epoxides mediate anti-inflammatory activities in addition to other pathways. Taken together, this indicates that the EDP-EAs and their stable derivatives may be utilized for treating neuroinflammatory conditions.

17.2.4 Vasoactivity of DHA Ethanolamide Epoxide (EDP-EA)

It is well known that CYP epoxygenases convert ω-3 PUFAs to PUFA epoxides. For example, DHA is converted to DHA epoxides (EDPs). These molecules are vasoactive in nature, and are more effective at relaxing pre-constricted vessels compared to CYP-derived AA epoxides (EETs) [59]. The Das lab demonstrated that CYP epoxygenase-mediated metabolism of DHEA produced a vasodilatory eCB epoxides, 19,20-EDP-EA. Compared to 19,20-EDP, as shown in Fig. 17.1, EDP-EA had a two-fold higher EC_{50} at relaxing bovine coronary arteries (Fig. 17.2) [13]. Although CYP-derived DHEA epoxides are vasoactive molecules, they do so with reduced potency relative to their parent molecule. Furthermore, 19,20-EDP-EA exerts anti-angiogenic effects in HMVEC cells. Angiogenesis plays several roles in pathological and non-pathological states. As eCBs are regarded as anti-cancer molecules through their anti-angiogenic effects, the Das lab tested the ability of 19,20-EDP-EA to inhibit angiogenesis in HMVEC cells. 1 μM and 3 μM 19,20-EDP-EA decreased tubulogensis by 31 and 75%, respectively. Previous to this study, the anti-angiogenic actions of the EDP metabolites were attributed to their inhibition of VEGF-stimulated cell migration via an unknown epoxyeicosanoic metabolic pathway [13].

17.2.5 ω-3 Endocannabinoid EPA Ethanolamide (EPEA)

The report that several fatty acid ethanolamides other than AEA have unique activities at the cannabinoid receptors sparked a study in 1996 by the Martin and Razdan groups, which sought to examine the structure-activity relationships of AEA analogs at CB1 [60, 61]. Previous to this study, AEA was the only endogenously produced fatty acid ethanolamide to bind and activate the cannabinoid receptors. By understanding the structural requirements necessary for ligands to bind CB1 it would be possible to develop more potent ligands for both biological and therapeutic applications. Cannabinoid structure-activity relationships (SAR) indicated that a phenolic hydroxyl, a lipophilic side chain, and an appropriately oriented carbocyclic ring system are required for cannabinoid receptor activity. One of the AEA analogs tested in their study was EPA ethanolamide (EPEA). Based on molecular dynamic simulations, EPEA was suggested to possess some affinity for CB1 [61].

Alongside these studies, the Di Marzo group determined the *in vivo* localization of EPEA in mouse brains. EPEA was detected at 0.18 ± 0.09 pmol/mg of brain lipid extract, which was the lowest level of all of the fatty acid ethanolamides detected in their study. EPEA was found at five-fold lower levels than DHEA [53]. Compared to EPEA, its parent molecule EPA was found at 443 ± 167 ng/g mice brain. However, dietary supplementation with EPA increased the endogenous levels of EPEA, thereby providing a dietary delivery method for elevating ω -3 eCBs [15]. Furthermore, when mice were fed a high ω -3 diet consisting of DHA and EPA phospholipids, the levels of EPEA increased in adipose tissue, corroborating the link between diet and the eCB system. More recently, a targeted lipidomics method using LC-MS/MS detected EPEA in rat brain (6.2 ± 1.6 pmol/g), heart (9.4 ± 6 pmol/g), kidney (37.1 ± 21 pmol/g), spleen (9.2 ± 4.1 pmol/g), liver (80.8 ± 20.1 pmol/g), and pig brain (2.2 ± 0.4 pmol/g) [13]. Interestingly, EPEA is found at a significantly lower level than DHEA

in rat brain (DHEA: 128.9 ± 27.9 pmol/g). Together, these findings support that supplementation with ω -3 fatty acids can modulate eCB levels and may increase the bioavailability of DHA and EPA for physiological function.

Although the biosynthesis of EPEA has not been proved, the well-studied transacylation-PDE pathway suggests that *n*-acyl ethanolamides are biosynthesized from glycerophospholipids via *N*-acylphosphatidyl ethanolamine (NAPE). Through this pathway, a Ca^{2+}-dependent *N*-acyltransferase and NAPE-hydrolyzing PLD render the fatty acid ethanolamide free from its membrane precursor [56]. This pathway is similar to the biosynthesis of AEA and may support the idea of a conserved biosynthesis for fatty acid ethanolamides.

17.2.6 ω-3 Endocannabinoid Epoxide Epoxyeicosatetraenoic Acid-Ethanolamide (EEQ-EA)

In 2017, the Das lab showed that EPEA, like DHEA, is a substrate for the CYP epoxygenase pathway. Using a targeted lipidomics LC-MS/MS approach they detected and quantified all five EPEA epoxide (EEQ-EA) regioisomers in rat brain, heart, kidney, spleen, liver tissues as well as in mouse microglial cells. They found that LPS-stimulated and EPEA-treated mouse microglia could convert EPEA to the EEQ-EAs via CYP epoxygenases. They further determined that one of the CYP isozymes responsible for this action is CYP2J2. Using recombinantly expressed proteins, CYP2J2-CPR Nanodiscs showed a favored production of 17,18-EEQ-EA, as shown in Fig. 17.1, compared to other regioisomers [13].

To further characterize EPEA and its epoxide metabolites, we were interested in testing whether the EEQ-EAs were eCBs. Previous studies consistently showed EPEA as a weak agonist for CB1 and CB2 [56]. Through the PRESTO-Tango assay, the Das lab showed that epoxidation of EPEA by CYPs led to an altered CB1 and CB2 activity compared to their parent molecule EPEA. For example, 17,18-EEQ-EA had a lower EC_{50} at CB2 than EPEA (EC_{50} 17,18-EEQ-EA: 1.4 nM and EC_{50} EPEA: 2.1 nM). However, compared to EPEA (EC_{50} CB1: 0.1 nM), 17,18-EEQ-EA had a lower potency at CB1 (EC_{50} CB1: 18.5 nM), thus showing a CB2-selectivity for 17,18-EEQ-EA [13] (Fig. 17.2). As mentioned above, discovering novel CB2-selective molecules may aid in the development of anti-inflammatory therapeutics that overcome any potential psychoactive activities that may be mediated through CB1 activation.

17.2.7 Anti-inflammatory Activity of EPA Ethanolamide Epoxides (EEQ-EAs)

Since EPA and EPEA have been shown to possess anti-inflammatory activities, the anti-inflammatory activities of EEQ-EAs were studied in brain microglia. When BV-2 microglia were stimulated with LPS, the levels of EPEA and total EEQ-EA were elevated. Interestingly, EPEA levels significantly surpassed DHEA levels after 12 h of LPS stimulation (DHEA: 6.7 ± 4.6 pmol/10^6 cells and EPEA: 2042 ± 939.2 pmol/10^6 cells). Furthermore, in LPS-stimulated BV-2 cells, 17,18-EEQ-EA (10 μM) dose-dependently reduced NO in a CB2-dependent manner, which was attenuated with CB2 antagonist, AM-630, treatment. In addition, 17,18-EEQ-EA dose-dependently decreased IL-6 and increased IL-10 (Fig. 17.2). Interestingly, 17,18-EEQ-EA was a more potent inhibitor of NO production than EPA epoxide (17,18-EEQ). This evidence shows that the CYP epoxygenase pathway may play be responsible for the anti-inflammatory nature of EPA through conversion of EPEA to its epoxide metabolite. Lastly, similar to 19,20-EDP-EA, the effects of 17,18-EEQ-EA were confirmed in freshly isolated piglet microglia cells, thereby corroborating the anti-inflammatory effects of 17,28-EEQ-EA in primary cells.

17.2.8 Vasoactivity of EPA Ethanolamide Epoxide (EEQ-EA)

Since it is known that CYP epoxygenases metabolizes EPA to vasoactive epoxide metabolites, the Das lab tested the vasodilatory activity of eCB EPEA and eCB epoxide 17,18-EEQ-EA in isolated bovine coronary arteries [59, 62]. Utilizing isometric tension measurements to monitor the ability of these molecules to relax preconstricted arteries, they determined that 17,18-EEQ-EA, like 19,20-EDP-EA, dose-dependently relaxed constricted bovine coronary arteries (Fig. 17.2). On the other hand, the ED_{50} values determined from 17,18-EEQ-EA was two-fold greater than the ED_{50} value of 17,18-EEQ (ED_{50} of 17,18-EEQ: 0.47 ± 0.01 μM and ED_{50} of 17,18-EEQ-EA: 1.1 ± 0.6 μM), thus showing that eCB epoxides are vasoactive but not as potent as their PUFA epoxides. It has been previously been shown that EPA epoxides act as endothelial-derived hyperpolarizing factors that inhibit platelet aggregation. To follow-up with these observations, the Das lab revealed that 17,18-EEQ-EA dose-dependently inhibits platelet aggregation and angiogenesis [13]. Overall these endocannabinoid epoxides are vasoactive.

17.3 Anti-tumorigenic Properties of the ω-3 Endocannabinoid Epoxides

A particular consequence of unresolved inflammation is the progression towards tumor formation. The microenvironment of a tumor is highly inflammatory and contains a carcinogenic milieu that promotes tumor progression via pro-inflammatory cytokines, growth factors, and several adhesion molecules [63]. Several studies have reported the anti-inflammatory, pro-resolving, and anti-tumorigenic activity of ω-3 fatty acids, DHA and EPA [64, 65]. Upon conversion to their epoxides, the EEQs and EDPs have been shown to be anti-proliferative, anti-tumorigenic, and can inhibit metastasis [66, 67]. The Das lab recently revealed that DHEA and EPEA epoxides display anti-cancer activities. EDP-EAs are not only abundant in osteosarcoma-derived lung tumors (20–80% increased levels compared to healthy lungs) but they also possess anti-inflammatory and anti-tumorigenic properties in an *in vitro* model of osteosarcoma [68]. More specifically, Roy et al. examined the ability of EDP-EA regioisomers 7,8-EDP-EA, 10,11-EDP-EA, and 13,14-EDP-EA to inhibit migration of cancer cells and induce apoptosis. Additionally, in 143B human osteosarcoma cells, 10,11-EDP-EA showed the highest ability to induce apoptosis in a CB1-dependent manner (EC_{50} at CB1: 0.43 nM). A time-dependent scratch assay revealed that 10,11-EDP-EA has anti-migratory potential, an indicator of metastasis. In order to increase the stability of 10,11-EDP-EA, derivatives were synthesized to prevent degradation by FAAH. Based on a previous characterization of CB1-binding and metabolic stability of AEA's functional groups, a *R*-1′-methyl isomer (10,11-EDP-IA), a cyclopropyl derivative (10,11-EDP-CA), and a *n*-propyl derivative (10,11-EDP-NA) was prepared. Through LC-MS/MS quantification of metabolites, the rate of hydrolysis of 10,11-EDP-EA to 10,11-EDP was decreased by nearly 100-fold with the derivatives 10,11-EDP-NA, 10,11-EDP-IA, and 10,11-EDP-CA. Furthermore, these stable derivatives were successful at inhibiting migration in osteosarcoma cells and reduce angiogenesis. Taken together, this novel class of eCB epoxides have shown potential to reduce tumor progression through activation of the CB1 receptor. In addition, this was one of the first studies to demonstrate the potential utility of cannabinoid-like molecules in the treatment of bone cancer. Furthermore, these studies provide the grounds for future work to determine the CB1-dependent anti-pain behavior of these molecules, which would be highly beneficial for the development of multi-modal bone cancer therapies.

17.4 Conclusions and Future Perspectives

The inflammatory response leads to release of lipid mediators that help in regulating the inflammation. There is a strong interest in identifying anti-inflammatory lipid mediators that can resolve the ongoing inflammation. In this chapter, we review a novel class of anti-inflammatory lipid mediators that emanate from the cross-talk of the two important biochemical pathways: the CYP-epoxygenase pathway and the endocannabinoid biosynthetic pathway. The ω-3 eCBs, DHEA and EPEA are metabolized by CYP epoxygenases to form the epoxides, EDP-EA and EEQ-EAs. These eCB epoxides are multifunctional molecules as they can target multiple receptors including the cannabinoid receptors and unknown epoxide receptor. Their effects on inflammation resolution, cancer and vasodilation have briefly been explored through multiple studies and show promise for the development of therapeutics for cerebrovascular and neuroinflammatory diseases.

Although evidence points to CB1 and CB2 receptors as the primary modulator of the anti-inflammatory and vasodilatory effects of the omega-3 eCB epoxides, the studies also indicate that the eCB epoxides are anti-inflammatory and vasoactive through an unknown receptor. In order to target hypertension and other vascular conditions through the eCB system, identification of this receptor and competing metabolic enzymes would be highly beneficial. Hence, it is important to establish the receptor systems through which these molecules elicit their physiological response. Additionally, it is critical to map all the primary synthetic and degradative pathways of these eCB epoxides. While it was shown that these eCB epoxides are substrates for two degradative pathways mediated by sEH and FAAH, it is possible that they are synthesized and stored in membrane and released by phospholipases. Current therapeutics, such as FAAH and sEH inhibitors, increase the endogenous levels of PUFA epoxides and eCBs, and have since shown promise for the treatment of inflammatory-based disease, hypertension, and nociceptive pain [21, 69, 70]. It needs to be evaluated if FAAH and sEH inhibitors increase the levels of these dual functional endocannabinoid epoxides.

Although it was shown that the ω-3 eCBs, DHEA and EPEA are metabolized by CYP epoxygenases to form the epoxides, the identity of the specific CYP enzyme is unclear. It has been shown that the ω-6 eCB 2-AG, AEA and ω-3 eCBs DHEA and EPEA are metabolized by CYP2J2 to produce epoxide metabolites [50, 71]. Several papers from Hollenberg laboratory have identified other CYPs such as CYP3A4 and CYP2D6 are involved in metabolizing eCBs [51]. However, there needs to be a systematic approach to delineate which CYPs are involved in the endocannabinoid metabolism in the body.

The EDP-EA and EEQ-EA metabolites were successfully detected in several tissues. Detection of lipid metabolites is often challenging as these lipid metabolites are unstable and the levels at which they are detected in blood and tissues are lower than their endogenous levels at the site of formation and site of action. The levels of EDP-EA and EEQ-EA in the tissues are within the pmol/g tissue range, however, their local concentration is likely to be greater than plasma levels. This is because they are locally produced and act in a localized manner on the receptors unlike most hormones and neuropeptides. For instance, it is hypothesized that eCBs are synthesized on-demand from their esterified membrane precursors in response to a variety of chemical and neuronal stimuli and are rapidly degraded by FAAH and sEH. Local measurement of lipid metabolite is technically challenging. As a result, the levels of these molecules extracted from tissues does not mirror the local concentrations in the intracellular and synaptic environment. In addition, it is important to note that many of these lipids are esterified in the membrane yet the levels that are reported within the literature reflect the abundance of free lipids often in healthy tissues. Future studies are needed to discern eCB and eCB epoxide levels in disease states as the expression of the enzymes such as CYP epoxy-

genases change during inflammation. Another cause for the differences in the levels of the lipid metabolites is the extraction efficiency. While large-scale lipidomics studies can identify a variety of lipid types, the solvent extraction method can create a bias against certain molecules based on their chemical structure and solubility. Although optimization of an efficient solvent system is imperative, detection limits and low extraction yields can occur when working on the pmol/g tissue scale.

In summary, the discovery of the eCB epoxides is the initial step in evaluating the role of CYP epoxygenase mediated metabolism of endocannabinoids to generate multi-functional lipid metabolites. There are other endocannabinoids such as noladin ether and endocannabinoid-like molecules such as *N*-arachidonoyl dopamine (NADA) and *N*-arachidonoyl serotonin (AA-5-HT) that closely interact with FAAH and CB1 [72–74]. Future investigations on the production of novel CYP-mediated bioactive eCB metabolites are imperative for a complete understanding of the CYP epoxygenase pathway. As these endocannabinoid epoxides are dual functional molecules exhibiting the classical epoxyeicosanoid and endocannabinoid activities, their bifunctional nature imparts the ability to simultaneously target multiple signaling pathways. This ability to target different pathways makes them more potent than other lipid metabolites. Hence, the discovery of these novel lipid metabolites also prompts the discovery of new scaffolds that can serve as templates for multi-target drugs. These therapies may be useful for the treatment of inflammatory pain and other conditions in which eCBs receptors are targeted.

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References

1. Matsuda LA, Lolait SJ, Brownstein MJ et al (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564. https://doi.org/10.1038/346561a0
2. Devane WA, Dysarz FA, Johnson RM et al (1988) Determination rat brain and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605–613
3. Fride E (2002) Endocannabinoids in the central nervous system – an overview. Prostaglandins Leukot Essent Fat Acids 66:221–233. https://doi.org/10.1054/plef.2001.0360
4. Bátkai S, Pacher P, Osei-Hyiaman D et al (2004) Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation 110:1996–2002. https://doi.org/10.1161/01.CIR.0000143230.23252.D2
5. Zelasko S, Arnold WR, Das A (2015) Endocannabinoid metabolism by cytochrome P450 monooxygenases. Prostaglandins Other Lipid Mediat 116–117:112–123. https://doi.org/10.1016/j.prostaglandins.2014.11.002
6. Galiegue S, Mary S, Marchand J et al (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232:54–61. https://doi.org/10.1111/j.1432-1033.1995.tb20780.x
7. Buckley NE, McCoy KL, Mezey É et al (2000) Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoid CB2receptor. Eur J Pharmacol 396:141–149. https://doi.org/10.1016/S0014-2999(00)00211-9
8. Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61–65. https://doi.org/10.1038/365061a0
9. Dhopeshwarkar A, Mackie K (2014) CB2 cannabinoid receptors as a therapeutic target–what does the future hold. Mol Pharmacol 86:430–437. https://doi.org/10.1124/mol.114.094649
10. Porter AC, Sauer J-MM, Knierman MD et al (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J Pharmacol Exp Ther 301:1020–1024. https://doi.org/10.1124/jpet.301.3.1020
11. Pertwee RG (2006) Cannabinoid pharmacology: the first 66 years. Br J Pharmacol 147:S163. https://doi.org/10.1038/sj.bjp.0706406
12. Carnevale L, Arango A, Arnold WR et al (2018) Endocannabinoid virodhamine is an endogenous inhibitor of human cardiovascular CYP2J2 epoxygenase. Biochemistry 57:6489–6499. https://doi.org/10.1021/acs.biochem.8b00691
13. McDougle DR, Watson JE, Abdeen AA et al (2017) Anti-inflammatory ω-3 endocannabinoid epoxides. Proc Natl Acad Sci U S A 114:E6034–E6043. 201610325. https://doi.org/10.1073/pnas.1610325114

14. Sagnella SM, Conn CE, Irena Krodkiewska XM, CJD (2011) Anandamide and analogous endocannabinoids: a lipid self-assembly study. Soft Matter 7:5319–5328. https://doi.org/10.1039/c1sm05141e
15. Wood JT, Williams JS, Pandarinathan L et al (2010) Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. J Lipid Res 51:1416–1423. https://doi.org/10.1194/jlr.M002436
16. Sharir H, Console-Bram L, Mundy C et al (2012) The endocannabinoids anandamide and virodhamine modulate the activity of the candidate cannabinoid receptor GPR55. J Neuroimmune Pharmacol 7:856–865. https://doi.org/10.1007/s11481-012-9351-6
17. Watanabe H, Vriens J, Prenen J et al (2003) Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature 424:434–438. https://doi.org/10.1038/nature01807
18. Cadas H, di Tomaso E, Piomelli D (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. J Neurosci 17:1226–1242
19. Sun Y-X, Tsuboi K, Okamoto Y et al (2004) Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. Biochem J 380:749–756. https://doi.org/10.1042/BJ20040031
20. Murataeva N, Straiker A, MacKie K (2014) Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. Br J Pharmacol 171:1379–1391. https://doi.org/10.1111/bph.12411
21. Cravatt BF, Lichtman AH (2003) Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. Curr Opin Chem Biol 7:469–475. https://doi.org/10.1016/S1367-5931(03)00079-6
22. Ahn K, Johnson DS, Cravatt BF (2010) NIH Public Access 4:763–784. https://doi.org/10.1517/17460440903018857.Fatty
23. Labar G, Bauvois C, Borel F et al (2010) Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. Chembiochem 11:218–227. https://doi.org/10.1002/cbic.200900621
24. Kay A, Michele MK, Benjamin CF (2011) Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. Chem Rev 108:1687–1707. https://doi.org/10.1021/cr0782067.Enzymatic
25. Deutsch DG, Goligorsky MS, Schmid PC et al (1997) Production and physiological actions of anandamide in the vasculature of the rat kidney. J Clin Invest 100:1538–1546. https://doi.org/10.1172/JCI119677
26. Horne EA, Stella N (2008) The ins and outs of endocannabinoid signaling in healthy and diseased brain. Futur Lipidol 3:435–452. https://doi.org/10.2217/17460875.3.4.435
27. Lu Y, Anderson HD (2017) Cannabinoid signaling in health and disease. Can J Physiol Pharmacol 95:311–327. https://doi.org/10.1139/cjpp-2016-0346
28. Chen S, Zhang H, Pu H et al (2014) Epoxy. Sci Rep 4:1–8. https://doi.org/10.1038/srep07458
29. Mievis S, Blum D, Ledent C (2011) Worsening of Huntington disease phenotype in CB1 receptor knockout mice. Neurobiol Dis 42:524–529. https://doi.org/10.1016/j.nbd.2011.03.006
30. Ravinet Trillou C, Delgorge C, Menet C et al (2004) CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. Int J Obes 28:640–648. https://doi.org/10.1038/sj.ijo.0802583
31. Thomas F, Gamage AHL (2013) The endocannabinoid system: role in energy regulation. Trends Pharmacol Sci 58:144–148. https://doi.org/10.1002/pbc.23367.The
32. Zimmer a M, Hohmann a G, Herkenham M, Bonner TI (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A 96:5780–5785. https://doi.org/10.1073/pnas.96.10.5780
33. Benito C, Tolón RM, Pazos MR et al (2008) Cannabinoid CB 2 receptors in human brain inflammation. Br J Pharmacol 153:277–285. https://doi.org/10.1038/sj.bjp.0707505
34. Di Marzo V (2008) Targeting the endocannabinoid system: to enhance or reduce. Nat Rev Drug Discov 7:438–455. https://doi.org/10.1038/nrd2553
35. Di Marzo V, Stella N, Zimmer A (2014) Endocannabinoid signalling and the deteriorating brain. Nat Rev Neurosci 16:30–42. https://doi.org/10.1038/nrn3876
36. Navarro G, Morales P, Rodríguez-Cueto C et al (2016) Targeting cannabinoid CB2receptors in the central nervous system. Medicinal chemistry approaches with focus on neurodegenerative disorders. Front Neurosci 10:1–11. https://doi.org/10.3389/fnins.2016.00406
37. Zipp F, Aktas O (2006) The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. Trends Neurosci 29:518–527. https://doi.org/10.1016/j.tins.2006.07.006
38. Kozak KR, Gupta RA, Moody JS et al (2002) 15-lipoxygenase metabolism of 2-arachidonylglycerol: generation of a peroxisome proliferator-activated receptor α agonist. J Biol Chem 277:23278–23286. https://doi.org/10.1074/jbc.M201084200
39. O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol 152:576–582. https://doi.org/10.1038/sj.bjp.0707423
40. Zygmunt PM, Petersson J, Andersson DA et al (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400:452–457
41. Rouzer CA, Marnett LJ (2011) Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. Chem Rev 111:5899–5921. https://doi.org/10.1021/cr2002799
42. Yang R, Fredman G, Krishnamoorthy S et al (2011) Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel

bioactive signals*. J Biol Chem 286:31532–31541. https://doi.org/10.1074/jbc.M111.237990
43. Aoki M, Matsumoto NM, Okubo Y, Ogawa R (2019) Bioelectrochemistry cytochrome P450 genes play central roles in transcriptional response by keratinocytes to a high-voltage alternating current electric fi eld. Bioelectrochemistry 126:163–171. https://doi.org/10.1016/j.bioelechem.2018.11.014
44. Snider NT, Nast JA, Tesmer LA, Hollenberg PF (2009) A cytochrome P450-derived epoxygenated metabolite of anandamide is a potent cannabinoid receptor 2-selective agonist. Mol Pharmacol 75:965–972. https://doi.org/10.1124/mol.108.053439
45. Snider NT, Walker VJ, Hollenberg PF (2010) Oxidation of the endogenous cannabinoid arachidonoyl ethanolamide by the cytochrome P450 monooxygenases: physiological and pharmacological implications. Pharmacol Rev 62:136–154. https://doi.org/10.1124/pr.109.001081
46. Walker VJ, Griffin AP, Hammar DK, Hollenberg PF (2016) Metabolism of anandamide by human cytochrome P450 2J2 in the reconstituted system and human intestinal microsomes. J Pharmacol Exp Ther 357:537–544. https://doi.org/10.1124/jpet.116.232553
47. Kalish BT, Kieran MW, Puder M, Panigrahy D (2013) The growing role of eicosanoids in tissue regeneration, repair, and wound healing. Prostaglandins Other Lipid Mediat 104–105:130–138. https://doi.org/10.1016/j.prostaglandins.2013.05.002
48. Node K, Huo Y, Ruan X et al (1999) Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 285(80):1276–1279. https://doi.org/10.1126/science.285.5431.1276
49. Zhao H, Chen J, Chai J et al (2017) Cytochrome P450 (CYP) epoxygenases as potential targets in the management of impaired diabetic wound healing. Lab Investig 97:782–791. https://doi.org/10.1038/labinvest.2017.21
50. McDougle DR, Kambalyal A, Meling DD, Das A (2014) Endocannabinoids anandamide and 2-Arachidonoylglycerol are substrates for human CYP2J2 Epoxygenase. J Pharmacol Exp Ther 351:616–627. https://doi.org/10.1124/jpet.114.216598
51. Snider NT, Kornilov AM, Kent UM, Hollenberg PF (2007) Anandamide metabolism by human liver and kidney microsomal cytochrome p450 enzymes to form hydroxyeicosatetraenoic and epoxyeicosatrienoic acid ethanolamides. J Pharmacol Exp Ther 321:590–597. https://doi.org/10.1124/jpet.107.119321.hydrolysis
52. Meunier B, de Visser SP, Shaik S (2004) Mechanism of oxidation reactions catalyzed by cytochrome P450 enzymes. Chem Rev 104:3947–3980. https://doi.org/10.1021/cr020443g
53. Berger A, Crozier G, Bisogno T et al (2001) Anandamide and diet: inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding N-acylethanolamines in piglets. Proc Natl Acad Sci 98:6402–6406. https://doi.org/10.1073/pnas.101119098
54. Dennis M (2010) Inflammatory hyperalgesia induces essential bioactive lipid production in the spinal cord. J Neurochem 114:981–993
55. Bisogno T, Delton-Vandenbroucke I, Milone A, Lagarde MDMV (1999) Biosynthesis and inactivation of N-arachidonoylethanolamine (anandamide) and N-docosahexaenoylethanolamine in bovine retina. Arch Biochem Biophys 370:300–307
56. Meijerink J, Balvers M, Witkamp R (2013) N-acyl amines of docosahexaenoic acid and other n-3 polyunsatured fatty acids – from fishy endocannabinoids to potential leads. Br J Pharmacol 169:772–783. https://doi.org/10.1111/bph.12030
57. Cui X, Kawashima H, Barclay TB et al (2001) Molecular cloning and regulation of expression of two novel mouse CYP4F genes: expression in peroxisome proliferator-activated receptor alpha-deficient mice upon lipopolysaccharide and clofibrate challenges. J Pharmacol Exp Ther 296:542–550
58. Maresz K, Carrier EJ, Ponomarev ED et al (2005) Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. J Neurochem 95:437–445. https://doi.org/10.1111/j.1471-4159.2005.03380.x
59. Zhang G, Panigrahy D, Mahakian LM et al (2013) Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proc Natl Acad Sci 110:6530–6535. https://doi.org/10.1073/pnas.1304321110
60. Hanuš L, Gopher A, Almog S, Mechoulam R (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 36:3032–3034. https://doi.org/10.1021/jm00072a026
61. Thomas BF, Adams IB, Mascarella SW et al (1996) Structure-activity analysis of anandamide analogs: relationship to a cannabinoid pharmacophore. J Med Chem 39:471–479. https://doi.org/10.1021/jm9505167
62. Morisseau C, Inceoglu B, Schmelzer K et al (2010) Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids. J Lipid Res 51:3481–3490. https://doi.org/10.1194/jlr.M006007
63. Coussens LM, Werb Z (2002) Inflammation and cancer. Pharmaceut Biotechnol 420(6917)860. Nature 420:860–867. https://doi.org/10.1038/nature01322.Inflammation
64. Jaudszus A, Gruen M, Watzl B et al (2013) Evaluation of suppressive and pro-resolving effects of EPA and DHA in human primary monocytes and T-helper cells. J Lipid Res 54:923–935. https://doi.org/10.1194/jlr.P031260
65. Laviano A, Rianda S, Molfino A, Fanelli FR (2013) Omega-3 fatty acids in cancer. Curr Opin Clin Nutr Metab Care 16:156–161. https://doi.org/10.1097/MCO.0b013e32835d2d99

66. Cui PH, Petrovic N, Murray M (2011) The ω-3 epoxide of eicosapentaenoic acid inhibits endothelial cell proliferation by p38 MAP kinase activation and cyclin D1/CDK4 down-regulation. Br J Pharmacol 162:1143–1155. https://doi.org/10.1111/j.1476-5381.2010.01113.x
67. Guodong Z, Dipak P, Lisa MM et al (2013) Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis. Proc Natl Acad Sci 110:6530–6535. https://doi.org/10.1073/pnas.1304321110/-/DCSupplemental. www.pnas.org/cgi/doi/10.1073/pnas.1304321110
68. Roy J, Watson JE, Hong IS et al (2018) Antitumorigenic properties of omega-3 endocannabinoid epoxides. J Med Chem 61:5569–5579. https://doi.org/10.1021/acs.jmedchem.8b00243
69. Hammock BD, Wagner Karen IB (2011) The soluble epoxide hydrolase as a pharmaceutical target for pain management. Pain Manag 50:383–386. https://doi.org/10.1097/FJC.0b013e3181506445
70. Sasso O, Wagner K, Morisseau C et al (2015) Peripheral FAAH and soluble epoxide hydrolase inhibitors are synergistically antinociceptive. Pharmacol Res 97:7–15. https://doi.org/10.1016/j.phrs.2015.04.001
71. Chen JK, Chen JK, Imig JD et al (2008) Identification of novel endogenous cytochrome P450 arachidonate metabolites with high affinity for cannabinoid receptors. J Biol Chem 283:24514–24524. https://doi.org/10.1074/jbc.M709873200
72. Bisogno T, Melck D, Bobrov M et al (2000) N-acyl-dopamines: novel synthetic CB1 cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem J 351:817. https://doi.org/10.1042/bj3510817
73. Fowler CJ, Tiger G, López-Rodríguez ML et al (2003) Inhibition of fatty acid amidohydrolase, the enzyme responsible for the metabolism of the endocannabinoid anandamide, by analogues of arachidonoyl-serotonin. J Enzyme Inhib Med Chem 18:225–231. https://doi.org/10.1080/1475636031000080216
74. Wang Y, Balvers MGJ, Hendriks HFJ et al (2017) Docosahexaenoyl serotonin emerges as most potent inhibitor of IL-17 and CCL-20 released by blood mononuclear cells from a series of N-acyl serotonins identified in human intestinal tissue. Biochim Biophys Acta Mol Cell Biol Lipids 1862:823–831. https://doi.org/10.1016/j.bbalip.2017.05.008

18 Overview of Lipid Biomarkers in Amyotrophic Lateral Sclerosis (ALS)

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Abstract

Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease involving motor neuron (MN) degeneration in the spinal cord, brain stem and primary motor cortex. The existence of inflammatory processes around MN and axonal degeneration in ALS has been shown. Unfortunately, none of the successful therapies in ALS animal models has improved clinical outcomes in patients with ALS. Therefore, the detection of blood biomarkers to be used as screening tools for disease onset and progression has been an expanding research area with few advances in the development of drugs for the treatment of ALS. In this review, we will address the available data analyzing regarding the relationship of lipid metabolism and lipid derived- products with ALS. We will address the advances on the studies about the role that lipids plays at the onset, progression and lifespan extension of ALS patients.

Keywords

ALS · Lipid biomarkers · Mass spectrometry · Cell signaling

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18.1 Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease without treatment and a short life expectancy upon clinical symptoms appear [1]. This underscores the necessity for an expansion of the current knowledge and underlying biological mechanisms driving disease. Recent research shows the presence of inflammatory processes around motor neurons (MN) and axonal degeneration, being evident through the accumulation of reactive astrocytes and activated microglia [2]. Many treatments have been tested on different ALS animal models. Unfortunately, no successful therapy with promising results in animal models has improved clinical outcomes in patients with ALS [3]. This is partly due to the lack of specific blood biomarkers, which can act as screening tools to identify individuals at risk of developing the disease, as well as the lack of clinical studies. The development of novel medical treatments has lead to an increase in life expectancy, especially in the western world. However, the former is accompanied by an increment on the appearance of cognitive dysfunctions related to aging in addition to the development of neurodegenerative diseases. Overall, these changes represent a significant challenge from the scientific and medical point of view. The current review aims to analyze the recent data

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obtained about lipid help in the development of drugs for the treatment of ALS.

18.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a multifactorial neurodegenerative disease caused by genetic and non-inheritable components leading to MN degeneration in the spinal cord, brain stem and primary motor cortex [1]. It is well established that ALS affects both cortical as well as cranial and spinal MN with variable evolution. The majority of ALS cases are sporadic, however there exists a 10% of familial cases, some of which arise from mutations of the protein Superoxide Dismutase 1(SOD1) [1]. The average age of onset of the disease is 55 years, beginning later in women than men, and with 90% of the patients dying within 5 years after the appearance of the clinical symptoms. For a definitive diagnosis of ALS the progressive spread of symptoms involving upper and lower motor neurons is required, according to the revised El Escorial and the Awaji criteria [4, 5]. The most used clinical scores for disease progression is the ALS functional rating scale-revised (ALSFRS-R) [5]. This scale is employed to evaluate the pace of disease progression, predict survival and assess the effects of disease-modifying drugs in therapeutic trials. The scale is based on clinical symptoms so it is imperative to discover biomarkers that allow early diagnosis of the disease. In addition, biomarkers will aid for therapeutic drug design to improve the quality of life of those affected due to the lack of effective pharmacological treatments for ALS [5]. The pathogenesis of ALS consists of two stages: an early neuroprotective stage and a later neurotoxic stage. Multiple mechanisms have been described for MN death including glutamate toxicity, mitochondrial dysfunction, protein misfolding and apoptosis [6]. However, ALS appears as a composite syndrome where the aberrant cellular pathways may not be ascribed solely from a protein misfolding issue, but cellular physiology aspects like deficiencies in RNA processing and compromised mitochondria homeostasis, increased oxidative stress, excitotoxicity, reduced neurotrophic support, and glial inflammatory responses [7]. Familiar ALS has been studied using active ($SOD1^{G93A}$, $SOD1^{G37R}$) mice mutants which have a phenotype characterized by progressive paralysis and death caused by the degeneration of MN, in addition to gliosis within the spinal cord, brain stem and cortex [6]. These features suggest a gain of toxic function of the mutant SOD might play a role in the neurodegenerative process. In addition, oxidative stress is an important contributor to neuronal death in ALS involving astrocytes [8]. Current research has shown that ALS is not a single cell type disease, since it involves microglia and astrocytes surrounding the MN. In fact, our laboratory reported astrocytes promote neuronal loss by mechanisms involving alterations in mitochondrial functionality, increased production of nitric oxide and nerve growth factor [2, 8–13]. Glial cells are the principle innate immune cell of the CNS and pathology associated with these cells is referred to as neuroinflammation, a hallmark of ALS [14, 15].

Importantly, there exists an increase of neurotoxic molecules such as pro-inflammatory cytokines, reactive oxygen species (ROS) and pro-inflammatory lipid-derived compounds. These molecules may cause further neuronal damage leading to further glial cell activation resulting in a positive feedback loop of neuroinflammation. Indeed, neuroinflammatory processes have been detected in ALS patients as well as in transgenic models of the disease [16]. Based on this background, many treatments aim to inhibit or reduce the pro-inflammatory action of microglia and astrocytes and counteract the progression of the disease. Unfortunately, none of the successful therapies in ALS animal models has improved clinical outcomes in patients with ALS [3]. This can be ascribed to different factors. For example, decreasing or deleting single pro-inflammatory factors such as TNF-α, IL1-β, and inducible nitric oxide synthase has had little-to-no effect on overall survival of $SOD1^{G93A}$ mice [8, 12]. These suggest that a multiplicity of pro-

inflammatory cytokines can compensate the absence of any single factor, and it is unlikely that continuing efforts to target a single factor in humans will provide significant therapeutic benefit in patients with ALS. Considering the importance of the disease and the fact that inflammatory processes are involved, the identification of blood biomarkers to identify individuals at risk of developing the disease or for their recruitment in clinical studies in order to analyze benefits in the development of drugs for the treatment of ALS would be of biological relevance. Below we will discuss the identification and use of lipid-derived products as biomarkers since research in the field and the optimization of lipidomic techniques are giving novel and important new data for using them as disease biomarkers.

18.3 Role of Lipids in Central Nervous System and ALS

Lipids are implicated in a wide variety of biological processes and can be classified into five categories: fatty acids, triacylglycerols (TAGs), phospholipids, sterol lipids and sphingolipids. The brain requires a near constant source of metabolites to maintain function, and contains the second highest concentration of lipids in the human body [17]. Although the consensus is that glucose metabolism almost completely satisfies the brain energy requirements, it has recently been shown that approximately 20% of the total energy requirement of the brain is met through the oxidation of fatty acids, and that this fatty acid oxidation may take place entirely in astrocytes [18]. Besides this fact, perhaps the most essential role for lipids in the brain is as components of the membrane cellular machinery. This is important in the brain since changes in membrane fluidity and many signaling processes occur in specific intracellular compartments. Importantly, fatty acids and their derivatives have been well characterized as drivers of intracellular signaling processes [19].

Two major polyunsaturated fatty acids (PUFAs) are present at high levels at the brain: arachidonic acid (AA) and docosahexaenoic acid (DHA). Since these PUFA are the precursors of important cell signaling molecules. As examples, DHA and AAs can be oxidized to give rise to prostaglandins or leukotrienes. The oxidation of DHA produces neuroprotectinD1, a signaling molecule that promotes cell survival under stress. The latter is part of the denominated specialized pro-resolving lipid mediators (SPM) giving to these PUFA precursors an important role in neuroinflammation [20, 21]. The presence of these compounds has been implicated in neuronal signaling processes controlling neurogenesis, brain vesicular activity, central glucose homeostasis, mood and cognition [22]. Other example is Prostaglandin E_2 (PGE_2), synthetized by Prostaglandin Endoperoxide H Synthase-2 (PGHS-2) from AA, which promotes inflammation after binding to its receptor [23]. Their role as biomarkers or footprints of ALS will be discussed later.

In many neurodegenerative diseases, an accumulation of ceramides has been observed. The latter is considered toxic, since it has been shown to promote neuronal death by oxidative stress and apoptosis in both animal models and patients [24–28]. Moreover it has been proposed in $SOD1^{G93A}$ mice that sphingomyelin-associated ROS production leads to MN death through the p75 factor [12]; in addition, it is reported that an increase of sphingomyelin in the spinal cord of ALS patients mediates MN death via oxidative stress [24]. From a structural-functional point of view, it has been shown that membrane fluidity in the brain and spinal cord decreases significantly over the course of disease in ALS mice [29]. In fact, neuronal membranes rich in phosphatidylethanolamine and phosphatidylserine are significantly less fluid. Thus, the increase in DHA results in more rigid membranes with an important impact of signaling activities in ALS brains [30].

Along with neuronal degeneration, alterations of lipid metabolism have been reported in ALS. Different studies have shown that muscle denervation has a role in the promotion of abnormal lipid metabolism. In fact, Body Mass

Index (BMI) at diagnosis remains the only metabolic independent prognostic factor for survival in ALS [31]. Studies to determine the role of lipid supplementation and serum lipid profile on ALS onset, progression or fate [4, 32–34] have been carried out, but with unfavorable outcomes. For example, supplementation with omega-3 fatty acids (e.g. eicosapentaenoic acid, EPA, before clinical symptoms appear, in SOD1^{G93A} mice increased the progression of the disease and shortened life span of the transgenic animals [35]. In addition, markers of oxidative stress such as 4-hydroxynonenal were also observed [36]. High fat diets exert a modest decrease in disease progression in a mouse model of ALS [37, 38]. In contrast to other pathologies, such as cardiovascular diseases, dyslipidemia is a good prognostic factor for ALS. When looking to ALS transgenic mice, they are leaner, hypolipidemic and present a higher metabolic intake of fatty acids in muscle than control animals [34].

Dyslipidemia, reduced body mass and increased resting energy expenditure are often present in ALS patients [39, 40]. Importantly, ALS is characterized by important alterations in energy homeostasis [37]. Leptin levels in ALS patients appeared significantly decreased, suggesting lower fat reserves [41]. Reports from the literature show a high LDL/HDL cholesterol ratio or elevated content of total cholesterol and triglycerides in ALS patients that were associated with better prognosis or slower disease progression [33, 42].

In summary, neuronal lipid metabolism in ALS is dysregulated affecting energy use, structural integrity, and signaling processes. Oxidative stress is increased since neurons metabolize a greater proportion of lipid substrates. When looking at the structural level, altered lipid metabolism leads to cytoskeletal defects and neuromuscular junction denervation. Finally, altered lipid metabolism disrupts important biological signaling processes, altering neurotransmitter synthesis and release and impairing intracellular transport.

18.4 Lipidomic Analysis in ALS Animal Models and Patients

Metabolomics studies search for small molecules present in cells, tissues or biological samples, whereas the observation of modifications in the levels of these molecules, in addition to physiological modifications of signaling pathways may help to elucidate where these changes are occurring, (e.g. intracellularly). When these molecules are lipids, the studies are called Lipidomics [43]. The chance that a small molecule (e.g. fatty acid) can be used as a biomarker relies on the existing communication between the brain and the periphery. The former must be accompanied to the capacity of different metabolites to cross the blood-brain barrier (BBB) and therefore be detected in plasma. In addition to the non-invasive capacity to obtain blood samples from patients, promotes that the metabolites present in plasma are an ideal source of biomarkers as molecular traces of the disease. Besides, in neurodegenerative diseases the hemato-encephalic barrier can be compromised, increasing the possibilities that these metabolites can be detected in plasma [43].

Lipidomic studies have shown that different classes of lipids can be used as biomarkers of ALS onset and progression when compared to healthy volunteers or other neurodegenerative patients, as well as to follow preliminary studies with pharmacological compounds that have impact on signaling pathways involved in neuroinflammation. Below we will discuss these biomarkers in terms of the complexity of the lipids analyzed and the oxidative status of these products.

18.4.1 Saturated and Unsaturated Fatty Acids

Nutritional status in conditions such as obesity and cancer can be followed by looking the blood palmitoleate to palmitate (16:1/16:0) and stearate to oleate (18:0/18:1) ratios [4, 44, 45]. Recent

studies have shown that levels of 16:1 and 18:1 fatty acids, as well as of the enzyme that introduces the instaurations at the carbon chain, stearoyl-CoA desaturase, are significantly increased in blood cells from ALS patients compared to healthy controls [4, 44, 45]. It is important to note that the ratio of different monounsaturated fatty acids was strongly increased in ALS patients. In particular, 16:1 and 18:1 levels increased significantly in blood cells, and higher oleate levels were also observed in serum [4]. Fatty acids levels in the blood correlated with ALSFRS-R, the 16:1/16:0 ratio in blood cells negatively correlated with ALSFRS-R decline over a six-month period [4]. Circulating free fatty acids were more abundant in ALS patients, suggesting increased lipid breakdown by adipocytes [46]. In fact, a decline of ALSFRS-R was also significantly associated with the levels of 16:1 itself. Survival rates were greater when associated to higher 16:1/16:0 ratios, with a prolonged life expectancy of almost 11 months in the population of patients with a high 16:1/16:0 ratio, as compared to patients with low 16:1/16:0 ratio. Higher levels of palmitoleate itself were also significantly associated with extended life span. In contrast to what was observed for the 16:1/16:0 ratio, no changes in survival were observed associated to the 18:1/18:0 ratio [4]. Moreover, BMI or circulating leptin content did not correlate with ALSFRS-R decline, nor prognosticated survival based on multivariate analysis. In fact, statistical analysis showed that blood cell 16:1/16:0 ratio was an independent prognostic factor for survival with age, BMI, site of onset and ALSFRS-R as variables [4]. The 16:1/16:0 index is an easy-to-handle parameter that predicts survival of ALS patients. The enzymatically and non-enzymatically oxidation of unsaturated fatty acids increase during oxidative stress and may reflect the presence of an inflammatory process. Lipid peroxidation susceptibility, assessed by the peroxidability index (PI) [47], was 16% lower in the blood cell fraction of ALS patients as compared to controls, independent of the omega-6/omega-3 (AA/EPA) index which did not show significant modification [4].

18.4.2 Complex Lipids

Complex lipids as triglycerides (TG), total cholesterol (TC), phospholipids (PL) were all analyzed as biomarkers of ALS onset, progression or drug effect on clinical symptoms [43]. The levels of TG and TC in ALS patients were comparable to those found in control subjects. A recent study analyzed the composition of lipids in cerebrospinal fluid (CSF) from ALS patients compared to controls [43]. In this study, the authors demonstrated that phosphatidylcholine PC(36:4), ceramide and glucosylceramide levels were higher in ALS patients than in control subjects. Bioinformatics and statistical analysis showed six different metabolites that can be followed as distinctive between ALS and control groups. These compound corresponded to the PC derivatives PC (36:2p), PC(36:4p), PC(40:6p), the sphingomyelin (SM) derivatives SM(d34:0), and SM(d39:1), and the TG (Triglyceride) (16:1/18:1/18:2) [43]. The PC and SM compounds levels were higher in ALS patients, in contrast to TG which was lower in ALS patients than in controls. It is worth noting the higher level of PC (36:4p and 36:4e) was the strongest discriminant factor identified by all the statistical approaches used [43]. However, and importantly, higher levels of SM(d43:2) were associated to a lesser decline of the ALSFRS-r score [43].

Similar studies were performed using transgenic ALS mice. In these experiments, the results were quite different from those of humans, highlighting the difficulties in transferring the data obtained in animals to humans. In this case the complex lipids, which were found to be discriminant between control and ALS mice, were PC (36:2), PC(36:4), PC(40:6). These three lipids were found in higher levels in the ALS groups than in controls [43].

We have previously discussed that accumulation of ceramides (Cer) are toxic for MN [16]. By contrast, increasing the formation and accumulation of Cer-derived agents may be protective by reducing ceramide synthesis thus limiting the direct toxic effect on MN [16]. The membrane lipids Glycosphingolipids (GSLs) are a heterogeneous lipid groups formed through the covalent

linkage of a glycan moiety to Cer, especially abundant in the CNS with important bioactive metabolic roles, growth factor signaling and participation on neuroinflammation [48, 49]. All of these activities are thought to participate in ALS disease pathogenesis. When cervical spinal cord gray and white matter samples from ALS patients were analyzed, the major isoforms of Cer, e.g. GalCer, GlcCer, LacCer, GM3, GM1, GL3, and SPM were significantly elevated [16]. This increase was not due to changes on the enzymes activities that catalyze the formation of these products. These results were confirmed by using an inhibitor of the enzyme that produces GlcCer, which increased Cer levels and accelerated disease progression in $SOD1^{G93A}$ mice [16].

18.4.3 Oxidized Lipids

Oxidized lipids can be used as blood biomarkers for diseases progression or drug treatments. Importantly, oxidized lipids involves enzymatic derived compounds with relevant biological and signaling actions. Arachidonic acid is the precursor of a wide variety of anti- or pro-inflammatory compounds when metabolized by the PGHS or Lipoxygenase (LOX) pathways. These compounds can be followed in small samples of blood and used as disease biomarkers [43, 50]. In fact, ALS mice as well as patients with sporadic ALS have increased levels of prostaglandin E_2 (PGE_2) [23]. This compound is formed by the action of PGE synthase-1 on PGH_2 synthesized by oxidation of AA by PGHS. Furthermore, the protein levels of microsomal PGE synthase-1 and PGHS-2, which catalyze PGE_2 biosynthesis, are significantly increased in the spinal cord of ALS mice [23]. In ALS patients, PGE_2 levels are increased in the serum and the CSF [51]. The pharmacological inhibition of PGE_2 receptor or the silencing of the gene coding for PGHS-2 can lower neuroinflammation in $SOD1^{G93A}$ mice, preserve motor functions and extend survival [52, 53]. Since PGE_2 can exert different biological actions depending of the tissue, its use as a blood or tissue disease biomarker needs further studies.

The involvement of AA metabolites in ALS was also supported by the increased mRNA and protein levels of 5-lipoxygenase (5-LOX) observed in $SOD1^{G93A}$ mice at 120 days of age. Of therapeutic interest, oral administration of the 5-LOX and tyrosine kinase inhibitors nordihydroguaiaretic acid, significantly extended lifespan and slowed motor dysfunction in this animal model [15]. We have recently reported changes on the levels of some of AA-derived compounds in $SOD1^{G93A}$ mice [50]. In addition, we have recently published changes in levels of LOX-derived products in $SOD1^{G93A}$ mice at different stages of the disease. We observed, when the clinical symptoms appear, a significant increase of 12-hydroxyeicosatetraenoic acid (12-HETE) in both plasma and brain whereas no changes were observed in age-matched non-transgenic mice. Similarly, 15-hydroxyeicosatetraenoic acid (15-HETE) levels were also higher in $SOD1^{G93A}$ brains. Prostaglandin levels were also increased at day 90 in plasma from SOD1G93A compared to non-Tg being similar in both types of animals at later stages of the disease [50].

Unexpected results also were reported when analyzing oxidized lipids in ALS mice models. In a recent study, the neuroprotective effect of EPA was analyzed [35]. The treatment with EPA resulted in an enhancement of neuroinflammation, faster disease progression and hastened death for $SOD1^{G93A}$ mice [35]. The authors propose that the observed results are due to the greater susceptibility to be peroxidized and therefore toxic end products of EPA.

18.5 Conclusions

ALS is a neurodegenerative disease, with a short life expectancy, for which efficient treatments are still missing. Since many factors affect the onset and progression of the disease, it is important to find biomarkers that may aid the design of drugs that specifically influence and mitigate the damage, prevent MN loss and neuromuscular denervation. The high presence at CNS, the capacity to cross the BBB and be found at plasma suggest that lipid products can be used as prognostics

indicators of ALS. The analysis we did in this review suggests that the benefits of detecting these biomarkers may help in designing and performing clinical trials whose aim should be not only increase life expectancy but also quality of life.

References

1. Al-Chalabi A, Hardiman O (2013) The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 9(11):617–628
2. Barbeito LH, Pehar M, Cassina P, Vargas MR, Peluffo H, Viera L, Estevez AG, Beckman JS (2004) A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. Brain Res Brain Res Rev 47(1–3):263–274
3. Gros-Louis F, Gaspar C, Rouleau GA (2006) Genetics of familial and sporadic amyotrophic lateral sclerosis. Biochim Biophys Acta 1762(11–12):956–972
4. Henriques A, Blasco H, Fleury MC, Corcia P, Echaniz-Laguna A, Robelin L, Rudolf G, Lequeu T, Bergaentzle M, Gachet C et al (2015) Blood cell palmitoleate-palmitate ratio is an independent prognostic factor for amyotrophic lateral sclerosis. PLoS One 10(7):e0131512
5. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron D (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Scler Other Motor Neuron Disord 1(5):293–299
6. Philips T, Rothstein JD (2015) Rodent models of amyotrophic lateral sclerosis. Curr Protoc Pharmacol 69(5):671–621
7. Rossi S, Cozzolino M, Carri MT (2016) Old versus new mechanisms in the pathogenesis of ALS. Brain Pathol 26(2):276–286
8. Cassina P, Cassina A, Pehar M, Castellanos R, Gandelman M, de Leon A, Robinson KM, Mason RP, Beckman JS, Barbeito L et al (2008) Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. J Neurosci 28(16):4115–4122
9. Miquel E, Cassina A, Martinez-Palma L, Bolatto C, Trias E, Gandelman M, Radi R, Barbeito L, Cassina P (2012) Modulation of astrocytic mitochondrial function by dichloroacetate improves survival and motor performance in inherited amyotrophic lateral sclerosis. PLoS One 7(4):e34776
10. Miquel E, Cassina A, Martinez-Palma L, Souza JM, Bolatto C, Rodriguez-Bottero S, Logan A, Smith RA, Murphy MP, Barbeito L et al (2014) Neuroprotective effects of the mitochondria-targeted antioxidant MitoQ in a model of inherited amyotrophic lateral sclerosis. Free Radic Biol Med 70:204–213
11. Pehar M, Cassina P, Vargas MR, Castellanos R, Viera L, Beckman JS, Estevez AG, Barbeito L (2004) Astrocytic production of nerve growth factor in motor neuron apoptosis: implications for amyotrophic lateral sclerosis. J Neurochem 89(2):464–473
12. Pehar M, Vargas MR, Robinson KM, Cassina P, Diaz-Amarilla PJ, Hagen TM, Radi R, Barbeito L, Beckman JS (2007) Mitochondrial superoxide production and nuclear factor erythroid 2-related factor 2 activation in p75 neurotrophin receptor-induced motor neuron apoptosis. J Neurosci 27(29):7777–7785
13. Vargas MR, Pehar M, Cassina P, Martinez-Palma L, Thompson JA, Beckman JS, Barbeito L (2005) Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: consequences for motor neuron survival. J Biol Chem 280(27):25571–25579
14. Boillee S, Cleveland DW (2008) Revisiting oxidative damage in ALS: microglia, Nox, and mutant SOD1. J Clin Invest 118(2):474–478
15. West M, Mhatre M, Ceballos A, Floyd RA, Grammas P, Gabbita SP, Hamdheydari L, Mai T, Mou S, Pye QN et al (2004) The arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid inhibits tumor necrosis factor alpha activation of microglia and extends survival of G93A-SOD1 transgenic mice. J Neurochem 91(1):133–143
16. Dodge JC, Treleaven CM, Pacheco J, Cooper S, Bao C, Abraham M, Cromwell M, Sardi SP, Chuang WL, Sidman RL et al (2015) Glycosphingolipids are modulators of disease pathogenesis in amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 112(26):8100–8105
17. Hamilton JA, Brunaldi K (2007) A model for fatty acid transport into the brain. J Mol Neurosci MN 33(1):12–17
18. Ebert D, Haller RG, Walton ME (2003) Energy contribution of octanoate to intact rat brain metabolism measured by 13C nuclear magnetic resonance spectroscopy. J Neurosci 23(13):5928–5935
19. Gutierrez J, Ballinger SW, rley-Usmar VM, Landar A (2006) Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells. Circ Res 99(9):924–932
20. Bazan NG, Molina MF, Gordon WC (2011) Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annu Rev Nutr 31:321–351
21. Bazan NG, Musto AE, Knott EJ (2011) Endogenous signaling by omega-3 docosahexaenoic acid-derived mediators sustains homeostatic synaptic and circuitry integrity. Mol Neurobiol 44(2):216–222
22. Bazinet RP, Laye S (2014) Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci 15(12):771–785
23. Miyagishi H, Kosuge Y, Takano A, Endo M, Nango H, Yamagata-Murayama S, Hirose D, Kano R, Tanaka Y, Ishige K et al (2017) Increased expression of 15-Hydroxyprostaglandin dehydrogenase in spinal

astrocytes during disease progression in a model of amyotrophic lateral sclerosis. Cell Mol Neurobiol 37(3):445–452
24. Cutler RG, Pedersen WA, Camandola S, Rothstein JD, Mattson MP (2002) Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress-induced death of motor neurons in amyotrophic lateral sclerosis. Ann Neurol 52(4):448–457
25. Brugg B, Michel PP, Agid Y, Ruberg M (1996) Ceramide induces apoptosis in cultured mesencephalic neurons. J Neurochem 66(2):733–739
26. France-Lanord V, Brugg B, Michel PP, Agid Y, Ruberg M (1997) Mitochondrial free radical signal in ceramide-dependent apoptosis: a putative mechanism for neuronal death in Parkinson's disease. J Neurochem 69(4):1612–1621
27. Bras J, Singleton A, Cookson MR, Hardy J (2008) Emerging pathways in genetic Parkinson's disease: potential role of ceramide metabolism in Lewy body disease. FEBS J 275(23):5767–5773
28. Car H, Zendzian-Piotrowska M, Fiedorowicz A, Prokopiuk S, Sadowska A, Kurek K (2012) The role of ceramides in selected brain pathologies: ischemia/hypoxia, Alzheimer disease. Postepy Hig Med Dosw 66:295–303
29. Miana-Mena FJ, Piedrafita E, Gonzalez-Mingot C, Larrode P, Munoz MJ, Martinez-Ballarin E, Reiter RJ, Osta R, Garcia JJ (2011) Levels of membrane fluidity in the spinal cord and the brain in an animal model of amyotrophic lateral sclerosis. J Bioenerg Biomembr 43(2):181–186
30. Ilieva EV, Ayala V, Jove M, Dalfo E, Cacabelos D, Povedano M, Bellmunt MJ, Ferrer I, Pamplona R, Portero-Otin M (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain J Neurol 130.(Pt 12:3111–3123
31. Reich-Slotky R, Andrews J, Cheng B, Buchsbaum R, Levy D, Kaufmann P, Thompson JL (2013) Body mass index (BMI) as predictor of ALSFRS-R score decline in ALS patients. Amyotroph Later Scler Frontotemporal Degener 14(3):212–216
32. Henriques A, Croixmarie V, Priestman DA, Rosenbohm A, Dirrig-Grosch S, D'Ambra E, Huebecker M, Hussain G, Boursier-Neyret C, Echaniz-Laguna A et al (2015) Amyotrophic lateral sclerosis and denervation alter sphingolipids and up-regulate glucosylceramide synthase. Hum Mol Genet 24(25):7390–7405
33. Dupuis L, Corcia P, Fergani A, Gonzalez De Aguilar JL, Bonnefont-Rousselot D, Bittar R, Seilhean D, Hauw JJ, Lacomblez L, Loeffler JP et al (2008) Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 70(13):1004–1009
34. Schmitt F, Hussain G, Dupuis L, Loeffler JP, Henriques A (2014) A plural role for lipids in motor neuron diseases: energy, signaling and structure. Front Cell Neurosci 8(25)
35. Yip PK, Pizzasegola C, Gladman S, Biggio ML, Marino M, Jayasinghe M, Ullah F, Dyall SC, Malaspina A, Bendotti C et al (2013) The omega-3 fatty acid eicosapentaenoic acid accelerates disease progression in a model of amyotrophic lateral sclerosis. PLoS One 8(4):e61626
36. Parakh S, Spencer DM, Halloran MA, Soo KY, Atkin JD (2013) Redox regulation in amyotrophic lateral sclerosis. Oxidative Med Cell Longev 2013:408681
37. Dupuis L, Oudart H, Rene F, Gonzalez de Aguilar JL, Loeffler JP (2004) Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. Proc Natl Acad Sci USA 101(30):11159–11164
38. Mattson MP, Cutler RG, Camandola S (2007) Energy intake and amyotrophic lateral sclerosis. NeuroMolecular Med 9(1):17–20
39. Desport JC, Preux PM, Magy L, Boirie Y, Vallat JM, Beaufrere B, Couratier P (2001) Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. Am J Clin Nutr 74(3):328–334
40. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP (2011) Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 10(1):75–82
41. Ngo ST, Steyn FJ, Huang L, Mantovani S, Pfluger CM, Woodruff TM, O'Sullivan JD, Henderson RD, McCombe PA (2015) Altered expression of metabolic proteins and adipokines in patients with amyotrophic lateral sclerosis. J Neurol Sci 357(1–2):22–27
42. Chio A, Calvo A, Ilardi A, Cavallo E, Moglia C, Mutani R, Palmo A, Galletti R, Marinou K, Papetti L et al (2009) Lower serum lipid levels are related to respiratory impairment in patients with ALS. Neurology 73(20):1681–1685
43. Blasco H, Veyrat-Durebex C, Bocca C, Patin F, Vourc'h P, Kouassi Nzoughet J, Lenaers G, Andres CR, Simard G, Corcia P et al (2017) Lipidomics reveals cerebrospinal-fluid signatures of ALS. Sci Rep 7(1):17652
44. Vinknes KJ, Elshorbagy AK, Drevon CA, Nurk E, Tell GS, Nygard O, Vollset SE, Refsum H (2013) Associations between plasma polyunsaturated fatty acids, plasma stearoyl-CoA desaturase indices and body fat. Obesity 21(9):E512–E519
45. Vinknes KJ, Elshorbagy AK, Nurk E, Drevon CA, Gjesdal CG, Tell GS, Nygard O, Vollset SE, Refsum H (2013) Plasma stearoyl-CoA desaturase indices: association with lifestyle, diet, and body composition. Obesity 21(3):E294–E302
46. Falcao-Pires I, Castro-Chaves P, Miranda-Silva D, Lourenco AP, Leite-Moreira AF (2012) Physiological, pathological and potential therapeutic roles of adipokines. Drug Discov Today 17(15–16):880–889
47. Spector AA (1999) Essentiality of fatty acids. Lipids 34(Suppl):1–3
48. Nordstrom V, Willershauser M, Herzer S, Rozman J, von Bohlen Und Halbach O, Meldner S, Rothermel U, Kaden S, Roth FC, Waldeck C et al (2013) Neuronal expression of glucosylceramide synthase in central nervous system regulates body weight and energy homeostasis. PLoS Biol 11(3):e1001506

49. Inokuchi J (2009) Neurotrophic and neuroprotective actions of an enhancer of ganglioside biosynthesis. Int Rev Neurobiol 85:319–336
50. Trostchansky A, Mastrogiovanni M, Miquel E, Rodriguez-Bottero S, Martinez-Palma L, Cassina P, Rubbo H (2018) Profile of arachidonic acid-derived inflammatory markers and its modulation by nitro-oleic acid in an inherited model of amyotrophic lateral sclerosis. Front Mol Neurosci 11(131)
51. Ilzecka J (2003) Prostaglandin E2 is increased in amyotrophic lateral sclerosis patients. Acta Neurol Scand 108(2):125–129
52. Pompl PN, Ho L, Bianchi M, McManus T, Qin W, Pasinetti GM (2003) A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. FASEB J 17(6):725–727
53. Klivenyi P, Kiaei M, Gardian G, Calingasan NY, Beal MF (2004) Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurochem 88(3):576–582

19 Flavonoids Ability to Disrupt Inflammation Mediated by Lipid and Cholesterol Oxidation

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Keywords

Flavonoids · Cardiovascular disease · Inflammation · Lipid oxidation · Cholesterol oxidation · LDL · Macrophages · Oxysterols · Resveratrol · Dietary antioxidants · Oxidative stress

19.1 Introduction

Flavonoids are plant secondary metabolites that act as protectants against harmful effects of UV-B radiation inasmuch as biotic stress, conferring at the same time pigmentation of fruits and leaves [67]. The term "flavonoid" refers to phenolics having a basic skeleton of diphenylpropane (C6-C3-C6), which consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle [25, 52]. Flavonoids are broken down into several different sub-categories based on their chemical structure. The main subclasses commonly found in food items are: flavonols, flavones, flavanones, flavan-3-ols, proanthocyanidins, and anthocyanins [44, 67]. Figure 19.1 depicts the major classification of flavonoids according to their chemical structure. Their occurrence in food matrices has been extensively reviewed [39, 44], and has been subject of extensive research in the last decades. Table 19.1 contains a few examples of compounds from each of the subcategory, with the fruit (berry) in which they are commonly found. The monomeric unit of flavonoids can dimerize and polymerize to form other important high molecular weight molecules; this is the case of proanthocyanidins, that are polymers of flavan-3-ols or flavanols. Not only do these compounds act as plant protectants, but they can also be very beneficial to human health. Cohorts studies performed in the early '90 have shown that dietary consumption of flavonoids was inversely associated with morbidity and mortality from coronary heart disease [31, 32]. These findings have opened an intensive field of research on the effects of flavonoids and flavonoids-rich food extracts in cardiovascular diseases (CVD) progression, particularly in the modulating CVD-associated oxidative stress and inflammation. In this short review, we will summarize the current findings in flavonoids beneficial effects in preventing CVD through inhibition of initial stages of CVD progression. Given the magnitude of scientific literature in the field, we will focus on two

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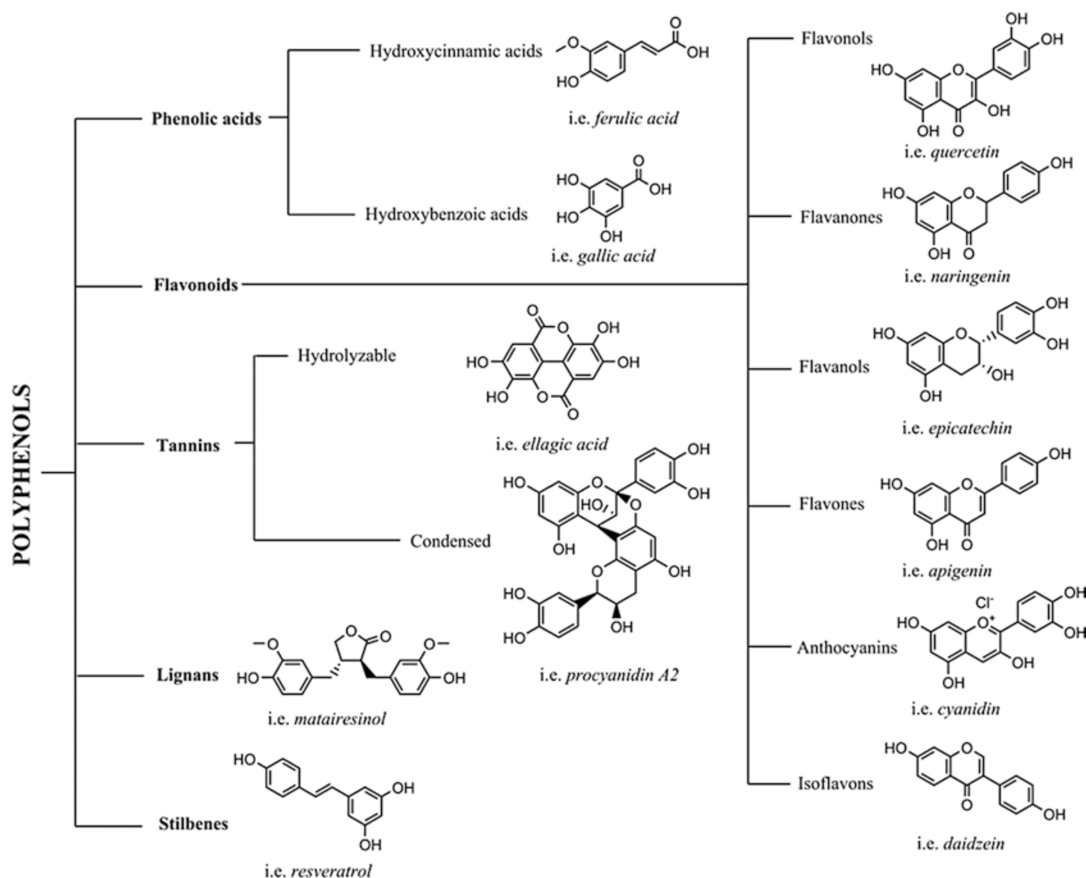

Fig. 19.1 Main classification of phenolic compounds

strictly mechanistic aspects: inhibition of chemical-induced LDL oxidation, and the effect of flavonoids in the monocyte/macrophages activation pathways.

19.2 Flavonoid Absorption and Bioavailability

The average US daily flavonoid intake is 189.7 mg/d, subdivided in flavan-3-ols (87%), flavanones (7.6%), flavonols (6.8%), and others [12]. Quercetin is the most important contributor to the estimated intake of flavonoids, mainly from the consumption of apples and onions [52]. Data regarding flavonoids bioavailability – i.e. the portion of the compound ingested that is digested, absorbed and metabolized – are still incomplete. Plasma or urine concentration of a certain compound is typically used to characterize its bioavailability [46]. In terms of flavonoids, the primary route of administration is ingestion. Once ingested, flavonoids undergo sulfation, methylation and glucuronidation in the small intestine and liver by respective enzymes [26, 44]. These metabolic forms are the ones commonly found in plasma, rather than the aglycone [10, 38, 44, 46, 64]. Several studies performed in humans have shown that after ingestion of flavonoid-rich food, maximum plasma levels are observed after 1.5–2 h; however, only sub- or low-micromolar concentration of the corresponding aglycone are retrieved. This is the case of green tea catechins (0.6–1.8 μM, depending on the considered compound) [74], quercetin from a vegetable and fruit-enriched diet (0.14 μM) [17], as well as from ingestion of apples and onions (1.5–3.4 μM) [33], among others. From these studies, it is clear that only a small percentage of flavonoids (<2%) of the initial intake is

Table 19.1 Individual flavonoids according to their subclass, as found in common berries

Flavonoid subclass	Compound	Berry species
Flavonols	Kaempferol	Blueberry, sour cherry, cranberry, raspberry, strawberry
	Quercetin	Blueberry, sour cherry, cranberry, raspberry, strawberry
	Myricetin	Blueberry, cranberry, strawberry
	Isoharmnetin	Sour cherry
Flavones	Apigenin	–
	Luteolin	–
Flavanones	Hesperitin	–
	Narigenin	Strawberry
Flavan-3-ols	(-)-Epicatechin	Blueberry, sour cherry, cranberry, raspberry, strawberry
	(-)-Epicatechin 3-gallate	Strawberry
	(-)-epigallocatechin	Cranberry, raspberry, strawberry
	(-)-epigallocatechin 3-gallate	Cranberry, raspberry, strawberry
	(+)-catechin	Blueberry, sour cherry, cranberry, raspberry, strawberry
	(+)-gallocatechin	Strawberry
Proanthocyanidins	Monomer	Blueberry, cranberry, raspberry
	Dimer	Blueberry, cranberry, raspberry
	Trimer	Blueberry, cranberry, raspberry
	4-6mers	Blueberry, cranberry, raspberry
	7-10mers	Blueberry, cranberry, raspberry
	Polymers	Blueberry, cranberry,
Anthocyanidins	Cyanidin	Blueberry, sour cherry, cranberry, raspberry, strawberry
	Delphinidin	Blueberry, cranberry, raspberry, strawberry
	Malvidin	Blueberry, cranberry, raspberry, strawberry
	Pelargonidin	Blueberry, cranberry, raspberry, strawberry
	Peonidin	Blueberry, sour cherry, cranberry, raspberry, strawberry
	Petunidin	Raspberry, strawberry

absorbed. Compounds that are not absorbed from the intestine migrate to the colon wherein they interact with the microflora. The flavonoids that re-enter circulation can now be broken into low aglycones by the microbiota and further into low molecular weight compounds that can be absorbed [64].

Different polyphenols have different absorption rates and metabolic conversions due to their variable structures. Regarding flavonols, studies performed in the Caco-2 model cell line have shown that the several glycosylated forms are poorly absorbed [15, 26]. Aglycones can pass through the gut wall because microflora hydrolyzes the sugar moiety [25]. However, there is also a compound specificity in the absorption mechanism, i.e. quercetin and daidzein glucosides seemed to be directly absorbed by active transport [26]. Not only does polyphenol structure affect the absorption, but so can the dose and food matrix in which they are contained. It has been found that the absorption efficiency can actually decrease as the dose increases, suggesting that there is an absorption saturation point [10]. Because of the overall poor absorption and low bioavailability of flavonoids, a variety of strategies have been explored in order to improve these inefficiencies. Increasing metabolic stability, intestinal absorption and changing the absorption site have been considered to improve flavonoid bioavailability [52, 64].

19.3 Lipid and Cholesterol Oxidation in LDL and Atherosclerosis

19.3.1 Chemical-Induced Oxidation of LDL

There is a generous literature regarding *ex vivo* or *in vitro* oxidation of LDL, and it is out of the scope of this chapter a detailed revision of the field, which can be found elsewhere [42]. Here,

we will summarize the main strategies used to assess LDL oxidation and how these experimental setups are relevant for flavonoids-centered antioxidant studies. LDL are complex macromolecules, made of a lipid core surrounded by a single non-exchangeable lipoprotein, known as apolipoprotein (ApoB). The average lipid core has been calculated to contain 600 molecules of free cholesterol, 1600 molecules of cholesteryl esters (mainly arachidonate and linoleate esters), 700 molecules of phospholipids (64% PC, 1.5% PE, 26% SM, and 11% LPC), 180 molecules of triacylglycerides (TAGs), and approximately 10 molecules of the lipophilic antioxidant α-tocopherol [37, 42]. Several exchangeable lipoproteins are also associated to LDL particle, and their function is to modulate interactions with specific cell receptors [7, 16]. In particular, high concentrations of ApoC-III in ApoB lipoproteins have been reported among patients with coronary disease and coronary atherosclerotic lesions, as well as metabolic syndrome and type 2 diabetes [16, 61].

Ex vivo and *in vitro* oxidation of LDL is an experimental strategy largely adopted to either characterize products of lipid oxidation, or assess the ability of antioxidants to dampen oxidation itself. Oxidation can be triggered via several mechanisms, including but not limited to free radicals generation, Fenton reaction, and peroxynitrite oxidation agents [19]. α,α′-azodiisobutyramidine dihydrochloride (AAPH) has been widely used as ROS inducer, since it degrades to highly reactive radical species that oxidized molecular oxygen to peroxy radicals [19]. Fenton reaction is triggered by micromolar solution of Cu^{2+} (or less commonly Fe^{2+}) salts, which are able to abstract a proton from preformed lipid peroxides to generate peroxyl radicals [28]. Finally, peroxynitrite is obtained from a variety of precursor, including linsidomine (SIN-1) [62, 65]. Oxidation of LDL can potentially affect each of its constituents, including the apoB, and cross-reactions between different fractions (i.e. protein/lipid chemical interaction) can exponentially multiply the chemistry of the oxidative products, to a variety of lipids with reported biological activity [42]. LDL can be mildly or highly oxidized (MM-LDL and the proper ox-LDL, respectively), although there is no general consensus about the active species present in each stage [11, 42, 50].

19.3.2 Cholesterol Oxidation in LDL

Cholesterol is a natural occurring compound that has several crucial functions within the body. It is a key component of all cellular membranes, a precursor to steroid hormones and is involved in many signal transduction pathways [27]. Cholesterol transport in humans is a complex process. LDL are the lipoproteins that carry esterified cholesterol from the liver to the peripheral tissue [71]. In humans, a balance of cholesterol is critical for many physiological processing, since accumulation of this molecules in cell membrane can affect the function of a variety of enzymes, transporters and receptors. Cholesterol is susceptible of being oxidized by naturally occurring oxidative agents, like reactive oxygen and nitrogen species (ROS and RNS, respectively), that can alter its chemical backbone by oxygenating carbons at several positions. Commonly, auto-oxidation of cholesterol (i.e. by chemical and physical means) occurs at position C-7 proximal to the C5-C6 double bond, or to the C-25 tertiary carbon [45, 47]. However, first oxidative derivatives can be further oxidized and go subsequent chemical rearrangements. To date, more than 80 compounds, broadly known as cholesterol oxidation products (COPs) have been identified. COPs are more polar than the parent cholesterol, thus present different chemical and biophysical properties in cell environment. Their different chemistry is the major source of health-associated issues, since they can interfere with cholesterol homeostasis at several level. It is not the scope of this chapter to detail the putative biological effects due to the exposure of COPs, as from cell, *in vivo* and (marginally) clinical studies. The reader can refer to our recent review [45] or other compelling works in the area. Briefly, *in vitro* works using several cell lines have reported that admin-

istration of high concentration of COPs – either individually or as a mixture of compounds – can trigger several physiological responses able to induce pro-inflammatory, pro-fibrogenic, and pro-apoptotic effects. A consistent body of literature has also assessed the cytotoxic, mutagenic and carcinogenic properties of COPs. As aforementioned, cholesterol homeostasis is strictly linked to COPs accumulation in fluids and cell compartments [45].

19.3.3 OxLDL Activate Monocytes/Macrophages Pathway

The mechanism by which LDL activates monocytes in the intima towards their conversion to macrophages is still controversial, although a rough phenomenological description has been provided [59]. There is scientific consensus that oxLDLs, rather than the parent LDL, are the primary stimulus leading endothelial activation and subsequent inflammatory response. Under particular conditions, LDL can cross the endothelial tissue of the blood vessels and being incorporated into the intima. According to the current response-to-injury hypothesis, oxidation of LDLs favors the expression of cell adhesion molecules by endothelial cells, such as the cell adhesion molecule-1 (ICAM-1) and the vascular adhesion molecule-1 (VCAM-1) [59]. These cell adhesion molecules favor LDL migration towards the intima. ROS and RNS-induced action on LDLs can also occur at the intima level [23, 40]. oxLDL-induced activation of macrophages is the second stage of atherosclerosis development. Both chemical and enzymatic oxidative processes act on the whole LDL chemical structure, including phospholipids, cholesterol (free and esterified), apolipoprotein and TAGs [11]. Lipids species derived from oxidation are responsible to “activate” monocytes, *via* specific interaction with membrane receptors. Extensive oxidation is necessary for LDL to bind macrophages receptors SR-A or CD36, including up to 50% cholesterol oxidation, apoB fragmentation, and diffused oxidation of unsaturated fatty acids [6, 37]. Isoprostane are one of the biomarkers of oxidative stress: PL-esterified isoprostanes are known to have pro-inflammatory action, and stimulate monocyte adhesion to the endothelial cell [1, 2]. Oxysterols, in particular 7-ketocholesterol, 7α-OH and 7β-OH cholesterol, and cholesterol epoxides are abundant in LDL, and are believed to exert cytotoxic effects on monocyte-macrophages ([5, 13]). Although toxic, ROS-induced COPs (particularly B-ring derivatives) seem unable to activate liver-X receptors (LXR-α and β) and peroxisome-proliferator-activated (PPARs) receptors in macrophages, and so trigger the anti-atherogenic response [6, 37, 69]. On the other side, enzymatic side-chain COPs like 22-OH, 25-OH and 27-OH cholesterols can trigger both LXRα and PPARs [69], which is explained by specific structural requirements needed for these ligand-receptor interactions to occur [36]. Other inflammatory mechanisms have been proposed for B-ring oxysterols, as 7-keto and 7-OH cholesterol isomers. A increasing literature body has demonstrated that B-ring oxysterols can stimulate inflammatory signaling in monocytes by interacting with Toll-like receptors [18], particularly TLR-4 [34]. 7β-OH cholesterol stimulate the increase of IL-8 secretion via the MEK/ERK1/2 pathway [69].

Oxidation of the fatty acid moiety of cholesteryl-esters also forms compounds – like 9-oxononanoyl cholesterol – able to activate adhesion of monocytes [5, 11]. These oxidative CE (OxCE) are also able to trigger inflammation through several mechanisms, including TLR-mediated response, as recently discussed in detail by Miller and Shyy [50].

The discussed inflammatory responses trigger the differentiation of monocytes, as well as their recruitment from the vascular vessels to the arterial wall, *via* trans-endothelial migration [43, 59]. The monocyte recruitment molecular process is still under debate, and involve several signaling mechanisms, as discussed elsewhere [43]. In the intima, monocytes then differentiate into macrophages, that uptake oxLDL *via* scavenging receptors, resulting in the transformation of macrophages to foam cells. Accumulation of foam cells and platelets is the boundary stone for

the formation of the atherosclerotic lesions known as fatty streaks.

19.4 Flavonoids Against LDL Oxidation

19.4.1 Mechanisms of Flavonoids Action Against Lipid Oxidation

Several studies in recent years have examined the relation between flavonoids and oxidation or inflammation. The powerful antioxidant activity exhibited by flavonoids is due to their action as free radical scavengers, singlet oxygen quenchers, and metal ion chelators [26]. Several *in vitro* systems have been used to evaluate the antioxidant potential of flavonoids. In this chapter, we will focus in LDL oxidation and macrophages activation.

LDL oxidation, as aforementioned, is the first critical step in triggering the inflammatory cascade that finally brings to atheroma formation and consequent CVD manifestation. Early works by Frankel's group [20, 48] have extensively explored the ability of fruits flavonoids extracts to dampen oxidation in LDL. Flavonoids possess lower redox potential than lipids, and are thus oxidized by ROS and RNS, resulting in more stable and less-reactive *ortho*-semiquinone radical compared to lipid radicals and peroxy radicals [3, 4, 29]. When evaluated individually, several pure flavonoids (catechin, quercetin, cyanidin, caffeic acid, and ellagic acid) showed up to 95% inhibition of Cu^{2+}-induced hexanal production in micromolar ranges (2.5–7.5 μM). However, specific binary and ternary mixtures of flavonoids showed antagonistic effects, particularly if caffeic and ellagic acids coexisted in equal amounts [48]. Dissimilarities among flavonoids AO activity can be explained taking into account differences in their chemical structures; particularly, structural variation in the C-ring and the oxidative state of C3 seemed to confer different reactivity [29, 48]. A structure-activity relationship (SAR) study by Vaya et al. [68] showed that the contribution of the different functional groups to the inhibition of LDL oxidation is well correlated to the energy needed to form the flavonoid radical by abstraction of a hydrogen atom. In other words, it is possible to classify flavonols according to their inhibition potential towards radical and copper induced oxidation, being more effective those compounds having two adjacent hydroxyls at the B-ring, like quercetin and catechin. Solubility and partitioning behavior of flavonoids could also play a role in defining their scavenging activity, considering that their effectiveness as LDL oxidative protectors relies in their ability to physically interact with the lipid core [48]. However, Paganga et al. [54] demonstrated that superiority of quercetin vs. catechin in inhibiting LDL peroxidation cannot be accounted to partitioning considerations, but to the exceptional ability of the former to chelate copper. Another interesting SAR study was performed by Yi and coworkers [75], where twenty-three 4-oxo-flavonoids (i.e. C-4 is oxidized to ketone function) were assessed against oxLDL-induced endothelial dysfunction. Similarly to the previous studies [48, 49, 68], flavonols – morin, myricetin, fisetin and quercetin – showed higher activity in micromolar range. The promising "hits" shared higher oxy-functionalization at the flavan ring: 3′,4′-*o*-dihydroxyl on B-ring, a 3-hydroxyl on C-ring, a 2,3-double bond and a 5,7-*m*-dihydroxyl on the A-ring were all required for the observed inhibitory effect.

19.4.2 Dietary Flavonoids and LDL Oxidation

A substantial amount of literature has used grape and wine as flavonoids-enriched matrix to leverage their LDL-protectant function, mainly supported by early hypothesis regarding the so-called "French paradox" formulated by epidemiologists in the early '80. The "French paradox" is the observation of low coronary heart disease (CHD) death rates despite high intake of dietary cholesterol and saturated fat in

southern French diet [58]. Red wine contains up to 250 mg/mL phenols, depending on type and variety [44]. Experiments performed using Petit Syrah wine showed that 5 μM concentration of individual catechin, myricetin, epicatechin, rutin and quercetin were able to inhibit LDL oxidation by Cu^{2+} in the range 60–75% [63], higher than α-tocopherol. More interesting, procyanidins (i.e. oligomeric forms of flavonols) were higher inhibitors of hexanal formation (up to 80% at 5 μM), which can be explain by their better ability to interact with the LDL constituents. A more extensive work performed on 14 grapes varieties demonstrated similar effects on the inhibition of hexanal generation, with the additional information that the inhibitory potential was positively correlated with the content of phenols, and in less extent anthocyanins and flavonols [49]. An interesting paper by Frémont and co-workers more deeply investigated the effects of wine extract and individual flavonoids in *in vitro* LDL oxidation by copper and AAPH [21]. Their results discriminated between chelating agents (resveratrol), which are active against Cu^{2+} oxidation, and radical scavengers (catechin) that inhibit AAPH lipid peroxide formation. Furthermore, in their studies highly unsaturated cholesteryl-esters, rather than phospholipids, were mostly affected by both oxidation mechanisms, and this was demonstrated by altered electrophoresis mobility of apoB after oxidation. These effects were negligible in white wines, which contain lower amounts of flavonoids, although imposing a longer grape-skin contact during winemaking can led to "red-like" white wines with analogue antioxidant capacity [22]. Beside grape, other flavonol-rich dietary extracts have been tested as antioxidants against LDL chemically-induced oxidation. Viana et al. [70] used an extract of *Vaccinium myrtillus* containing anthocyan, catechin, chalcone and other flavonoids to dampen Cu^{2+}-induced LDL oxidation. They showed a significant increase of the *lag* phase of lipid peroxidation associated with the presence of vitamin E, indicating that the effects of flavonoids are limited to the early stages of oxidation, when intrinsic antioxidants are still present. Similar results have been obtained with flavonoids extract from different species of berries [30, 51], as well as other fruits [44].

An obvious following step in CVD-related flavonoids research are *in vivo* and clinical studies. Various studies have exposed either animal models or humans subjects to flavonoids-enriched diets, recording controversial results that partially debunked the "anti-oxidative claim" [44]. Part of this controversy is due to the lack of reliable biomarkers for measuring plasma antioxidant activity, lack of long-term studies and underestimation of gut and liver biotransformation [44, 73]. A few examples of *in vivo* works follow, and a good critical review can be found elsewhere [44].

O'Reilly and co-workers coupled *in vitro* assessment of a flavonoid mixture against Cu^{2+} oxidation of LDL with dietary intervention study. All the tested flavonoids – including quercetin, luteolin, catechin, and kaempferol, among others – showed sub-micromolar ability to halve TBARS value and decrease LDL peroxides. Surprisingly, when 32 subjects were exposed to a high-flavonoids diet, no significant effects were found in *ex vivo* LDL oxidation [53]. From the *in vitro* effects on LDL, Pignatelli and others [56] administered red and white wine to 20 healthy volunteers, finding a significant reduction in plasma conjugate dienes, as well as urinary PGF-2α-III, a marker of oxidative stress. Flavonols from concentrated cranberry juice decreased plasma oxLDL and cell adhesion molecules ICAM-1 and VCAM-1 in 30 men, over three successive periods of 4 weeks [60]. In their review, Lotito and collaborators [44] list a few works that failed to demonstrate *in vivo* efficacy of flavonoids in inhibit plasma oxidation. The conclusion is that, although epidemiology studies are fairly convincing about their antioxidant effects, is unlikely that flavonoids *per se* acts against LDL oxidation, given their poor bioavailability. Likely, observed effects are due to upregulation of endogenous antioxidants enzymes, like those of the uric acid biosynthetic pathway.

19.4.3 Flavonoids and Inflammatory Activation Pathways

The immune system is integrated by a highly complex regulated group of cells that may interact in a cell-cell manner and may also respond to intercellular messages including hormones and cytokines. Diet, pharmacological agents, pollutants, and food chemicals display remarkable actions, at both pharmacological and biochemical level, that affect the function of inflammatory and immune cells, including macrophages [24]. We already discussed the function of monocytes and derived-macrophages in triggering the inflammatory effect during the formation of the atheroma and CVD progression. Macrophage can proliferate in the presence of a specific growth factor, M-CSF, and in the presence of an inducer such as lipopolysaccharide (LPS), they stop proliferating adopt a phenotype that is characterized by the expression of early cytokines and NO [14]. Several flavonoids – as genistein – can interact with enzymes and receptors involved in the generation of the inflammatory processes, especially tyrosine and serine-threonine protein kinases. In monocytes, those are responsible for the production of cytokines, such as TNF-α, IL-6 and IL-1β. Kaempferol and quercetin inhibitory effects against tyrosine kinases was reflected in a significant antiproliferative effects on M-CSF-activated macrophages [14]. Aglycones and conjugated-derivative showed anti-inflammatory activity: an interesting study by Kawai and co-workers [38] found that quercetin-3-glucoronide and quercetin disrupt expression of CD36 in murine macrophages cell lines. In vivo studies on mice showed that epigallocatechin-3-gallate can inhibit LPS-induced and interferon-γ-activated nitric oxide synthase (iNOS) gene expression, inasmuch as iNOS activity in cultured macrophages, and so reducing nitric oxide production and subsequently oxidative stress [8]. Direct inactivation of iNOS was explained also by the ability of certain flavonoids to bind arginine and the iNOS cofactor tetrahydrobiopterin [8]. A more extended study performed by Hämäläinen and co-workers [35] showed that several flavonoids – including kaempferol, genistein, quercetin and dadzein – were able to reduce STAT-1 and NF-*k*B activation (both iNOS transcription factors), and suppress iNOS expression, whereas others (including naringenin and pelargonidin) had no effect on STAT-1. A similar mechanism has been proposed to explain the strong inhibition of NO generation and iNOS expression in activated macrophages by resveratrol [66], although a similar study contradicts these findings [72]. Resveratrol can dampen the pro-inflammatory phenotype by activating sirtuin-1, a type III histone deacetylase that suppresses NF-*k*B factor [55]. Resveratrol downstream regulation mode of the mitochondrial biogenesis *via* sirtuin-1 activation seems to be strongly dose-dependent [9, 57]. It is important to notice that these *in vitro* studies, as well others [41] were performed at relatively high concentration of resveratrol, far from the ones normally observed in plasma after ingestion (see Sect. 19.2).

19.5 Conclusions

A few considerations follow from the above review. First, several epidemiology studies conducted in the last decades have demonstrated health benefits due to regular intake of flavonoid-rich foods. Second, the chemistry of flavonoids makes them prone to show radical-scavenging and metal-chelating properties, that can be successfully used to dampen *in vitro* lipid oxidation, in particular Cu^{2+} and free radical-induced oxidation of plasma LDL. This has been achieved both using fruit and vegetable extracts, as well as individual pure flavonoids standards, with inhibitory concentration in the order of 5–10 μM. However, clinical studies performed in healthy subjects were only partially able to replicate these *in vitro* and *ex vivo* findings by administration of a variety of dietary regimes with high content in flavonoids. The lack of *in vitro-in vivo* correlation can be attributed to several factors, that can be summarized in: i) reduced adsorption (0 to 1–2 μM in plasma) and short lifetime, that make unlikely an actual antioxidant effect, ii) biotransformation to

flavonoids-conjugates, with unknown or reduced biological activity. In order to positively merge the *in vitro* observations with the diffused epidemiological studies, research should be focused in validate those speculated mechanisms in more physiological settings. Among various aspects worth of research interests, the authors believe that priority should be given to a deep understanding of the "fate" of flavonoids in our organism, a critical effort that should consider adsorption, bioavailability, and the actions of intrinsic metabolism, inasmuch as gut microbiota. Also, the "flavonoids effect" should be better contextualized in the overall diet, thus facilitating the comprehension of combined effects between other macro and micro-nutrients, that can potentially act as synergistic/antagonist. Thus, a holistic rather than a criterion-based approach can led to more realistic outcomes and expectations on the consumer.

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References

1. Berliner JA, Gharavi NM (2008) Endothelial cell regulation by phospholipid oxidation products. Free Radic Biol Med 45(2):119–123
2. Berliner JA, Leitinger N, Tsimikas S (2009) The role of oxidized phospholipids in atherosclerosis. J Lipid Res 50(Suppl):S207–S212
3. Bors W, Michel C, Stettmaier K (2001) Structure-activity relationships governing antioxidant capacities of plant polyphenols. In: Methods in enzymology. Elsevier, Amsterdam, pp 166–180
4. Bors W, Saran M (1987) Radical scavenging by flavonoid antioxidants. Free Radic Res Commun 2(4–6):289–294
5. Brown AJ, Dean RT, Jessup W (1996) Free and esterified oxysterol: formation during copper-oxidation of low density lipoprotein and uptake by macrophages. J Lipid Res 37(2):320–335
6. Brown AJ, Jessup W (1999) Oxysterols and atherosclerosis. Atherosclerosis 142(1):1–28
7. Campos H, Perlov D, Khoo C, Sacks FM (2001) Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia. J Lipid Res 42(8):1239–1249
8. Chan MM-Y, Fong D, Ho C-T, Huang H-I (1997) Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. Biochem Pharmacol 54(12):1281–1286
9. Chang YP, Ka SM, Hsu WH, Chen A, Chao LK, Lin CC, Hsieh CC, Chen MC, Chiu HW, Ho CL (2015) Resveratrol inhibits NLRP3 inflammasome activation by preserving mitochondrial integrity and augmenting autophagy. J Cell Physiol 230(7):1567–1579
10. Charron CS, Kurilich AC, Clevidence BA, Simon PW, Harrison DJ, Britz SJ, Baer DJ, Novotny JA (2009) Bioavailability of anthocyanins from purple carrot juice: effects of acylation and plant matrix. J Agric Food Chem 57(4):1226–1230
11. Choi SH, Sviridov D, Miller YI (2017) Oxidized cholesteryl esters and inflammation. Biochim Biophys Acta Mol Cell Biol Lipids 1862(4):393–397
12. Chun OK, Chung SJ, Song WO (2007) Estimated dietary flavonoid intake and major food sources of US adults. J Nutr 137(5):1244–1252
13. Clare K, Hardwick SJ, Carpenter KL, Weeratunge N, Mitchinson MJ (1995) Toxicity of oxysterols to human monocyte-macrophages. Atherosclerosis 118(1):67–75
14. Comalada M, Ballester I, Bailon E, Sierra S, Xaus J, Galvez J, de Medina FS, Zarzuelo A (2006) Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure–activity relationship. Biochem Pharmacol 72(8):1010–1021
15. Dai J-y, Yang J-l, Li C (2008) Transport and metabolism of flavonoids from Chinese herbal remedy Xiaochaihu-tang across human intestinal Caco-2 cell monolayers. Acta Pharmacol Sin 29(9):1086–1093
16. Davidsson P, Hulthe J, Fagerberg B, Olsson B-M, Hallberg C, Dahllöf B, Camejo G (2005) A proteomic study of the apolipoproteins in LDL subclasses in patients with the metabolic syndrome and type 2 diabetes. J Lipid Res 46(9):1999–2006
17. Erlund I (2004) Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr Res 24(10):851–874
18. Erridge C, Webb DJ, Spickett CM (2007) 25-hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol upregulate interleukin-8 expression independently of toll-like receptor 1, 2, 4 or 6 signalling in human macrophages. Free Radic Res 41(3):260–266
19. Esterbauer H, Jiirgens G (1993) Mechanistic and genetic aspects of susceptibility of LDL to oxidation. Curr Opin Lipidol 4(2):114–124
20. Frankel E, German J, Kinsella J, Parks E, Kanner J (1993) Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 341(8843):454–457
21. Frémont L, Belguendouz L, Delpal S (1999) Antioxidant activity of resveratrol and alcohol-free

wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci 64(26):2511–2521
22. Fuhrman B, Volkova N, Suraski A, Aviram M (2001) White wine with red wine-like properties: increased extraction of grape skin polyphenols improves the antioxidant capacity of the derived white wine. J Agric Food Chem 49(7):3164–3168
23. Förstermann U, Xia N, Li H (2017) Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. Circ Res 120(4):713–735
24. García-Lafuente A, Guillamón E, Villares A, Rostagno MA, Martínez JA (2009) Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflamm Res 58(9):537–552
25. Georgiev V, Ananga A, Tsolova V (2014) Recent advances and uses of grape flavonoids as nutraceuticals. Nutrients 6(1):391–415
26. González R, Ballester I, López-Posadas R, Suárez M, Zarzuelo A, Martinez-Augustin O, Medina FSD (2011) Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 51(4):331–362
27. Guizzetti M, Costa LG (2005) Disruption of cholesterol homeostasis in the developing brain as a potential mechanism contributing to the developmental neurotoxicity of ethanol: an hypothesis. Med Hypotheses 64(3):563–567
28. Gutteridge JM (1986) Iron promoters of the Fenton reaction and lipid peroxidation can be released from haemoglobin by peroxides. FEBS Lett 201(2):291–295
29. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13(10):572–584
30. Heinonen IM, Meyer AS, Frankel EN (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J Agric Food Chem 46(10):4107–4112
31. Hertog MG, Feskens EJ, Kromhout D, Hollman P, Katan M (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 342(8878):1007–1011
32. Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A, Nedeljkovic S (1995) Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Arch Intern Med 155(4):381–386
33. Hollman PC, Van Trijp JM, Buysman MN, vd Gaag MS, Mengelers MJ, De Vries JH, Katan MB (1997) Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett 418(1–2):152–156
34. Huang J-D, Amaral J, Lee JW, Rodriguez IR (2014) 7-Ketocholesterol-induced inflammation signals mostly through the TLR4 receptor both in vitro and in vivo. PLoS One 9(7):e100985
35. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E (2007) Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediat Inflamm 2007:45673
36. Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ (1999) Structural requirements of ligands for the oxysterol liver X receptors LXRα and LXRβ. Proc Natl Acad Sci 96(1):266–271
37. Jessup W, Kritharides L, Stocker R (2004) Lipid oxidation in atherogenesis: an overview. Biochem Soc Trans 32(1):134–138
38. Kawai Y, Nishikawa T, Shiba Y, Saito S, Murota K, Shibata N, Kobayashi M, Kanayama M, Uchida K, Terao J (2008) Macrophage as a target of quercetin glucuronides in human atherosclerotic arteries implication in the anti-atherosclerotic mechanism of dietary flavonoids. J Biol Chem 283(14):9424–9434
39. Lairon D, Amiot MJ (1999) Flavonoids in food and natural antioxidants in wine. Curr Opin Lipidol 10(1):23–28
40. Leeuwenburgh C, Hardy MM, Hazen SL, Wagner P, Oh-ishi S, Steinbrecher UP, Heinecke JW (1997) Reactive nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. J Biol Chem 272(3):1433–1436
41. Leiro J, Alvarez E, Arranz JA, Laguna R, Uriarte E, Orallo F (2004) Effects of cis-resveratrol on inflammatory murine macrophages: antioxidant activity and down-regulation of inflammatory genes. J Leukoc Biol 75(6):1156–1165
42. Levitan I, Volkov S, Subbaiah PV (2010) Oxidized LDL: diversity, patterns of recognition, and pathophysiology. Antioxid Redox Signal 13(1):39–75
43. Ley K, Miller YI, Hedrick CC (2011) Monocyte and macrophage dynamics during atherogenesis. Arterioscler Thromb Vasc Biol 31(7):1506–1516
44. Lotito SB, Frei B (2006) Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med 41(12):1727–1746
45. Maldonado-Pereira L, Schweiss M, Barnaba C, Medina-Meza IG (2018) The role of cholesterol oxidation products in food toxicity. Food Chem Toxicol 118:908–939
46. McGhie TK, Walton MC (2007) The bioavailability and absorption of anthocyanins: towards a better understanding. Mol Nutr Food Res 51(6):702–713
47. Medina-Meza IG, Barnaba C (2013) Kinetics of cholesterol oxidation in model systems and foods: current status. Food Eng Rev 5(3):171–184
48. Meyer AS, Heinonen M, Frankel EN (1998) Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. Food Chem 61(1–2):71–75
49. Meyer AS, Yi O-S, Pearson DA, Waterhouse AL, Frankel EN (1997) Inhibition of human low-density

lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (Vitis vinifera). J Agric Food Chem 45(5):1638–1643
50. Miller YI, Shyy JY-J (2017) Context-dependent role of oxidized lipids and lipoproteins in inflammation. Trends Endocrinol Metab 28(2):143–152
51. Miranda-Rottmann S, Aspillaga AA, Pérez DD, Vasquez L, Martinez AL, Leighton F (2002) Juice and phenolic fractions of the berry *Aristotelia chilensis* inhibit LDL oxidation in vitro and protect human endothelial cells against oxidative stress. J Agric Food Chem 50(26):7542–7547
52. Nijveldt RJ, Van Nood E, Van Hoorn DE, Boelens PG, Van Norren K, Van Leeuwen PA (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74(4):418–425
53. O'Reilly JD, Sanders TA, Wiseman H (2000) Flavonoids protect against oxidative damage to LDL in vitro: use in selection of a flavonoid rich diet and relevance to LDL oxidation resistance ex vivo? Free Radic Res 33(4):419–426
54. Paganga G, Al-Hashim H, Khodr H, Scott BC, Aruoma OI, Hider RC, Halliwell B, Rice-Evans CA (1996) Mechanisms of antioxidant activities of quercetin and catechin. Redox Rep 2(6):359–364
55. Pan W, Yu H, Huang S, Zhu P (2016) Resveratrol protects against TNF-α-induced injury in human umbilical endothelial cells through promoting sirtuin-1-induced repression of NF-KB and p38 MAPK. PLoS One 11(1):e0147034
56. Pignatelli P, Ghiselli A, Buchetti B, Carnevale R, Natella F, Germano G, Fimognari F, Di Santo S, Lenti L, Violi F (2006) Polyphenols synergistically inhibit oxidative stress in subjects given red and white wine. Atherosclerosis 188(1):77–83
57. Price NL, Gomes AP, Ling AJ, Duarte FV, Martin-Montalvo A, North BJ, Agarwal B, Ye L, Ramadori G, Teodoro JS (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab 15(5):675–690
58. Renaud S d, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339(8808):1523–1526
59. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340(2):115–126
60. Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C (2008) Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. Br J Nutr 99(2):352–359
61. Sacks FM (2015) The crucial roles of apolipoproteins E and C-III in apoB lipoprotein metabolism in normolipidemia and hypertriglyceridemia. Curr Opin Lipidol 26(1):56–63
62. Shafiee M, Carbonneau M-A, Urban N, Descomps B, Leger CL (2003) Grape and grape seed extract capacities at protecting LDL against oxidation generated by Cu 2+, AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extracts or compounds. Free Radic Res 37(5):573–584
63. Teissedre PL, Frankel EN, Waterhouse AL, Peleg H, German JB (1996) Inhibition ofIn vitrohuman LDL oxidation by phenolic antioxidants from grapes and wines. J Sci Food Agric 70(1):55–61
64. Thilakarathna SH, Rupasinghe HP (2013) Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 5(9):3367–3387
65. Thomas SR, Davies MJ, Stocker R (1998) Oxidation and antioxidation of human low-density lipoprotein and plasma exposed to 3-morpholinosydnonimine and reagent peroxynitrite. Chem Res Toxicol 11(5):484–494
66. Tsai SH, Lin-Shiau SY, Lin JK (1999) Suppression of nitric oxide synthase and the down-regulation of the activation of NFκB in macrophages by resveratrol. Br J Pharmacol 126(3):673–680
67. Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. Nutrients 2(12):1231–1246
68. Vaya J, Mahmood S, Goldblum A, Aviram M, Volkova N, Shaalan A, Musa R, Tamir S (2003) Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. Phytochemistry 62(1):89–99
69. Vejux A, Lizard G (2009) Cytotoxic effects of oxysterols associated with human diseases: induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. Mol Asp Med 30(3):153–170
70. Viana M, Barbas C, Bonet B, Bonet MV, Castro M, Fraile MV, Herrera E (1996) In vitro effects of a flavonoid-rich extract on LDL oxidation. Atherosclerosis 123(1–2):83–91
71. Von Eckardstein A, Nofer J-R, Assmann G (2001) High density lipoproteins and arteriosclerosis: role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 21(1):13–27
72. Wadsworth TL, Koop DR (1999) Effects of the wine polyphenolics quercetin and resveratrol on pro-inflammatory cytokine expression in RAW 264.7 macrophages. Biochem Pharmacol 57(8):941–949
73. Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. Am J Clin Nutr 81(1): 243S–255S
74. Wiseman S, Mulder T, Rietveld A (2001) Tea flavonoids: bioavailability in vivo and effects on cell signaling pathways in vitro. Antioxid Redox Signal 3(6):1009–1021
75. Yi L, Jin X, Chen C-Y, Fu Y-J, Zhang T, Chang H, Zhou Y, Zhu J-D, Zhang Q-Y, Mi M-T (2011) Chemical structures of 4-oxo-flavonoids in relation to inhibition of oxidized low-density lipoprotein (LDL)-induced vascular endothelial dysfunction. Int J Mol Sci 12(9):5471–5489

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