Inflammatory Processes in the Dental Pulp

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7.1 Introduction

Innate and adaptive immune mechanisms are prevalent in the dental pulp and are a key feature of its defense capacity to minimize the effects of injurious challenge. Thus, the pulp shows similarities to many of the other connective tissues of the body but perhaps differs due to its noncompliant environment where the rigid covering shell of hard mineralized tissue constrains the pulp tissue swelling. The architecture of the tissue provides further constraints clinically and limits attempts to remove and repair the causes of the injury. Bacterial infection of the dental pulp represents the most common injurious challenge to the tissue due to the effects of dental caries, clinical operative procedures, and trauma. As a consequence, a mixed microbial flora, particularly including gram-negative, anaerobic bacteria, is present in the diseased pulp [1].

Inflammatory processes are important in the host's immune response to injurious challenge and represent a broad array of cellular and molecular events. These processes aim to both recruit circulating immunocompetent cells from the vasculature to eliminate pathogens and necrotic tissue debris and stimulate responses by resident cells in the pulp to minimize tissue damage and initiate reparative and regenerative events. Innate immune responses will particularly trigger local cytokine production and promote an influx of phagocytic leukocytes as part of the proinflammatory response. The relatively noncompliant and non-self-cleansing environment of the pulp may often lead to infections becoming chronic, and adaptive immune responses can also come into play. The latter process leads to T- and B-cell recruitment and activation and adds further complexity to the inflammatory response. Although clearly these various responses are defensive in nature, both the combination of their complexity and the constraints imposed by the structure of the tooth can lead to exacerbation of tissue injury and compromise tooth vitality. There is also increasing evidence of the sequestration of a variety of bioactive molecules within the dentin matrix [2] and their release during carious matrix dissolution will further complicate the cellular signaling taking place in the pulp. Clearly, while it is possible to generalize about the various defense responses occurring in the diseased and infected pulp, each individual case will be unique in terms of the extent of disease activity and the consequent involvement and timing of the various defense responses. Thus, the clinical management of pulpal inflammation can be a significant challenge.

Although there is a good clinical appreciation of the impact of inflammation on disease progression and treatment outcomes (see Chap. 9), the correlations between the biological events of pulp inflammation and the clinical presentation

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of disease progression are currently poorly understood. This represents a significant challenge to clinical diagnosis and management of pulpal disease [3, 4], particularly as regenerative approaches to therapeutically promote tooth vitality emerge [5–10]. The importance of understanding the cellular and molecular basis of pulpal inflammatory processes is now further emphasized with the recognition that there is considerable cross talk between inflammatory and regenerative events. Traditionally, tissue defense and repair/regeneration have been considered as distinct processes but clearly, these processes should now be considered as working in tandem.

7.2 The Pulpal Environment and Injury Responses

Dental caries represents the most prevalent infectious disease globally and affects the majority of the population. While not a life-threatening disease, caries has significant impact economically, nutritionally, and in terms of pain and quality of life. In health, the architecture of the tooth protects the pulp well from the infectious influences of the oral cavity. Carious infection of the tooth, however, soon exposes the pulp to bacteria and their products. Traumatic injury to the tooth, although physical in nature, provides indirect exposure of the pulp to these bacterial influences since the tooth is constantly bathed in bacteria-containing oral secretions. Increasing identification of tooth wear in the population is also a risk factor for pulpal infection with dentin exposure due to erosion, abrasion, and attrition opening diffusion pathways to the pulp.

The nature of the bacterial challenge will vary depending on disease progression and the extent of existing tooth tissue loss. At earlier stages, relatively small bacterial products may begin to diffuse within the tubules to the pulp, but with increasing disease progression, permeability of the tissues will increase and allow intact bacteria to migrate and colonize the deeper areas of the dentin and pulp (Fig. 7.1a–c). Thus, both the range of bacterial pathogenic challenges and their intensity may show considerable variation during the course of disease. As well as the challenge from intact bacteria, both cellular breakdown products and metabolites will likely contribute. Thus, cell membrane degradation products from gram-positive and gram-negative microbes, such as lipoteichoic acids, lipopolysaccharides, and DNA as well as other cell-derived products, may all contribute to the challenge posed by bacteria. Bacterial metabolites such as the weak organic acids produced during carbohydrate fermentation are well-established as major factors in tooth tissue degradation during caries, and these may also contribute to the insult caused by the bacteria. It is important, however, to recognize that there may also be an indirect bacterial challenge posed through the action of these bacterial metabolites on the dental tissues. Carious demineralization of dentin by bacterial acids will be accompanied by the dissolution of a significant proportion of the noncollagenous extracellular matrix of dentin. These dentin matrix components are now recognized to comprise a diverse range of molecules; a number of which include structural matrix molecules while others include cytokines, growth factors, and inflammatory mediators [2, 11]. Proteomic analysis of dentin already indicates the presence of up to nearly 300 distinct proteins [12, 13], and many of these display bioactive properties capable of signaling a multitude of cellular events in both tissue-resident cells and those recruited to the pulp as a part of the immune defense and wound healing processes. Mineralized tissues provide a unique environment in that expression of bioactive molecules by their formative cells frequently leads to their subsequent sequestration in the extracellular matrix in a fossilized state. This is especially true of dentin, which shows limited remodeling, unlike bone, and these sequestrated molecules may remain with their bioactivity in a protected state until the matrix is demineralized during injurious events, such as caries. While bacteria and their products may initiate defense responses classically associated with many of the other tissues of the body, superimposition of the effects of dentin matrix components released during carious demineralization may significantly

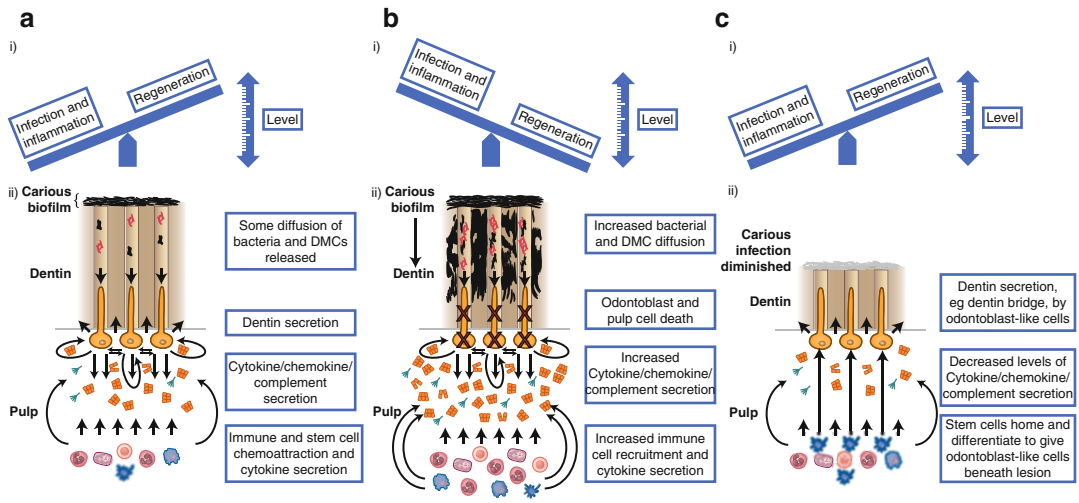

Fig. 7.1 (**a**) Early stage of carious disease with minimal hard tissue involvement. (*i*) Inflammation and infection are at relatively low levels which enable and promote tissue regenerative mechanisms, such as reactionary dentinogenesis. (*ii*) Bacteria and their products, as well as released dentin matrix components (*DMCs*), diffuse within the dentinal tubules where they are detected by odontoblasts, which can then elicit reactionary dentinogenic events and cytokine and complement secretion. Immune and potentially stem cells can be attracted to the site beneath the lesion at relatively low levels where they contribute further to proinflammatory mediator production. (**b**) Chronic and later stages of carious disease with increasing hard tissue involvement. (*i*) Relatively high levels of infection and inflammation lead to the impeding of tissue regenerative events. (*ii*) Increased amounts of bacteria and their products, as well as released DMCs, diffuse down the dentinal tubules where they signal odontoblast death. Relatively high levels of cytokines and immune system cells are present in the infected pulpal tissue. (**c**) Resolution of infection and modulation of inflammation, e.g., following clinical intervention. (*i*) Dental tissue regenerative events, such as reparative dentinogenesis, are enabled as infection and inflammation levels are decreased. (*ii*) Progenitor cells are recruited and differentiate to give a new population of odontoblast-like cells. Potential sources of progenitor cells include dental pulp stem cells (*DPSCs*). Low-level proinflammatory mediators, e.g., complement, cytokines, and reactive oxygen species (*ROS*), may promote signaling of these events. *Arrows* within pulp indicate signaling or secretory activity

modulate the pulpal and immune responses (Fig. 7.1a–c). Indeed, antibacterial activity displayed by some of these dentin matrix components [14] may modify the nature or intensity of the bacterial challenge to the pulp. Some signaling pathways are common to a number of cell types and their processes, which lead to more unpredictable effects of the combined challenges from bacterial and dentin matrix component exposure. For example, p38 mitogen-activated protein kinase (MAPK) signaling has been implicated in the control of odontoblast secretory activity during tertiary dentinogenesis [15]. Exposure to reactive oxygen species (ROS), which are generated during bacterial challenge, can also activate MAPK and NF-κB signaling pathways [16, 17]. These pathways can be initiated through a variety of cellular stresses, such as cytokine and bacterial LPS exposure as well as heat shock [18, 19].

Traditionally, wound generation and healing in the body's tissues follow a distinct chronological pattern with defense mechanisms initiated first, and once clearance of the injurious challenge has been largely achieved, healing processes are invoked (Fig. 7.1a–c). Such a pattern may be less distinct in the dentin-pulp where significant release of pro-regenerative factors at the time of tissue injury may lead to competing influences of defense and regeneration or healing occurring alongside one another. In such circumstances, the relative intensities of these competing influences may direct the outcomes of tissue events, although other factors may also affect outcomes. Tertiary dentinogenesis represents a repair response of the dentin-pulp, ultimately

aimed at tissue regeneration if conditions are permissive. This repair process may be further subclassified into reactionary and reparative dentinogenesis depending on whether the formative cells are upregulated surviving postmitotic primary odontoblasts or a new generation of odontoblasts-like cells arising from differentiation of stem/progenitor cells due to local death of the primary odontoblasts (see Chap. 2). Clearly, the complexity of these two processes differs significantly, and in the context of competing tissue defense and healing influences, simple upregulation of secretory activity of an existing population of odontoblasts (reactionary dentinogenesis) may be more easily achieved. During reparative dentinogenesis, involvement of pulp-derived mesenchymal stem cells (MSCs) may influence defense events through their immunomodulatory properties [20–22]. Thus, it is important that inflammation and repair/regeneration are considered as overlapping and interrelated processes.

7.3 Environmental Sensing by Odontoblasts and Pulp Cells

The main role of odontoblasts has long been considered to be that of dentin matrix secretion, and morphologically, these cells are well adapted to this function. However, it is becoming increasingly apparent that odontoblasts have much broader roles in the defense of the tooth and environmental sensing (Fig. 7.1a–c). This is emphasized by the histological structure of the dentin-pulp where the intricate and elaborate permeation of dentin matrix by the odontoblast process and its lateral branches [23] ensures that the cell communicates intimately with its extracellular matrix. Thus, the odontoblasts are well positioned to detect invading bacteria and their products, as well as dentin matrix components released during carious demineralization, at an early stage of the disease process. While odontoblasts are likely to be the first cells of the pulp that come into contact with the bacterial pathogens and their components, other pulpal cells will also subsequently be exposed to these stimuli. Indeed, recent evidence implicates odontoblasts, pulpal fibroblasts, and endothelial cells in the detection of exposure to bacterial pathogens. Consequently, these cells should be regarded as a constitutive part of the pulp's defense response to bacterial pathogens [16].

7.4 Innate and Adaptive Immune Responses

Both innate and adaptive immune responses encompass a complex range of cellular and molecular events. While the earlier responses to bacteria and other injurious challenges generally reflect innate immunity, the transition to adaptive immunity is a gradual one as infections become chronic and will vary in the same way as disease progression in each individual patient varies. Thus, at later stages of disease progression, adaptive responses will likely be superimposed on innate responses. This situation provides significant challenges to the identification of suitable targets for diagnosis or therapeutic intervention. In describing the innate and adaptive immune responses of the pulp, it is difficult to categorize or assign the molecular and cellular changes observed in the tissue as being distinct to either of these responses. Instead, it is probably more helpful for the reader to consider these changes in a chronological order in relation to disease progression. In this way, it is perhaps easier to understand their involvement in the clinical presentation of pulpal inflammation (see Chap. 9).

7.4.1 Bacterial Pathogen Recognition

Pattern recognition receptors (PRRs) are a group of cell membrane- and endosome-bound receptors, which can recognize ligands (pathogen-associated molecular patterns or PAMPs) that are broadly shared by pathogens but which are distinct from host molecules [24]. The Toll-like receptors (TLRs) are a key family of PRRs, which play a central role within the innate immune system in the recognition of their ligands or PAMPs. These ligands predominantly

include the surface components of bacteria, including lipopolysaccharides (LPS), lipoteichoic acids (LTS), flagellin, peptidoglycans, and lipoproteins as well as nucleic acid ligands from bacterial or viral pathogens. TLR-1 to TLR-6 and TLR-9 expression has been detected in odontoblasts and pulpal fibroblasts, and binding of these PRRs to their respective ligands initiates an acute inflammatory response, leading to activation of cells and release of proinflammatory mediators [16, 25–31]. These molecules include those associated predominantly with the vascular responses of the pulp including histamine, endothelin, serotonin, and neuropeptides and a broad array of cytokines and chemokines with potent cellular signaling properties (Fig. 7.1a–c). Other PRRs include the cytoplasmic NOD-like receptors (NLRs) and retinoic acid-inducible gene (RIG)-like receptors (RLRs) [32], although minimal information is currently available on their involvement in dentin-pulp-mediated inflammation.

7.4.2 Early Vascular Responses

An early feature of pulpal inflammation is changes to the vascular flow in the pulp with vasodilation and increases in blood flow. These changes are associated with increased fluid and plasma protein exudation and recruitment of leukocytes. Fluid exudation or edema during acute inflammation classically gives rise to swelling in soft tissues, although, as noted previously, such swelling is constrained in the pulp by the covering hard shell of mineralized dentin. Key molecular mediators of these vascular responses may include histamine, endothelin, neuropeptides, and serotonin. Both in vitro [33, 34] and in vivo [35, 36] studies indicate that histamine can produce vasodilation and reductions in blood flow in the pulp. Endothelin-1, a vasoconstrictor, and its receptors are constitutively expressed in odontoblasts and dental papilla of the developing teeth [37], and its application to pulp causes a decrease in blood flow [38]. A number of neuropeptides have been reported in pulp including substance P, calcitonin gene-related peptide, neurokinin A, neurokinin K, neuropeptide K, neuropeptide Y, somatostatin, and vasoactive intestinal peptide [39], and while these are largely associated with neural structures, some neuropeptides have been reported to be expressed in pulp fibroblasts [40, 41]. A number of these neuropeptides are vasodilators, while others are vasoconstrictors. Their involvement in neurogenic inflammation is complex [39], but these neuropeptides may provide novel therapeutic targets for the control of both pain and inflammation simultaneously [42]. Serotonin, a vasoconstrictor, can stimulate prostaglandin E_2 (PGE_2) [43] and prostacyclin (PGI_2) [44] production as well as increase blood flow in the pulp tissue [45]. PGE_2 and PGI_2 are among a broader family of prostaglandins, which have been implicated in pulpal inflammation [46–48]. Interestingly, histamine synergistically activates COX-2 (one of the cyclooxygenase enzymes involved in prostaglandin generation) expression and PGE_2 production in pulp fibroblasts through a TLR2-mediated process [49].

Several molecular cascade systems, based on plasma proteins, act in parallel to these cell-derived mediators to further initiate and propagate the acute inflammatory response. The complement system is activated by bacteria, with the outcome being the lysis of the bacterial membrane. Early reports have provided rather variable evidence for the contribution of complement activation in the pulp [50–54]; however, these data may reflect the difficulties in detecting the rather transient presence of these proteins during the inflammatory process. Many inflammatory mediators have relatively short half-lives, which in part explains why acute inflammation readily subsides in some tissues once the stimulus has been removed [55]. However, in the pulp, the problems of elimination of the infectious agents and their components ensure that the stimulus will often be ongoing. Complement will likely play a role in leukocyte recruitment (see next section) in the pulp as well as potentially progenitor cell recruitment for subsequent regenerative events [56] (Fig. 7.1a–c). The other molecular cascade systems closely associated with inflammation are the clotting and fibrinolytic systems. Dental pulp has long been recognized to show

fibrinolytic activity [57] and the fibrinolytic system may contribute to early wound organization during pulp healing. In the inflamed pulp, gene transcript and protein levels of tissue-type plasminogen activator are increased significantly [58] and are upregulated in the presence of proinflammatory cytokines [58, 59]. As well as allowing plasminogen cleavage to plasmin for fibrin clot lysis, the proteolytic action of plasmin may breakdown C3 facilitating initiation of the complement cascade.

Even at the level of the vascular responses to injurious challenges, the complexity and interrelationships of inflammatory mediator involvement in the pulp are apparent, and our understanding of these events is currently limited.

7.4.3 Leukocyte Recruitment

Recruitment of leukocytes to sites of inflammation is an important aspect of pathogen elimination through phagocytosis and degranulation mechanisms. Increases in vascular permeability facilitate their migration through the endothelial lining, and such extravasation is carefully regulated by the action of molecules involved in their adhesion and transmigration. These molecules include integrins, selectins, endothelial adhesion molecules, and the cell adhesion molecules (intercellular adhesion molecules 1–5, ICAM 1–5; vascular cell adhesion molecule 1, VCAM-1; junctional adhesion molecules, JAMs; platelet endothelial cell adhesion molecule 1, PECAM-1; endothelial cell adhesion molecule, ECAM). While some of these adhesion molecules are constitutively expressed in odontoblasts and other pulp cells in health [60, 61] for the maintenance of tissue architecture, expression of others increases significantly during episodes of inflammation. For instance, weak reactivity for E- and P-selectins in the healthy pulp is strongly upregulated following injury [62, 63].

Recruitment of leukocytes and other cells to sites of inflammation involves attraction along gradients of chemotactic molecules (Fig. 7.1a–c). These chemotactic molecules are diverse in their origins, perhaps reflecting to some extent the lack of specificity of their influences on cell type. Bacterial components are chemotactic to neutrophils in the pulp [64], and components of the dentin matrix released during carious demineralization are chemotactic to both inflammatory cells [65–68] and resident pulp cells [69]. Recognition that the composition of the dentin matrix reflects the expression of a diverse range of molecules by odontoblasts in addition to the well-established structural extracellular matrix components [2, 11] explains why these matrix components have immunomodulatory effects. Certain interleukins are basally expressed by odontoblasts [70] leading to their sequestration within the dentin matrix, and a complex cocktail of pro- and anti-inflammatory molecules have been detected in the dentin matrix [71]. Several inflammatory mediators also show chemotactic properties including complement components C3a and C5a, the arachidonic acid metabolites, and the leukotrienes (especially leukotriene B_4 – LTB_4). Growth factors, cytokines, and chemokines also modulate chemotaxis. The chemotactic effects of some of these molecules influence migration of both pulp progenitor and inflammatory cells [56, 69] highlighting the interplay between inflammatory defense and regenerative events in this tissue following injury (Fig. 7.1a–c).

7.4.4 Cytokine and Chemokine Mediation of Inflammatory and Post-injury Events

PAMP recognition by TLRs on odontoblasts and pulpal fibroblasts results in activation of the nuclear factor kappa B (NF-κB) intracellular signaling pathway, which is central to regulation of the molecular inflammatory response in many cell types [16, 25–31]. A range of cytokines and chemokines are produced as a result of activation of NF-κB signaling, and these molecules regulate much of the immune and inflammatory response. These cytokines and chemokines are synthesized by a variety of immune and tissue structural cells in response to infectious and traumatic challenge and have potent cellular signaling properties. Binding of these molecules to specific cell surface

receptors further modulates target-cell gene expression and molecular responses via second messenger signaling mechanisms [72, 73]. The actions of these cytokines and chemokines are often synergistic with stimulation of a cascade of release of other related molecules following their initial receptor binding [74]. The immunomodulatory actions of these cytokines and chemokines will impact on both innate and adaptive immune processes, including extravasation, leukocyte recruitment, cell activation and differentiation, and antibody production, as well as regenerative events associated with the wound healing response.

The archetypal proinflammatory regulatory cytokines include interleukin-1α and interleukin-1β (IL-1α,IL-β) and tumor necrosis factor-α (TNF-α), which have been demonstrated to play important roles in pulp's response to bacterial challenge [66, 67, 75–81]. Dentin matrix dissolution during caries may also stimulate expression of both TNF-α and IL-1β by macrophages [65] emphasizing that environmental sensing and defense mechanisms in the pulp may be broad ranging. The cascades of inflammatory mediator released after early PAMP-PRP interaction are well illustrated by the induction of the proinflammatory cytokine, IL-8 (which is central to neutrophil recruitment and activation), by stimulation with bacterial components or due to IL-1β and TNF-α exposure [70]. Upregulation of IL-8 has been reported in carious human pulp tissue [71, 78, 82], and the constitutive expression of this cytokine in odontoblasts [70] also highlights the complexity of the cellular interrelationships taking place in the defense of the pulp. Both gene and antibody array technologies have allowed demonstration of increased levels of several inflammatory cytokines and S100 transcripts and proteins in carious compared with healthy pulpal tissue [71, 78, 83]. These data are corroborated by other reports demonstrating increased interleukin levels in bacterially challenged pulpal tissue, including increases in IL-4 [84], IL-6 [85], and IL-10 [84]. The release of proinflammatory cytokines within the diseased pulp will have wide-ranging effects generally aimed at reinforcing control of the pathogenic challenge and subsequently resolution of inflammatory processes and stimulation of regenerative events. Among these proinflammatory effects will be the development of chemotactic gradients which promote the recruitment and activation of immune system cells [86, 87] to underpin the innate and adaptive immune responses (Fig. 7.1a–c). These chemotactic mechanisms will operate in tandem with those described previously for leukocyte recruitment to sites of inflammation.

7.4.5 Immune Cell Mediation of Innate and Adaptive Immune Responses

T- and B-lymphocytes, plasma cells, neutrophils, and macrophages are observed to infiltrate the pulp in increasing numbers as carious disease progresses [88, 89] (Fig. 7.1a–c). These cells constitute the effectors of the innate and adaptive immune responses; the latter of which will become increasingly superimposed on the former as caries extends deeply and more extensively into the dentin-pulp and the inflammation becomes more chronic in nature. As caries extends, the immune cell infiltrate in the pulp will also increase and will change from being more focal and localized to a much more extensive presentation. Such changes reflect the transition from more acute to chronic inflammation (Fig. 7.1a–c).

A prime role for the neutrophils and macrophages is that of phagocytosis, especially during the earlier acute phase of inflammation when bacterial pathogens are first encountered. Extravasation of natural killer (NK) cells to sites of inflammation in response to cytokines [90] allows their interaction with immature dendritic cells (DCs), which can lead to reciprocal activation and increased cytokine production by these cells [91]. NK cells likely contribute to further cytokine production during caries including that of IFN-γ [84], which can activate macrophages to stimulate phagocytosis as well as promoting T-cell responses [92]. Tissue-resident DCs are found in the pulp and following PAMP recognition; immature DCs will undergo maturation after which they will likely function in antigen

presentation to naïve T cells. T cells have been shown to be present in healthy pulp [93] with CD8+ T cells predominating [88, 89, 94]. An immunosurveillance role is generally assumed for these cells. This contrasts with B cells, which appear to be largely absent from the healthy pulp [89, 93] as also are their plasma cell progeny [51]. However, with establishment of deeper infection within the dentin-pulp, the initial inflammatory cell infiltrate of neutrophils and monocytes intensifies with accumulation of helper T (T_H) cells, cytotoxic T (T_C) cells, regulatory T (T_{reg}) cells, B cells, and plasma cells as adaptive immune defenses develop [88]. While these immune cells are recruited to the tissue for defense purposes, their ability to achieve clearance of the infection is frequently insufficient, and tissue destruction will often result collaterally. Such tissue destruction may in part be a direct result of the immune cells' scavenging actions on bacterial pathogens during which release of degradative enzymes and molecules, such as matrix metalloproteinases (MMPs), and reactive oxygen species (ROS) can negatively impact on the host tissue extracellular environment. ROS, which include superoxide anions, hydrogen peroxide, and hydroxyl radicals, can exacerbate tissue injury due to their damaging effects on DNA, proteins, and lipids. Apoptosis can result from cell exposure to ROS through activation of mitogen-activated protein kinase (MAPK) and NF-κB signaling pathways [16, 17]. These pathways can be initiated through a variety of cellular stresses, such as cytokine and bacterial LPS exposure, and heat shock [18, 19] highlighting the many opportunities for their activation during infection and inflammation in the pulp. Triggering of these pathways will further stimulate immune cell activity and contribute to an increasing accumulation of inflammatory mediators. It is abundantly clear that the cellular responses initiated during the innate responses associated with acute inflammation and their increasing complexity as adaptive immune responses come into play during chronic inflammation are still poorly defined in the pulp as disease progresses. While many observational studies have reported the presence of various immune cells and their inflammatory mediators in carious pulpal tissue, few functional studies have provided a clear picture of the specific functional activities of these cells and their complex interrelationships. This potentially reflects the individual variation in disease progression in the pulp, which is controlled by diverse factors. Significant challenges still exist to our understanding of the innate and adaptive immune defenses in the pulp, and this constrains clinical approaches to management of pulpal infection and inflammation.

7.4.6 Anti-inflammatory Activities and Inflammation Resolution

In ideal circumstances, immune defense following injurious challenge to a tissue will lead to elimination of the infecting agent and ultimately provides a conducive environment within which wound healing can occur. Such circumstances are not easily achieved in the dentin-pulp with its noncompliant environment and the exposure of the tooth to the oral cavity with its complex microflora and abundant supply of nutrients. Nevertheless, mechanisms for the regulation of inflammation require both suppression and activation of responses. Identification of several anti-inflammatory and pro-resolving mediators has started to clarify how inflammation may be suppressed after it has achieved its purpose.

The lipoxins are metabolites of arachidonic acid, which negatively regulate the actions of the leukotrienes inhibiting neutrophil chemotaxis and adhesion as well as stimulating apoptotic cell phagocytosis by macrophages and other anti-inflammatory actions [95]. Although there have been no reports of lipoxins in pulp to date, they represent an interesting potential target for control of pulpal inflammation together with the other families of anti-inflammatory mediators described later in this chapter. Three distinct families of anti-inflammatory pro-resolving lipid mediators are now recognized: resolvins, protectins, and maresins [96]. These families target distinct cell populations by interaction with specific receptors and contribute to the overall resolution

of inflammation. Resolvins suppress proinflammatory mediator production and regulate neutrophil movement to sites of inflammation. In a rodent model of pulpal infection and inflammation, resolving E1 (RvE1) application led to a decrease in inflammation at 24 and 72 h [97]. Protectins can block T-cell migration and secretion of TNF-α and IFN-γ and promote T-cell apoptosis as well as upregulating the chemokine receptor CCR5 on neutrophils to suppress chemokine signaling. The recently discovered maresins are produced by macrophages and inhibit proinflammatory mediator production by LTA4 hydrolase [98]. The actions of these various specialized pro-resolving anti-inflammatory mediators are only starting to be elucidated, and little information exists on their involvement in pulpal inflammation. Nevertheless, they represent exciting targets for the modulation of inflammatory activity, and the use of analogs may potentially provide novel therapeutic tools for clinical management of inflammation.

7.4.7 Inflammation-Regeneration Cross Talk in Dentin-Pulp After Injury

The specialized environment of the dentin-pulp can lead to competing pathogenic influences during disease in terms of concomitant release of proinflammatory and pro-reparative/regenerative factors as identified earlier in this chapter. Clearly, a balance in favor of tissue repair is the goal of clinical management of pulpal disease, but this may be unrealistic when relatively high levels of infection and inflammation persist. Current evidence suggests that reparative and regenerative processes ensue only after significant control or resolution of infection and inflammation has occurred [99–101] (Fig. 7.1a–c). Many of the potential cellular signaling mediators present in the post-injury tissue milieu demonstrate pleiotropic effects, which can show dose dependency and contribute to the balance of tissue outcomes. Thus, cytokines and growth factors, such as TNF-α and TGF-β as well as released dentin matrix components, can have detrimental effects on pulpal tissue and induce cell death if present at relatively high concentrations during the infectious and inflammatory processes [66, 68, 102, 103] in contrast to their beneficial effects at lower concentrations. While therapeutic targeting of inflammation may be attractive to change the balance of tissue events, infection control may prove more effective since the inflammatory challenge will continue as long as bacterial involvement persists. However, eradication of bacterial infection of the dental tissues, especially while minimizing host cell damage, has long been a challenge to operative dentistry and endodontics. It is therefore important to better understand the interplay between inflammation and repair/regeneration to guide decision making in the management of dental disease.

It has recently been emphasized that inflammation is an important prerequisite to enable repair and regeneration to subsequently ensue [104]. This may reflect both the need for defense processes to create a conducive environment for repair/regeneration and, also, the pleiotropic effects of some of the proinflammatory mediators, which may impact on repair/regenerative events. A number of proinflammatory mediators can promote degradative events in the oral and dental tissues during their defense responses to pathogenic challenge, for example, in bone resorption in periapical lesions [66, 105]. However, some cytokines, such as TNF-α, can also stimulate pro-regenerative/reparative signaling, including via p38 MAPK pathway activation, leading to odontoblast-like differentiation of dental pulp stem cells with increased dentin phosphoprotein (DPP) and dentin sialoprotein (DSP) expression and tertiary dentinogenesis [106]. The importance of this inflammation-regeneration interplay is further emphasized by the correlation of p38 MAPK signaling with initiation of tertiary dentinogenesis [15]. Such molecular switching is fundamental to the upregulation of odontoblast secretory activity and dentin deposition during the wound healing responses in the pulp. Other proinflammatory mediators, including IL-1β, may also contribute to the interplay of events in the pulp post-injury. IL-1β can stimulate mineralized bone matrix formation by

osteoblasts while inhibiting proliferation and differentiation of bone marrow mesenchymal stem cells (BMMSCs) [107]. In the liver, IL-1β can induce the normally quiescent hepatocytes to proliferate, thereby contributing to regeneration of this organ [107]. In the context of the dentin-pulp, proinflammatory cytokines may stimulate wound healing in surviving odontoblasts at earlier stages of disease when the pathogenic challenge is relatively less intense. However, during chronic disease, these cytokines may suppress odontoblast-like cell differentiation from stem/progenitor cells until infection is controlled and a more conducive tissue environment for wound healing prevails (Fig. 7.1a–c).

Other inflammatory mediators may also influence post-injury events in the pulp. While immune cell-derived ROS can contribute to tissue damage, at relatively low levels, these molecules can promote stem/progenitor cell differentiation and mineralization [108]. Clearly, there is a complex interplay occurring between the various molecules mediating events in the pulp post-injury, and the relative concentrations of these molecules may be key to the cellular signaling outcomes. Thus, at relatively low concentrations, these molecules may stimulate regenerative/reparative events, including cellular recruitment, differentiation, and matrix secretion. At higher concentrations, however, such events may be impeded through signaling inhibition and tissue degradative processes (Fig. 7.1a–c). Elucidation of these inflammation-regeneration interplay relationships is complicated by the various origins of these mediator molecules. Contributory sources will be tissue resident and immune cells as well as dentin matrix sequestrated pools released during carious dissolution. The relative contributions from these various sources will fluctuate with both the rate of disease progression (and hence, carious matrix dissolution) and the extent of cellular signaling taking place in the tissue (Fig. 7.1a–c). Some of the cytokine receptors responsible for triggering of cellular responses are common to both the immune/inflammatory and stem cells, which may explain the pleiotropic effects of these cytokines. For example, C-X-C chemokine receptor 4 (CXCR4) is expressed on lymphocytes and granulocytes and is also involved in stem cell recruitment [109, 110]. Both stromal cell-derived factor-1 (SDF-1)/CXCL12 and its receptor are expressed in pulp and are upregulated during disease [111, 112]. The sharing of common cytokine receptors between immune and stem cells probably reflects evolutionary conservation of cellular signaling processes. Recruitment of both cell types is required post-injury, and the extent of injury or infection may be the prime determinant of the level of involvement of these different cell types [113]. Further regulatory control of tissue events in the inflammatory milieu may also be triggered through modulation of stem cell cytokine receptor expression. For instance, increased cytokine levels can modulate the surface expression of CXCR4 on stem cells [109]. Such modulation could result in the suppression of regenerative/reparative responses during active inflammation.

In addition, mesenchymal stem cells (MSCs) can display immunomodulatory effects in tissues further demonstrating the dynamic interplay between inflammation and regeneration [114]. Dampening of excessive inflammatory responses by MSCs through modulation of immune cells will likely involve a number of mechanisms. One such process has recently been identified in which MSCs have been demonstrated to inhibit the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in monocytes/macrophages by decreasing ROS generation [115]. Although the scope of the immunomodulatory properties of dental pulp stem cells is still being explored [20–22], their contribution to the inflammation-regeneration interplay in pulp is already evident. Notably, TLR binding to stem cells, including those from pulp, can activate the NF-κB proinflammatory signaling cascade resulting in suppression of their differentiation [25, 26]. In addition, LPS binding to TLR4 in stem cells from the apical papilla (SCAPs) induces IL-6, IL-8, and TNF-α production in a time-dependent manner, and this can be suppressed by treatment with the transcription factor nuclear factor I C (NFIC) [116].

The influences of cell death on the tissue environment should also be considered in the context

of the interplay between inflammation and regeneration. Pulp-capping agents, such as calcium hydroxide and mineral trioxide aggregate (MTA), have long been used to stimulate reparative dentinogenesis following pulpal disease. Although the precise mechanisms of action of these agents remain controversial, it has been suggested that hydroxyl ion release from the material [117] leads to high pH conditions locally in the tissue resulting in cell necrosis [118, 119]. Chemical irritation of vital pulp tissue beneath the area of necrosis was proposed to stimulate reparative processes. Other possible mechanisms of action, including the local dissolution of growth factors and cytokines from the dentin matrix [120, 121], have also been proposed. It is now known that necrotic cells release low levels of proinflammatory mediators [122–125], and these may promote regenerative/reparative events if the levels of release do not become excessive. Increased cytokine release (IL-1α, IL-1β, IL-2, IL-6, and IL-8) from mineralizing cells has also been reported following exposure to MTA [126–128]. The release of low levels of cytokines from necrotic and mineralizing cells, in addition to dentin matrix dissolution of these molecules, may provide concentrations which favor regeneration/repair rather than promoting significant inflammation during milder disease conditions in the tissues. As the disease stimulus becomes more intense, the increasing levels of these cytokines released may then tip the balance towards more chronic inflammation.

It is clear from this that there are many complex molecular responses and interactions occurring in the diseased pulp. Identification of the involvement of these molecules is important both to our understanding of inflammatory and regenerative/reparative events, the identification of diagnostic markers, and the development of novel clinical therapeutic strategies to maintain pulp vitality. As new technologies become available, opportunities for more sensitive molecular profiling of diseased tissues arise. High-throughput transcriptional profiling and subsequent bioinformatic analyses represent one such technology. The use of this approach to investigate carious pulp tissue identified that inflammation was the predominant ontological tissue response upregulated; however, several other activated processes were also detected [129]. Notably, scrutiny of pro-regenerative/reparative responses indicated that the cytokine adrenomedullin (ADM) was also upregulated in carious pulp tissue. This molecule has wide-ranging effects, including immunomodulatory and antibacterial capabilities, and can stimulate hard tissue cell differentiation and mineralization [130–133]. We have now demonstrated similar effects for ADM in pulp [134]. Interestingly, ADM is a part of the neuropeptide family released during dental neuro-inflammatory events [135], and as discussed previously, these neuropeptides can exhibit anti-inflammatory actions [136, 137], and therefore ADM may contribute to the inflammation-regeneration interplay in diseased pulp. Exploitation of other new tissue-profiling technologies will undoubtedly further contribute to our understanding of the interplay of tissue events in the diseased pulp at the molecular level and identify further diagnostic and therapeutic molecular targets.

7.5 Future Directions

The environmental sensing and defense roles of odontoblasts and pulpal fibroblasts provide an exquisite system to detect pathogenic challenges which subsequently lead to release of a wide variety of regulatory cytokines and chemokines [138] that signal subsequent inflammatory, immune, and regenerative responses in pulp. The sequestration of many bioactive molecules and proinflammatory mediators within the dentin matrix in a fossilized state provides a further level of modulation once these molecules are released during carious dissolution of the dental tissues. It has become clear that a complex interplay can take place between all of these molecules and that inflammation and regeneration/repair are not distinct, but are intimately interlinked. The cross talk and balance between inflammation and regeneration/repair are dependent on both the presence and concentrations of the various signaling mediators. Thus, in a slowly progressing carious lesion tissue, conditions may be conducive to

regenerative/reparative events, which become suppressed as the injurious challenge increases with advancing disease (Fig. 7.1a–c). While there is still considerable scope to better understand the involvement of many of the signaling mediators in both inflammation and regeneration/repair, there is also now a significant opportunity to therapeutically target some of the inflammatory mediators to dampen their effects. For instance, the use of antioxidants, such as N-acetyl cysteine (NAC), in conjunction with dental restorative materials may limit the activation of key proinflammatory signaling pathways, including NK-κB activation, with subsequent effects on cytokine release which may then favor regenerative events within the dentin-pulp [139]. In addition, the anti-inflammatory actions of TGF-β1 [30, 140] may complement its stimulatory effects in reparative dentinogenesis. Furthermore, targeted upregulation of the transcription factor NFIC, which is important in tooth root development, can suppress cytokine production by pulp stem cells [116]. These and many other therapeutic routes offer exciting possibilities to modulate the inflammatory responses in the diseased pulp and to tip the balance of tissue responses towards preservation of pulp vitality and tissue repair.

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