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David Artis *Editors*

The Th2 Type Immune Response in Health and Disease

From Host Defense and Allergy to Metabolic Homeostasis and Beyond

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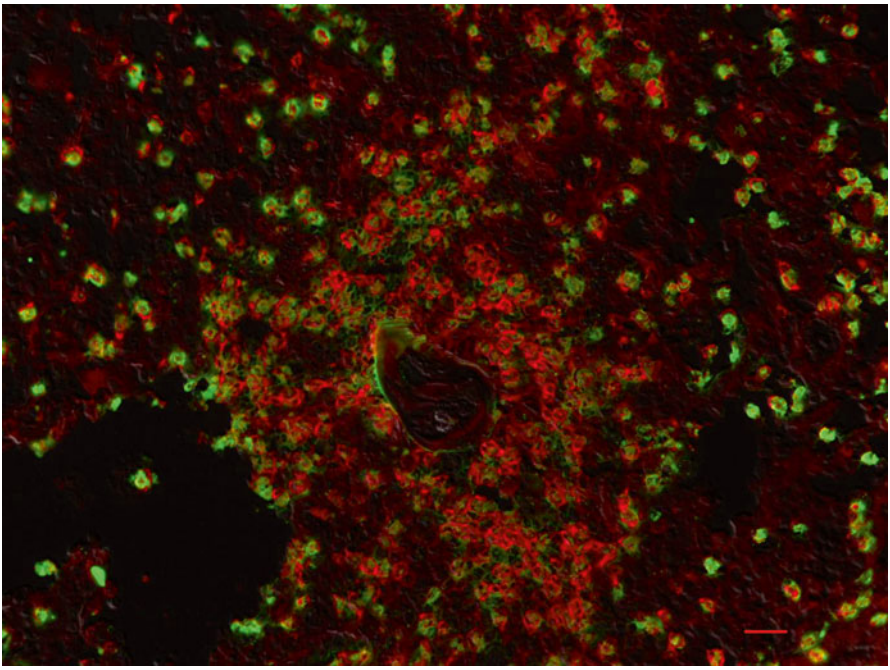

Chen F et al. Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion. Nat Immunol. 2014;10:938–46

Introduction

The immune response that develops after helminth infection in humans and mice exhibits a characteristic pattern of secreted proteins, called cytokines. They include interleukins (IL)-4, IL-5, IL-9, and IL-13. This signature motif helps define what is now referred to as the type 2 immune response. It is similar to the allergic immune response and also has significant similarities to the response occurring during sterile inflammation. It is, however, quite different from the type 1 response triggered by microbial pathogens that instead includes elevations in TNF, IL-12, IL-17, and IFN-γ. Understanding the events that trigger the initiation of the type 2 immune response and how the associated cell lineages interact to coordinate an effective immune response is one of the most rapidly developing areas in immunology today. The potential for translational applications resulting in new clinical therapeutics seems high. In the chapters of this book, world-renowned authorities have been brought together to provide a synthesis in our understanding of this dynamic and exciting field of study.

Type 2 immunity is initiated through signaling pathways quite different from the type 1 immune response initiated following infection with many microbial pathogens. The type 1 response is largely activated through pathogen-associated molecular patterns (PAMPS), conserved microbial structures that bind pattern recognition receptors (PRRs), such as toll-like receptors, which then trigger a cascade of signaling pathways leading to specific activation phenotypes of both innate immune cells, such as macrophages, and antigen-specific T and B cells. In contrast, danger-associated molecular patterns (DAMPS) seem to play a predominant role in the development of the type 2 immune response. Host immune and nonimmune cells can produce DAMPS, which are essentially endogenous signals that alert neighboring cells to cell damage and potential danger. Trefoil factor 2 and adenosine, interacting with the A2B adenosine receptor, have recently been identified as DAMPS that are released from damaged epithelial cells and have the capability of triggering cytokine alarmins, particularly IL-33 [1, 2]. IL-33 along with other cytokines, including IL-25 and thymic stromal lymphopoietin (TSLP), then triggers activation of various innate immune cells that, acting together, initiate the type 2 innate immune response [3–5]. For example, the rather scarce basophils are increasingly

being recognized as significant players in the development of the type 2 immune response, in some cases providing an essential early source of IL-4. In Chap. 1, Dr. Voehringer discusses these granulocytes in detail and compares their mechanisms of activation and function to eosinophils and mast cells. The recently identified Group 2 innate lymphoid cells (ILC2s) are also important sources of type 2 cytokines and are often activated at later stages of the response, potentially driven in part by T cells. Dr. Tait Wonjo in Chap. 2 reviews recent studies investigating the role of ILC2s in resistance to helminths and also in tissue repair and homeostasis. Each of these innate immune cells has specific activities and functions but also, perhaps as a result of the common signaling pathways (e.g., STAT6) activated in these different cell lineages, show similar characteristics. Recent studies suggest that even neutrophils can differentiate into an alternatively activated (N2) cell phenotype specific to the type 2 immune response [6]. Although granulocytes, mast cells, and ILC2s all play significant roles in the development of the type 2 immune response, including providing important sources of type 2 cytokines, it is clear that dendritic cells play the essential role in Ag-specific Th2 cell activation and differentiation. Dr. Lambrecht discusses dendritic cells in the context of the type 2 immune response in Chap. 3. This chapter dovetails nicely with Chap. 4 by Dr. Pearce, which focuses on how these T cells develop in the context of helminth infection and how they shape and augment the protective response. Dr. Pearce also provides a discussion of B cells and how antibodies may contribute to helminth trapping and ultimately parasite eradication.

Also associated with the type 2 immune is the activation of T regulatory cells, which are now being harnessed for the treatment of a variety of inflammatory diseases. Tregs are induced during helminth infection and can control type 2, as well as type 1 immunity, mitigating harmful type 2-mediated pathology including fibrosis. The development and role of Tregs during the type 2 immune response is described in detail by Dr. Maizels in Chap. 5. The activation of Treg cells in the context of the type 2 immune response results in a formidable regulatory response that includes controlling effects of Th2 cytokines as well as Treg cells on harmful inflammatory responses. The type 2 cytokines help shape and amplify a response that can mediate host protection against large multicellular parasites. This protection can take the form of both resistance and tolerance. Resistance mechanisms can lead directly to parasite damage and/or expulsion. In Chap. 6, Dr. Loukas discusses new approaches that are being implemented to augment resistance through vaccine development and further outlines their associated challenges. Tolerance includes the activation of cells to express specific molecules that mitigate the tissue damage that would otherwise occur as these parasites transit through vital organs. These tolerance mechanisms include factors that both control harmful inflammation and that directly promote wound healing. Dr. Nair in Chap. 7 discusses tissue remodeling effects of type 2 immune responses with a particular focus on alternatively activated macrophages, which are increasingly recognized as central players in helminth-induced wound healing. Dr. Loke in Chap. 8 discusses how tolerance mechanisms might be harnessed to control harmful inflammation associated with autoimmune diseases ranging from diabetes to inflammatory bowel disease. He discusses recent

studies involving both experimental models and clinical applications. Immunity is also capable of profoundly affecting metabolism. Dr. Chawla provides an intriguing chapter at the crossroads of immunity and metabolism, discussing recent discoveries elucidating the intricate links between these formerly distinct disciplines, and the potential for therapeutically promoting metabolic health through activation of type 2 responses (Chap. 9).

Taken together, increased understanding of the type 2 immune response has broad implications. Its significance ranges from the potential for improved development of anti-helminth vaccines to the control of harmful inflammation that can lead to both autoimmune pathogenesis and metabolic disorders associated with obesity. However, our understanding of the type 2 immune remains at a rudimentary level and considerable research is required before we can fully exploit this ancient response for the development of novel and effective immunotherapies. The chapters in the book provide an important platform for the development of new insights into how this fascinating immune response works and how we may harness its components for novel vaccines and for targeted treatments associated with harmful type 1 and type 2 inflammation.

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Regulation and Function of Basophil, Eosinophil, and Mast Cell Responses

David Voehringer

Introduction

Eosinophils, basophils, and mast cells are effector cells of the innate immune system and generally associated with type 2 immunity in response to allergens and helminth infections. In addition, all three cell types are implicated in a variety of other biological functions that will not be covered in this chapter. About 130 years ago, Paul Ehrlich described that eosinophils, basophils, and mast cells can be distinguished based on their characteristic staining of cytoplasmic granules with organic dyes. The granules store various pro-inflammatory effector molecules that can be released within minutes after activation of the cell. Eosinophils, basophils, and mast cells can further express Th2-associated cytokines including IL-4 and IL-13 that are critical for induction of effector functions in other cells such as mucus secretion by goblet cells, collagen production by fibroblasts, activation of smooth muscle cells, class switch recombination to IgE in B cells, secretion of chemokines from endothelial cells, or differentiation of alternatively activated macrophages. In addition to their pro-inflammatory role during the active phase of the immune response, eosinophils, basophils, and mast cells may contribute to resolution of inflammation, tissue remodeling, and restoration. This chapter describes the regulation of development, homeostasis, and effector functions of these three cell types in type 2 immune responses.

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Development and General Characteristics of Mast Cells, Basophils, and Eosinophils

Mast cells were named by Paul Ehrlich as fattened cells (Mastzellen) since he thought that the numerous cytoplasmic granules store nutrients and it took some time until the true nature of the granules was revealed [1]. The first steps of mast cell development take place in the bone marrow where mast cell precursors are generated from common myeloid progenitors (CMP) or from more differentiated granulocyte/monocyte progenitors (GMP) with a potential intermediate cell type (basophil/mast cell progenitor, BMCP) that has the potential to generate both mast cells and basophils (Fig. 1) [2, 3]. The development of mast cells is strictly dependent on expression of the receptor tyrosine kinase c-KIT and the KIT ligand. Mice with loss-of-function mutations in the *c-Kit* gene or *Kitlg* gene lack mast cells. In addition, these mouse strains have other defects since c-KIT is expressed not only by mast cells but also by

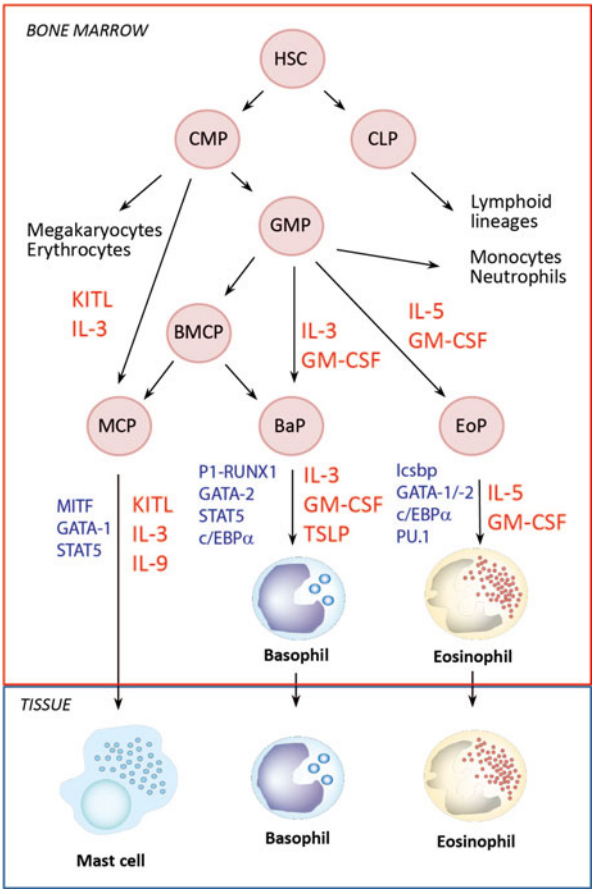

Fig. 1 Development of mast cells, basophils, and eosinophils. Schematic illustration of the cell populations, transcription factors (*blue*), and cytokines (*red*) that promote the development of eosinophils, basophils, and mast cells. *HSC* hematopoietic stem cell, *CMP* common myeloid progenitor, *CLP* common lymphoid progenitor, *GMP* granulocyte/monocyte progenitor, *BMCP* basophil/mast cell progenitor, *MCP* mast cell progenitor, *EoP* eosinophil progenitor, *BaP* basophil progenitor

many other cell types [4]. On the other hand, constitutively active mutants of c-KIT cause mastocytosis in many different mammalian species. The number of mast cells in peripheral tissues is rather low under steady-state conditions but inflammatory conditions can lead to increased mast cell numbers and this response is believed to be mainly caused by cytokines like KITL, IL-3, and IL-9. Transcription factors that are involved in mast cell development include MITF, GATA-1, and STAT5. The final steps of mast cell development occur outside the bone marrow. Mast cell precursors leave the bone marrow and migrate to peripheral tissues where they finish their maturation. Mast cells are mainly located in barrier tissues like the skin and the mucosa of lung and intestine suggesting that they serve as sentinels for rapid responses to invading pathogens. Their lifespan ranges from weeks to months and mature mast cells can even undergo further proliferation. The final tissue localization determines the expression pattern of mast cell-associated proteases that are stored in cytoplasmic granules and constitute up to 50 % of the total protein mass of the cell. Mast cells and basophils express the metalloprotease carboxypeptidase A3 in addition to several different serine proteases of the chymase and tryptase family [5]. Murine mast cells are divided in two subgroups: mucosal mast cells (MMC) and connective tissue mast cells (CTMC) which both express distinct sets of proteases. Human mast cells express fewer proteases and are grouped in tryptase-expressing mast cells (M_T, mainly mucosal) and tryptase and chymotryptase-expressing mast cells (M_{TC}, mainly connective tissue). Other stored molecules in cytoplasmic granules include histamine, proteoglycans, and some cytokines [5].

Basophils are the least abundant population of granulocytes and constitute less than 1 % of leukocytes in the peripheral blood. The basophil lineage develops mainly from GMPs in the bone marrow [3]. Basophils finish their development in the bone marrow and enter the blood and peripheral tissues as fully matured cells (Fig. 1). The transcription factors STAT5, GATA-2, c/EBPα, and P1-RUNX1 play a critical role during basophil development. In addition, a basophil/mast cell-committed progenitor was found in the murine spleen and may also exist in the bone marrow suggesting that basophils and mast cells evolved from a common progenitor cell [3]. Indeed, a mast cell/basophil-like cell could be identified in the sea squirt *Styela plicata* [6]. The functional similarity between mast cells and basophils is reflected by the fact that both cell types express the high-affinity receptor for IgE (FcεRI), histamine, potent lipid mediators, proteases, and similar sets of chemokines and cytokines (Table 1). However, in contrast to mast cells, basophils do not require c-KIT for development. IL-3 is the main cytokine that promotes basophilia although high levels of TSLP can also expand the basophil population in mice [7]. Interestingly, IL-3 and TSLP are dispensable for basophil development under steady-state conditions since basophils are still present in mice that cannot respond to both cytokines [7]. Basophils have a lifespan of about 60 h in the spleen of naïve mice [8]. But the lifespan could be increased in inflamed tissue with high levels of IL-3, GM-CSF, or TSLP. Basophils are smaller than mast cells and contain an indented nucleus. Although basophils and mast cells express a similar set of effector molecules, murine mast cell-associated protease (mMCP) 8 and mMCP11 are only expressed in basophils. Human basophils do not express these proteases but they can be distinguished from mast cell by high expression levels of basogranulin or CD203c.

Table 1 Similarities and differences between mast cells, basophils, and eosinophils. Some genes were only reported to be expressed in either human (h) or murine (m) cells

	Mast cells	Basophils	Eosinophils
Morphology of nucleus	Round	Indented, segmented	Segmented
Cytokines that promote development	KITL, IL-3, IL-9	IL-3, TSLP, GM-CSF	IL-5, GM-CSF
Critical transcription factors for development	MITF, GATA-1, STAT5	P1-Runx1, c/EBPα, GATA-2, STAT5, IRF-8	GATA-1, GATA-2, c/EBPα, PU.1, IRF-8
Lifespan (steady state)	Several weeks	60 h	36 h
Activating receptors	FcεRI, FcγRI (h), FcγRIIA (h), FcγRIIIA (m), IL3R, IL18R, IL33R, CD200R2-4 (m), TLR4 (m)	FcεRI, FcγRI (h), FcγRIIA (h), FcγRIIIA (m), IL3R, IL18R, IL33R, CD200R2-4 (m), TLR2 (m)	FcγRIIIA (m), FcγRIIA (h), several IgA receptors, PIR-A
Inhibitory receptors	FcγRIIb (m), CD200R1, gp49B, SIRP-α, PIR-B, Siglec-5 and -8 (h),	FcγRIIb, CD200R1, gp49B, SIRP-α, Siglec-5 and -8 (h)	FcγRIIb (m), Siglec-F (m), Siglec-8 (h), PIR-B
Chemoattractants the cells respond to	LTB_4, PGE_2, KITL, SDF-1α (CXCL12)	Anaphylatoxins (C3a, C5a), IGF-1, IGF-2	Eotaxins (CCL11, CCL24, CCL26), RANTES (CCL5), LTB_4, LTD_2, PGD_2, PAF, anaphylatoxins (C3a, C5a)
Effector molecules	Histamine, serotonin (m), lipid mediators (LTB_4, LTC_4, PGD_2, PAF), proteases (chymases, tryptases, CPA), cytokines (IL-4, IL-5, IL-13, and many more), several chemokines	Histamine, serotonin (m), lipid mediators (LTB_4, LTC_4, PAF), proteases (chymases, tryptases, CPA), cytokines (IL-4, IL-5, IL-13, and many more), several chemokines	MBP, EPX, ECP (h), EDN (h), EARs (m)

Eosinophils develop in the bone marrow from eosinophil-committed progenitors (EoP) which are IL-5Rα$^+$ and derived from GMP (Fig. 1). The eosinophil lineage is critically dependent on the transcription factor GATA-1 since an engineered point-mutation in the GATA-1 promoter leads to a selective loss of the eosinophil lineage in ΔdblGata mice [9]. The eosinophil lineage is also lost in mice that lack the transcription factor IRF-8. In addition, the transcription factors c/EBPα, PU.1, and GATA-2 promote eosinophil development and maturation from uncommitted

precursors. Eosinophil maturation in the bone marrow is associated with up-regulation of the chemokine receptor CCR3 and L-selectin (CD62L) [10]. Eosinophils leave the bone marrow as fully matured cells with a condensed nucleus and numerous cytoplasmic granules that can be stained with the red dye eosin named after "eos" the Greek goddess of the dawn. IL-5 is the major cytokine that drives eosinophil development and prolongs their lifespan. Th2 cells and ILC2 are probably the main sources of IL-5 in vivo. IL-5, IL-3, and GM-CSF are closely related cytokines that bind to receptors composed of a cytokine-specific alpha chain and a common beta chain which transduces the signal and is used by all three receptors. Eosinophils express receptors for IL-5 and GM-CSF while basophils express receptors for IL-3 and GM-CSF. IL-5 and GM-CSF have synergistic effects on eosinophils and can prolong their lifespan which is about 30 h in the spleen of naïve mice [10]. Eosinophils can be identified and isolated by flow cytometry based on their high side -scatter profile and high expression of CCR3 and the sialic acid-binding immunoglobulin-like receptor Siglec-F (in mice) or Siglec-8 (in humans).

The granules of eosinophils contain cytotoxic proteins and ribonucleases. Furthermore, human eosinophils contain so-called Charcot-Leyden crystals (CLC) which are aggregates of galectin-10 with unknown function. The crystalloid core of the eosinophil-specific granules is formed by major basic protein (MBP) that is expressed in two isoforms. The matrix contains mainly eosinophil peroxidase (EPX) and in human eosinophils eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) which are members of the RNase A superfamily. Mouse eosinophils express four orthologs of ECP and EDN which are named eosinophil-associated ribonucleases (EARs). The RNase activity is however not required for the cytotoxic activity of ECP [11]. EDN was shown to reduce the infectivity of RNA viruses in vitro, promote the chemotaxis of immature dendritic cells, and enhance Th2 responses in vivo [12]. The cytoplasm of eosinophils also contains lipid bodies where leukotrienes and prostaglandins are synthesized.

Although development and basic characteristics are very similar between mouse and human eosinophils, there are also some differences that may explain discrepancies of eosinophil in vivo functions between mouse and man [13].

Modes of Activation

Mast cells and basophils express a similar set of activating receptors on the cell surface. The high-affinity receptor for IgE (FcεRI) binds IgE with 10^{-10} M and is expressed on mast cells and basophils as a trimeric complex with an IgE-binding α chain without signaling capacity and a signal-transducing complex consisting of a β chain with four transmembrane domains and a disulfide-linked homodimer of the FcRγ chain which is also part of other activating Fc receptors (Fig. 2). The β and γ chains of the FcεRI and the two adaptor proteins LAT and NTAL contain ITAM motifs that are phosphorylated by tyrosine kinases upon receptor cross-linking and initiate signaling cascades which ultimately lead to degranulation, expression of

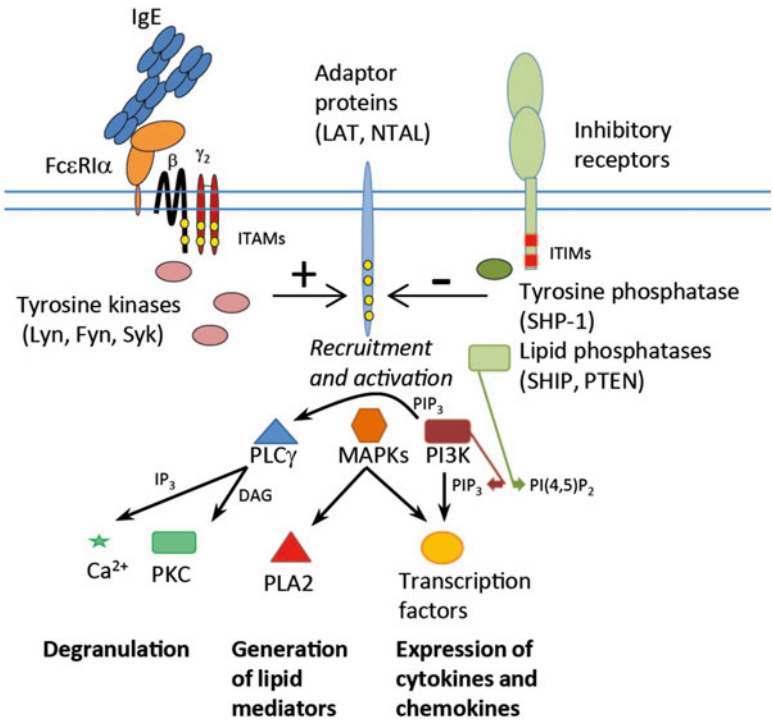

Fig. 2 Mast cell and basophil activation by FcεRI and its counteraction by inhibitory receptors. Cross-linking of FcεRI-bound IgE leads to phosphorylation of immunoreceptor tyrosine activation motifs (ITAMs, *yellow circles*) and initiates three major signaling cascades that ultimately lead to degranulation, expression of cytokines and chemokines, and secretion of lipid mediators. Other surface receptors with inhibitory motifs (ITIMs) recruit phosphatases that block the signaling cascades by de-phosphorylation of ITAMs and phosphatidylinositol-(3–5)-trisphosphate (PIP3)

cytokines and chemokines, and synthesis of arachidonic acid-derived lipid mediators like leukotriene B_4 (LTB_4), LTC_4, and prostaglandin D2 (PGD_2) (reviewed in [14]). Interestingly, FcRγ is also required as part of the IL-3 receptor complex for induction of IL-4 production in basophils [15]. Other cell types including eosinophils, monocytes, and dendritic cells can express a dimeric version of FcεRI that lacks the β chain and can only poorly activate the cells. Serum concentration of IgE is about 10 ng/ml which is roughly 100,000 times lower than the concentration of IgG. Furthermore, free IgE has a short half-life of only 2 days. However, mast cells and basophils can efficiently capture IgE and thereby get sensitized for long periods of time due to the low dissociation rate of IgE from FcεRI. Mast cells and basophils can also be activated by murine FcγRIIIA (low-affinity IgG receptor) and by human FcγRI (high-affinity IgG receptor) or human FcγRIIA (low-affinity IgG receptors) [16]. Further activating receptors on the cell surface of basophils and mast cells

include receptors for cytokines of the type I hematopoietin family (IL-3, GM-CSF, TSLP) and the IL-1 family (IL-18, IL-33), many different G-protein-coupled receptors including receptors for the anaphylatoxins C3a and C5a and a variety of pattern recognition receptors such as Toll-like receptors, C-type lectin receptors, and others (Table 1). Stimulation of murine basophils with IL-3 or TSLP results in different gene expression profiles indicating that basophils can adapt their effector function to the local cytokine milieu [7]. Mast cells and basophils can further be directly activated for example by proteases secreted from house dust mites or hookworms [17] and by IPSE/alpha-1, a glycoprotein from *Schistosoma mansoni* that activates the cells in an IgE-dependent but antigen-independent manner [18].

Surprisingly, the modes of eosinophil activation are not well defined. Eosinophils express various activating and inhibitory receptors and can be activated in vitro by cross-linking of receptors for IgG and IgA or by soluble factors including IL-5, GM-CSF, eotaxins, C3a, C5a, platelet activating factor (PAF), and leukotriene B4. In addition, eosinophils can be activated via β_2 integrins, especially Mac-1 (CD11b/CD18). Activated murine eosinophils down-regulate L-selectin (CD62L) and up-regulate Siglec-F [10]. Numerous studies revealed that human and mouse eosinophils respond differently to activating stimuli which may explain in part conflicting results from studies in both species [13].

Prolonged activation of mast cells, basophils, and eosinophils can cause severe tissue damage. Therefore, inhibitory receptors play an important role to counteract the stimulatory function of the activating receptors. Various inhibitory receptors are expressed on the cell surface including FcγRIIB, gp49B, and SIRP-α. Many of them contain ITIM motifs in the cytoplasmic tail and recruit phosphatases which shut off the signaling cascades (Fig. 2).

Effector Functions

The early phase of type 2 immune responses is often characterized by secretion of TSLP, IL-33, and IL-25 from tissue-resident cells that respond quickly to invading parasites or allergens (Fig. 3). TSLP can directly act on dendritic cells and induce their differentiation to Th2-promoting antigen-presenting cells. IL-33 can activate mast cells, basophils, and eosinophils. In addition, IL-33 and IL-25 promote the expansion and activation of type 2 innate lymphoid cells that are a major source of IL-5 and IL-13 but also produce some IL-4. Mast cells can induce the release of TSLP, IL-25, and IL-33 in response to infection with gastrointestinal helminths suggesting that mast cells may contribute to the orchestration of the early phase of type 2 immunity independently of IgE [19]. Mast cells, basophils, and eosinophils can present antigen to naïve CD4 T cells and secrete IL-4 under certain conditions but they are largely dispensable for Th2 polarization. The main function of mast cells, basophils, and eosinophils is, however, to serve as effector cells during the acute and late phase of the immune response against parasites and allergens.

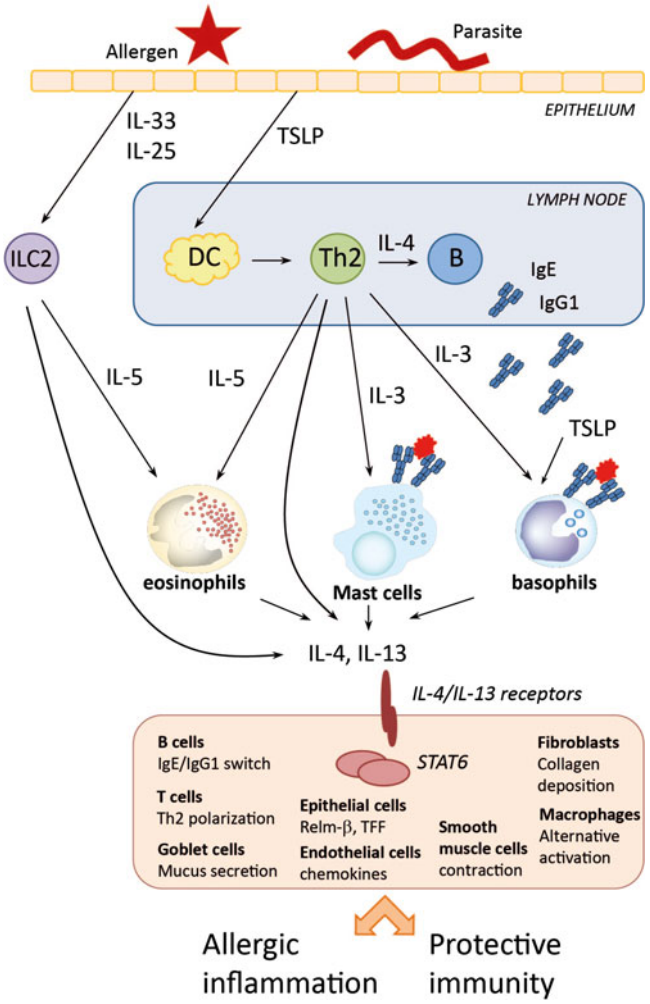

Fig. 3 Participation of eosinophils, basophils, and mast cells as source of IL-4 and IL-13 for STAT6-dependent effector pathways. Allergens and helminths cause rapid release of IL-25, IL-33, and TSLP from cells in barrier tissues like skin and mucosa. This leads to mobilization of type 2 innate lymphoid cells (ILC2) and activation of dendritic cells (DC) that promote differentiation of Th2 cells which are required as source of IL-4 for IgE and IgG1 production by B cells. IL-4 and IL-13 from eosinophils, basophils, and mast cells act on a variety of different target cells that contribute to protective immunity or allergic inflammation by expression of distinct sets of STAT6-regulated genes. Other effector molecules of eosinophils, basophils, and mast cells are not depicted

Mast Cells

Systemic activation of mast cells causes anaphylaxis, the most severe and often fatal form of an allergic response which is characterized by a rapid drop in blood pressure and body temperature. These symptoms are mainly caused by antigen-mediated cross-linking of receptor-bound IgE on sensitized mast cells which leads to degranulation and release of the vasodilating substance histamine. Mast cells also play a major role in local allergic reactions of barrier tissues like lung, skin, or intestine. Within minutes of activation they cause local inflammation, the so-called type I hypersensitivity reaction, by release of proteases and histamine. In addition, they produce cytokines, chemokines, and lipid mediators that lead to further recruitment of Th2 cells, granulocytes, and monocytes which then contribute to the allergic late phase reaction, also named type IV hypersensitivity reaction.

On the other hand, mast cells have important beneficial functions for the host. They can degrade venom toxins [20] and contribute to protective type 2 immunity against some helminths and ticks [5]. The observed protective function against gastrointestinal helminths is mainly based on studies with KIT-mutant mouse strains or mice that are deficient for certain mast cell-associated proteases. The molecular mechanisms of mast cell-mediated protection are poorly understood. Mast cells may help to trap larval stages of tissue-dwelling helminths in granulomas or directly damage larvae by release of proteases. Furthermore, secretion of IL-4 and IL-13 from mast cells could promote collagen deposition by fibroblasts, induce mucus production from goblet cells, activate smooth muscle cells, and stimulate release of effector molecules from intestinal epithelial cells which generates an inhospitable environment for adult worms in the intestinal lumen. Mast cells were further shown in a mouse model to be important for resistance against secondary infestation with ticks. Unexpectedly, the expression of activating Fc receptors on mast cells was not required for this protection [21].

Basophils

Basophils were detected in the lung of asthma patients and could play an important role during the late phase reaction [22]. They are also recruited to the skin in some inflammatory skin diseases like atopic dermatitis or urticaria [23]. Studies in mice have shown that basophils are essential for IgE-mediated chronic allergic inflammation of the skin independently of mast cells [24]. However, it remains unclear to what extent basophils contribute to the pathology of inflammatory skin diseases and which basophil-derived effector molecules might be involved in this process. Furthermore, it is not known which chemotactic signals promote basophil recruitment to the lung or skin. In contrast to mast cells, basophils play no major role for anaphylaxis in the mouse [25].

Despite their pro-inflammatory function in certain allergic responses, basophils can also help to protect against helminths and ticks. This activity is thought to depend on pathogen-specific antibodies that bind to activating Fc receptors on basophils and mediate rapid activation upon antigen encounter. By release of IL-4 and IL-13 basophils may induce the protective pathways against helminths described above. In addition, both cytokines can promote the differentiation of alternatively activated macrophages which are also involved in protection against helminths and further serve to repair and remodel damaged tissues. Basophils also produce other cytokines including IL-5 which mobilizes eosinophils. The physiological function of basophil-derived proteases and other effector molecules remains to be established.

Eosinophils

Allergic disorders with local accumulation of eosinophils in lung and skin include allergic asthma and atopic dermatitis, respectively. Eosinophils are mainly mobilized by IL-5 and recruited into tissues by the C-C chemokines eotaxin-1, -2, and -3 (CCL11, CCL24, and CCL26, respectively) which bind to the receptor CCR3 although eosinophils can also respond to RANTES (CCL5) and many other chemokines [13]. In addition, chemotaxis is induced by the anaphylatoxin C5a and the lipid mediators PAF, LTB_4, LTD_2 and PGD_2. Eosinophils can cause tissue damage by release of their cytotoxic proteins and generation of reactive oxygen species. MBP and EPX can directly activate mast cells. Eosinophils can express the Th2-associated cytokines IL-4, IL-5, and IL-13 and thereby promote the late phase response of allergic inflammation. Studies in eosinophil-deficient mice showed that eosinophils can promote effector T-cell recruitment, cytokine production, and mucus secretion in models of allergic lung inflammation (reviewed in [26]). Eosinophils are further involved in tissue fibrosis by secretion of TGF-β during the chronic stages of the response. However, eosinophils may also dampen the allergic response by degradation of histamine, leukotrienes, and PAF (reviewed in [27]). Direct evidence for the pro-inflammatory role of eosinophils in asthma comes from a study where anti-IL-5 treatment of patients with eosinophilic asthma efficiently depleted eosinophils and ameliorated clinic symptoms [28, 29].

Helminth infections often lead to a significant increase of circulating eosinophils suggesting that eosinophils are involved in protection against these parasites. Indeed, IL-5-transgenic mice that contain large numbers of eosinophils in most tissues can directly kill the L3 larval stage of *Nippostrongylus brasiliensis* in the skin [30]. However, this protective effect was not observed with other helminth species and eosinophil-deficient mice show unimpaired expulsion of *N. brasiliensis* during primary infection. Interestingly, eosinophils contributed to protective immunity during secondary infection. This suggests that helminth-specific antibodies or memory T cells are involved in activation of eosinophils. In vitro studies showed that human eosinophils can directly kill Schistosomula larvae and this activity was dependent on activation by IgG or IgA [31]. Surprisingly, the size and number of

granulomas formed around *S. mansoni* eggs was not different between wild-type and eosinophil-deficient mice [32]. It appears from studies in eosinophil-deficient mice that protective immunity against helminths is not critically dependent on eosinophils and can be mediated by redundant mechanisms.

Conclusion

Mast cells, basophils, and eosinophils are myeloid effector cells with potent pro-inflammatory functions during type 2 immune responses. Mast cells and basophils have a similar gene expression profile and both cell types can be efficiently activated by cross-linking of FcεRI-bound IgE. Basophils and eosinophils complete their development in the bone marrow and have a lifespan of a few days while mast cells finish their maturation in the tissue and live for several weeks. All three cell types store effector molecules in cytoplasmic granules which are rapidly released after activation. In addition, they can secrete the Th2-associated cytokines IL-4 and IL-13 upon stimulation and thereby contribute to activation of STAT6-dependent target genes that mediate protective immunity against helminths and ticks but also promote the inflammatory response against allergens.

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Innate Lymphoid Cells: An Emerging Population in Type 2 Inflammation

Elia D. Tait Wojno

Abbreviations

AAM	Alternatively activated macrophage
ACT 1	NF-κB activator 1
AD	Atopic dermatitis
APC	Antigen-presenting cell
Areg	Amphiregulin
BAL	Bronchoalveolar lavage fluid
CRS	Chronic rhinosinusitis
CRTH2	Chemoattractant receptor-homologous molecule expressed on T helper type 2 cells
CysLT2	Cysteinyl leukotriene receptor 2
DC	Dendritic cell
DR3	Death receptor 3
EGFR	Epidermal growth factor receptor
FPR2/ALX	Formyl peptide receptor 2/lipoxin A_4 receptor
γc	γ-Chain
GATA3	GATA binding protein 3
GFI1	Growth factor-independent 1 transcription repressor
ID2	Inhibitor of DNA binding 2
IFN-γ	Interferon-γ

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Ig	Immunoglobulin
IL	Interleukin
ILC	Innate lymphoid cell
ILC1	Group 1 innate lymphoid cell
ILC2	Group 2 innate lymphoid cell
ILC3	Group 3 innate lymphoid cell
LTD_4	Leukotriene D_4
LTi	Lymphoid tissue inducer
LXA_4	Lipoxin A_4
MHC II	Major histocompatibility class II
MPP^{type2}	Multipotent progenitor type 2
NFIL3	Nuclear factor interleukin 3 regulated
NK	Natural killer
PGD_2	Prostaglandin D_2
PLZF	Promyelocytic leukemia zinc finger protein
R	Receptor
RORα	Retinoic acid receptor-related orphan receptor α
RORγt	Retinoic acid receptor-related orphan receptor γ
SCF	Stem cell factor
STAT3	Signal transducer and activator of transcription 3
T-bet	T-box expressed in T cells
TCF1	Transcription factor 1
T_H2	T helper type 2
TL1A	Tumor necrosis factor-like ligand 1A
TNF	Tumor necrosis factor
TOX	Thymocyte selection-associated high mobility group box
TSLP	Thymic stromal lymphopoietin
VIP	Vasoactive intestinal peptide

Introduction

Type 2 immune responses play a key role in the initiation, maintenance, resolution, or prevention of numerous human disease states, including infection with parasitic helminths, allergic diseases, fibrosis, and metabolic disorders [1–5]. Type 2 cytokine responses drive protective immunity to parasitic helminths as well as pathologic allergic inflammation associated with diseases such as asthma, atopic dermatitis (AD), and food allergy [1–5]. In addition, these responses are also associated with tissue remodeling and repair and metabolic homeostasis [1–5]. Thus, type 2 immune responses are linked to a wide array of diseases that together are responsible for a significant public health and economic burden worldwide, and a better understanding of the regulation of type 2 inflammation has the potential to inform treatment and management of many of these diseases [1–5].

Type 2 immune responses begin with the production of epithelial cell-derived cytokines including interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin (TSLP) and antigen presentation by antigen-presenting cells (APCs) such as dendritic cells (DCs) and macrophages [1–3, 6, 7]. These early responses in turn promote production of the cytokines IL-4, IL-5, IL-9, and IL-13, development and activation of $CD4^+$ T helper type 2 (T_H2) cells, antigen-specific immunoglobulin (Ig)E production, and recruitment of innate effector cell populations such as eosinophils, mast cells, and basophils to the epithelial barrier [1–3, 6]. Together, these responses act back upon the epithelial barrier to regulate effector mechanisms such as mucus production, smooth muscle contractility, and barrier permeability that mediate helminth expulsion, result in signs and symptoms of type 2 inflammation, or contribute to reparative or homeostatic processes [1–5].

While a significant body of literature describes how responses by adaptive T and B cells promote type 2 cytokine responses, less is known regarding the regulation of innate immune responses that drive the initiation, maintenance, and resolution of these responses [1, 7]. Importantly, studies in the last 5 years have identified a novel subset of innate immune cells, the group 2 innate lymphoid cells (ILC2s), that is found in humans and mice in multiple tissues and is critical in the development of type 2 cytokine responses [3, 7–11]. This chapter describes recent advances in our understanding of the development and activation of ILC2s and how these cells contribute to type 2 inflammation in the context of helminth infection and allergy. Additionally, emerging work is discussed that describes alternative roles of ILC2s in promoting tissue remodeling and metabolic homeostasis. In particular, recent studies are highlighted that reveal how ILC2 responses could be targeted therapeutically to treat diseases in which ILC2-associated type 2 inflammation plays a role.

Identification and Definition of ILC2s

ILC2s are a newly described subset of innate cells within the ILC family, which includes classical natural killer (NK) cells and lymphoid tissue-inducer (LTi) cells [3, 7–17]. All ILCs lack expression of cell lineage markers associated with T cells, B cells, DCs, macrophages, and granulocytes, but do express CD90 (Thy1), the stem cell factor (SCF) receptor c-Kit, CD25 (IL-2 receptor (R)α), and CD127 (IL-7Rα) [3, 7–17]. As NK cells appear to have distinct developmental and functional characteristics when compared to other ILC subsets described below [3, 7–17], here the term "ILC" will be used to refer to only non-NK cell members of the ILC family.

The ILC family is currently categorized into three major subsets. These subsets are comparable to the three major $CD4^+$ T helper lineages and are distinguished by their differential requirements for transcription factors during development and expression of distinct transcription factors and effector cytokines by mature cells [8]. The group 1 ILCs (ILC1s) include newly described innate cells that are T-box expressed in T cells (T-bet)-dependent, produce interferon-γ (IFN-γ), and promote

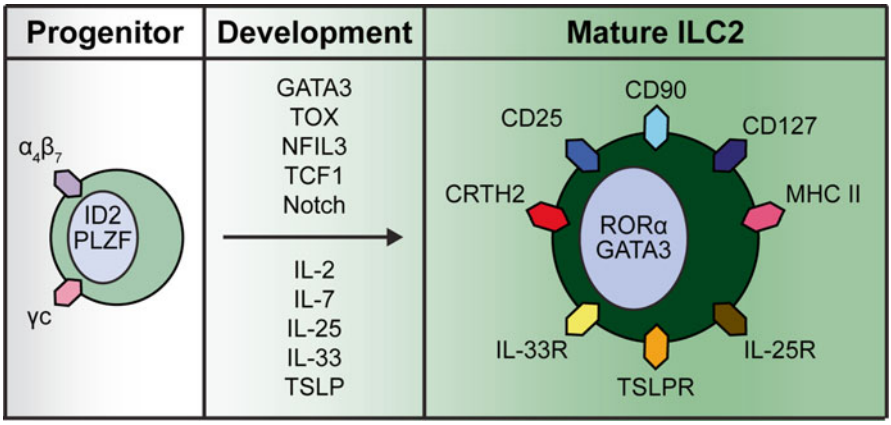

Fig. 1 A network of transcription factors and cytokines directs ILC2 development. ILC2s arise from a CLP that expresses the integrin $\alpha_4\beta_7$, the common γc receptor, and the transcription factors ID2 and PLZF. Signals from the cytokines IL-2, IL-7, IL-25, IL-33, and TSLP and the transcription factors GATA3, TOX, NFIL3, and TCF1, along with Notch signaling, guide the differentiation, maturation, and activation of ILC2s. These mature ILC2s are negative for lineage markers of T and B cells, monocytes, macrophages, and innate granulocytes, but they do express CD90 (Thy1), CD25, CD127, IL-25R, IL-33R, TSLPR, and CRTH2

immunity to intracellular pathogens and intestinal inflammation (classical NK cells are also categorized into this group). The group 3 ILCs (ILC3s) include retinoic acid receptor-related orphan receptor γ (RORγt)-dependent LTis and other RORγt-dependent cells that respond to IL-23, produce IL-17A and/or IL-22, and support lymphogenesis in the fetus and in adults, immunity to extracellular bacteria, and inflammation at multiple mucosal and barrier surfaces [8, 10, 12, 13, 15–19].

In contrast, ILC2s are dependent upon and express the transcription factors retinoic acid receptor-related orphan receptor α (RORα) [20, 21] and GATA binding protein 3 (GATA3) [22–27] (Fig. 1). These cells respond specifically to the epithelial cell-derived cytokines IL-25, IL-33, and TSLP and the tumor necrosis factor (TNF) family member TNF-like ligand 1A (TL1A) to produce IL-4, IL-5, IL-9, IL-13, and/or the epidermal growth factor receptor (EGFR) ligand amphiregulin (Areg) [3, 7–11]. These effector functions then support the development of type 2 inflammation in the context of immunity and allergic disease, and also contribute to the ability of ILC2s to maintain tissue homeostasis by promoting wound healing, tissue remodeling, and metabolic homeostasis [3, 7–11, 28–48].

While the categorization of ILCs into the distinct subsets described above has been useful in providing a rubric for understanding the developmental requirements and effector functions of these cells, it remains possible that additional innate cell subsets exist, or that there may be functional plasticity among different subsets of ILCs [8, 10]. For example, cells termed multipotent progenitor type 2 (MPP^{type2})

cells were originally thought to be an ILC-like population that promoted type 2 immunity to helminth infection [49]. However, subsequent studies showed that these cells were distinct from ILC2s, as MPP^{type2} cells responded preferentially to IL-25 and exhibited a progenitor-like phenotype and function, while ILC2s responded preferentially to IL-33 and were terminally differentiated [50]. Regarding plasticity of ILC subsets, recent studies have shown that murine and human ILC3s could respond to IL-12 and IL-18, which mediates dynamic expression of RORγt and T-bet [51], loss of IL-17 and IL-22 expression, and acquisition of the ability to produce IFN-γ [51–54]. However, it remains unclear whether ILC2s demonstrate similar functional plasticity, and further studies will be required to fully dissect how ILC2s respond to changing environmental cues to modulate their characteristic effector functions.

Requirements for the Development of ILC2s

Following the identification of ILC2s and other ILC subsets, there has been tremendous interest in understanding the pathways that lead to the development and differentiation of ILC2s. All ILCs are derived from a bone marrow-resident common lymphoid progenitor that expresses the integrin $\alpha_4\beta_7$ [55–58]. Downstream of this precursor, the development of the three ILC subsets has been shown to be dependent on the transcription factors inhibitor of DNA binding 2 (ID2) [55, 58–60] and promyelocytic leukemia zinc finger protein (PLZF) [59]. In addition, ILC development was dependent upon the common γ-chain (γc or CD132), IL-7, the Notch pathway, and the transcription factors thymocyte selection-associated high mobility group box protein (TOX), nuclear factor, interleukin 3 regulated (NFIL3), GATA3, and transcription factor 1 (TCF1) [8, 10, 19, 27, 28, 31, 35, 55, 59–68] (Fig. 1).

The transcription factor GATA3 is particularly key for the development and maintenance of ILC2s [22–27]. While deletion of this factor at the earliest stages of ILC development in hematopoietic stem cells prevented the development of all ILCs [27, 68], deletion of GATA3 in downstream precursors only prevented the development of ILC2s, suggesting that sustained GATA3 expression is uniquely required for ILC2 differentiation [22–27, 68]. Other transcription factors coordinate the effects of GATA3 on ILC2 development, including growth factor-independent 1 transcription repressor (GFI1), a transcription factor that targets the *Gata3* gene and helps to maintain GATA3 expression [69]. In addition to GATA3, other transcriptional regulators contribute to the development of ILC2s [20, 21]. Together, these findings suggest a model in which multiple transcription factors converge in a regulatory network that controls the development of ILC2s (Fig. 1).

Regulation of ILC2 Effector Functions

Studies in multiple models and in humans suggest that ILC2s promote type 2 inflammation that contributes to protective immunity to helminth parasites and allergic disease, while supporting the maintenance of tissue remodeling mechanisms and metabolic homeostasis through production of IL-4, IL-5, IL-9, IL-13, and Areg and interactions with other innate and adaptive immune cells [3, 7–11]. The factors that drive the acquisition of ILC2 effector functions include a diverse array of cytokines and lipids derived from epithelial cells and other immune cells that are produced in response to helminth parasites, viruses, and fungi as well as allergens [3, 7–11]. In particular, the epithelial cell-derived cytokines IL-25, IL-33, and TSLP are critically important for ILC2 development, activation, and acquisition of effector function [25, 28–30, 32, 34, 39, 42–44, 48, 50, 70–77]. Other cytokines, including the γc cytokines IL-2, IL-4, and IL-7, also support the development of ILC2s and promote their activation [3, 7–11, 77–80]. Two recent studies have shown that the TNF family member TL1A acted on ILC2s that express death receptor 3 (DR3) to drive allergic inflammation in the lung and protective immunity to helminth parasites [40, 41]. Finally, autocrine signaling by IL-9 produced by ILC2s was demonstrated to be important in promoting ILC2 survival [45] (Fig. 2).

In addition to responding to cytokine stimuli, ILC2s also respond directly to bioactive lipids of the eicosanoid family that are produced in the context of allergic inflammation. Specifically, ILC2s express the prostaglandin D_2 (PGD_2) receptor chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2), and ligation of CRTH2 in vitro elicited chemotaxis of ILC2s and production of IL-5 and IL-13 [81–84]. ILC2s also responded to leukotriene D_4 (LTD_4) [85] and were inhibited by lipoxin A_4 (LXA_4) [81]. Together, these studies suggest that numerous proteins and lipids regulate ILC2 activation and effector function. However, additional studies will be required to more completely define the factors that regulate ILC2 activities in various tissues during the steady state or in the context of inflammation (Fig. 2).

ILC2s Contribute to Protective Immunity to Helminth Parasites

While the role of type 2 cytokines in mediating helminth expulsion has been appreciated for many years, the critical source of these cytokines in vivo was previously poorly defined [1, 2, 4, 6, 7, 86]. *Nippostrongylus brasiliensis* is a mouse-adapted intestinal nematode parasite used as a model of hookworm infection [87], and protective immunity in mice is dependent upon IL-13-mediated changes in the intestinal epithelium, including an increase in mucus production and changes in smooth muscle contractility, that lead to parasite expulsion [86]. Seminal work in 2006 showed that an innate cell population that expressed c-Kit and produced type 2 cytokines was required for resistance to *N. brasiliensis*, providing the first indication that innate cells, rather than adaptive $CD4^+$ T cells, were required as a source of type 2 cytokines during helminth infection [88].

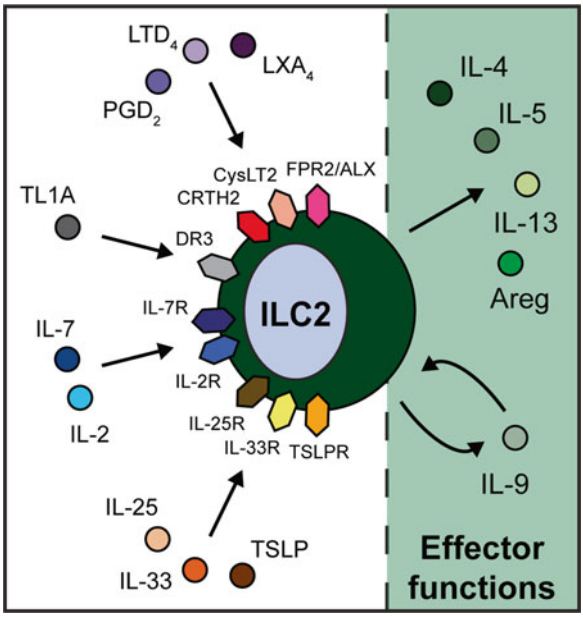

Fig. 2 Cytokines and bioactive lipids regulate ILC2 acquisition of effector function. The epithelial cell-derived cytokines IL-25, IL-33, and TSLP act on ILC2s, driving cell proliferation and production of the type 2 cytokines IL-4, IL-5, IL-9, and IL-13. IL-9 produced by ILC2s, in addition to other γc cytokines such as IL-2 and IL-7, promote ILC2 proliferation and survival. In addition, the TNF family member TL1A signals to its receptor, DR3, on the surface of ILC2s to elicit expression of effector cytokines. Finally, ILC2s also respond to the eicosanoids PGD_2, LTD_4, and LXA_4 through the receptors CRTH2, cysteinyl leukotriene receptor 2 (CysLT2), and formyl peptide receptor 2/lipoxin A_4 receptor (FPR2/ALX), respectively. PGD_2 and LTD_4 drive the activation of ILC2s, while LXA_4 limits ILC2 responses

Subsequent studies formally defined this innate cell population as innate helper cells, nuocytes, or innate helper type 2 cells, again in the context of infection with *N. brasiliensis* [28–30], and these cells are now universally referred to as ILC2s [8]. During *N. brasiliensis* infection, ILC2s are the dominant producers of IL-13, and mice lacking ILC2s or IL-13 failed to efficiently expel parasites [28–30]. Adoptive transfer of IL-13-expressing ILC2s to mice deficient in ILC2s or IL-13 was able to promote parasite expulsion, thus identifying ILC2s as key players in mediating interactions between the immune system and the epithelial barrier that are required for protection against helminth parasites [28–30] (Fig. 3). While it remains unclear exactly how ILC2s contribute to protective immune responses during infection with helminths aside from *N. brasiliensis*, there are studies that suggest that ILC2s do contribute to immunity to diverse helminth species, such as *Strongyloides venezuelensis* [89, 90]. Similarly, Areg has been shown to be required for expulsion of the nematode parasites *Trichuris muris* [91], and ILC2s are a predominant source of Areg in some contexts [35], suggesting that ILC2s may contribute to immunity to *T. muris* as well.

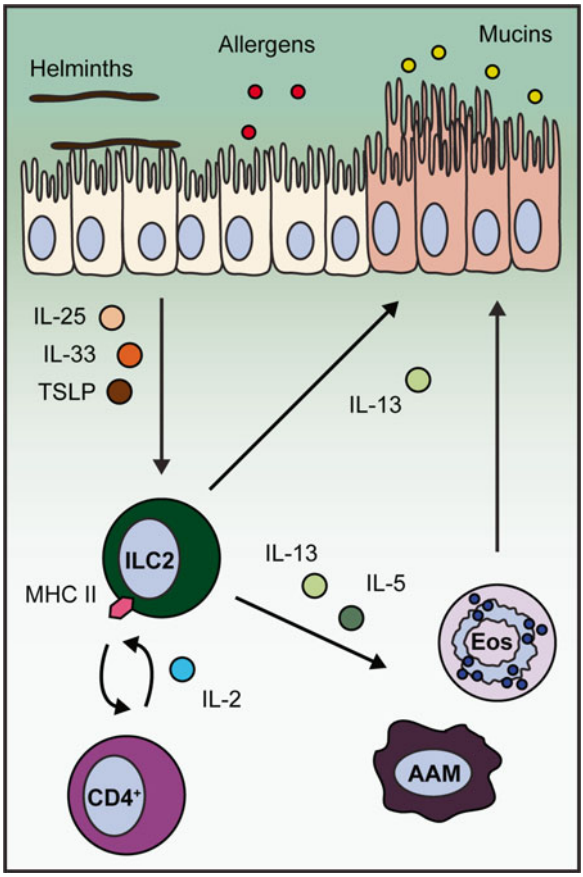

Fig. 3 ILC2s respond to cues from the epithelium and interact with innate and adaptive cells to promote type 2 inflammation. Following exposure to helminth antigens or allergens, epithelial cells produce the cytokines IL-25, IL-33, and TSLP. ILC2s respond to these cytokines and express IL-5 and IL-13. IL-5 and IL-13 act on other innate cell types such as eosinophils and macrophages to promote tissue eosinophilia and alternative activation of macrophages. These innate responses, coupled with the action of ILC2-derived IL-13 directly on the epithelium, serve to mediate changes in epithelial barrier physiology that contribute to increased mucin production and smooth muscle contractility. In addition, activated ILC2s interact with $CD4^+$ T cells via expression of MHC II, which drives T-cell production of IL-2 that acts back on ILC2s to support their continued proliferation, activation, and survival. Together, these ILC2-centric pathways contribute to type 2 inflammation that is protective during helminth infection and pathologic in the context of allergic disease

Numerous factors and pathways regulate ILC2 function during infection with helminth parasites. The cytokines IL-25 and IL-33 were required for the activation of these cells and subsequent parasite expulsion [28–30, 32], and expression of the signaling molecule NF-κB activator 1 (ACT1) in epithelial cells was critical for the efficient production of IL-25 and IL-33 following infection [92]. Another cytokine, TL1A, also elicited ILC2 responses in the context of helminth infection [40, 41]. Recent work has shown that eicosanoids can also regulate ILC2 responses following

helminth infection, as the PGD2 receptor CRTH2 mediated ILC2 accumulation and type 2 inflammation in the lung of mice that had been infected with *N. brasiliensis* [84]. A variety of transcription factors are required for optimal ILC2 responses. During *N. brasiliensis* infection, GATA3 was required for ILC2 development and function [22, 23], GFI1 regulated ILC2 responsiveness to IL-33 and supported maintained GATA3 expression [69], and TCF1 promoted ILC2 expansion [31]. Finally, very recent evidence suggests that interactions with T cells during infection are important in supporting ILC2 responses. Through expression of major histocompatibility class II (MHC II), ILC2s interacted with and activated naïve $CD4^+$ T cells, resulting in the production of Tcell-derived IL-2 that supported ILC2 expansion, effector function, and parasite expulsion [33]. Collectively, these data show that ILC2s are regulated by various cytokines and transcription factors in order to allow ILC2s to interact with other immune cells and the epithelium to mediate protective immunity to helminth infection (Fig. 3).

ILC2s Promote Allergic Inflammation

ILC2s are potent sources of type 2 cytokines and are subject to complex regulation by a variety of pathways and factors [3, 7–11]. Thus, it is not surprising that ILC2 responses are not solely protective during helminth infection, but can also drive pathologic type 2 cytokine-associated responses associated with allergy [3, 7–11]. A significant body of work now supports a key role for ILC2s in the initiation and maintenance of allergic inflammation at mucosal and barrier surfaces in the context of multiple allergic diseases, including allergic asthma, allergic airway inflammation, chronic rhinosinusitis (CRS), AD, and food allergy [3, 7–11].

Shortly after the discovery of murine ILC2s in the intestine and fat-associated lymphoid clusters [28–30], these cells were also identified in the murine lung [35, 93]. Numerous studies have now established that IL-25, IL-33, and/or TSLP can elicit ILC2-derived IL-5 and IL-13 production that contributes to airway hyperresponsiveness and allergic airway inflammation in various murine models [20, 24, 26, 31, 37–41, 69, 74–76, 78, 85, 94–98] (Fig. 3). Notably, bioactive lipids such as eicosanoids can also promote ILC2 responses that regulate type 2 inflammation in the lung. For example, murine lung ILC2s responded to LTD_4 by producing IL-4 and IL-5, which was associated with eosinophilia induced by exposure to *Alternaria* species [85]. Similarly, human ILC2s express the receptor for LXA_4, which regulated IL-13 production by ILC2s [81].

Importantly, new studies suggest that ILC2s contribute to allergic inflammation in the lung through a variety of mechanisms in addition to their ability to produce IL-5 and IL-13. For instance, in response to IL-2 signals, murine lung ILC2s produced IL-9 that was necessary for their survival and effector function in response to challenge with papain [99]. This dependence on IL-2 signaling suggests that ILC2 activities are closely tied with those of T cells, which are the primary source of IL-2 [100]. In support of this concept, recent work has revealed that ILC2s and T cells interact to coordinately drive allergic lung inflammation. In vitro co-culture of ILC2s

and CD4+ T cells led to Tcell proliferation and type 2 cytokine production, and co-transfer of these cells into mice that lacked both T cells and ILC2s drove allergic airway inflammation in response to ovalbumin or the cysteine protease bromelain [101] (Fig. 3). In addition, following exposure to papain, IL-13 from ILC2s promoted DC migration to the draining lymph node and priming of naïve T cells [102].

Notably, there is significant evidence to suggest that ILC2s play a role in asthma and upper and lower allergic airway inflammation in humans. Allergic rhinitis is characterized by type 2 cytokine responses in the upper airways and can be associated with the development of CRS [103]. Nasal polyps, a hallmark of CRS, had an enriched population of CRTH2-expressing ILC2s that responded to IL-25, IL-33, and TSLP [25, 34, 104, 105]. Additionally, ILC2s have been identified in the human adult and fetal lung that expressed IL-33R, CRTH2, and/or CD161 [34, 35, 81, 84], and levels of epithelial cell-derived cytokines and eicosanoids that activate ILC2s were elevated in the lung tissues of human asthmatics [81, 106–109]. Finally, ILC2s isolated from the peripheral blood of asthmatics were more numerous than ILC2s in the peripheral blood of healthy controls, and they also produced more IL-5 and IL-13 in response to stimulation [110]. Taken together, these studies indicate that eicosanoids and epithelial cell-derived cytokines activate ILC2s to produce cytokines and interact with innate and adaptive immune cells, leading to allergic airway inflammation in mice and humans (Fig. 3).

The importance of ILC2s in mediating type 2 inflammation in the upper and lower airways suggests that these cells contribute to other atopic diseases at different tissues sites. In support of this idea, levels of the epithelial cell-derived cytokines that activate ILC2s, including IL-25, IL-33, and TSLP, have been shown to be increased in the skin of patients with AD [111–114] and in the intestine of patients with food allergy [115–117]. Indeed, the ILC2 population was expanded in AD and AD-like lesions in humans and mice, respectively [43, 77], where these cells responded to IL-2, IL-25, IL-33, and TSLP, produced IL-5 and IL-13, and interacted with innate granulocyte populations to mediate allergic inflammation [44, 73, 77, 118]. While a role for ILC2s in food allergy has not yet been described, ILC2s in the intestine could drive inflammation in response to IL-25 in a murine model of oxazalone-induced colitis [119], suggesting that further research investigating the contribution of ILC2s to type 2 inflammation in the gastrointestinal tract is warranted. Collectively, studies in murine models of allergic disease and in human patients with allergic disease suggest that ILC2s play a key role in driving allergic inflammation at multiple mucosal and barrier surfaces, and that these cells and their effector functions could be targeted in the treatment of upper and lower allergic airway inflammation, AD, and potentially food allergy in humans.

ILC2s Support Tissue Remodeling and Wound Healing

While the role for ILC2s in promoting pathologic and protective type 2 inflammation is now well-established [3, 7–11], new data are emerging that highlight additional functions of ILC2s. In particular, ILC2s appear to contribute to tissue

remodeling, wound healing, and tissue homeostasis in a number of contexts [3, 7–11]. A role for ILC2s in tissue remodeling was initially described during influenza A virus infection in mice, in which ILC2 depletion led to a defect in the ability of the lung epithelium to repair itself following virus-induced tissue damage [35]. In this context, lung ILC2s produced the EGFR ligand Areg, which was critical in mediating efficient repair of the lung tissue [35]. A recent study supports the idea that ILC2s have tissue-protective roles in the lung during infection with other pathogens, as IL-9 promoted survival of ILC2s in the lung that produced Areg and contributed to tissue repair following migration of the helminth *N. brasiliensis* through the lung [45]. The tissue-protective effects of ILC2s do not appear to be limited to the lung. Recent evidence suggests that cholangiocytes, the epithelial cells of the bile duct, also derive protective benefits from ILC2s. In response to IL-33, expansion of an IL-13-producing ILC2 population supported cholangiocyte proliferation in a murine model of biliary atresia [120]. Interestingly, this cholangiocyte hyperplasia was also associated with an increase in cholangiocarcinoma in mice, suggesting that precise regulation of ILC2-dependent tissue repair mechanisms is required to avoid pathologic outcomes [120].

In keeping with this idea, emerging evidence suggests that ILC2s can also contribute to dysregulated tissue remodeling associated with fibrosis and pathology [42, 72]. Following challenge with *Schistosoma mansoni* eggs, the development of pulmonary fibrosis was dependent upon IL-25-elicited ILC2s that produced IL-13, and the same study showed that ILC2 frequency and number were increased in the bronchoalveolar lavage (BAL) fluid of human patients suffering from idiopathic pulmonary fibrosis [48]. Also, in a model of hepatic fibrosis, ILC2s that responded to IL-33 and produced IL-13 were important in driving fibrotic processes [72]. Thus, similar to the capacity of ILC2s to contribute to both protective immune responses against helminth parasites and detrimental type 2 responses during allergic inflammation, the ability of ILC2s to drive tissue remodeling processes can be either beneficial or pathological. While the regulation of these activities is likely tissue- and disease-specific, further work is required to dissect the pathways that influence ILC2-mediated tissue remodeling and to determine whether these cells and their activities could be targeted to regulate tissue remodeling and homeostasis in various disease states.

ILC2s Promote Metabolic Homeostasis

Cutting edge studies in ILC2 biology have begun to focus on how ILC2s contribute to the maintenance of metabolic homeostasis [42, 46, 47, 121, 122]. Previous work has demonstrated that a type 2-polarized cytokine environment in adipose tissue elicits alternatively activated macrophage (AAM) activities and the accumulation of eosinophils, which in turn are associated with metabolic homeostasis [5, 123–125]. In contrast, classical activation of macrophages and T_H1-polarized inflammation in the adipose is associated with obesity, insulin resistance, and metabolic disease

[126–130]. These previous findings, coupled with the observations that ILC2s are potent sources of type 2 cytokines in other tissues [3, 7–11], prompted an investigation of the role of ILC2s in maintaining type 2 cytokine responses that support metabolic homeostasis.

An initial study demonstrated that IL-13 played a role in limiting hyperglycemia, which is associated with insulin resistance. IL-13 limited glucose production by inhibiting genes involved in gluconeogenesis in the liver via signal transduction and activator of transcription 3 (STAT3) [121], suggesting that sources of IL-13, such as ILC2s, might contribute to glucose homeostasis. In addition, IL-5-responsive eosinophils in the adipose have been associated with the maintenance of healthy adipose tissue function in the lean state, and another study has shown that IL-33-elicited ILC2s that produced IL-5 maintained eosinophil and AAM populations in the fat [46]. Supporting a role for ILC2 effector functions in promoting metabolic homeostasis in the adipose, IL-25 treatment resulted in an increase in ILC2 populations in obese mice, associated with the expansion of eosinophil and AAM in the adipose, increased weight loss, and improved glucose tolerance. Similarly, ILC2 depletion in obese *Rag1*$^{-/-}$ mice resulted in increased weight gain and glucose intolerance, and transfer of ILC2s to obese mice led to weight loss and improved insulin sensitivity [42].

In addition to their role in regulating immune cells that contribute to metabolic homeostasis in the fat, ILC2s that reside in the intestine produced IL-5 following caloric intake in response to vasoactive intestinal peptide (VIP), a neuropeptide produced following feeding that is tied to circadian rhythms. These data thus establish a link between ILC2s, caloric intake, and eosinophil responses that have been associated with metabolic homeostasis [47]. A very recent publication has also shown that IL-33-responsive ILC2s in the fat regulate adiposity and the recruitment of beige adipocytes that control caloric expenditure [122]. Together, these studies suggest that ILC2s may be key regulators of metabolic processes in the steady state. Of note, a very recent study has shown that micronutrient deficiencies appear to influence ILC2 responses that mediate protective immunity against pathogens [131], suggesting that ILC2s are also key players in coordinating nutrition and metabolism during infection and inflammation. Further studies will be needed to better understand how ILC2s sense nutritional status to regulate metabolic homeostasis during the steady state and following encounters with pathogens.

Conclusions and Future Directions

A rapidly advancing body of work highlights the key role that ILC2s play in protective and pathologic type 2 immune responses in the context of helminth infection, allergic disease, tissue remodeling and repair, and metabolic homeostasis [3, 7–11]. Together, these studies provide new insight into how the innate immune system contributes to type 2 cytokine responses that participate in a variety of key biologic processes [1]. However, numerous outstanding questions remain regarding the development, effector function, and regulation of ILC2s. The precise developmental

paths that lead to ILC2 hematopoiesis remain unclear, and how ILC2 populations turn over in the steady state and change in the course of aging is unknown. Also, it seems likely that cytokines and factors in addition to the epithelial cell-derived cytokines and eicosanoids shape ILC2 responses. In particular, the pathways that negatively regulate ILC2 responses and the mechanisms that control ILC2 migration are poorly understood. Similarly, ILC2s likely produce cytokines or other factors in addition to type 2 cytokines and Areg. Further work will be required to more comprehensively profile the effector molecules produced by ILC2s and how these cells interact directly and indirectly with epithelial cells and innate and adaptive immune cells. Most importantly, our current understanding of ILC2 biology suggests that these cells and their effector functions could be targeted therapeutically in human diseases in which type 2 cytokine responses play a role, including helminth infection, allergic disease, fibrosis, and metabolic disease. Future studies that investigate ILC2 responses in humans and in murine models of human diseases could result in the development of innovative new therapies that target innate immune pathways involved in the pathogenesis of multiple inflammatory diseases.

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Dendritic Cells and Type 2 Inflammation

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Introduction

The immune system is constantly exposed to a large variety of antigens and has to distinguish pathogens from harmless antigens. Dendritic cells continuously scan the environment to sense potentially harmful pathogens and substances. They are professional antigen-presenting cells (APCs) that have the capacity to capture foreign antigen, migrate to the draining lymph nodes, present Ag to naïve T cells, and initiate an immune response. It is very established how DCs initiate a protective Th1, Th17, and/or CTL immune response against pathogens, tailored to clear the inciting pathogen [1]. By expressing pattern recognition receptors for foreign antigens, and by expressing a plethora of antigen uptake receptors, DCs can sense the nature of the pathogen and process a large variety of antigens for presentation to naïve T cells. In this process they display peptide-MHC complexes on their surface (signal 1 for T cell activation), display costimulatory molecules (signal 2), and produce the polarizing cytokines (signal 3) for driving the expansion and differentiation of

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various T cell subsets. Not surprisingly, mice lacking DCs have strong defects in antibacterial, antifungal, and antiviral immunity that depend on Th1, Th17, and CTL response [2, 3]. It has been much more controversial if and how DCs play a crucial role in the Th2 type inflammation that develops in response to environmental allergens (like house dust mite (HDM), cockroach allergen, tree and grass pollen, fungal spores, and animal dander), tissue-dwelling parasites like helminthes and nematodes, adjuvants like aluminum hydroxide, and experimental agents like the enzyme papain [4, 5]. Th2 type inflammation that is commonly seen in allergic inflammation and surrounding tissue-dwelling parasites or their secreted products is typically rich in eosinophils, mast cells, basophils, and alternatively activated macrophages ("M2 macrophages") and is controlled by adaptive Th2 immune cells that produce IL-4, IL-5, and IL-13 in collaboration with innate lymphoid type II cells (ILC2) that mainly produce IL-5 and IL-13 (Fig. 1) [6]. As our immune system constantly encounters environmental allergens, and as helminth-infected individuals that are left untreated often carry their parasites for life, chronicity is another feature of type 2 inflammation. Very commonly, type 2 inflammation therefore leads to tissue remodeling and deposition of extracellular matrix components at sites of allergen exposure or helminth residence. As exposure is so chronic and not particularly damaging to the host, allergens and helminths are also good inducers of T regulatory cells (Tregs) and often Th2 immunity is very well balanced by induced Treg cells [7–9].

Dendritic Cells Are Necessary and Sufficient to Induce Th2 Immunity

The role of DCs in inducing Th2 immunity has mainly been studied in models of asthma and allergic rhinitis [6], as well as in models employing the proteinase papain [10–12]. When allergic individuals encounter certain allergens, they develop a Th2-dominated inflammatory immune response against the allergen, whereas healthy individuals mount a balanced Th1 and Treg response [13, 14]. In allergic asthma, persistent inhalation of allergens leads to chronic eosinophil-rich inflammation, goblet cell metaplasia, and bronchial hyperreactivity and eventually to airway obstruction. Through production of the cytokines IL-4, IL-5, and IL-13, Th2 lymphocytes can control the many features of allergic disease, such as IgE synthesis, eosinophil expansion and activation, mucus overproduction, and hyperreactivity of smooth muscle cells typically seen in asthma [15, 16]. Although asthma was initially thought to be controlled by adaptive Th2 immunity exclusively, recent insights have demonstrated that innate immune cells like innate lymphoid type 2 cells (ILC2s) can also produce IL-5 and IL-13 and contribute to airway inflammation [17–19]. Additional cells like Th17 cells, γδ T cells, and Th9 cells could also play a role in disease, particularly in severe forms that are resistant to current therapies with inhaled steroids [20–22].

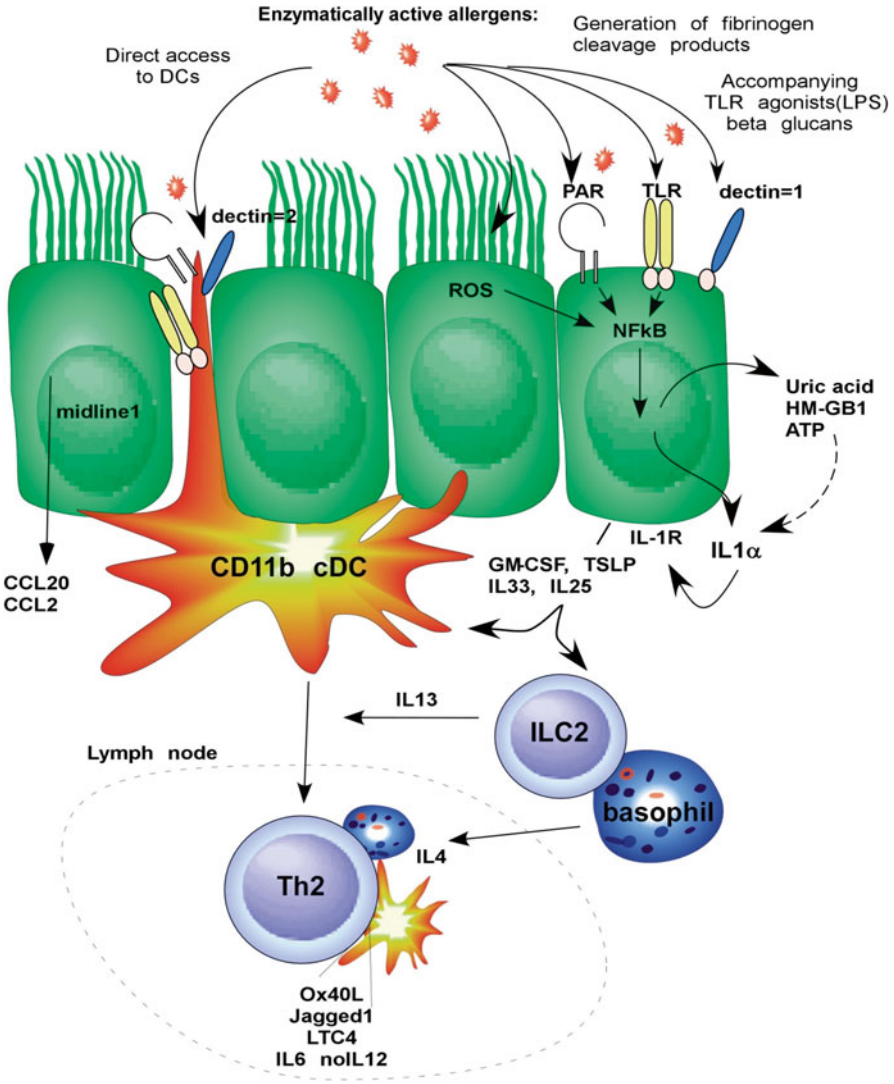

Fig. 1 Enzymatically active allergens

Before any form of adaptive immunity is induced to inhaled allergens, the allergen must get through the natural barriers of the body (skin, mucus membranes) and reach the cells of the immune system that are recirculating in the central lymphoid organs. DCs are one of the first immune cells that will come into contact with allergens at mucosal surfaces [23, 24]. In the lungs, intestine, and skin, DCs sit at the basolateral side of epithelial cells and can sample luminal antigens directly by extending dendrites across the epithelial barrier [25–28]. Studies in mice and

humans have shown that lung DCs and gut DCs express tight junction proteins, suggesting that they form tight junctions with epithelial cells to maintain the epithelial barrier function during antigen sampling [29–31]. In a study employing dual-photon microscopy to visualize lung DC behavior in situ, the sampling activity of lung DCs was shown to be inhomogeneously distributed at distinct sites of the lungs. DCs in the alveolar regions project dendrites across epithelial barriers and excel in antigen uptake. In contrast, antigen-bearing DCs accumulate near the conducting airways, in the same region as activated T cells, and rarely send processes across the airway epithelium [32].

After antigen uptake, the main function of DCs is to migrate to the draining lymph nodes and present the processed antigen to T cells, leading to clonal expansion of antigen-specific T cells. Since allergy is dominated by a Th2 lymphocyte response, it is not surprising that DCs have a central role in inducing an allergic response, and compelling evidence now proves this proposition. Antigen-loaded GM-CSF-cultured BMDCs or splenic DCs administered directly in the lung can sensitize mice, leading to a Th2-skewed inflammation upon antigen challenge [33, 34]. This adoptive transfer model also has some induction of Th17 cells, mimicking the mixed Th2/Th17 profile of human asthmatics [34]. When DCs are repeatedly injected into the lungs, there is even induction of irreversible airway remodeling, characterized by deposition of extracellular matrix components under the basement membrane [35]. Similarly, DCs originating from the lungs of allergen-exposed mice are also sufficient to induce sensitization when transferred to naïve recipients [36–38].

In addition to these studies demonstrating that DCs are sufficiently capable of inducing Th2 immunity in the lung, recent studies have also demonstrated that lung DCs are necessary for induction of Th2 immunity to allergens. Depletion of lung DCs in CD11c-DTR transgenic mice during the first exposure of mice to inhaled allergen HDM abolished Th2 cytokine production and cardinal features of asthma [38]. Also in models of sensitization to the enzymatic model allergen papain and to helminth allergens, DCs seem to be required for optimal Th2 immunity [10–12, 39]. Whether innate ILC2 and DCs have functional interactions in asthma remains to be investigated, although such interactions failed to be found in the skin during the steady state. Along the same lines, depletion of CD11c high cells did not affect the induction of ILC2 in a Th2-dependent model of helminth infection [40].

In the context of allergy and helminth infection, several reports have proposed that basophils and eosinophils can also have antigen-presenting capacity and can be sufficient for the induction of allergic responses [10, 41–43]. In these studies, an antibody to the high-affinity IgE receptor FcεRI was however used to deplete basophils, potentially also depleting DCs [38]. Direct evidence that human basophils act as APCs for Th2 immunity has also been hard to find [44]. It is more likely that basophils cooperate with DCs to prime for Th2 immunity, perhaps by serving as an early source of IL-4 [38, 45]. Studies directly comparing eosinophils with DCs have also failed to find a strong APC function in lung eosinophils, except maybe for primed Th2 cells [46, 47].

Different Subtypes of DCs Induce Th2 Immunity

It is currently believed that various tasks of DCs in the immune system are performed by different subsets of DCs, and these concepts are now also slowly emerging in the Th2 field. DCs can be subgrouped based on their ontogeny, differential tissue distribution, surface marker expression, and function [48]. In the steady state, DCs in mice and human are broadly divided into conventional (c)DCs and plasmacytoid (p) DCs. pDCs express Siglec H, the bone marrow stromal antigen-1, B220, and Ly6C and are potent producers of type I interferons in response to viral infections. cDCs form the predominant group of DCs and are further subcategorized into lymphoid tissue-resident $CD8^+$ and $CD8^-$ $CD11b^+$ cDCs and the non-lymphoid $CD103^+$ and $CD11b^+$ cDCs, found o.a. in lungs, skin, and gut, and migrate to the draining nodes when encountering a foreign antigen. Both $CD8^+$ and $CD103^+$ cDCs depend on the transcription factors IRF8 and Batf3 and excel in cross-presentation of exogenous antigens to $CD8^+$ T cells [49]. The $CD11b^+$ cDCs depend on the transcription factor IRF4 and Notch/RBPjκ, and excel in presentation to CD4 T cells. Though these DC subsets are present during the steady state, the situation changes during inflammation, when monocytes migrate to the local tissue and give rise to mo-DCs, or sometimes called inflammatory DCs [48, 50].

Recent studies have addressed whether DC subsets of the lung are differentially able to induce Th1, Th17, or Th2 responses. Ex vivo-sorted subsets of $CD103^+$ and $CD11b^+$ cDC differentially induced, respectively, Th1 and Th2 responses to the model allergen OVA [51]. This problem has also been addressed directly in vivo, but it is hard to sort the different subsets under conditions of inflammation. During inflammation, monocyte-derived DCs are rapidly recruited to the site of inflammation in a CCR2-dependent fashion. The monocytic marker Ly6C has often been used to identify $CD11b^+$ Mo-DC from $CD11b^+$ cDCs. However, as this receptor is downregulated when mo-DCs enter the inflamed tissue, staining with customary surface markers failed to distinguish mo-DC from CD11b cDCs. In search for better markers, we and others recently described that mo-DCs, but not CD11b cDCs, expressed the FcεRI (recognized by MAR-1), CD64, and MerTK [37, 52]. Using this strategy to separate all DC subsets, DC subsets were sorted from the LN of HDM-sensitized mice and both $CD11b^+$ cDC and $CD64^+$ moDC, but not $CD103^+$ DCs, could sensitize acceptor mice after adoptive intratracheal transfer. Mice deficient in Flt3L lacked all cDC subsets and failed to induce Th2 immunity to low doses of HDM allergen. However, $Ftl3L^{-/-}$ mice still mounted Th2 immunity when a high dose of HDM was used, thus recruiting moDCs to the lung. Depletion of $CD103^+$ using langerin-DTR mice did not abolish cardinal features of asthma, confirming that CD103+ DCs play a redundant role in the HDM-driven asthma model [37]. Recent work suggests that CD103+ cDCs might even promote tolerance to inhaled allergens [53–56]. The fact that $CD11b^+$ cDCs seem to be necessary for Th2 immunity was also recently confirmed in *CD11c*Cre x $Irf4^{fl/fl}$ mice that selectively lack this subset of cDCs. These mice failed to mount Th2 immunity driven by OVA and the Th2 adjuvant alum [57].

Following injection of papain in the skin, basophils collaborate with DCs to induce Th2 immunity (*see* Fig. 1) [10, 42]. Papain is captured by CD11b$^+$ cDCs that also express macrophage galactose type C-type lectin 2 (CD301b) in the skin and various mucosae [58]. Using the promoter of this gene to drive expression of the diphtheria receptor, it was shown that conditional depletion of CD301b$^+$ CD11b$^+$ cDCs abolished the early generation of IL-4-producing Th2 cells to papain as well as to alum and to *Nippostrongylus brasiliensis*, although IgE responses to the parasite were intact [11]. Similarly, *CD11c*Cre x *Irf4*$^{fl/fl}$ mice lack PDL-1$^+$ CD301b$^+$ cDCs in the skin-draining lymph nodes and there was a defect on Th2 response generation to papain [12]. Despite these reports, others have shown that DCs are not necessary to mount Th2 immunity to papain. The most likely explanation is that with very high doses of allergen, there might be other APCs that come into play [42].

Dendritic Cells Are Also Required for Effector Th2 Immune Responses to Allergens

Dendritic cells are not only necessary and sufficient for inducing Th2 immunity in naïve animals, studies in mice from which DCs can be conditionally depleted have shown that they are also non-redundant during the challenge phase and upon repeated encounters of already primed mice to inhaled allergens [35, 59, 60]. In area of eosinophil-rich type 2 inflammation, DCs have an activated phenotype expressing higher levels of costimulatory molecules OX40L, CD80, CD86, PDL-1, and PDL-2 [59]. At this stage of the immune response, they are closely located to effector T cells around the airways and large blood vessels [32, 61]. Here they might serve as an important source of chemokines that can attract effector T cells to the lungs [37, 62]. Also allergen-specific IgE and IgG1, through stimulation of FcεRI and FcγRIII, respectively, have a strongly enhancing function as they target inhaled allergens to DCs in already primed mice, thus boosting Th2 immunity further [63, 64].

Even when moDCs can induce Th2 immunity when adoptively transferred to other mice, it is much more likely that they play a more predominant role during the effector phase of the response. MoDCs were shown to be poorly migratory to the draining nodes due to lack of CCR7 expression, and therefore lung moDCs would be predicted to mainly interact with effector Th2 cells that migrate back to the lung [65]. In support of this model, moDCs produced chemokines attracting effector Th cells [37, 62]. They are also the predominant APC expressing FcεRI and RcγRIII. The exact role of PDL-2 expression on DCs is currently unclear. PDL-2 was identified as a marker for the IRF-4-dependent Th2 inducing DCs of the skin [12].

Direct Activation of DCs in Response to Allergen Exposure

In the steady state, DCs are immature and in order to initiate an immune response, DCs need to be activated to become mature and migrate to the LN, in a CCR7-dependent manner. DCs are equipped with a large variety of PPR for sensing the local environment, including TLRs, C-type lectin receptors, NOD-like receptors, and RIG-I-like receptors [66, 67]. These PPRs have evolved to detect a variety of molecular patterns on microbes, called PAMPS, or molecules released by necrotic cells, called DAMPS. DCs also express various cytokine receptors that can induce activation upon autocrine or paracrine secretion. Evidence is emerging that most allergens are capable of activating different classes of PRRs, but the outcome on Th immunity might differ per allergen [66, 67]. For example, glycan structures in the peanut glycoallergen ara h 1 can mediate DC activation and Th2 inducting through DC-SIGN, whereas others have shown that triggering of DC-SIGN by the HDM allergen Der p 1 promotes Th1 immunity [68, 69]. The mannose receptor on DCs mediates internalization of a variety of allergens through their carbohydrate moieties, leading to Th2 polarization [70]. Triggering of the C-type lectin receptor dectin-2 on DCs by HDM induces the production of cysteinyl-leukotrienes (CysLT). Subsequently, CysLTs affect the function of DCs in an autocrine manner via positive effects on CysLT(1)R receptor and negative effects via CysLT(2)R. Mice deficient in CysLT(2)R or adoptive transfer of DCs lacking CysLT(2)R developed markedly enhanced Th2 immunity to HDM. One of the main HDM allergens, Der p 2, is a functional homolog of a MD-2, the lipopolysaccharide (LPS)-binding component of the Toll-like receptor (TLR) 4 signaling complex, and in this way amplifies TLR4 signaling and DC activation [71]. Within helminthes, there are also specific molecules that directly trigger Th2 responses by DCs. Omega-1 is a constituent of *Schistosoma mansoni* soluble egg antigen (SEA) that triggers DCs to promote Th2 immunity [72, 73]. Mechanistically, omega-1 is internalized via its glycans by the mannose receptor (MR) on DCs and subsequently impairs protein synthesis by degrading both ribosomal and messenger RNA through an endonuclease activity.

How exactly activated DC prime for Th2 immune responses after migrating to the draining nodes is less clear, as the cytokine driving Th2 differentiation like IL-4 is not produced by DCs, in contrast to IL-12, TGF-β, IL-23, and IL-6 that are involved in Th1 and Th17 differentiation. It could be that induction of Th2 immunity is the default pathway when DCs fail to produce Th1- or Th17-polarizing cytokines (*see* Fig. 2). Alternatively, ligands acting via Notch receptors on Th cells might provide a direct polarizing signal for Th2 immune induction [74, 75]. Also the B7 family costimulatory molecule PDL2 was identified as a marker of Th2-inducing DCs, although it remains to be shown if signaling via PD1 on T cells boosts Th2 immunity [12]. After migrating to the draining lymph node, the current concept is that DCs move to the T cell zone to activate T cells. In case of Th2 immunity, the

validity of this proposition was recently challenged in a model of *H. polygyrus* infection. In the draining lymph nodes of infected mice, B cells were shown to secrete CXCL13 and recruited CXCR5-expressing DCs and T cells, resulting in DC-T interactions outside of the T cell zone. These interactions appeared to be necessary for optimal Th2 development, as mice that lacked CXCR5 on either T cells or DCs had impaired Th2 immunity to *H. polygyrus* infection [76]. It remains to be investigated whether this model is applicable to all Th2-dependent immune responses.

Indirect Activation of Lung DCs by Epithelial Cells

Although DCs express PPRs and are strategically localized to sense the environment directly, recent studies have now shown that the epithelium is equally important in activating DCs in response to allergens (*see* Fig. 2) [77]. This is most certainly

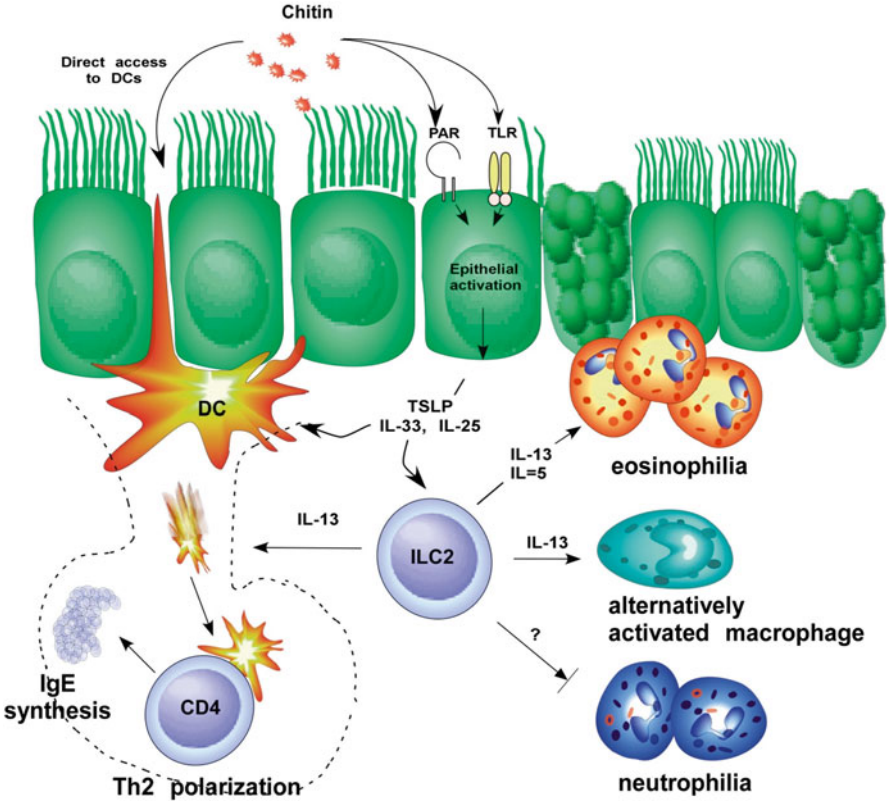

Fig. 2 Enzymatically active allergens (papain)

the case when one considers the environmental adjuvants like diesel particles, fine dust particles, and toxic gases like NO_2 that can all promote Th2/TH17 sensitization by DCs. By expressing a variety of TLRs, C-type lectin, and protease -activated PARs, epithelial cells are not merely a physical barrier to the outside world [78]. We and others have recently shown that TLR4 triggering on epithelial cells is essential for DC activation. In radiation-chimera mice lacking TLR4 on either radiosensitive hematopoietic or radioresistant epithelial cells, DC activation and migration relied on signals given by the radioresistant cells upon HDM administration. Conversely, HDM-driven allergic asthma could not be induced when the epithelial cell lacked TLR4 [25, 75]. The effects of TLR4 triggering appear to be dose dependent, as exposure to high-dose endotoxin can also suppress the development of allergy to HDM [79]. Although it is beyond the scope of this review article to discuss in detail how exactly epithelial cells activate lung DCs, we want to point out that cytokines like TSLP, IL-33, IL-25, and GM-CSF are made in a TLR4-dependent manner by airway epithelial cells and are crucial in causing DC activation and allergic Th2 sensitization. The relative importance of either of these cytokines depends on the type of allergen or the type of respiratory adjuvant [80, 81]. TSLP is not only made by epithelial cells, but it can also be made by DCs directly and act on DCs and T cells to promote Th2 immunity [82]. In mice lacking STAT5 only in DCs, there was a severe defect in Th2 immune response induction that was similar to TSLPR-deficient mice, suggesting that the main action of TSLP is through effects on DCs that require STAT5 to respond to it [83]. IL-33 also mainly affects DC and ILC2 activation. The production of the cytokine IL-33 by lung epithelial cells is closely regulated, and suppressed by apoptotic cell recognition and clearance in homeostatic conditions [84]. Epithelial cells produce trefoil factor 2 (TFF-2) in response to allergen exposure and this boosts IL-33 production in epithelial cells and DCs further [85]. IL-33 can also be released by necrotic cells, but few studies support the idea that there is predominant necrotic cell death in asthma. IL-33 can also be produced by lung macrophages and DCs, in a process greatly facilitated by allergen-specific IgG1 immune complexes triggering the FcγRIII receptor [64].

In addition to the established role for innate pro-Th2 cytokines, we and others have also recently demonstrated a crucial role for IL-1α in epithelial-DC cross talk in the context of HDM allergy. In an NLRP3 inflammasome- and caspase-1-independent manner, HDM triggered IL-1α production by epithelial cells, and acted in an autocrine fashion leading to IL-33, TSLP, and GM-CSF release. Cytokines downstream of IL-1α like GM-CSF and IL-33 contributed to DC activation, and therefore, IL-1R-deficient mice were resistant to HDM-driven asthma [86]. Contrasting the predominant role for IL-1α in causing DC activation, others have found, in a model of NO_2-enhanced airway inflammation, that IL-1β and caspase-1 were mediating the adjuvant effects on DC activation and Th2 development [87].

How exactly the innate pro-Th2 cytokines are made is a matter of intense study. In addition to producing cytokines, ECs may also activate DCs by producing DAMPS in response to allergens. For example, EC can induce DC activation by uric acid (UA) production, which is released in response to inhaled HDM in both mice and humans, and is involved in boosting cytokine production [88]. Although UA crystals are classical activators of the NLRP3 inflammasome, HDM- and UA-induced

Th2 responses in the lung were intact in *Nlrp3*-deficient or *Asc*-deficient mice. Another inflammasome NLRP10, thought to negatively regulate other inflammasomes, was shown to be involved in Th2 immune response induction by regulating the capacity of DCs to migrate to the draining nodes [89].

A Role for Inflammatory DCs in Human Allergy

Monocyte-derived DCs have been grown in vitro for decades, but have been hard to trace in human samples. A recent study however identified and broadly characterized InflDCs recovered from the synovial fluid of rheumatoid arthritic patients and tumor ascites from cancer patients. In addition to CD11c and HLA-DR, these cells expressed CD206, CD11b, Sirpα, CD14, CD1a, and FcεR1. Microarray analysis showed that the inflDCs were enriched for an in vitro mo-DC gene signature, suggesting that they were derived from monocytes and the in vivo counterparts of moDCs. The infDCs in this study produced Th17-polarizing cytokines and preferentially induced Th17 cells. Whether infDCs T helper lymphocyte polarization depends on the nature of the pathologic condition remains to be investigated [50].

In addition to being present in the allergic lungs, inflammatory FcεR1+ DCs are also present in the skin of atopic dermatitis patients, where they are called inflammatory dendritic epidermal cells (IDECs) [90]. Cells with a similar phenotype have also been described in the lungs of humans undergoing lung surgery, but it is not yet known whether asthmatics have more of these moDCs [91]. In any case, circulating and tissue-resident DCs of asthmatic and allergic patients have higher levels of FcεRI armed with IgE [92, 93].

A Central Role for DCs in Inducting Tolerance in Response to Allergens

Foxp3$^+$ regulatory T cells are masters in dampening immune responses in an allergen-specific manner and thereby mediate peripheral tolerance and inhibit allergy. Regulatory T cells are classified into two categories: thymus-derived T_{reg} cells (tT_{reg} cells) and peripheral derived T_{reg} cells (pT_{reg} cells). pT_{reg} cells can develop de novo in lymphoid organs from naïve CD4 T cells, whereas tT_{reg} cells develop in the thymus. Induction of pT_{reg} cells requires TCR signaling and weak co-stimulation in the presence of TGF-b and is facilitated by RA [94]. RA is a metabolite of vitamin A and key enzymes responsible for its conversion are the retinal dehydrogenase (RALDHs), encoded by the *Aldh1a1*, *-2*, and *-3* genes. Certain lung DC subsets express *Aldh1a2* and therefore have the ability to produce RA, thus contributing to tolerance induction [54, 95]. Most interestingly, mice that lack pT_{reg} cells because of a deficiency in the intronic foxp3 enhancer CNS1 develop spontaneous Th2

inflammation and asthma [94]. Dendritic cells play a central role in regulating tolerance in several ways. They can mediate deletion and anergy of T cells, and have the ability to maintain homeostasis and induce de novo generation of pT_{reg} cells [96]. In the context of allergic inflammation, several studies have recently been published aiming at identifying the DC subset responsible for induction of pT_{reg} cells that damp allergic exacerbations. Almost a decade ago, evidence was provided that pDCs could initiate a tolerogenic response during allergic sensitization by inducing regulatory T cells. Induction of regulatory T cells most likely occurred in the draining lymph nodes, as pDCs could take up antigen and migrated here after allergen administration. pDC depletion using anti-Gr-1 or anti-BST2 broke tolerance against inhaled harmless antigens and adoptively transferred pDCs protected against asthma development [97, 98]. In support of this finding, administration of the hematopoietic growth factor Fms-like tyrosine kinase receptor-3 ligand (Flt3L) suppresses airway inflammation [99, 100] by enhancing the number of pDCs in the lungs [101]. Although depleting antibodies to Gr-1 and BST2 have often been used to study the role of pDC in vivo, these receptors are also broadly expressed on other immune cells, particularly during inflammation. New DT-based mouse models have recently been developed that allow specific elimination of pDCs [102]. The group of Malissen created a mouse in which an IRES-DTR-GFP cassette was introduced into the *Siglech* locus, which encodes the pDC-specific receptor Siglec H. These mice were deficient for Siglec H and pDC could be specifically ablated after DT administration. Using these mice, pDC depletion indeed showed to hamper pT_{reg} cell induction, confirming earlier findings for a role of pDCs in pT_{reg} cell induction [103]. In an attempt to identify pDC subsets, Lombardi et al. revealed that pDC can be subdivided into $CD8\alpha^{-}\beta^{-}$, $CD8\alpha^{+}\beta^{-}$, and $CD8\alpha^{+}\beta^{+}$ cells. While the $CD8\alpha^{-}\beta^{-}$ promoted AHR, adoptively transferred $CD8\alpha^{+}\beta^{-}$ and $CD8\alpha^{+}\beta^{+}$ were tolerogenic. The CD8-expressing pDCs exhibited high RALDH activity and promoted the differentiation of naïve $CD4^{+}$ T cells into $Foxp3^{+}$ regulatory T cells [104]. Although all these studies revealed that pDC can induce pT_{reg} cells that damp allergic reactions, a recent study by Khare failed to find this link. In this study, a tolerization model was used in which mice were treated for 10 consecutive days with 1 % OVA aerosols. This led to the de novo production of foxp3 regulatory T cells in adoptively transferred $foxp3^{-}CD4^{+}$ T cells originating from $DO11xRAG2^{-/-}$ mice. This pT_{reg} cell induction was drastically reduced upon depletion of $CD11c^{+}$ cells using CD11c-DTR-eGFP mice. Detailed analysis revealed that $CD103^{+}$ and a proportion of $CD11b^{+}$ DCs were depleted upon DT administration, while numbers of pDCs were increased. Using co-culture assays of sorted DC subsets and gene-deficient mice, $CD103^{+}$ DCs were shown to mediate induction of pT_{reg} cells in tolerized mice. In addition, $CD103^{+}$ DCs, but not pDCs, upregulated *aldh1a2* in tolerized mice, showing a role for $CD103^{+}$ DCs in inducing tolerance in this specific model [54]. This notion is also supported by other studies [53, 55]. In addition to DCs, a recent publication showed that tissue-resident lung macrophages can also promote the development of pT_{reg} cells in the steady state and express high levels of TGFβ and RALDH [9].

Macrophages Prevent DC Activation Upon Allergen Encounter

Besides the role for regulatory T cells in maintaining tolerance during allergy, recent studies have now described a role for resident macrophages in maintaining a pulmonary tolerogenic environment by inhibiting DC activation [105–107]. At least two types of macrophages reside in the airways of mice during steady state: alveolar macrophages and interstitial macrophages. Alveolar macrophages are most abundantly present and are enriched in the airway lumen, whereas interstitial macrophages are present in the intersitium. Both subtypes express F4/80, while the alveolar macrophages express high levels of CD11c and SiglecF. In the steady state, IM were capable of producing high levels of IL-10 and inhibited AHR induced by OVA-pulsed BMDCs. Moreover, depletion of IM using anti-F4/80 led to Th2 responses against harmless inhaled antigens, while AM depletion using liposomal clodronate did not induce eosinophilia [106]. Macrophages were shown to mediate tolerance through the transcription factor hypoxia-inducible factor (HIF)1a, which is well known for its prominent role in hypoxia. Mice that lacked HIF1a in LysM-expressing cells—mostly AM, IM, and a proportion of cDCs—had exacerbated AHR in response to inhaled allergens. Allergen sensitization induced by ova-loaded BMDCs could be inhibited when sorted HIF1a-sufficient IM, but not AM or HIF1a-deficient IM, were co-cultured with the BMDCs prior to intratracheal administration. Mechanistically, Myd88-dependent activation of the HIF1a signaling led to inhibition of DC function, leading to reduced airway inflammation in response to allergens [105]. The ability of pulmonary macrophages to prevent DC activation in response to inhaled allergens has recently also been demonstrated in rats. AM were depleted using clodronate liposomes, and replaced with AM from naïve or sensitized rats. Naïve AM abolished DC activation and Th2 polarization [107]. The role for IM has not been addressed in rats in this study; however, overall, these novel studies suggest that pulmonary macrophages can keep the airways in an tolerogenic state by preventing DC activation in response to inhaled allergens. These recent studies largely support the work of Holt et al. reaching the same conclusions using alveolar macrophages to suppress DC activation [108].

Concluding Remarks

Over the last year, much progress has been gathered on how lung dendritic cells initiate and maintain Th2 immunity and tolerance to allergens and how this function is influenced by lung macrophages and lung epithelial cells. This conceptual framework will be crucial in understanding the many genome-wide association studies and gene-by-environment interactions that are currently ongoing in large cohorts of children. Although much progress has been made in mouse studies, translational studies in human asthmatics are still grossly lacking.

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Th2 Cell Responses in Immunity and Inflammation Following Helminth Infection

Edward J. Pearce

Introduction

Helminth parasites are multicellular pathogens from two distinct phyla—the Platyhelminthes (flatworms) and Nematoda (roundworms). As a group they infect billions of people, largely in the poorest parts of the world where infection transmission is supported by underdeveloped sanitation and poor vector control [1]. While helminth infections have relatively low fatality rates they are increasingly recognized to be the cause of severe morbidity, and as such have recently gained heightened recognition as important but neglected tropical diseases [2, 3].

Despite enormous organismal heterogeneity and life cycle complexity amongst parasitic helminths, these pathogens are united immunologically by the fact that they nearly always induce pronounced Th2 immune responses. The origins of our understanding of this fact date to the observations that elevated IgE levels and eosinophilia are strong indications of helminth infections (see [4–6]). In the 1980s it became clear that the expression of IL-4 and IL-5, cytokines that control immunoglobulin isotype class switching to IgE in B cells, and increased eosinophil release from the bone marrow and survival in the periphery, are linked and characteristic of a subset of $CD4^+$T cells defined as T helper type 2 cells (Th2 cells) [7]. From this finding, it was a relatively straightforward step to the realization that the dominant response to helminth parasites was likely to be Th2 in nature [8]. Helminth infections are often chronic, and sometimes associated with the development of severe pathology, and early work linked Th2 response development during infection with the parasitic flatworm *Schistosoma mansoni* to the onset of disease [9, 10], so there was initially some debate as to whether Th2 responses serve any protective function

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in the context of helminthic disease [11]. However, it quickly became clear that Th2 cells regulate a spectrum of protective responses that allow animals to eradicate, or live with, helminth parasites [12–15].

The "Th2 Response": Innate and Adaptive Components

Put most simply, Th2 cells are MHC class II restricted CD4 T cells which make physiologically relevant levels of IL-4, IL-5, and/or IL-13. In humans and mice, the IL-4 allele is in a clustered locus with IL-5 and IL-13 and although each allele can be expressed independently, all three are often coordinately regulated, such that production of these "Th2 cytokines" is considered to be a mark of a Th2 response [16, 17]. In detail, the situation is more complex and the production of IL-4, IL-5, and IL-13 is often accompanied by the expression of additional genes, such as *Il9*, *Il10*, and *Areg*, that add to the overall Th2 signature (e.g. [18, 19]). While IL-4 is not strictly necessary for the development of Th2 cells, it is strongly supportive of the expansion and establishment of Th2 cell responses [20]. Th cells were named based on their perceived role in helping B cells to make antibody, and the production of IgG1 and IgE by B cells requires T cell help including the provision of IL-4, and increased amounts of specific and non-specific antibodies in these classes typify helminth infections [21]. It is now clear that a subset of Th cells, T follicular helper (Tfh) cells, which differ from naïve or effector cells in being specialized to enter B cell follicles and germinal centers, is specialized for helping B cells [22]. In helminth infections these cells share with Th2 cells the ability to make IL-4 (thus accounting for their ability to induce class switching to IgG1 and IgE production) but additionally produce IL-21 [23–25], a cytokine that is critical for plasma cell differentiation.

Helminth infection-induced immune responses in which Th2 cells are prominent are best considered "type 2" responses, since they are usually characterized by the additional participation of a range of cells of the innate immune system, including eosinophils, basophils, mast cells, and type 2 innate lymphoid cells (ILC2s), all of which have the potential to produce one or more of the canonical Th2 cytokines [26–30].

The Protective Roles of Type 2 Responses During Helminth Infection

Immunity to helminth infections is manifested in at least three ways (Fig. 1): (1) The resolution of a primary infection; (2) Resistance to reinfection; and (3) Protection of vital functions during chronic, immunologically unresolvable infection. Specific examples of these types of immunity will be discussed below.

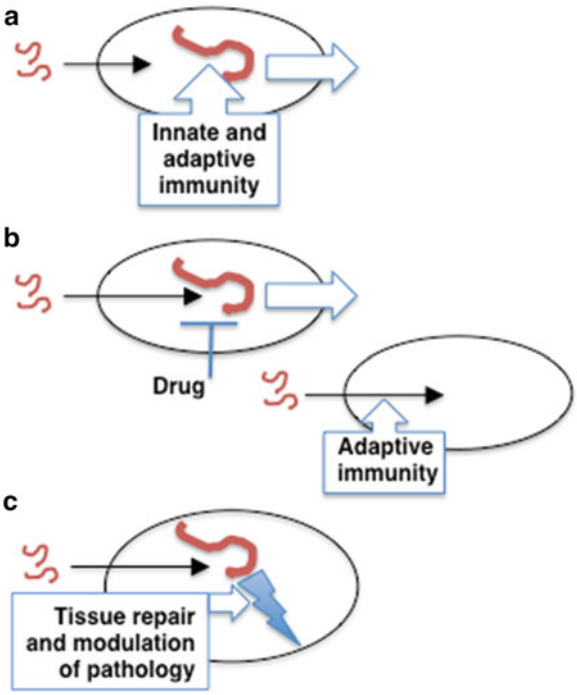

Fig. 1 Immunity to helminth infections is manifested in at least three ways. (**a**) The resolution of a primary infection. Some intestinal nematodes are killed as a result of the effects of IL-4/IL-13 produced by innate type 2 cells and Th2 cells on intestinal epithelial cells, including goblet cells, and on associated musculature. (**b**) Following drug treatment of some helminth infections, hosts are resistant to reinfection. Immunity may be mediated by cytokines from Th2 cells and cooperating innate system cells, through mechanisms similar to those that mediate resolution of primary infection, but enhanced by faster kinetics associated with the secondary immune response. Immunity is additionally more potent in some cases because of antibody that has developed as a result of initial infection and clearance, and because of the rapid recruitment of cells such M2 macrophages to the immune reaction that targets incoming larval parasites and prevents their establishment. (**c**) Protection of vital functions during chronic, immunologically unresolvable infection. In these settings, the immune response promotes tissue repair and sequesters parasites and any toxic molecules that they may make (indicated by the *lightning symbol*). In these settings, excessive tissue damage is prevented by immune system intrinsic regulatory mechanisms and immunomodulatory molecules released by the parasites themselves

Functionally, protective responses in each of these categories are largely mediated by the direct or indirect effect of type 2 cytokines on other cells, which assume effector functions under their influence. The primary effector cells of immunity to helminth infections are B cells (the antibody products of which can work in conjunction with other cell types), macrophages, granulocytes, epithelial cells, and muscle cells.

Resolution of Primary Infection

The resolution of primary infection is perhaps the exception rather than the rule since many parasitic helminths cause chronic infection—indeed, chronicity is often considered a hallmark of helminth infection. Nevertheless, various intestinal nematodes, including the whipworm *Trichuris muris* and the hookworm *Nippostrongylus brasiliensis*, are immunologically expelled by murine hosts shortly after adult worms establish in the intestine and initiate egg production (Fig. 1a). These protective responses are often heavily dependent on the early production of IL-13 and/or IL-4, and the ability of these cytokines to stimulate intestinal smooth muscle contraction [31, 32], altered intestinal epithelial cell function leading to increased luminal fluid flow [33, 34], increased epithelial cell turnover [35], and goblet cell expression of effector RELMβ, which inhibits parasite feeding and chemotaxis [36, 37], and Muc5ac, which is directly detrimental to *T. muris* and *N. brasiliensis*, as well as to another intestinal nematode *Trichinella spiralis* [38] (Fig. 2). Together these effects lead to worm expulsion. These processes can be stimulated by IL-4/IL-13 made by innate type 2 cells, or by Th2 cells and therefore additionally play essential roles in the spectrum of protective responses from those that begin to work during early primary infection to those recalled in immune animals upon re-exposure to infection. Interestingly, the ability of innate type 2 cells to participate in resolution of primary infection, as discussed below, is dependent on the presence of $CD4^+$ T cells (discussed in [28, 39]). Thus there is an intimate reciprocal link between innate and adaptive immunity during the development of responses that will lead to expulsion of primary infections and resistance to secondary infection.

Innate type 2 cells are present in naïve mice, poised to produce Th2 cytokines upon appropriate stimulation, and are mobilized within hours/days during the early stage of the response to helminth parasites. Because of the kinetics of the innate type 2 response, which occurs at a time when the adaptive Th2 response is at its very earliest stage of development, there has been a focus on whether the cells that make up this response are able to mediate innate protective responses against helminths and/or to help promote Th2 cell responses, by perhaps secreting IL-4 and/or IL-13. Of special current interest in this regard are ILC2s (Fig. 2). Discovered only recently, these cells are derived from lymphoid progenitors, but do not express markers of other immune cell lineages, or clonotypic antigen receptors [40–42]. ILC2s produce IL-5 and IL-13, exist throughout the body and play important homeostatic roles (e.g. [43]). However, ILC2s can become activated and increase in numbers in response to helminth infection, a response that is mediated by IL-25 and IL-33 [28, 40–42]. These cytokines are released by epithelial cells (and possibly other cells such as mast cells and macrophages) in response to necrotic damage or other signals (e.g. Trefoil Factor 2 [44]), and through their effects on ILC2s acts as "alarmins" to initiate innate responses [28] (Fig. 2). Conceptually, this mode of action fits well with the idea of intestinal or skin epithelial surfaces being broached by invading or migrating helminth larvae. Indeed, ILC2s are engaged rapidly following infection with *N. brasiliensis* [42, 45], and stimulated by IL-25 released by intestinal epithelial

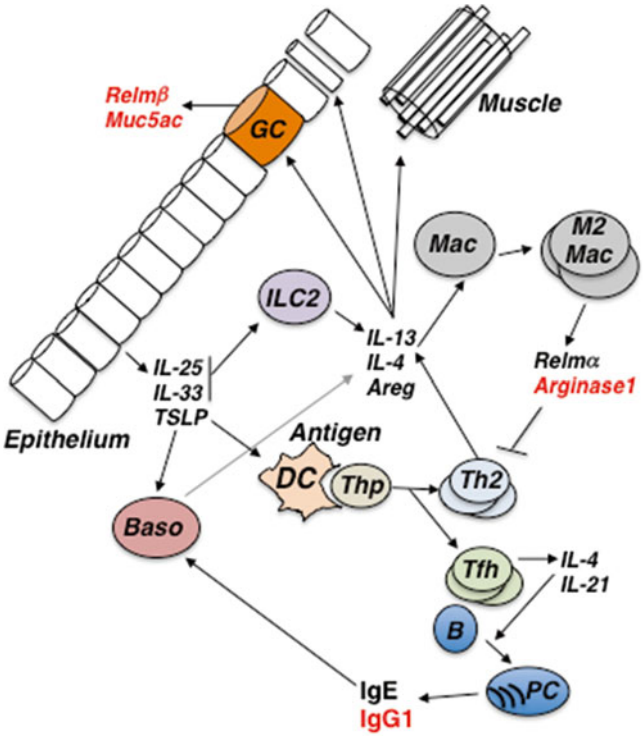

Fig. 2 Innate and adaptive type 2 immunity is tightly integrated. Innate responses to helminth parasites can be initiated by epithelial cell damage, leading to the production of the alarmins IL-25, IL-33, and TSLP. IL-25 and IL-33 can activate ILC2s to make IL-13 (but not IL-4), which has marked effects on epithelial cell (EC), goblet cell (GC), and muscle cell biology that together can promote the expulsion of intestinal parasites. IL-13 also alternatively activates macrophages, which can then proliferate and begin making mediators that lead to parasite damage, but which also promote tissue healing and regulate Th2 cell responses to prevent overt immunopathology. TSLP made by epithelial cells can promote basophil hematopoiesis and activation, and by inhibiting IL-12 production by DCs promotes the induction of Th2 cell responses. Many helminths also produce molecules that limit the ability of DCs to make IL-12. T-cell response initiation involves the extensive proliferation of T cells that are specific for the target antigens and the emergence of Th2 cells that make IL-13 and IL-4, and of Tfh cells that make IL-4 and IL-21 and are specialized to help IgG1 and IgE B-cell responses. Th2 cells can move into sites of infection where the cytokines that they make perpetuate effects on epithelial cells, muscle cells, and macrophages initiated by ILC2s. T cells also sustain ILC2 populations. Eosinophils are not depicted in this figure. However, these cells participate in type 2 immunity due to the strong production of IL-5 by ILC2s and Th2 cells. Mast cells are also absent from the figure, but would be expected to participate in type 2 responses. Mediators in *red* have been shown to have detrimental effects on helminth parasites. *PC* plasma cell. *Areg* amphiregulin, a cytokine made by Th2 cells and ILC2s that promotes epithelial cell turnover

cells [46]. In the absence of IL-25, or IL-25R, mice have fewer ILC2s and exhibit delayed parasite expulsion kinetics [41, 45]. However, injection of IL-25 into infected WT mice or transfer of activated WT ILC2s into infected IL-25R-deficient infected mice is sufficient to mediate rapid expulsion through an IL-13-dependent mechanism [41, 45]. Interestingly, in mice lacking the IL-33R (T1/TS2), or in which T1/TS2 is blocked, expansion of the ILC2 population following infection with *N. brasiliensis* fails to occur, and the mice are unable to rapidly clear the parasite [41, 44], indicating that despite their similar roles in promoting ILC2 activation, IL-33 and IL-25 must each have essential non-redundant functions in immunity, although what these are is currently unclear. IL-33 has also been shown to play a role in immunity to the nematode parasite to *T. spiralis* [47], and to be able to induce immunity to *T. muris* [48].

Multi-potent projenitor type 2 cells ($MMP^{type\ 2}$), IL-25-dependent but T1/ST2-negative and IL-33-independent cells associated with type 2 immunity but distinct from ILC2s, have been implicated in resistance to *T. muris* [49, 50]. As their name suggests, these cells have the potential to differentiate into other cell types, including basophils, monocytes, mast cells, and macrophages, and are thought to promote the expression of Th2 immunity in part through extramedullary hematopoiesis to produce cell types that contribute to protection.

Basophils, circulating cells that enter tissues from the blood, are also activated early following helminth infection during which they can rapidly accumulate in affected tissues and enter reactive lymphoid organs [51]. Based on the outcome of depletion by antibodies directed at the FcεR, basophils were implicated as antigen-presenting cells responsible for activating naïve $CD4^+$ T cells during the development of Th2 responses following infection with helminths [52]. However, the more recent use of engineered mice in which basophils are deleted with high specificity has shown that these cells are dispensable for polarized Th2 responses elicited by *N. brasiliensis* or *S. mansoni* [53]. Nevertheless, IL-4 and IL-13 from these cells contribute to the expulsion of primary *N. brasiliensis* infection [54]. Basophils also play an important role in the clearance of primary *T. muris* infection [55]. Immunity to this parasite is dependent on TSLP, another alarmin made by epithelial cells. In contrast to the situation in WT mice, peripheral basophil numbers along with Th2 responses and associated downstream effector functions (discussed below) fail to develop in infected $Tslpr^{-/-}$ mice, and adult parasites persist as a chronic infection. However, transfer of WT basophils into infected $Tslpr^{-/-}$ mice is able to partially restore the spectrum of type 2 immune responses and resulting worm expulsion [55]. This study revealed that TSLP can selectively promote basophil hematopoiesis and the emergence of a population of basophils that differs transcriptionally from basophils elicited by IL-3 (Fig. 2). TSLP has other important functions in Th2 immunity, as discussed further below.

Mast cells are found throughout the body, especially adjacent to epithelia. Mastocytosis is a common feature of helminth infections [56], and mast cells have been implicated in resistance to the nematodes *T. spiralis* [57] and *Strongyloides* spp. [58, 59]. There is recent evidence that mast cell-deficient mice have diminished Th2 responses and are more susceptible to primary infection with *T. muris* and to the trichostrongyle nematode *H. polygyrus* (once known as *Nematospiroides dubius*,

and more recently referred to as *H. polygyrus bakeri*) [60], due to a failure of ILC2 activation resulting from a deficit in IL-25, IL-33, and TSLP production by epithelial cells. Thus mast cells may play an important role in initiating type 2 immunity by provoking the production of alarmins, although the mechanism underlying this response is unclear at present.

Resistance to Reinfection

The second important role for type 2 responses in helminth infection is in adaptive immunity to reinfection. This is well illustrated by infection with *H. polygyrus*. Primary infection in mice with this intestinal nematode can be chronic even when the host mounts a type 2 response, but in these cases chemotherapy leaves the cured host immunologically resistant to secondary infection [61] (Fig. 1b).

There is a long-standing recognition that, while IL-4 and IL-13 made by memory Th2 cells may contribute heavily to resistance to secondary *H. polygyrus* infection by directly modulating epithelial cell, muscle cell, and macrophage responses [62], antibody is also playing a crucial role in adaptive immunity in this system [21]. Thus μMT mice and JhD mice, which lack B cells, and AID mice, which have B cells but are unable to secrete antibodies, are unable to resist secondary infection with *H. polygyrus*, despite developing what for the most part appear to be normal Th2 responses [63–65]. Moreover, IgG1 antibody from animals immune to *H. polygyrus* is able to passively confer immunity to naïve animals [63, 65, 66], and mice deficient in IL-21 fail to develop IgG1 secreting plasma cells and subsequently are unable to resist reinfection following drug cure [67]. Antibodies are also recognized to be important for protection against primary infection with *T. spiralis*, or *H. polygyrus* following maternal transfer in milk from immune mothers to offspring [68, 69].

Parasite-specific antibodies have also been shown to be capable of conferring protection against a broad spectrum of other helminth infections following experimental passive transfer, even in cases where there is not demonstrable role for antibody in naturally acquired immunity (reviewed in [21]). However, some of these findings have been difficult to reproduce, a problem that may be ultimately due to differences in antibody titer between different experiments. This would be consistent with the fact that there is a correlation between the efficacy of immune serum in passive immunization and the number of times the donor animals have been infected/boosted [21, 61], since titer would be expected to rise with each boost. In a real world setting, the role of immunologic boosting due to the death of existing parasites and reinfection has been postulated in human immunity to infection against schistosomes [70, 71]; in this infection, resistance can develop following drug treatment, and is correlated with the amount of IgE antibody against key parasite antigens [72].

Antibodies exert protective roles through a variety of mechanisms that vary depending on the infection and life stage targeted. For example, in the intestine, antibodies promote the entrapment of *T. spiralis* worms in mucus, leading to

expulsion [73], whereas in tissues antibody can mediate FcR-dependent cytotoxic effects by neutrophils and eosinophils, as illustrated in the case of immunity to the nematode *Strongyloides stercoralis* [74]. In *H. polygyrus* infection, antibodies associated with FcR on basophils allow the antigen-specific production of IL-4/IL-13 during challenge infection [75], leading to the induction of protective intestinal responses linked to the activation of epithelial cells, goblet cells, and muscle cells (Fig. 2), which together promote expulsion of worms from the gut [15]. Moreover, basophils can mediate protection against secondary infection with *N. brasiliensis* independently of Th2 cells [76].

In addition to antibody, macrophages also play a crucial role in immunity to *H. polygyrus*. These cells exist throughout the body as resident components of most tissues. These cells are embryonically derived, seeded into tissues in utero, and maintained by in situ proliferation [77, 78]. It is well established that during inflammation, additional macrophages of hematopoietic origin can develop from monocytes recruited from the bone marrow [77]. Macrophages play crucial roles in immunity and can adopt different activation states depending on context. Interferon-γ in combination with TLR agonists promotes M1 (or classical) activation, whereas IL-4 and IL-13 promote M2 (or alternative) activation by the IL-4Rα [79, 80]. From the host defense standpoint, M1 macrophages are inflammatory. In contrast, M2 macrophages are pro-angiogenic and pro-fibrotic, and make a range of molecules that serve to modulate inflammation, promote tissue repair, and regulate adaptive immunity [80, 81] (Fig. 2). Recent work has revealed that increases in macrophage numbers at sites of infection with helminth parasites can additionally, or exclusively, be driven by IL-4-stimulated proliferation of local macrophages [82] (Fig. 2), a finding that has spurred significant re-examination of the origin of these cells in different inflammatory settings.

M1 macrophages can kill growing schistosomes, and may be important for immunity to these pathogens in certain experimental vaccination settings where deliberately induced Th1 responses are protective [83]. However, M2 macrophages dominate during naturally developing type 2 responses during helminth infection, and play a significant protective role in some instances, such as *H. polygyrus* infection. In this case, ingested infectious larvae invade the intestinal wall before emerging into the intestinal lumen to grow into adult parasites. Whilst in their tissue invasive life stages, the parasites become foci of granulomatous inflammation, which by definition involves macrophages. Global deletion of macrophages during this period of a challenge infection in infected and cured mice effectively ablates protective immunity [62]. Immunity in this setting is sensitive to inhibition of arginase1, which is strongly expressed by M2 macrophages, and it appears as though larvae are being killed through a mechanism that induces metabolic stress [62]. M2 macrophages also play a role in inducing the IL-4/IL-13-dependent smooth muscle contractions that lead to the expulsion of *N. brasiliensis* worms [31].

Recently, M2 macrophages were shown to be capable of cooperating with neutrophils to kill *S. stercoralis* larvae in vivo [84]. While not restricted to roles in type 2 immunity, neutrophils do participate in inflammation associated with

helminth infections [15], playing a role in the promotion of type 2 immune responses during *N. brasiliensis* infection, for example [85]. However, they can assume pathologic roles in settings where type 2 immunity is less robust and Th17 responses emerge. This has been well documented in mouse strains which are genetically susceptible to developing acutely lethal disease when infected with *S. mansoni*, and in strains which normally develop chronic infection with this parasite, but which have been immunized prior to infection with schistosome egg antigens in complete Freund's adjuvant [86, 87].

The Protection of Vital Functions During Chronic Infection

The third role for protective type 2 responses is to allow host survival during chronic infection (Fig. 1c). This is the case during infection with the parasitic flatworm *S. mansoni*. Despite mounting a strong type 2 response during infection with this parasite, the host is unable to clear infection, which consequently is chronic. Nevertheless, loss of function of IL-4 in this system leads to severe morbidity and death associated with excessive inflammation in the absence of any increase in infectious burden [14]. During infection with this organism, eggs produced by the parasites (living in the portal vasculature) can become trapped in the sinusoids of the liver where they act as foci for $CD4^+$ T-cell-dependent granulomatous inflammation, a process that serves a critical host-protective role by participating in the sequestration of parasite eggs and the toxins that they secrete [88]. In the absence of IL-4 or IL-4Rα, schistosomiasis is acutely lethal [14, 89, 90], and this phenotype is recapitulated in mice that lack IL-4Rα expression on macrophages [89]; this indicates that the protective effects of IL-4 are mediated by macrophages and therefore, presumably, that M2 activation is critical. A failure to heal damage caused by the transit of parasite eggs into the gut lumen appears to be at least partially responsible for increased morbidity and mortality in the absence of M2 macrophages, although the emergence of M1 macrophages and associated inflammation also appears to play a contributing role [89]. A role for M2 macrophages in controlling acute tissue damage has also been noted in mice infected with the *N. brasiliensis*. Following transcutaneous infection, larval *N. brasiliensis* migrate through the lungs prior to entering the digestive system and maturing as adult worms in the intestine. In wild-type mice, pulmonary migration is associated with rapidly developing hemorrhage, inflammation, and reduced lung function, that resolves coincidently with the appearance of M2 macrophages at the site, but fails to resolve and rather is lethal in mice that lack IL-4Rα or are depleted of macrophages [91].

M2 macrophages also play an important role in regulating the intensity of the immune response to the benefit of the host. Several IL-4-induced genes are implicated in this process. For example, in *S. mansoni* infection, Relmα negatively regulates $CD4^+$ T-cell responses and in so doing prevents the development of severe type 2-associated immunopathology [92, 93] and Arginase1 produced by macrophages suppresses Th2 cell cytokine production and associated downstream inflammation

and fibrosis [94, 95]. Moreover, following exposure to the filarial nematode *Brugia malayi*, M2 macrophages develop the ability to potently suppress the proliferation of other cells through a cell contact-dependent mechanism that is presumably distinct from those mediated by Arginase1 or Relmα [96].

In the steady state, eosinophils have a clear role in adipose metabolic homeostasis [97]. During infections with helminths they increase in number and accumulate at tissue sites of invasion and inflammation. However, it has been difficult to assign eosinophils a defining role in either immunity or immunopathology commensurate with the magnitude at which they are involved in the response, and there remains a sense that the primary role of these cells during infection is yet to be discovered. Nevertheless, eosinophils have been shown to be capable of killing helminth larvae of various types [98], and can, along with M2 macrophages, make mediators such as Relmα, that regulate the intensity of type 2 inflammation [92].

The Modulation of Th2 Responses and Associated Inflammation During Chronic Helminth Infection: Everyone Benefits?

During chronic helminth infections caused by schistosomes and by filarial nematodes, Th2 responses peak during early stages of infection and then decline despite the fact that parasites, and therefore parasite antigens, persist [99–102] (Fig. 3). This process is reminiscent of the development of $CD8^+$ T-cell exhaustion during chronic viral infection [103]. It has been argued that loss of Th2 cell function over time in helminth infections reflects the development of adaptive immunologic tolerance to parasite antigens [104], resulting from persistent antigenic stimulation [105], and/or

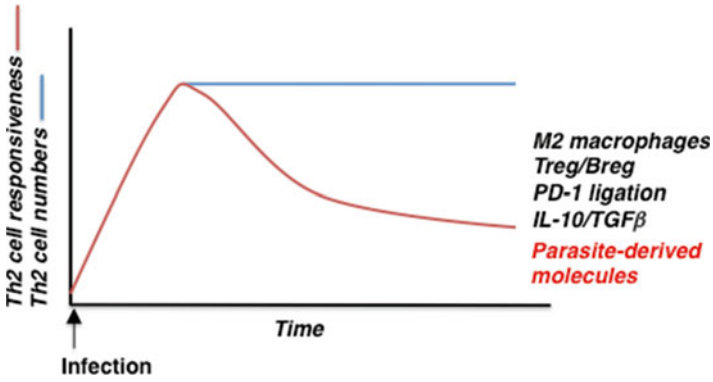

Fig. 3 The modulation of Th2 responses and associated inflammation during chronic helminth infection. Th2 cell responsiveness declines during chronic antigen despite the persistence of parasites. Immunomodulation does not reflect the loss of Th2 cells, but rather their regulation by M2 macrophages, Treg and Breg cells, cytokines, inhibitory receptor ligation, and parasite-derived molecules

extrinsic processes in which hyporesponsiveness is imposed by other cells such as M2 macrophages (discussed above, and [106]) or regulatory T (and B) cells (discussed by Maizels in "Regulatory T Cell Control of Type 2 Inflammation", and [107, 108]) (Fig. 3). The regulatory cytokines TGFβ and IL-10 have been implicated in this process [109], and IL-10 serves the additional function of suppressing residual Th1 responses that can occur in certain helminth infections, and therefore further polarizes the adaptive response in the Th2 direction [110, 111] (Fig. 3).

Downregulation of Th2 responses during chronic infection is generally thought of as being advantageous in settings where the immune response is incapable of clearing the infection and Th2 cells are causing immunopathology. This is the case in schistosomiasis, where despite the fact that the type 2 response plays a vital tissue-protective role there is a risk that the inflammatory and wound healing components of this type of immunity can themselves become pathological. Specifically, ongoing schistosome egg deposition and focused production of the pro-fibrotic cytokine IL-13 (which is linked to protective IL-4 production) in the liver can lead to severe fibrosis with portal hypertension [112]. In the absence of appropriate regulatory mechanisms, these processes can become overwhelmingly damaging (e.g. [94]).

Antibody can also play a protective role during helminth infection by regulating inflammation [21]. This is apparent in chronic *S. mansoni* infection in B-cell-deficient mice, in which immunopathology is exaggerated, leading to greater morbidity and mortality than is the case in infected wild-type mice [113]. Mechanistically, immunoregulation by antibody is likely to reflect the binding of IgG1-containing immune complexes to macrophages [114], with resultant anti-inflammatory effects, since in other systems macrophages that interact with immune complexes assume marked regulatory roles [115, 116], by producing IL-10 and TGF-β1, two cytokines which play important roles in regulating inflammation during schistosomiasis [117]. More broadly, the roles of B cells and antibody in survival during chronic schistosomiasis may reflect a mechanism analogous to that mediated by intravenous immunoglobulin therapy (IVIG), which is used successfully in humans for the treatment of autoimmune diseases [118]. Recent work has shown that the canonical type 2 cytokine IL-4 induces the increased expression of FcγRIIB on monocytes in humans and mice, and that mice which lack IL-4 or FcγRIIB are not protected against inflammation by IVIG [119].

In some cases, Th2 response downregulation favors parasite persistence. For example, reversal of hyporesponsiveness by blockade of the inhibitory receptor PD-1 expressed by Th2 cells during chronic infection with the filarial worm *Litomosoides sigmodontis* infection allows the expression of effective antiparasite immune responses [120]. The realization that type 2 immunity is often tightly regulated during helminth infections, and that these processes can favor parasite survival, led to the realization that parasites are able to produce molecules that strongly influence the immune response [121] (Fig. 3). The characterization of these molecules, and the possibility that they might be developed for therapeutic use in conditions where immune responses (particularly type 2 responses) are pathogenic, such as allergy and asthma,

is a subject of considerable current interest (discussed by Loukas in "Developments in the Design of Anti-helminth Vaccines," and [122]). The fact that many inflammatory conditions are alleviated by helminth infection attests to the promise of this approach [123].

How Do Helminth Antigens Promote Type 2 Responses?

One of the greatest advances in our understanding of the how type 2 immunity develops following exposure to helminth parasites has come from recent work on the "alarmins," IL-25, IL-33 and TSLP, cytokines made by damaged or activated epithelial cells that trigger innate immune responses and help orchestrate complementary adaptive immune responses (Fig. 2). IL-25 and IL-33 have been discussed above. The third alarmin, TSLP, has at least 2 known functions in type 2 immunity. The first is to suppress IL-12 production, thereby favoring the induction of Th2 responses [124]. TSLP is critical for type 2 response development during infection with *T. muris* [55, 124] but not during infection with *H. polygyrus*, *N. brasiliensis* [125] or *S. mansoni* [126], which may reflect differences in the inherent abilities of these parasites to suppress the production of IL-12 by DCs [125]. The second function of TSLP is to promote basophil hematopoiesis that is independent of IL-3, the cytokine conventionally associated with basophilia [55]. Wild-type basophils induced by TSLP, which are functionally distinct from IL-3-elicited basophils, are capable of restoring immunity to *T. muris* in otherwise susceptible $Tslpr^{-/-}$ mice [55].

The fact that IL-4 is essential for Th2 cell differentiation in vitro led to much speculation that an innate source of IL-4 would be critical for Th2 cell development in vivo, and consistent with this there have been many reports that type 2 innate cells are key players in Th2 cell activation. However, early observations showed that DCs exposed to helminth antigens preferentially induce Th2 cell differentiation, suggesting that despite the ability of many other cell types to contribute to type 2 immunity, direct contact of parasite products with these APCs is sufficient for Th2 polarization [127, 128]. The subsequent establishment of the primary importance of DCs in Th2 response development during helminth infections [129, 130], and the molecular identification of helminth products such as nematode chitin [131], and *S. mansoni* Omega 1, that possess Th2 adjuvanticity and are, at least in the case of the latter, able to drive Th2 cell development through effects on DCs [132, 133], has led to considerable interest in specific pathways activated in DCs that condition them to preferentially induce Th2 responses [29]. A major focus of this work has been on the identification of lectins that recognize and permit the uptake of helminth glycoproteins [134, 135], with mannose receptor being implicated as playing a major role in this process [136]. A detailed discussion of the role of distinct dendritic cell subtypes in Th2 immune response induction can be found elsewhere in this volume in the chapter "Dendritic Cells and Type 2 Inflammation" by Lambrecht.

Summary

The response to helminth infections involves the engagement of innate type 2 cells that in the steady state play important roles in metabolic homeostasis and sterile wound healing, and the overlapping initiation of adaptive immune responses in which Th2 cells, in an antigen-specific manner, make many of the same cytokines that are made by the innate type 2 cells. The type 2 cytokines IL-4 and IL-13 made during these innate and adaptive responses activate a variety of other cell types, notably including macrophages, that play crucial roles in parasite expulsion, or in establishing an environment in which infected hosts can survive in the face of ongoing tissue damage associated with worm persistence. A key feature of the adaptive response is the emergence of B cells making helminth-specific antibodies that can interact with other cell types, or act directly, to provide protection against further infection. During chronic infection with helminths, regulatory mechanisms develop, in part stimulated by immunomodulatory parasite products, that promote host and (directly or indirectly) parasite survival and have beneficial effects that can ameliorate unrelated inflammatory conditions. Rapid advancements in our understanding of type 2 immunity raise the possibility of the rationale development of new immunologic approaches for preventing or treating helminth infections, and developing approaches to minimize the effects of the inflammatory diseases that emerge in areas where helminth parasite transmission is prevented.

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Regulatory T-Cell Control of Type 2 Inflammation

Rick M. Maizels

Introduction

The adaptive immune response to challenge by exogenous pathogens or toxins, or exposure to innocuous commensals and environmental proteins, is orchestrated above all by the compartment of $CD4^+$ T lymphocytes (Fig. 1). These provide firstly a cell population with a vast array of antigen-specific receptors enabling each foreign specificity to be recognized and attacked, meanwhile generating an immunological memory for any future engagement. This population constitutes many forms of potent effector cell phenotypes which can drive inflammation, recruit and drive differentiation of other immunocytes, and promote inflammation and pathogen clearance. Critically, however, $CD4^+$ cells also include suppressive regulatory cell subsets which dampen reactivity and ensure a return to homeostatic balance once danger has passed. The interaction, and interchange, between these effector and regulatory populations is as critical in the setting of Th2 inflammation as in any other area of immunology, as detailed below.

Understanding the interaction and mechanism of Treg suppression is of fundamental importance for the control of immunopathology, infection, tumors, and transplantation [1, 2]. Exciting new therapies can be envisaged which Tregs may be induced or expanded to control major autoimmune diseases, promote allograft tolerance or resolve inflammatory bowel conditions [3]. Equally importantly, new insights may lead to the repression of Tregs in cancer settings where they are detrimental. In a similar manner, in the context of the Th2 response, there is a need to

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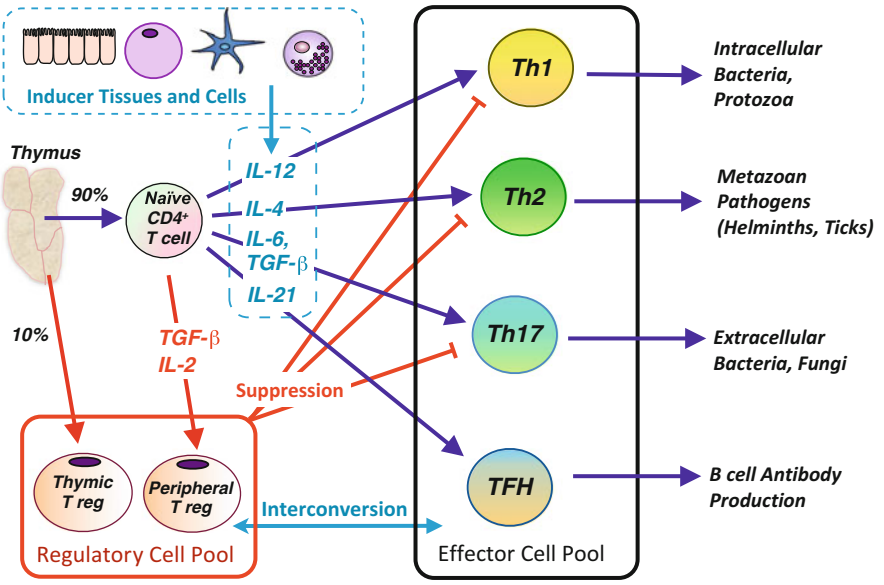

Fig. 1 CD4+ T cells: decision makers and executors. T-cell responses are induced by professional antigen-presenting cells, innate lymphoid cells, granulocytes, and inflamed tissues releasing key cytokines including IL-4, IL-6, IL-12, IL-21, and TGF-β. These drive differentiation into Th1 subsets. Regulatory cells develop as around 10 % of T cells leaving the thymus; the pool of thymic Tregs is boosted by peripheral induction of Tregs through IL-2 and TGF-β. These Tregs can suppress responsiveness by each of the T effector cell subsets. Under certain circumstances, Tregs can convert to effector function, or effector cells may switch to regulatory function

both enhance and inhibit Treg activity, to respectively ameliorate Th2 pathologies such as allergy, and to boost the protective Th2 response to helminth parasite infection, as discussed in this chapter.

Th2 and T-Cell Subsets

The classical dichotomy of CD4+ T-cell subsets into Th1 and Th2 was established in the 1980s and remains a guiding paradigm in immunology, separating the more aggressive IFN-γ-mediated inflammatory cellular pathway suitable for clearing intracellular pathogens from a more antibody-dominated mode attacking multicellular parasites [4]. In particular, the Th2 mode of responsiveness is closely associated with IgE and allergy, as well as with a range of innate cell types such as eosinophils, mast cells, and goblet cells which are responsible for localized or systemic inflammatory conditions. Each of these components of the Th2 network are activated by key cytokines produced by Th2 cells, most predominantly IL-4 which itself induces Th2 differentiation and B-cell isotype switching [5]. Many functions of IL-4 are replicated by IL-13, as receptors for these two cytokines share the IL-4Rα receptor

chain [6, 7], for example driving macrophages into the alternatively activated state, and promoting goblet cell hyperplasia [8]. The cytokines IL-3, IL-5, and IL-9 are also major products of Th2 cells, inducing differentiation and expansion of basophils (IL-3) [9], eosinophils (IL-5) [10], and mast cells (both IL-3 and IL-9) [9, 11], while IL-10 [12] is a multi-potential cytokine with both inductive and suppressive features as detailed in a later section.

Importantly, the Th2 response does not operate in isolation but in conjunction, competition, or antagonism with a number of other T-cell types (Fig. 1). The Th2 phenotype overlaps with more recently defined, distinct subsets including the T-follicular helper cell (TFH) [13, 14] and cells dedicated to the production of IL-9 ("Th9" cells) [15]. There is a both competition and antagonism with more contrasting Th phenotypes, the Th1 mentioned above (for example through IFN-γ-mediated inhibition of Th2 differentiation) as well as newer Th17 [16, 17] and Th22 [18] subsets. However, the most potent antagonist of Th2 responsiveness and the focus of this chapter is the repressive pathway of regulatory T cells (Tregs) which block the effector phase of the Th2 response.

The Broader Type 2 Network

Th2 effector responses rely directly upon a portfolio of cell types from the immune system (e.g. dendritic cells, macrophages, and granulocytes) as well as non-immunological (e.g. epithelial) tissues (Fig. 2). As mentioned above, this responder network is driven and bound together by the two cytokines which signal through the IL-4Rα chain, IL-4, and IL-13 [8]. Crucially, these cytokines are produced not only by activated Th2 cells, but also a range of innate immune cells including basophils [19, 20], eosinophils [21], mast cells [22], and recently defined innate lymphoid cells [23–26]. An even wider range of cell subsets and tissues respond through IL-4Rα signaling, including T cells differentiating from the naïve state, B cells and granulocytes, and epithelial cells (Fig. 2). In particular, the macrophage compartment responds in a distinct fashion to IL-4/IL-13, by adopting an "alternative activation" mode, distinct from IFN-γ-mediated classical activation [27, 28]. Alternatively activated macrophages adopt a contrasting metabolic state of β-oxidation of fatty acids rather than glycolysis associated with classical activation [21], and produce high levels of a chitinase-like protein, Ym1 (Chi3L3), a resistin-like molecule (RELMα), and the enzyme arginase-1 [29]. Arginase-1 competes for the same substrate, L-arginine, that is required by inducible nitric oxide synthase (iNOS) in classically activated macrophages, reinforcing the diametrical relationship between these two phenotypes. Thus, the alternatively activated macrophage is both a hallmark of the innate Type 2 response, and a reflection of a dominant Type 2 environment.

Additional interest has recently focused on the role of innate lymphoid cells in the initiation and expression of the Type 2 response [24–26]. These lack markers of major hematopoietic lineages, such as B cell, T cell, or myeloid surface proteins, but

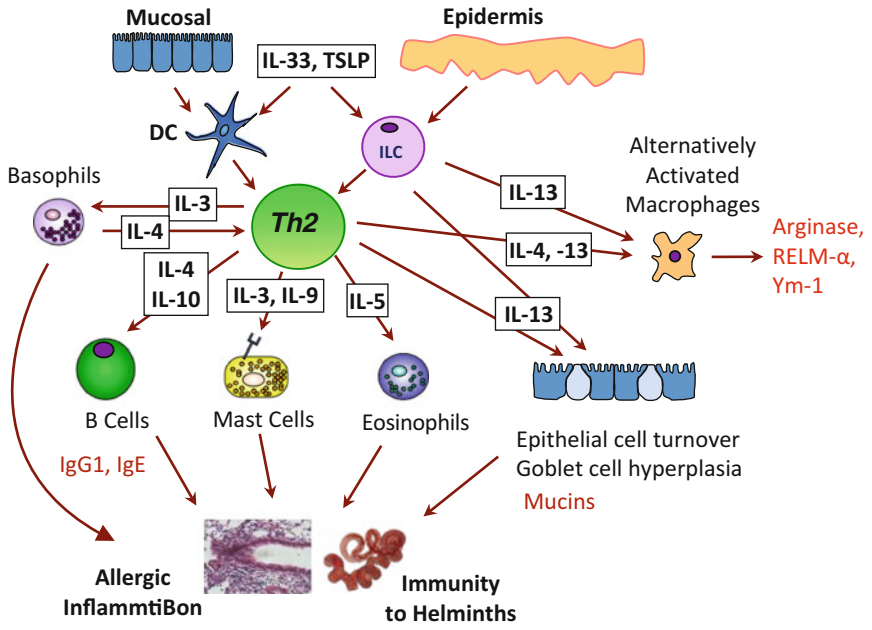

Fig. 2 Innate Type 2 mechanisms: inducers and effectors. The innate system induces Th2 immune responses through activated dendritic cells and innate lymphoid cells reacting to key alarmin cytokines (such as IL-33 and TSLP) released from barrier surface cells, e.g. in the mucosa or skin. Activated innate cells release key cytokines, including IL-13 which can directly drive alternative activation of macrophages (marked by the production of Arginase-1, RELMα, and Ym1). T cells driven to the Th2 mode of differentiation produce a wider range of cytokines, which further activate innate partners such as basophils, mast cells, eosinophils, and goblet cells. Some of these cell types strengthen the Th2 response by producing additional IL-4. B cells also respond to Th2-derived IL-4 and IL-10 which co-regulate the production of IgG1 and IgE. The combination of these cytokines and effector populations result in beneficial outcomes of parasite expulsion as well as detrimental syndromes such as allergy and asthma

produce key cytokines at early stages of an immune response, which may be IFN-γ in the case of ILC1 cells, IL-4, IL-5 and IL-13 (from ILC2s) or IL-17/IL-22 (from ILC3s) [30]. Type 2 cytokines from ILC2s can generate a T-cell-independent eosinophilia and alternative activation of macrophages, as well help induce the adaptive Th2 response to antigen. Hence, Type 2 immunity encompasses parallel innate and adaptive arms which can work independently, or in sequence and in concert.

Type 2 Inflammation

In many immunological lexicons, inflammation is synonymous with Th1/Th17-dependent outcomes including leukocyte infiltration into tissues, edematous permeabilization of the vasculature, and systemic effects on body temperature and metabolism.

However, a specific set of inflammatory processes are mediated by the Th2 pathway. These include, most conspicuously, allergic inflammation which in an IgE-dependent manner releases a battery of vasoactive mediators, recruits eosinophils into barrier tissues (particularly the lung), and promotes copious goblet cell hyperplasia and mucus production. Chronic inflammatory disorder can lead to tissue remodeling and airway remodeling, leading to an aggravated asthmatic state. Other Th2-associated pathologies include ulcerative colitis in humans, in which IL-13 is predominant [31], skin inflammatory conditions such as atopic dermatitis [32], and the most dramatic of all—systemic anaphylaxis, a life-threatening condition mediated by IgE hypersensitivity to allergens. In each of these settings, immunological intervention by manipulating the balance of T-cell subsets represents an attractive but untested strategy to alleviate human disease.

The interconnection between innate and adaptive mechanisms is nowhere plainer than in the chemokine system, mediated by short-range chemotactic factors which induce tissue infiltration and amplify inflammation [33]. Committed Th2 cells initiate expression of CCR3, CCR4 and CCR8, as well as CCR7 which is required for T-cell entry into and egress from peripheral tissue [34, 35]. CCR3 is expressed by eosinophils, mast cells and basophils as well as Th2 cells [36], and is the principal receptor for eotaxin (CCL11), illustrating the commonality between different subunits of the Type 2 response. CCR4 binds to two Type-2-associated chemokines, CCL17 (TARC, thymus, and activation-regulated chemokine) and CCL22 (MDC, Macrophage-derived chemokine), each of which are induced by IL-4 or IL-13, and are highly upregulated in allergic asthma and eczema. CCR5 is also shared by eosinophils, basophils and T cells and is bound by CCL5 (RANTES), while CCR8 was reported to be most closely associated with Th2 cells producing IL-5 [37] or IL-10 [38]. These, and many additional, chemokine interactions can result in intense recruitment of cells to a localized focus of inflammation, which may be centered around an invading parasite, a sterile wound or even a reaction to a harmless allergenic particle.

Regulatory T Cells

Regulatory T cells (Tregs) are now universally recognized as the key policing population that ensures immunological integrity and balance in a challenging environment [39]. The archetypal marker of Tregs is the transcription factor Foxp3, and mutation of this locus leads to dysregulated immunity and severe inflammatory disease [40]. Two pathways lead to the development of this suppressive phenotype: in the thymus, a subset of around 10 % of all cells exiting into the periphery are $Foxp3^+$ regulatory cells, carrying an apparently stable epigenetic imprint that maintains their function. A second pathway acts upon the other 90 % of potentially pro-inflammatory $CD4^+$ T cells in the periphery: they can be induced into the regulatory phenotype by an appropriate environment, for example by exposure to TGF-β at the time of TCR engagement [41]. This subset is thought to be less stable and more

prone to revert to an effector status. These two lineages are now termed thymic and peripheral regulatory T cells (tTreg and pTreg respectively) [42].

The role of Tregs was first established in model systems by their ability to suppress autoimmunity [1] and colitic disease [43, 44], and by the fact that Treg-deficient mice rapidly succumb to uncontrolled systemic inflammation [45]. Pathology in the absence of Tregs is particularly intense in the intestinal tract, indicating a key role in controlling responsiveness to commensal bacteria and food antigens [46]. Thus, Tregs may not only block autoimmunity against self-antigens but also deleterious responsiveness to harmless exogenous specificities [47]. The importance of the latter type, presumably induced in the periphery rather than the thymus, is demonstrated by the intestinal pathology in mice lacking the CNS-1 intronic control region of Foxp3, which is targeted by the TGF-β pathway for induction of pTregs [48]. Interestingly, in these latter mice, intestinal inflammation showed excessive Th2 characteristics.

Thus, while autoimmunity and colitis are predominantly Th1/Th17-dependent pathologies, Tregs are also able to suppress Th2 responses, as confirmed by elevated Type 2 cytokines in Foxp3-deficient mice [45, 49]. Interestingly, Tregs may need to adopt some of the characteristics of the effector population they block for suppression to take place: for example, Th17-mediated colitis is prevented by Tregs that express Stat3, a signaling molecule involved in Th17 function [50], perhaps to share the same migratory properties as the effector population in question. Similarly, Th1 reactions (e.g. to *Mycobacterium tuberculosis*) are repressed by Tregs that must express the canonical Th1-associated transcription factor Tbet, which drives expression (through Stat1) of CXCR3 and allows Tregs to infiltrate the same tissues as the Th1 effector population [51]. Most germane to the focus of this article, Th2 responses are most potently suppressed by Tregs expressing IRF4, a Th2-associated transcription factor, as indicated by selective enhancement of Th2 cytokines, and augmented IgE production, in mice carrying a Treg-specific deletion of IRF4 [52].

Molecular Pathways of Suppression of the Th2 Response

Tregs were originally defined by their ability to directly abrogate the pathological effect of T cells in vivo, for example when either or both are transferred into a T-cell-deficient mouse [53, 54]. Subsequently, in vitro suppression assays demonstrated inhibition of effector T-cell proliferation when co-incubated with Tregs [55], demonstrating direct Treg–Teff interactions in the suppressive process. In both in vivo and in vitro settings, a series of key mechanistic suppressive pathways have now been identified [56–58].

Foremost among these mechanisms is the release of suppressive cytokines, which act on a broad range of target cells and tissues, with potential for systemic effects beyond the site of Treg activation. The two principal suppressive cytokines, discussed below, are TGF-β and IL-10 which have been extensively reviewed elsewhere [59]. In addition, IL-35 is a further immunoregulatory cytokine produced by

Tregs that can exert similar down-modulatory effects on helper T cells independently of TGF-β or IL-10 [60]. In each case, these cytokines also act in a feed-forward loop to induce further Tregs of the corresponding subtype, i.e. Foxp3^{+} pTreg, IL-10^{+} Tr1, and Tr35 [56].

Tregs are marked by high expression of CD25, the high-affinity IL-2 receptor, and by consuming IL-2 in their vicinity may depress levels of this growth factor which is required by newly activated effector T cells. Indeed, the ability of Tregs to block proliferative responses in vitro has been directly ascribed to IL-2 depletion by cells in culture [55], and effector cells may be driven to apoptosis by the lack of IL-2 [61]. With the recent discovery that innate lymphoid cells are also IL-2-dependent [24], Tregs may also be able to restrain undesired innate reactivity through similar withdrawal of an essential growth factor.

The second generic set of suppressive mechanisms operate through cell signaling at the interface between Tregs and their target cells. Among these is TGF-β, which may be immobilized at the cell surface, allowing directed delivery of inhibitory signals through the TGF-β receptor on any interacting cell [62]. The most important surface signaling molecule associated with downregulation, however, is CTLA-4. This member of the CD28 co-stimulatory family acts as an inhibitor of activation of T cells, primarily by interfering with positive signaling to effector cells encountering cognate antigen at the priming phase; CTLA-4 acts by removing ligand from the co-stimulatory molecules CD80 and CD86 [63]. Blocking CTLA-4 antibody abrogates the ability of Tregs to suppress in vitro [64] and in vivo [65], and the use of a Foxp3^{Cre}-mediated Treg-specific deletion of CTLA-4 confirmed that intrinsic expression of this molecule by Tregs in vivo was essential to avoid a panoply of inflammatory outcomes, including excessive IgE production [66].

Thirdly, some Tregs can exert direct cytotoxic effects on responder populations, via the release onto the surface of the target cell of Granzyme B, a serine protease more widely associated with CD8^{+} cytotoxic T cells and NK cells. In mice, while wild-type and perforin-deficient Tregs were able to prolong skin allograft survival in vivo, Granzyme B-deficient Tregs failed to do so [67], pointing to a specific role for this cytotoxic pathway in Treg function.

Fourthly, another close-range means by which Tregs can inhibit the effector arm of immunity is through the generation of adenosine in the local environment [68]. Adenosine binds to the A2A purinergic receptor of responding T cells leading to an inhibitory state of high intracellular cAMP; Tregs express two key ectoenzymes, CD39 and CD73 which convert ATP thorough AMP to adenosine [69], with CD39 (which metabolizes ATP to AMP) upregulated on Tregs [70] and CD73 (which dephosphorylates AMP to adenosine) induced on all T-cell subsets by the action of TGF-β [71]

Finally, Tregs can act indirectly by modulating accessory cells of the innate immune system, in particular dendritic cells, leading either to tolerization of the effector T-cell population or in some cases their conversion into additional regulatory phenotype cells for more complete suppression. For example, when Tregs and Teffs are co-cultured with DCs, Tregs preferentially bind and aggregate (through LFA-1) around the DCs, obstructing access for the effector T cells, while also induc-

ing a downshift in CD80/86 co-stimulatory molecules such that any subsequent interaction with naïve T cells will be intrinsically tolerogenic [72].

The Role of TGF-β

TGF-β is one of the central cytokines in the dynamics of T-cell development and regulation [73, 74]. Co-incident ligation of the TCR and the TGF-β receptor induces expression of Foxp3 in nonregulatory CD4+ T cells and drives them into the regulatory compartment; hence pTregs are highly TGF-β-dependent. In contrast, TGF-β is not required for thymic Treg development, although it acts to stabilize and sustain tTregs in vivo [75].

TGF-β also acts on effector T-cell populations in a context-dependent fashion, with a correspondingly varied set of outcomes consistent with its biological role as an inducer of differentiation rather than a specialized immunosuppressive cytokine. In the absence of an inflammatory milieu, TGF-β will induce Foxp3 expression in naïve CD4+ T cells, and drive them into a functionally suppressive Treg phenotype [76, 77]. However, in the presence of inflammatory cytokines such as IL-6, naïve T cells exposed to TGF-β develop along the Th17 pathway [78, 79] (Fig. 1), while if IL-4 is predominant, Th9 expression is favored [15, 80].

TGF-β is also an important inducer of tissue repair pathways, and represents an important interface between immunity and wound healing [81]. Hence, its therapeutic application is not straightforward, as it can act in different settings to promote inflammation or repair, or indeed act to excess as in the case of fibrosis in damaged tissue.

Role of IL-10

IL-10 is viewed as a canonical immunoregulatory cytokine, as evident by the disseminated inflammatory disease that occur in IL-10-deficient mice [59]. It is produced by a range of immune and innate cells, but in particular by T cells, and is a member of a cytokine family (including IL-22) with broader roles in maintaining barrier integrity [82]. IL-10 is expressed both by conventional $Foxp3^+$ Tregs as well as by a subset of $Foxp3^-$ IL-10-producing T cells which are often regarded as a further, Tr1, regulatory subset [83]. In some Th2 settings, such as in mice developing hepatic granulomas around schistosome eggs, IL-10 protects animals from lethal inflammation although it does not dampen the overall level of Th2 responsiveness [84, 85]. In this regard, Th2 immunity is relatively inured to suppression by IL-10, but many other cell types such as dendritic cells and granulocytes are downmodulated by this cytokine, which may therefore play an overall dampening role in Th2 pathologies [86].

IL-10 is also responsible for promoting Th2 responsiveness in vivo, most probably through the differential repression of Th1 leading to reduced IFNγ levels.

In the absence of IL-10, protective Th2 responses to helminth infection can be ablated [87]. In addition, IL-10 can suppress Th17 responses [88], again promoting Th2 dominance. Hence, overexpression of IL-10 can provoke symptoms of airway hyper-responsiveness, presumably by unbalancing the homeostatic role of IFN-γ and inhibiting competing T-cell subsets [89].

Activation and Migration of Tregs

For Tregs to exert a suppressive effect, they must co-locate themselves to the site of immune activation and/or inflammation, requiring the expression of a suite of integrin markers and chemokine receptors. It is likely that these are upregulated following "activation" of Tregs, although the activation state(s) of this population remain poorly defined. Like all T cells, Tregs may be stimulated by cognate antigen through their TCR, but as thymic-derived Tregs may be in constant contact with self antigen, other signals such as TLR ligation or other inflammatory input may also be required to trigger functional suppressive effects. A prominent marker associated with Treg activation is CD103, the $\alpha_E\beta_7$ integrin associated with infiltration of mucosal tissues through binding E-cadherin [90], and highly elevated in Tregs infiltrating colonic tumors [91]. Alongside CD103, activated Tregs raise levels of ICOS, and monoclonal antibody blockade of ICOS function in diabetes-prone mice accelerated disease onset and reduced Treg numbers in the inflamed pancreas [92].

CD103 is upregulated by stimulation with TGF-β [93], which is a potent inducer and sustainer of Treg populations [75]. Activated Tregs also express CCR7 as well as L-selectin (CD62L) which are required for entry into lymph nodes where they may influence dendritic cells and potential effector T-cell populations, while CCR9 may be required for Tregs to enter inflamed tissue as reported in the intestinal setting [94], just as CCR6 is necessary for Tregs to suppress Th17-mediated inflammation in autoimmune disease [95]. Other key factors now known to determine Treg infiltration include GATA-3, which as well as being the central transcription factor for committed Th2 cells, is required in activated Foxp3$^+$ Tregs for both migration into intestinal tissue and to maintain a fully suppressive phenotype [96].

Treg Suppression of Type 2 Inflammation in Allergy

It is now clear that Tregs can control Th2 responsiveness in a wide range of pathological settings [97–101]. Allergic airway inflammation, provoked in mice by allergen sensitization with the Th2-biasing adjuvant alum, can be alleviated by the transfer of Tregs [102, 103] which express Foxp3 [104]; in these reports, protection was associated with IL-10 production. Further evidence for Treg suppression of Th2 inflammation in the airways was found in mice carrying various infections including bacteria such as *Mycobacterium vaccae* [105] and *Helicobacter pylori* [106] as well as by

helminth parasites (see below). Thus, a regulatory T-cell population from *M. vaccae*-treated mice transfers protection against airway inflammation into recipient mice, which is blocked by antibodies to either IL-10 or TGF-β [105], while in the case of *H. pylori*-induced suppression it was shown that depletion of Foxp3$^+$ Tregs from the transferred population completely ablated protection in recipient mice [106].

Consistent with these studies in laboratory mice, investigations in human patients argue that Tregs are a decisive factor in susceptibility to allergy against pollen or house dust mite [107, 108], cow's milk [109], and bee venom [110]. From these studies, the balance between regulatory and effector subsets appeared to determine allergic or healthy outcomes to allergen exposure, and dynamic shifts in this balance switched patients between one status and the other. Localization of Tregs is a further important factor, with a deficiency in skin Tregs evident in atopic dermatitis patients [111]. In human neonates, homeostasis has yet to be established with regulatory populations slower to gain function than the effector population [112], while in patients with steroid-resistant asthma the correct balance can be induced by administration of vitamin D [86]. Again, IL-10 has been identified as an important suppressive mediator [86, 110].

Tregs in Ulcerative Colitis

Ulcerative colitis (UC) is one of the two main forms of inflammatory bowel disease, together with Crohn's disease [113]. UC is often considered to represent an "atypical" Th2-mediated pathology due to high levels of IL-5 and IL-13 together with more "Type 1" cytokines such as TNF, while Crohn's disease is a more conventional Th1/Th17-dependent syndrome. While the evidence from mouse models of colitis unequivocally demonstrate the absolute necessity for Tregs in controlling intestinal inflammation [114], the relationship in human UC is less well documented. For example, while peripheral blood Tregs are sparser and less potent in UC patients [115], there does not appear to be a local deficiency of Foxp3$^+$ Tregs in the intestinal tissue [116]. However, a more recent study of patients receiving leukocytapheresis therapy found that Treg:Teff ratios increased in cases entering remission, but not in those failing to respond to treatment [117]. While no mechanistic insights are yet available into whether this represents a direct Treg:Th2 interaction, these data support the general concept that the balance between Tregs and Teffs is pivotal in both types of inflammatory bowel disease [118].

Tregs in Helminth Infection

Among the strongest known inducers of Th2 responsiveness are the metazoan parasites, both helminth worms (e.g. nematodes and trematodes) and ectoparasites such as mites, ticks and biting insects [8]. Notably, many helminth parasites also promote

Treg activation and expansion, a property they may have evolved precisely to contain host-protective but parasite-damaging Th2 immunity [119, 120]. Thus, the frequency of Foxp3$^+$ Tregs increases in mice infected with the filarial parasites *Brugia malayi* [121] and *Litomosoides sigmodontis* [122–124], as well as with the intestinal nematodes *Heligmosomoides polygyrus* [125–127] and *Strongyloides ratti* [128]. Likewise, Treg expansion occurs in *Schistosoma mansoni*-infected mice [129], as well as recipients of schistosome egg antigen [123] although in the latter setting alongside a parallel Th2 response.

In *L. sigmodontis*-infected mice, Tregs also increased expression of ICOS [130], while CD103 is also sharply upregulated on Tregs during *H. polygyrus* and *S. mansoni* infections [126, 127, 129]. ICOS-deficient mice show impaired frequencies of intestinal Tregs in the latter setting [131]. In the case of *B. malayi*, live parasites are required for Treg expansion as injection of heat-killed organisms did not reproduce this effect [121], while Treg induction can be replicated by exposure of naive T cells in vitro, or mice in vivo, to secreted ("HES") products of *H. polygyrus* which can ligate and signal through the TGF-β receptor [132]. The ability of the HES products to drive Treg differentiation argues that their expansion in helminth infection may be actively promoted by parasites, and not simply reflect the physiological accompaniment of the anti-parasite effector response.

Tregs driven by helminth infection appear activated and potently suppress pathological reactions such as allergic airway inflammation in mice [125, 133]. Activation may occur through parasite products such as HES [132], or through TLR ligands as in the case of Schistosome parasites [134, 135].

Manipulation of Treg populations in mice have provided further insights into the ability of Tregs to control Th2 immunity. Thus, depletion of Tregs with anti-CD25 antibody enhances host immunity to infection, resulting in lower worm burdens in mice infected with *L. sigmondontis*. Expression of effective immunity also required neutralizing antibodies to GITR or CTLA-4 [122, 124] to restore Th2 responsiveness in a population silenced by prior Treg activity. In *Trichuris muris* infection, while anti-CD25 treatment exacerbated pathology, no change in worm numbers resulted [136].

As yet, few studies have reported results with various genetic depletion models for Tregs. In the case of *H. polygyrus*, no difference in worm burden was noted in diphtheria toxin-treated C57BL/6 DEREG mice at day 14 of infection [137]; however, Th2 responsiveness was boosted and as genetically resistant strains (e.g. SJL) with stronger Th2 reactivity do not expel parasites until after 2 weeks of infection [138], the time point assayed may not have been optimal. In the case of *S. ratti*, early depletion of Tregs significantly reduced worm loads, but if depletion was delayed to day 4 of infection, no significant difference in infection was observed [128].

Human helminth infections also present abundant evidence of Treg expansion, with elevated Foxp3$^+$ T-cell frequencies in cross-sectional surveys of Bancroftian filariasis [139] while Foxp3 expression correlated positively with schistosome infection intensity in children [140]. Following anti-schistosome therapy, Foxp3 levels fell in patients [141], while patients developing more severe immunopathology of schistosomiasis were found to bear lower steady-state Treg frequencies

[142]. Functional studies on human populations infected with the highly prevalent soil-transmitted helminths (such as hookworm and *Ascaris*) revealed Tregs able to suppress bystander responses to malaria and BCG [143], echoing concerns that helminth immunomodulation may compromise vaccine efficacy.

A long-standing observation in human filariasis is a marked antigen-specific hyporesponsiveness in chronically infected patients; generally these cases are asymptomatic carriers of the transmission stage (microfilariae), indicating a state of tolerance to the parasite that may be maintained through the action of Tregs [144]. It is interesting therefore that in a recent study, Tregs from asymptomatic microfilaremic patients were found to show more active suppression than cells from individuals who had developed pathology as a result of heightened immune reactivity to the parasite [145].

More broadly, the dampened immune responsiveness of helminth-infected humans is reflected in epidemiological studies showing that these individuals have lower levels of skin test atopy (a broad measure of allergic reactivity) [146, 147] and anti-nuclear antibody (an early marker of autoimmune reactivity) [148]. Moreover, in both settings, anthelmintic treatment can result in increased reactivity, arguing for a causal effect of helminth infection on bystander responses to harmless antigen specificities. As a result, a concept has emerged that helminth-promoted Treg activity acts more broadly to down-modulate detrimental immune reactivities, in a revised version of the "Hygiene Hypothesis" [149].

An interesting parallel between human and rodent settings is the importance of the regulatory network in restraining IgE responses. In mice with Treg deficiencies, IgE levels can be excessively high as in the case of the CNS-1 deficiency which impairs pTreg induction [48]. Likewise, high IgE is one of the signs of IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome resulting from mutations at the Foxp3 locus [40]. In human helminth infections, asymptomatic "hyporesponsive" patients with strongest evidence of Treg activity have low IgE but very high IgG4 levels [150], while B-cell switching to IgG4 results from IL-10, TGF-β, and GITR expression from Tr1 and Foxp3$^+$ Tregs [151, 152]. Hence, elevated total serum IgG4 can represent a biomarker for a highly immunoregulated state in humans [153].

Plasticity and Interconversion of Th2 and Treg

The rigid divisions between T-cell subsets are recognized as being, in reality, quite fluid [154]. Tregs are not an immutably fixed population, and many examples are known of Tregs producing classical "inflammatory" cytokines or even switching their status altogether. In particular, Foxp3 expression may quantitatively but not fully repress effector functions of the cell. In mice with an altered Foxp3 locus which results in lower protein production per cell, Tregs preferentially expressed IL-4 and Th2-associated pathologies (elevated autoantibody, lymphoproliferation, and blephartis) ensued [155].

A striking example of the reprogramming of Treg cells toward a Th2 function was reported in a setting that deletes Dicer (a key microRNA processing enzyme) within Foxp3-expressing lineages: these show both Th1 (IFN-γ^+) and Th2 (IL-4^+) progeny of the Foxp3^+ population arise in vivo, accompanying severe inflammatory disease [156]. However, it is not clear whether in normal Dicer-sufficient Tregs, such conversion is permitted to occur.

In further mouse models, the switch from Tregs to inflammatory cells can be tracked by using transgenic constructs such as Rosa26-YFP combined with Cre recombinase under the Foxp3 promoter, to permanently mark Foxp3-expressing cells whatever their future fate. In one such system, 15 % of peripheral YFP$^+$ cells no longer expressed Foxp3, a proportion that increased in mice with the autoimmune-prone NOD genetic background [157]. Moreover, adoptive transfer of these "ex-Treg" cells into diabetes-prone recipients induced rapid onset of disease [157]. In another model system, Treg cytokine production associated with Th1/Th17 can be evoked by immunization with CpG ligand for TLR9; again conversion occurs among Tregs marked by GFP expression under the Foxp3 promoter [158], providing further evidence that cells previously committed to the Treg compartment are able to change their fate [159].

In the intestinal tract, an instance of localized conversion in the Peyer's patches occurs, in which Tregs lose Foxp3 expression and re-differentiate into the follicular helper T-cell (TFH) phenotype, specifically, providing help for IgA switching of B cells in germinal centers [160]. Although the TFH subset does not neatly correspond to Th1, Th2, or Th17 (as TFH express Bcl-6 rather than T-bet, GATA-3 or RORγt as the dominant transcription factor), they produce IL-4 and can perhaps be viewed as closest to Th2, which produce additional B-cell growth and switch factors (e.g. IL-5, IL-6, and IL-10). Indeed, TFH can also arise from Th2 cells during helminth infection [161] and in this instance retain GATA-3 expression [162]

Treg-Orientated Therapy

Therapy to modulate Tregs may have several objectives, and accordingly adopt varying strategies. For many allergies and autoimmune diseases, the objective may be to generate or sustain antigen-specific Tregs. This may require as little as an exogenous delivery of vitamin D, which proves beneficial in cases of steroid-resistant asthma [163], while antigen-specific strategies such as specific immunotherapy of allergy by repeated low-dose allergen exposure may recruit endogenous cognate Tregs, or convert effector cells to a Treg status [98, 164–166]. For colitis and other conditions in which multiple targets (e.g. commensal bacteria) are involved, a broader bystander strategy is required to raise the overall level of Treg activity. As one example, airway allergy can be suppressed in mice by administering a simple IL-2 complex which preferentially expands Tregs [167].

In contrast, in many infection settings, the objective is to counter Tregs and potentiate the host immune system, as illustrated in some of the helminth parasite

models [122, 124, 128]. A number of possible avenues can be explored beyond simple depletion strategies, and as we discover more about the biochemical and epigenetic basis of Treg function, means may become available to convert Tregs into more appropriate effector cells at the level of intracellular signaling. In addition, we have already learned that ablating Treg activity may not be sufficient in a chronic setting in which the effector population has become anergized; here it is necessary to manipulate the co-stimulatory pathways to rejuvenate immunity [120]. Interestingly, this latter strategy is one showing most promising results in tumor immunotherapy [168, 169].

Conclusions

Tregs are a physiological, and potent, means of repressing Type 2 responsiveness by dampening effector Th2 reactivity and acting directly on other cell types (particularly dendritic cells and macrophages), recruiting them into the regulatory network. While Tregs play a vital role in maintaining body health, they are part of a delicate balance within the immune system which not infrequently goes awry. Consequently, understanding the role of Tregs in modulating or permitting Th2-mediated immunopathologies is a crucial task which will illuminate the pathway to future therapy of allergic disorders and other maladies. On the other side of the coin, where Tregs are restraining a potentially protective Th2 response, as in the case of chronic helminth infections, new strategies need to be tested to alter the regulatory:effector balance in the patient to allow anti-parasite immunity to be fully expressed, while safeguarding them from the risk of allergic or other immunopathologies. These challenges are now being addressed.

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Developments in the Design of Anti-helminth Vaccines

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Parasitic Helminth Infections: Global Health Impact

Parasitic helminths are among the most debilitating infectious agents of humans, yet they remain neglected tropical diseases with no effective vaccines that can protect humans from infection. However, the development of efficacious vaccines against some parasites of livestock, along with advances in the understanding of the mechanisms of protective immunity to helminths, provide optimism that anti-helminth vaccines will be developed to limit the burden of human disease. Epidemiological studies of humans and experimental animal models have highlighted the importance of Type-2 immunity in natural and acquired resistance against most helminth species. Here, we describe the progress in the development of vaccines against major parasites of livestock (cestodes, ascarids, blood-feeding nematodes and trematodes) and the major causes of helminthiases of humans (hookworm and schistosomiasis), focusing on how understanding of host immunology and parasite biology has lead to the rational design and subsequent trials of candidate antihelminth vaccines.

Unlike bacterial, fungal, protozoan and viral pathogens, most helminths do not proliferate within their hosts and hence the severity of the infection depends on the level of initial exposure to the parasite. While infections with some helminthes, such as the STHs, do not commonly result in death, infected individuals experience significant morbidity, particularly when infections cause high worm burdens. School-age children are particularly susceptible to intense infections and consequently suffer from impaired nutrition, growth, memory and cognition and

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educational performance. In addition, individuals are often "polyparasitized" by chronic infection with multiple helminth species, and these infections can exacerbate the severity of extraneous diseases such as malaria, HIV, and even cancer, justifying the implementation of large-scale control and elimination programs for helminths in endemic areas.

Current Treatment Methods for Parasitic Helminth Infection

Anthelmintic Drugs

In developed countries over the last century, helminths have been mostly eliminated as a health risk for humans by the reduction of poverty, implementation of health education and improved sanitation measures and widespread availability of anthelmintic drugs. However, in developing countries, waiting for poverty reduction and urbanization to occur is not feasible, leading to the implementation of large-scale anthelmintic chemotherapy efforts. Beginning in the 1990s [1, 2], these "deworming" strategies focused on school-age children and used single doses of mebendazole or albendazole to limit prevalence of infection with STHs. However, mass deworming programs face significant challenges, such as the focus on school-age children that excludes other populations that are at risk of these infections. Such control programs require careful and challenging optimization of community involvement and participation and subsequent evaluation of the effectiveness of these treatment methods. In addition, it is likely that the reliance on albendazole and mebendazole for the control of STHs will have less of an impact on reducing hookworm transmission, given that hookworm infection intensities are at their highest in adults [3]. Lastly, the arsenal of drugs to treat human helminth infections are severely limited, with very few new drugs in the development pipeline, and the drugs used currently do not protect against reinfection [4]. This could cause major problems for existing drug administration programs if drug resistance develops and spreads, as has been seen for nematode parasites of ruminants [5]. Hence, future efforts in the design of new helminth infection control measures that can limit the incidences of reinfection should include the development and integration of effective anti-helminth vaccines.

Anti-helminth Vaccines

Efforts to develop anti-helminth vaccines for use in humans and livestock have persisted for many years, with steady progress made in the development of efficacious vaccines in ruminants and several promising vaccine candidates against human helminth diseases. Nevertheless, a vaccine that protects humans against any species of helminth remains elusive. In order to develop such a vaccine, more needs to be

known about how the mechanisms involved in acquisition (or lack thereof) of long-lasting immunity, and how the parasite itself may interfere with this process. Since Type 2 immune responses are implicated with enhancing innate and acquired immunity to helminths, determined by the use of experimental animal models and human immune-epidemiological investigations, it is logical that targeting the Type 2 arm of the immune response will yield the most effective vaccines. The following sections review the progress made in vaccine development, including the identification of candidate antigens, mechanisms of induced protective immunity, implementation in vaccine trials in humans and livestock, and the future for vaccine design.

Vaccines Against Parasitic Helminths: An Overview

After the first effective vaccine against a helminth species of cattle was developed in the 1950s, it was anticipated that many more vaccines would follow, however this has proven to be much more difficult for other helminth species. In contrast to vaccines against bacteria and viruses that replicate in their host, vaccines against helminths need not achieve sterile immunity and require only lowering of the parasite burdens below pathogenic levels, thereby limiting transmission. Most vaccination strategies have involved the use of either (1) attenuated or irradiation-killed infectious parasite stages, (2) mixtures of helminth somatic antigens, or (3) purified recombinant parasite antigens. Each strategy has its advantages and disadvantages in terms of efficacy and practicality and in general, are quite successful at demonstrating immune protection in experimental animal models, domestic animals, and livestock.

Lessons from Animal Models of Helminth Infections

Experimental animal models have been used extensively in studies investigating both the natural protective immune responses elicited by helminths, and for vaccination studies. Much of what we know about the immune mechanisms of how parasitic helminth infections elicit protective Type 2 cytokine responses that mediate parasite expulsion has been elucidated from rodent models and is discussed in detail in other chapters of this book. One limitation of using rodents for vaccine studies is that many of the important human pathogenic helminths (*Ascaris*, hookworm, and *Schistosoma haematobium*) do not have suitable small animal models based on parasite life cycle or natural chronicity of infection. However, hamsters can be used as models for urogenital schistosomiasis and some species of hookworms, and *Ascaris suum* (pig parasite) and *Nippostrongylus brasiliensis* are useful models to study human ascariasis and hookworm infection, respectively, in mice. Together, the use of rodent models is vital for the development of rational anti-helminth vaccines and examples of how these animal models have aided in the design of vaccines against helminths are outlined in later sections.

Vaccines in Livestock

Helminth infections are a huge problem in the livestock industry, as helminths are among the most common infectious agents of ruminants and cause immense economic losses, when reduced productivity and cost of anthelmintic drugs are considered. Thus, much effort has been put into the development of anti-helminth vaccines that limit reinfection rates and prevent the continuous development of drug-resistant parasites. Tapeworms, ascarids, blood-feeding nematodes and trematodes are some of the most common infectious agents for animals of veterinary importance and the following subsections discuss successes and failures of vaccine development in livestock and how our understanding (or lack of understanding) of the nature of protective immune responses has guided rational vaccine design.

Cestodes (Tapeworms)

Tapeworms of veterinary importance have a global distribution and infect pigs or cattle as intermediate hosts, or in the case of *Echinococcus granulosus* infect dogs and other carnivorous predators as definitive hosts. Humans are infected by ingesting undercooked meat from the intermediate hosts and hence the development of vaccines in these hosts will prevent zoonotic parasite transmission. Animals infected with cestodes display strong immunity to reinfection, though the primary infection persists via concomitant immunity, which is thought to be antibody-driven [6]. Studies in mice and pigs have demonstrated that the initial immune response to *Taenia* infection is of a T-helper Type 1 (Th1) phenotype, characterized by increased IFNγ and TNFα expression, but little change in IL-4 or IgG1 levels [7–9]. These Type 1 cytokine responses are thought to be responsible for immune protection against *Taenia solium* vaccination [10] and Type 2 immune responses are correlated with increased susceptibility to disease [11]. Similar to what occurs in livestock, *E. granulosus* infection of dogs results in a mixed Type 1/Type 2 cytokine response and the IgG2, IFNγ and IL-12-dependent Th1 immune response is implicated for increased protection in mice [11]. Together, this suggests that promoting a robust Type 1 cytokine response is a rational strategy for developing cestode vaccines. The last 25 years has seen the development of highly effective stage-specific recombinant protein vaccines against cestode parasites of livestock and has provided a model for the future design of vaccines against parasites of humans [12–16]. While the efficacious vaccines against *T. ovis*, *T. solium*, *T. saginata*, and *E. granulosus* are not commercially available for use in the livestock industry, hope remains that increased commercial interest and resolution of practicality issues will result in the use of these new tools to control cestode infections. Clearly however, research involving cestode vaccines has highlighted that targeting the Type 2 immune response for vaccine development is not appropriate for all helminth species.

Ascarids

Ascarids such as *Ascaris suum* are important pathogens of pigs, where infection occurs following ingestion of eggs that hatch and migrate via the liver to the lungs and intestine. Eosinophilia occurs in the liver and lung and can lead to liver fibrosis or eosinophilic pneumonia if infections become chronic. The importance of Type 2 immune responses and eosinophilia in controlling infections with the parasite are unclear, although primary infections of pigs results in natural clearance of the parasite from the intestine, correlating with increased IgG1 titers but not with eosinophil numbers [17], suggesting that Type 2 immune responses may be involved in immune protection via eosinophil-independent mechanisms. Mast cells and basophils degranulate in response to *A. suum* antigens, suggesting that these granulocytes may be involved in regulating Type 2 cytokine-dependent inflammation or anti-parasite resistance [18, 19]. Early efforts to develop *A. suum* vaccines used irradiated parasite eggs and yielded promising results [20, 21]. More recent efforts to develop recombinant protein vaccines against *Ascaris* have involved the use of both murine and porcine models and highlighted the importance of humoral immunity, particularly IgG1 responses, in the development of vaccination-mediated immune protection. These vaccines elicited either mixed Th1/Th2 cytokine responses in mice [22–24], or biased Type 2 cytokine responses in pigs [25] though these vaccines did not significantly protect from the initial appearance of parasites in the gut. In order to develop more efficacious vaccines, efforts should be focused on antigens released by the larval stage of the parasite prior to or during the lung/liver migration phase.

Blood-Feeding Nematodes

Haemonchus contortus is a gastrointestinal parasite of sheep and goats that is related to the hookworm species infective to humans such as *N. americanus*. Similar to other parasites of veterinary importance, drug resistance is a major problem for *Haemonchus* infections, and there have been significant efforts in recent years in developing a vaccine against this parasite [26]. Infections with *Haemonchus* can result in self-cure, which leads to robust protective immunity to reinfection that is dependent on Th2 cytokine responses, such as IL-4, IL-10, mast cells, and IgE [27, 28]. For vaccination against *Haemonchus*, it is critical to target young lambs (<6 months) as it is the period in which the lambs harbor potentially lethal worm burdens due to an immature immune system [29]. Parasite-specific IgE levels and Type 2 cytokine responses are increased greatly during *Haemonchus* infection in adult sheep [30], but not in young lambs, suggesting that protective immunity is IgE-mediated [31]. The immunological mechanisms behind the inability of young lambs to mount protective Type 2 immune responses remain incompletely understood [32]. *Haemonchus* larval and cuticular extracts have been trialed as vaccines, with mixed success [33], although immunization of sheep with the larval antigen

Hc-sL3 results in a Th2 immune response and significant protection against infection [34]. Substantial efforts have been made to utilize "hidden" *Haemonchus* antigens to vaccinate sheep. Two of these, H11—a gut membrane protease, and H-gal-GP—a gut membrane protein complex, are leading candidates for commercial vaccine development, either alone or in combination [35, 36], although more work is needed to optimize production of recombinant forms of these proteins. Lastly, because of the biological and pathological similarities between *Haemonchus* and hookworms infective to humans, it is hoped that progress in development of vaccines against human hookworm will also aid in the development of vaccines with veterinary importance, or vice versa.

Trematodes

Fasciola hepatica is a trematode pathogen of sheep and cattle with wide geographical prevalence and is considered an emerging threat to human health. These parasites are responsible for billions of dollars of losses annually in the agriculture industry. Livestock can be treated with the drug triclabendazole, but resistance to this drug is emerging in Europe and Australia. *Fasciola* infection of animals elicits a mixed Type 1/Type 2 and regulatory T cell (Treg) immune response in its host, leading to chronic infection [37]. Sheep and cattle do not develop robust naturally acquired resistance to reinfection [37], although there is evidence that resistance to infection can be conferred by passive transfer of immune cells [38]. This transferred protective immunity was not correlated with a Th2 immune response and cattle could be "trickle" infected to establish chronic infections in the face of ongoing Type 2 immune responses. The remarkable ability of *Fasciola* to withstand an onslaught of Type 2 immune-dependent effector mechanisms is owed to the inherent ability of the parasite to produce immunoregulatory excretory/secretory (ES) proteins [39], which in turn limits the potential harmful pathological effects of unrestrained Th2 cytokine-mediated inflammation.

There has been intense interest in developing vaccines against *Fasciola*. Vaccination studies in ruminants have yielded mixed success and in some cases have attempted to use vaccine candidates that are shared with other trematode parasites such as *S. haematobium* [37]. Vaccination with the tegument protein thioredoxin provided partial protection in rabbits, but was weakly immunogenic in cattle [40, 41]. Glutathione-S-transferases were trialed as a vaccine against fasciolids based on their efficacy in preventing *S. haematobium* infection, though these vaccine trials have again yielded mixed results [42, 43]. Similarly, fatty-acid binding proteins (FABPs) were trialed as cross-reactive vaccines to protect against schistosomes and *Fasciola*, and while some FABPs were effective in rabbits [44, 45], the results in livestock have been mixed despite some encouraging results with the FABP Sm14 [46]. Vaccination with cathepsin L1 and L2 peptidases, proteins that cleave antibodies bound to the parasite surface, provided significant degrees of protection in sheep and cattle [47]. Vaccine development strategies such as these that target the immunogenic proteins that *Fasciola* produces, and the sophisticated

mechanisms that the worm uses to evade the immune system will potentially lead to the development of more efficacious vaccines that have the potential for protecting against multiple trematode species.

Recent Developments in the Design of Human Helminth Vaccines: Focus on Hookworm and Schistosomiasis

While research into helminth vaccines of veterinary importance have focused on limiting infections with a variety of diverse helminth species, there has been a relative focus in efforts to develop vaccines against the most harmful parasites of humans. Together, hookworms and schistosomes are two of the most prevalent and important human infections worldwide, due to their chronic nature and detrimental impact on disability-adjusted life years. By targeting these key parasitic infections, it is hoped that helminth-induced morbidity such as blood loss and fibrotic inflammation will be lessened by efficacious vaccines. The optimal strategy for vaccine delivery would be to administer them in early childhood, thereby limiting the anemia, malnutrition, impaired physical and cognitive development that each of these infections causes in early life. However, vaccine development in humans faces significant challenges, not the least of which is the difficulty and limitations of studying natural immune responses in humans in a controlled experimental manner.

Hookworm Infection and Natural Immunity

Infections with the hookworms *N. americanus*, *Ancylostoma duodenale*, and *A. ceylanicum* are widespread in impoverished and tropical regions of the world. Chronic infection causes continuous blood loss from the intestine and depletion of iron and protein stores due to the parasite feeding. While hookworm infection can be effectively treated with anthelmintic drugs, reinfection often occurs rapidly after treatment [4], necessitating the development of vaccines. However, this has been an enormous challenge for helminth vaccinologists, primarily because the evidence for natural, acquired immunity to hookworm infection in humans is limited and there is evidence that both the frequency and intensity of infections with hookworms can increase with age [48]. Hookworms can survive in humans for years due to sophisticated immunomodulation mechanisms from the very first instances of infection. Nevertheless, there have been numerous efforts to develop vaccines against hookworms of humans and animals using a variety of approaches.

Hookworm-infected individuals display an immune profile similar to that observed with most other helminth infections, i.e. Type 2 cytokine responses, elevated IgG1, IgG4 and IgE titers, eosinophilia, and mastocytosis [49]. However, this naturally acquired immune response is only partially effective at reducing worm burdens, though there have been observations of positive correlations between IL-5

and IgE titers and reduced fecal egg counts [50]. In the few studies that have utilized controlled, experimental infections with hookworms in humans, it was demonstrated that *Necator* induces parasite-specific Type 2 cytokine responses (IL-4, IL-5, IL-9, and IL-13) at the site of infection and in the periphery, along with increased regulatory (IL-10, TGF-β) and Type 1 cytokine responses (TNF-α, IFN-γ) [51–54]. It remains possible that induction of this diverse regulatory and proinflammatory cytokine profile by *Necator*, rather than a wholly Type 2-biased immune response, is responsible for the establishment of chronic infections and this may in part be due to immunomodulatory proteins released by hookworms [55]. Thus, vaccines that protect against hookworm infection may be designed to target immune evasion strategies used by the worm, or antigens released by the vulnerable larval stages of the parasite soon after infection.

Hookworm Vaccines

The first vaccine against a hookworm was developed in the 1930s, which was a live *A. caninum* larval vaccine that protected laboratory dogs against repeated infections [56]. Later efforts used irradiated-attenuated infective third-stage larvae (irL3), which also caused significant reductions in parasite burden after challenge infection [56]. A canine hookworm vaccine consisting of *A. caninum* irL3 was marketed during the 1970s, providing high levels of antibody-mediated protection against disease, but was eventually discontinued due to logistical issues relating to production cost, storage requirements, and incomplete protection efficacy [57, 58]. In more recent studies, it was shown that the antibody-mediated protection induced by irL3 vaccine was associated with induction of Th2 cytokine responses against specific antigens secreted by *A. caninum* L3, suggesting that at least part of the success of the irL3 vaccine could be attributed to its effective induction of a polarized, antigen-specific Type 2 cytokine response [59].

More recent efforts to develop vaccines against hookworms have focused on identifying (1) soluble factors that L3 produce upon host entry, and (2) proteins involved in blood feeding of the adult worm, in an effort to develop a recombinant protein vaccine that reduces the ability of the worm to parasitize the host (Fig. 1). Incubating L3 in vitro with serum provokes the release of three major protein components: a zinc metalloprotease (*Ac*-MTP-1), *Ancylostoma* Secreted Protein (ASP)-1 and ASP-2 [60], all of which have been subsequently produced as recombinant proteins. Critically, in studies with dogs vaccinated against *A. caninum* L3, it was determined that *Ac*-ASP-2 was the predominant antigen to which the antibody response was directed and when *Ac*-ASP-2 was used to vaccinate dogs or hamsters against experimental hookworm challenge, high levels of protective immunity were achieved [59]. Similar proteins were subsequently isolated from the human hookworm *N. americanus* [61], which were advanced into clinical development given the encouraging vaccine efficacy data of *Ac*-ASP-2 in animals, and human studies from *N. americanus* endemic areas where titers of anti-ASP-2 IgE were associated

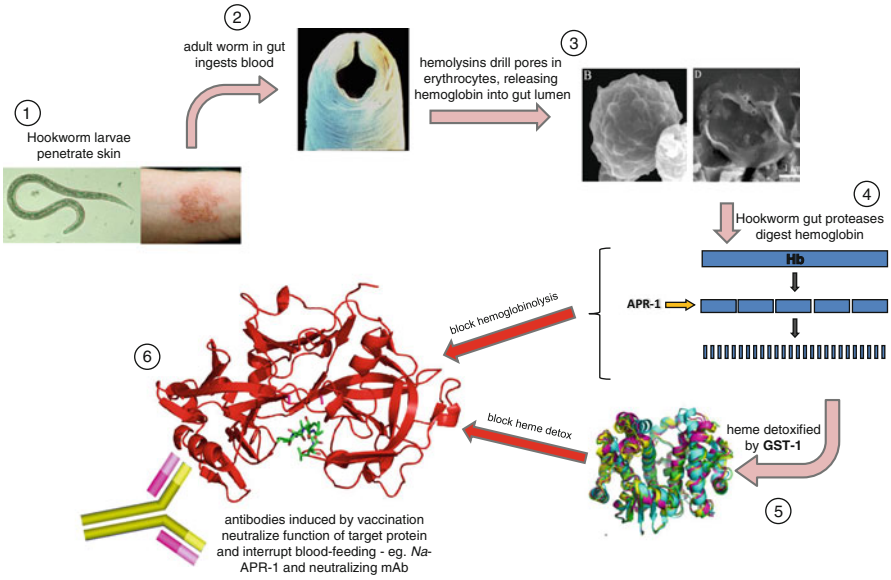

Fig. 1 *Necator americanus* degradation of host blood components by the activities of parasite hemolysins and hemoglobinases lining the parasite gut membrane, and detoxification of free heme. *Arrows* represent examples where antibodies to recombinant hookworm vaccine antigens have been shown to neutralize the enzymatic activity of the target protein

with a reduced risk of acquiring severe hookworm infection [62]. In a phase 1 study in hookworm naïve people, *Na*-ASP-2 was well-tolerated and immunogenic [63]. However, a follow-up safety and immunogenicity trial of this vaccine in adults from a hookworm endemic area in rural Brazil had to be stopped when 3/10 participants developed generalized urticarial reactions following injection of the vaccine [64]. These urticarial reactions were likely a result of pre-existing anti-*Na*-ASP2 IgE levels due to prior hookworm infections, raising important implications for the development of vaccines against helminths, given the potent ability of helminths and helminth-derived proteins to induce biased Th2 immune responses. Current efforts to reduce the allergenic capacity of *Na*-ASP-2, while retaining its immunogenicity, may yield modified vaccines that could re-enter the clinic if proven effective in animal models and safe in hookworm naïve and endemic populations [65]. Alternatively, a *Na*-ASP-2-based vaccine may be more suitable for delivery to young children, without previous exposure to hookworm infections.

Since *Na*-ASP-2 has been shelved as a vaccine candidate, more recent vaccination strategies have focused on targeting the feeding and metabolic requirements of the intestinal adult stage of the parasite, since blood feeding causes the major detrimental sequelae of hookworm disease. *Necator* depends on host hemoglobin for nutrition, and the two leading vaccine candidates for hookworm are an aspartic protease (*Na*-APR-1) which aids in hemoglobin proteolysis, and a glutathione-S-transferase (*Na*-GST-1) which detoxifies one of the toxic by-products of hemoglobin

digestion, heme [66]. Challenge studies in laboratory animals demonstrated that both recombinant *Na*-GST-1 and *Na*-APR-1 can induce protective efficacy against hookworm infection (Fig. 1) [67–70]. The Human Hookworm Vaccine Initiative (HHVI), a partnership between the Sabin Vaccine Institute and academic researchers and institutions is currently developing a vaccine that contains both *Na*-GST-1 and *Na-APR-1*, formulated with an aluminum hydroxide adjuvant for clinical testing in humans. Both vaccine candidates will be tested in Phase 1 trials in hookworm-naïve adults and children, then combined into a single product and retested in phase 2b and 3 trials in hookworm-endemic regions to evaluate its efficacy at reducing blood loss and anemia. Encouragingly, individuals living in hookworm endemic regions do not display detectable IgE against *Na*-APR-1 [68], suggesting that urticarial reactions are likely not to occur. Whether these vaccine antigens will induce high levels of antigen-specific antibodies in humans, protective Type 2 cytokine responses and reductions in worm burdens and intestinal blood loss remains to be seen.

Schistosomes and Natural Immunity

Schistosomes such as *S. mansoni*, *S. haematobium*, and *S. japonicum* are among the most important parasites of humans in terms of their impact on global human health [71–73]. *S. haematobium* is the most prevalent, causing urinary tract schistosomiasis and *S. mansoni* and *S. japonicum* are the major causes of intestinal schistosomiasis [73]. While schistosomiasis can be treated with drugs such as praziquantel, this does not protect against reinfection [74] and could lead to the emergence of drug resistance [75]. This, along with the high disease burden caused by these flatworms and the fact that humans living in endemic areas can become resistant or partially immune to reinfection over time [76] has led to a strong justification for the development of anti-schistosome vaccines [74, 77, 78].

Most experimental research into the immune response to schistosome infection has been focused on *S. mansoni*, in both rodent models and in humans. The immune response to *S. mansoni* is complex, with several tissues infected and multiple sites of egg deposition. In mice, initial infection results in a moderate Type 1 cytokine response as the parasite transits from the skin to the liver via the blood circulation [79]. After maturation, the worms migrate to the intestine where they lay eggs that are shed in the stool, or recirculate back to the liver where they deposit and induce a robust Type 2 cytokine response [80]. This Type 2 cytokine response elicits fibrotic granuloma formation, rich in collagen, Th2 cells, alternatively activated macrophages (AAMacs), B cells and eosinophils, that acts to sequester the egg from the surrounding tissue. This Type 2-mediated granuloma formation is critical for the prevention of excessive tissue damage in the liver, as genetic absence of Type 2 cytokine, such as IL-4, causes lethality of infection due to cachexia [81]. The protective effect of IL-4 is dependent on AAMacs, which mediate wound repair and resolution of inflammation [82]. Further, Type 2 cytokine expression is critical for the forma-

tion of class-switched anti-schistosome IgG1 antibodies that protect in later stages of infection, or during subsequent infections [83, 84]. IgE is also implicated in immune protection against schistosomiasis in humans [85]. Similar Type 2 regulatory immune responses could mediate fibrotic pathology in humans, since patients presenting with severe liver fibrosis exhibit elevated Type 2 cytokines, and low fibrosis individuals displayed higher levels of IFN-γ [86, 87]. Hence, Type 2 immune responses are critical for immune regulation during schistosome infection and are a rational target for the development of human anti-schistosome vaccines. However, evidence from people living in schistosome-endemic areas of Brazil paints a more complex story, where some individuals are putatively resistant (PR) to infection despite years of constant exposure and have no previous use of anthelmintic drugs [88, 89]. These PR individuals mount distinct immune responses to *S. mansoni* compared to people with drug-induced anti-parasitic resistance, displaying both a Type 1 and Type 2 cytokine profile, while chronically infected individuals exhibited a predominant Type 2 response [76, 90, 91]. Hence, it is hypothesized that a mixed immune response, including Type 1 responses to schistosomula antigens and Type 2 responses to adult worm and egg antigens may be optimal for anti-schistosome resistance.

Schistosome Vaccines

Vaccination of animal models of schistosome infection, such as rodents, pigs, and baboons with radiation-attenuated larvae confers robust and long-lasting immunity against reinfection [77]. Importantly, multiple recombinant protein vaccines have been developed that can elicit modest levels of protective immunity in animal models [77]. Observations made in studies involving the PR cohorts in Brazil have made significant discoveries related to potential vaccine antigens that elicit appropriate immune responses. Two of these antigens, the tegumental proteins *Sm*-TSP-2 and Sm29 were identified by their ability to be strongly recognized by serum antibodies from PR patients, but not chronically infected individuals [88, 92]. The tetraspanin *Sm*-TSP-2, which is expressed on the surface of live worms and is essential for parasite tegument development and maturation [93], provided high levels of protection as a recombinant vaccine in mice and is strongly recognized by serum IgG1 and IgG3 from PR individuals [88]. Thus, *Sm*-TSP-2 is currently being developed as a recombinant protein vaccine to prevent heavy infections with *S. mansoni* in humans [94]. Related *S. mansoni* tetraspanin proteins also show promise as vaccines, including Sm23 and *Sm*-TSP-1 [95–97]. Additional schistosome tegument proteins such as Sm29 and Sm28 have been assessed in trials in mice and hamsters as recombinant vaccines with promising results [98–100], and Sm28 showed some protective efficacy in primates [101], though its efficacy in humans remains unclear. A related protein from *S. haematobium* (Sh28) has entered clinical trials in Europe and West Africa [102], though reports of its efficacy are not known at this stage. Recent findings using *S. mansoni* GPI-anchored proteins from the parasite tegument as vaccines showed significant protection against challenge infection, which correlated with a mixed Th1/Th2 cytokine response [103].

Other schistosome tegument proteins with potential as vaccine antigens have been identified, such as calpain, which is the target antigen of a specific protective $CD4^+$ Th1 cell clone that induces macrophage-dependent killing of schistosomula [104]. Calpain, also known as Smp80, has been shown to protect via antibody-dependent mechanisms against experimental infection in mice and baboons and shows great promise as a subunit vaccine for *S. mansoni* infection [105, 106]. The FABP Sm14, as described in "Trematodes," has been assessed as a recombinant protein vaccine displaying high vaccine efficacy in some mouse trials [107], but reduced efficacy in others [108]. Interest in developing and improving the efficacy of the Sm14 vaccine is high, particularly given its potential uses for improving human and veterinary health as a combined schistosome and *Fasciola* vaccine [107, 109]. Paramyosin has also been tested as a vaccine candidate, providing significant protection against challenge infection and is currently undergoing production as a *S. japonicum* transmission-blocking vaccine for use in water buffaloes in Asia [110]. Together, it is clear that there is intense research into the development of anti-schistosome vaccines, however very little is known about the nature of the protective immune mechanisms that a vaccine would need to elicit to provide optimal resistance to infection, though targeting the humoral immune system by eliciting neutralizing antibodies (particularly IgG1) against key proteins that larval schistosomes require for their intra-mammalian development is a rational strategy.

Conclusions

The development of vaccines that protect against helminth infections requires the integration of efforts to understand the immunology of helminth infections, the biology of the parasite, and the epidemiological implications of transmission in endemic areas. Research using rodent models has taught us a lot about the nature of the protective immune responses that develop naturally in resistant animals. Type 2 immune responses are central to the immune-mediated protection against infection with most species of helminth. Information about the initiation and regulation of Type 2 immune responses in mice has been successfully translated to some modes of helminth infection of veterinary importance, leading to the development of vaccines that work. However, our severely limited understanding of how humans control helminth infections, and our even less developed understanding of the immune responses to vaccines and the effector mechanisms by which vaccines exert their anti-parasitic effects has hindered the development of vaccines against helminths that cause immense problems for human health globally.

Clearly, there has been significant progress made into the early stages of development of vaccines that protect humans, companion animals, and livestock against helminthic infections. However, significant challenges still await helminth vaccinologists before vaccines that protect against human helminth infections become a reality. Recent advances in helminth genomics, proteomics, and immunomics have opened up a wealth of information available for the helminth vaccinologists to mine

[111], which could aid in the identification of the next generation of candidate helminth vaccine antigens. This, along with the availability of high-throughput protein expression techniques such as in vitro translation using prokaryotic or eukaryotic ribosomes, has the potential to transform vaccinate candidate discovery and expedite the elucidation of interactions between these factors and human immunoglobulins or immune cells and proteins. Multivalent vaccines such as those proposed to protect against both hookworm and schistosomiasis [71] would be an enormous leap forward by improving the logistics for widespread vaccine delivery to affected areas. In addition, optimal control of these parasites in human populations in endemic areas will require the integration of public health improvement measures such as improved sanitation and health education, along with strategic administration of anthelmintic drugs alongside vaccines.

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Tissue Remodeling and Repair During Type 2 Inflammation

Alexander J. Chan, Jessica C. Jang, and Meera G. Nair

Abbreviations

AAM	Alternatively activated macrophage
CAM	Classically activated macrophage
DTR	Diphtheria toxin receptor
ECM	Extracellular matrix
EGF	Epidermal growth factor
FAP	Fibro/adipogenic progenitor
FGF	Fibroblast growth factor
IGF	Insulin-like growth factor-1
ILC	Innate lymphoid cell
LAP	Latency associated protein
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cell
RELM	Resistin-like molecule
PDGF	Platelet-derived growth factor
TGF-β	Transforming growth factor β
TIMP	Tissue inhibitor of metalloproteinase
Th	T helper
TREM2	Triggering receptor expressed on myeloid cell 2
TSLP	Thymic stromal lymphopoietin
VEGF	Vascular endothelial growth factor

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Introduction

Response to injury and subsequent wound repair is an essential survival mechanism that occurs in all multicellular organisms at varying degrees of complexity. In mammals, several cellular and molecular pathways have evolved to coordinate wound healing. Whether it is an external injury of epithelial surfaces, internal organ wounds such as vessel rupture, or invasive pathogen infection, the body must react rapidly to prevent excessive infection, fatal inflammation, or organ failure. Although better known for its function in defense against pathogens, the immune response also orchestrates critical steps in wound healing via activation of innate and adaptive immune cells to generate cytokines and tissue growth factors. In particular, the T helper type 2 cytokine (Th2) pathway (Table 1), characterized by the cytokines IL-4 and IL-13, is an essential mediator of wound healing and acts to downregulate excessive inflammation while promoting tissue repair.

Our understanding of the various cell-types and derived factors that mediate wound healing have benefited from several injury models including external wounds (e.g., skin biopsies or burn models), internal organ wounds (e.g., chemically induced injuries) or infection (e.g., helminth) (Table 2). Additionally, aberrant wound healing occurs in several clinical conditions, such as diabetes, where ulcers result from defective wound healing, or systemic sclerosis, where an overactive wound healing response causes pathologic scar tissue formation, known as fibrosis. Understanding the critical balance necessary for optimal tissue repair, including protection from excessive blood loss or pathogen invasion, while limiting fibrosis, could provide new therapeutic avenues for wound healing.

Table 1 Glossary of wound healing terms

Initiation
• Th2 cytokines: secreted proteins, such as IL-4 and IL-13, that are expressed and secreted by T helper 2 cells and trigger the wound healing process
Remodeling
• Tissue remodeling: process of reorganizing tissue through angiogenesis (blood vessel formation) and breakdown/reformation of the ECM
• AAM: macrophages that respond specifically to Th2 cytokines and express arginase1, RELMα, YM1, TREM2, and growth factors to induce tissue remodeling and repair
• Extracellular matrix: component of the connective tissue that provides the structural support for new tissues. Collagen comprises the majority of the ECM
Repair
• Tissue repair/fibrogenesis: proliferation of fibroblasts and formation of the fibrous connective tissue, supported by the ECM
• Tissue regeneration: complete renewal of damaged tissue involving replication and/or differentiation of stem cells, resulting in restoration of tissue to its previous normal state
• Fibrosis: aberrant tissue repair, leading to the formation of excess connective tissue and scars. The antagonist to tissue regeneration
• Myogenesis: the formation of muscular tissue
• Stem cells: multipotent progenitor cells with the potential to differentiate into various cells as part of tissue regeneration

Table 2 Models to study tissue repair and remodeling

Injury model	Description	References
Chemical injury	The use of cytotoxic chemicals to inflict injury. Examples include bleomycin for lung fibrosis, carbon tetrachloride for liver fibrosis, and cardiotoxin for muscle necrosis	[24, 60, 80]
Colon punch biopsy	Flexible biopsy forceps attached to an endoscope creates lesions in the colon mucosal layer	[58]
Evans blue assay	An azo dye that binds to serum albumin. Large or inappropriately healed wounds will show increased dye uptake due to vascular leakage	[60]
Helminth infection	The hookworm *Nippostrongylus brasiliensis* colonizes the lung as part of its lifecycle and the active burrowing through the tissue causes acute damage and inflammation	[6, 10, 81] (Fig. 2)
	The trematode *Schistosoma mansoni* colonizes the intestine while the eggs are trapped in the liver where they induce significant fibrosis	
Skin wound	Predominantly used to study epidermal injury, biopsy punchers (punch biopsy) or a scalpel (excisional wound) creates similar size wounds. Wound repair can be monitored over time or excised for histological analysis	[30, 31]
Burn model	Can be used to study hypertrophic scarring. Mice are exposed to extremely hot water (~90 °C)	[5]

The four stages of wound healing consist of coagulation, inflammation, tissue remodeling, and tissue repair/fibrogenesis (Table 1) [1]. Following initial injury, activated platelets convert fibrinogen to fibrin forming a clot to prevent excessive blood loss. Within hours, neutrophils emigrate from nearby tissue, killing microbes via free radicals or phagolysosomes and causing inflammation. Activated macrophages control microbes but also perform housekeeping functions to clear cell debris and produce tissue remodeling factors allowing angiogenesis (blood vessel formation) for waste removal and access to nutrients and oxygen. Activated fibroblasts at the edge of the wound proliferate and migrate into the wound to replace the clot with fibrous granulation tissue, effectively sealing off the wound from potential secondary infection. Finally, fibrogenesis is characterized by production of the collagen-rich extracellular matrix (ECM) to build the appropriate foundation for the new tissue formed by fibroblast and epithelial cell proliferation.

The transition from one stage of wound healing to the next requires a tightly regulated balance between inflammation and tissue repair, relying heavily on Th2 cytokine signaling. Any imbalance can cause insufficient wound closure or excessive ECM deposition and chronic inflammation, leading to detrimental fibrosis [2]. In this chapter, we will explore how the Th2 immune response contributes to wound healing, focusing on the cell-types that (I) generate the appropriate type 2 cytokine milieu for wound healing, (II) respond to Th2 cytokines to secrete cytokines or growth factors, and (III) mediate tissue repair (Fig. 1). We will conclude with the clinical implications of modulating the Th2 cytokine response in wound healing and fibrosis (IV).

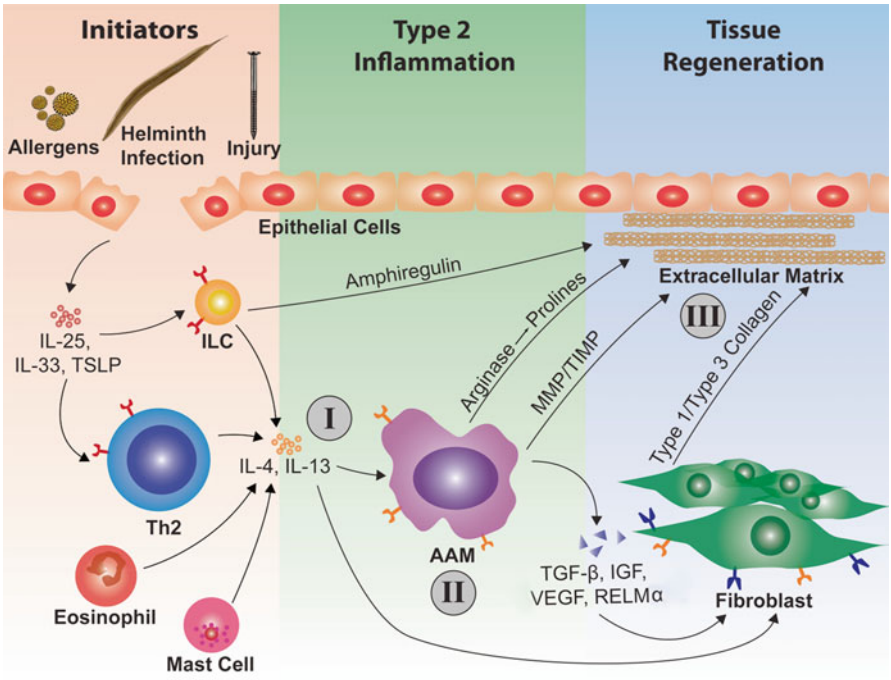

Fig. 1 Model of Th2 cytokine-induced tissue repair. (I) Allergens, helminth infection, or direct injury induce epithelial-derived cytokines IL-25, IL-33, and TSLP that activate the production of Th2 cytokines. (II) The Th2 cytokines IL-4 and IL-13 promote AAM activation. (III) Together with other factors secreted by innate and adaptive immune cells, AAMs act on fibroblasts to mediate ECM deposition for the foundation of new tissue

Th2 Cytokine Producers Following Injury

The Th2 immune response, characterized by the production of IL-4 and IL-13, is predominantly induced by helminths, chronic infection, or allergens. Given that helminths are large multicellular pathogens that cause significant tissue damage, it is perhaps not surprising that infection triggers a wound healing Th2 immune response to either "wall off" the infection by granulation tissue, or to repair the wounds resulting from these destructive tissue invasive pathogens [3]. In the context of chronic allergic inflammation such as asthma, the excessive Th2 cytokine environment promotes lung angiogenesis, collagen deposition and epithelial cell and fibroblast proliferation, all processes that occur in wound healing. The study of helminth infection and allergic inflammation has therefore provided valuable insight into understanding how Th2 cytokines mediate wound healing.

Following injury, the stage of wound healing can dictate what T helper cell response is initiated. The acute immune response involves the activation of proinflammatory cells, notably Th1/Th17 cells, that protect the host from potential pathogen invasion. Studies using a burn model (Table 2) have shown that the initial Th1

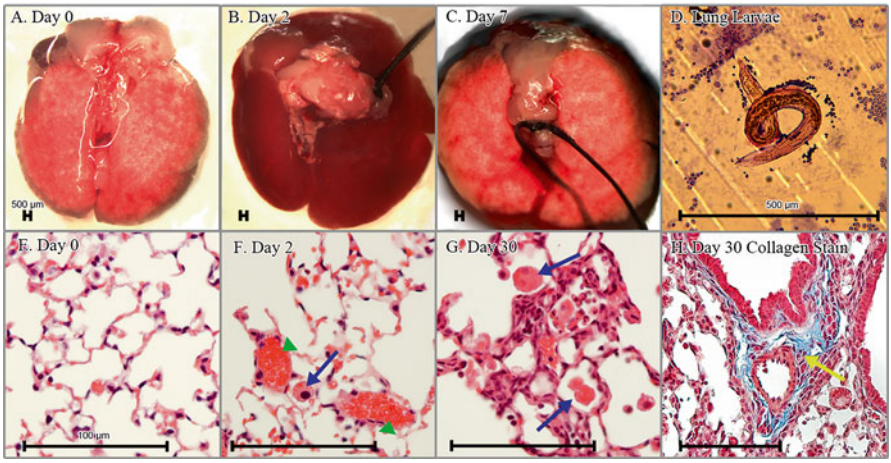

Fig. 2 *Nippostrongylus brasiliensis* infection as a model of lung tissue injury and repair. Mice were left naïve (Day 0) or infected with 500 larvae, sacrificed at indicated time-points and examined macroscopically (**a**–**c**) or by H and E-staining (**d**–**g**). Compared to naïve lungs (**a**, **e**), day 2-infected lungs exhibit excessive hemorrhaging (**f**, *green arrow*) as a consequence of the parasite colonization of the lung tissue (**d**). By day 7, the lungs show remarkable wound repair (**c**) and gross morphology is equivalent to naïve mice (**a**). However, at day 30, chronic tissue remodeling results in macrophage activation (**g**, *blue arrow*) and excessive collagen deposition evident by Masson's trichrome staining (**h**, *yellow arrow* indicates blue collagen)

response protects against opportunistic microbial infection, but eventually shifts to a Th2 response starting day 3 post injury perhaps out of necessity to keep destructive inflammation at bay [4, 5]. Consistent with this, lung tissue injuries caused by hookworm infection or treatment with the cytotoxic chemical bleomycin (Table 2), drive an acute Th17 cell response that can be fatal if not counter-regulated by a Th2 cytokine response [6–9]. Following infection with the hookworm, *Nippostrongylus brasiliensis* [6, 10], the burrowing of the worms in the lung tissue between 1 and 2 days results in hemorrhaging that is resolved by day 7 (Fig. 2a–c). Chen et al. showed that the anti-inflammatory cytokine IL-10 produced by Th2 cells participated in this wound resolution by inhibiting IL-17A, a key cytokine in recruiting neutrophils to the site of injury [6]. In addition to downregulating Th1/Th17 cell responses, Th2 cytokines mediate wound healing by inducing expression of proteins that promote fibrogenesis [10, 11]. However, if dysregulated, excessive type 2 inflammation can drive fibrotic diseases, as observed in the chronically inflamed lungs following hookworm infection (Fig. 2g, h).

IL-33, TSLP, and IL-25

IL-33, thymic stromal lymphopoietin (TSLP) and IL-25 have recently emerged as critical initiators of the Th2 immune response. Allergens, helminth infections and chemical signals can cause disruption to the epithelial barrier resulting in the

production of these cytokines. IL-33 is released by necrotic epithelial cells and provides the initial signal that promotes Th2 cell recruitment. Following helminth infection or exposure to allergens, TSLP promotes Th2 cell maturation in several ways, which include inducing dendritic cell surface expression of Th2- promoting OX40L, inhibition of Th1/Th17 immune responses and mediating basophil activation to produce Th2 cytokines [12–15]. Likewise, IL-25 is produced by activated T cells and epithelial cells and mediates the production of IL-4 while inhibiting Th1/Th17 cytokine responses [16, 17].

Th2 Cytokine Producers

$CD4^+$ Th2 cells produce the cytokines IL-4, IL-5, IL-9, IL-13, and IL-21 [3]. IL-4 and IL-13 mediate macrophage alternative activation and directly act on fibroblasts to promote tissue repair and wound healing. Despite the fact that both IL-4 and IL-13 bind to IL-4Rα and signal through STAT-6, IL-13 is a stronger pro-fibrotic signal than IL-4 [18]. This is likely due to a number of different factors, including the specificity of IL-13 for the receptor IL-13Rα_2. When bound to IL-13Rα_2, IL-13 triggers activation of the promoter for TGF-β, a growth factor that stimulates fibroblasts to synthesize components of the ECM such as collagen [19]. IL-13 also increases collagen accumulation by inducing fibroblasts to decrease secretion of matrix metalloproteinases (MMP), which have the ability to break down collagen, and increase expression of tissue inhibitor of metalloproteinases (TIMP) [20, 21].

In addition to $CD4^+$ Th2 cells, innate lymphoid cells (ILC) produce Th2 cytokines and have recently emerged as critical mediators of tissue remodeling and repair. The group 2 ILCs, also activated by IL-25 and IL-33, orchestrate $CD4^+$ Th2 cell differentiation via production of IL-4, IL-5, and IL-13 [17, 22]. Supporting their role in wound healing, ILC depletion resulted in impaired lung function, tissue destruction and fatality in a flu infection of mice [23]. ILCs express several wound healing genes, including the ECM proteins decorin, asporin, and dermatopontin, and the epidermal growth factor (EGF) family protein amphiregulin [23]. Exogenous addition of amphiregulin to ILC deficient mice restored lung function and mediated repair. Consistent with an important role in tissue repair, another study employing carbon tetrachloride to induce liver fibrosis showed that amphiregulin promoted expression of pro-fibrotic signals (TIMP-1, connective tissue growth factor, α-smooth muscle actin) and fibroblast survival and proliferation [24]. These recent studies on ILCs suggest there is still much to be understood about their role not only in immunity, but also in homeostasis and injury repair.

Other than ILCs and Th2 cells, granulocytes such as mast cells can also contribute to wound healing via production of Th2 cytokines. Following skin biopsies from healthy volunteers, mast cells proliferated and were major contributors of IL-4 production in the wound [25]. By producing IL-4 and TGF-β, mast cells regulate proliferation and differentiation of fibroblasts into myofibroblasts [26], which

are smooth muscle cell precursors. Mast cells also activate myofibroblasts to synthesize collagen, MMPs, fibronectin, and proteoglycans, all necessary components for the formation of new tissue [26, 27]. Furthermore, other granulocytes such as eosinophils and basophils also promote an optimal Th2 cytokine environment for wound healing.

In summary, the first stage of wound healing involves the participation of innate and adaptive immune cells that produce Th2 cytokines and other factors that direct wound healing. The generation of a type 2 cytokine milieu will mediate the next critical steps in this process, notably the alternative activation of macrophages and tissue regeneration.

Alternatively Activated Macrophages in Tissue Remodeling and Fibrogenesis

Macrophages are innate phagocytic cells that provide one of the first lines of host defense against microbes. This primordial function exists in metazoan organisms as primitive as starfish. Elie Metchnikoff was awarded the Nobel prize in 1908 for the discovery of macrophages when using thorns to inflict wounds on starfish larvae [28]. In alignment with the context in which they were discovered, macrophages are critically involved in mediating wound repair. In contrast to the classically activated macrophages (CAM) that are induced by IFNγ and kill pathogens, macrophages that are alternatively activated by IL-4 and IL-13 (AAM) are more typically associated with wound healing functions [29]. AAMs promote wound healing via a variety of mechanisms, from regulating the inflammatory response and engulfing cellular debris to expressing an array of wound healing genes.

Macrophage depletion studies following skin punch biopsies in mice revealed important roles for these cells in all stages of wound healing. Two independent studies employing either LysMCre or CD11b-Diphtheria toxin receptor (DTR) transgenic mice to deplete macrophages at selected time-points following skin injury, demonstrated that macrophages downregulated wound hemorrhaging and promoted the formation of vascularized granulation tissue, fibrogenesis, and reepithelialization [30, 31].

Several studies have demonstrated that AAMs rather than CAMs are necessary for wound healing. Following infection with trematode *Schistosoma mansoni*, which infects the intestine and the liver, macrophage-specific IL-4Rα$^{-/-}$ mice (LysMCre-IL-4Rα$^{-/-}$) died from infection suggesting an essential protective role for AAMs in *Schistosoma*-induced organ injury [32]. In acute lung injury following hookworm infection, both IL-4Rα$^{-/-}$ mice and CD11b-DTR transgenic mice suffered from exacerbated lung hemorrhaging that was ameliorated with the transfer of IL-4 responsive macrophages [6]. In addition to promoting AAM activation, IL-4 is also a strong proliferative factor for tissue macrophages [33]. Mechanistically, AAMs may mediate wound healing via several pathways summarized below.

AAM Signature Genes

In the past decade, gene expression studies employing Th2 cytokine deficient mice (STAT-6$^{-/-}$, IL-4Rα$^{-/-}$, or IL-4$^{-/-}$) in wound or helminth infection models have identified several AAM-specific genes that are critically dependent on Th2 cytokines [34–37], including Arginase1, RELMα, and Ym1.

Arginase1 is a cytosolic enzyme expressed by AAMs and is the counterpart enzyme to nitric oxide synthase, which is expressed by CAMs. Whereas nitric oxide synthase breaks down arginine to produce microbicidal nitric oxide, AAM-expressed arginase uses arginine to generate ornithine and urea. As such, arginase expression by AAMs is anti-inflammatory and inhibits CAM function and nitric oxide production. In addition, ornithine, generated from arginase, can be enzymatically processed to generate polyamines (spermine and spermidine) and prolines, which participate in essential wound healing steps [38]. Polyamines promote cell proliferation, while prolines contribute to the stereochemistry and stability of collagen triple helices that are necessary for ECM deposition [39]. Supportive of a tissue repair function for arginase, a recent skin excision study showed that pharmacological inhibition of arginase or deletion of arginase in macrophage and endothelial cells (Tie2Cre-Arg1 transgenic mice) caused delayed wound healing, increased inflammation and defective ECM deposition [40]. Surprisingly, in another study, *Schistosoma* infection of macrophage-specific arginase deficient mice (LysMCre-Arg1 transgenic mice) caused increased liver fibrosis and Th2 cell responses [41]. This suggests that the involvement of arginase in tissue repair is complex and the outcome may depend on the tissue site or other downstream factors such as T cells. In humans, arginase expression is upregulated in asthmatic patients [42], and the clinical significance in Th2 inflammation or asthma-induced fibrosis has yet to be determined.

Resistin-like molecule-α (RELM-α) is a cysteine-rich secreted protein that is highly induced in Th2 inflammatory conditions including allergic airway inflammation [43, 44], bleomycin-induced lung fibrosis [45, 46] and helminth infection [34–36]. Mechanistically, RELMα may promote tissue remodeling by stimulating cell proliferation, mediating tissue vascularization or inducing collagen production. However, in response to helminths *Nippostrongylus* or *Schistosoma*, RELMα$^{-/-}$ mice exhibited exacerbated type 2 inflammation and fibrosis, and this was in part due to RELMα-mediated downregulation of Th2 cell responses [47, 48]. Similar to the function of Arginase1, the overall effects of RELMα on wound healing may depend on RELMα expression level, the inflammatory milieu and the downstream cellular targets. In humans, the homologous protein RELMβ is expressed in patients with scleroderma and idiopathic pulmonary fibrosis [49, 50], suggesting potential clinical implications for the study of this protein family in fibrosis.

Ym1 belongs to the family of chitinase-like proteins. Chitinases bind and cleave chitin, an abundant polysaccharide present in fungi and other invertebrates that may contribute to allergic inflammation and asthma. However, Ym1 binds chitin but has no chitinase activity. Putative functions for Ym1 include the promotion of Th2 cell responses [51]. In contrast, the homologous protein AMCase, which has functional chitinase activity, acts to reduce allergic airway inflammation through the breakdown

of the potential allergen chitin [52]. In humans, chitinase-like protein YKL-40 is upregulated in asthma and airway fibrosis [53]. Given the expression of these proteins in Th2 cytokine inflammation, investigating whether chitinases or chitinase-like proteins regulate these responses may identify new targets to promote wound healing or reduce allergic inflammation and fibrosis.

Growth Factors

AAMs express a number of growth factors that stimulate the activation and proliferation of many cell-types necessary for wound healing. In *Nippostrongylus*-induced acute lung injury, macrophages suppressed excessive hemorrhaging via expression of insulin-like growth factor-1 (IGF-1). IGF-1 expression is induced by Th2 cytokines and can contribute to wound healing by activating fibroblasts [54]. Likewise, platelet-derived growth factors (PDGF) stimulate the early proliferation of fibroblasts, as well as their differentiation into myofibroblasts [54]. In excision wound injury, $CCR2^{+}$ inflammatory monocytes were an essential early source of vascular endothelial growth factor (VEGF) that promotes angiogenesis [55]. In another study, delayed wound healing following macrophage depletion was correlated with reduced TGF-β expression [31]. TGF-β contributes to tissue repair both by acting on immune cells to inhibit inflammation while promoting fibroblast activation and collagen synthesis. In homeostatic conditions, TGF-β function is inhibited when bound to the latency-associated protein (LAP) [56]. Several proteases including MMP-9 can activate TGF-β by cleaving the LAP. Since MMP-9 is expressed by AAMs in response to IL-13, macrophages mediate fibroblast activation and collagen synthesis in a two-step process involving TGF-β expression and subsequent activation by MMPs. In addition, MMP-9 can induce keratinocyte migration to the wound edge, allowing healing of epidermal skin wounds. AAMs also express metalloproteinases MMP-12, MMP-19 and inhibitor TIMP-1 that counter-regulate each other to balance collagen levels and ECM deposition for optimal tissue repair.

TREM2

Triggering receptor expressed on myeloid cell 2—(TREM2)—is expressed on the surface of myeloid cells and promotes anti-inflammatory and phagocytic function [57]. In a colonic wound model, TREM2 expression was increased in AAMs and was essential for optimal wound healing by promoting epithelial cell proliferation while limiting inflammatory cytokine expression [58]. AAMs also express other inhibitory receptors such as PD-L2, however their contribution to wound healing is unclear [59].

In summary, AAMs produce a myriad of factors that contribute to wound healing. In addition to an anti-inflammatory function, macrophages also activate non-immune cells such as fibroblasts to mediate tissue repair, the final stage in wound healing.

Fibrogenesis and Tissue Regeneration

Fibrogenesis, the final stage in wound healing involves four major cell-types: endothelial cells, fibroblasts, myofibroblasts, and epithelial cells. These cells are activated by cytokines, chemokines, and growth factors to lay down the ECM foundation for new tissue. The Th2 response instructs this critical process by promoting endothelial cell mediated vascularization of the new tissue, stimulating fibroblasts to lay down new collagen, and inducing myogenesis (muscular tissue formation) for wound contraction in muscle injuries [9, 60].

Angiogenesis is necessary to provide nutrients and resources for new tissue, as well as remove waste and debris from the site of injury. It is during the tissue remodeling stage that nearby endothelial cells degrade their current basement membrane in order to branch out and form new vessels into the site of injury. When IL-4 and IL-13 are bound to the IL-4Rα receptor on endothelial cells, the expression of vascular cell adhesion protein 1 and formation of vascular tubules occurs [61]. Likewise, angiogenic factors such as TGF-β, fibroblast growth factor (FGF) and VEGF are continuously secreted to promote the recruitment and proliferation of endothelial cells from existing capillaries. During this stage, the basement membrane is slowly reconstructed, weaving through the ECM to support the newly formed tissue [62].

In response to Th2 cytokines and growth factors such as TGF-β, fibroblasts proliferate and synthesize collagen precursors procollagen 1 (COL1a1) and procollagen 3 (COL3a1) through activation of the SMAD pathway [63]. The synthesis of collagen allows replacement of the temporary platelet clot with a more structured foundation. Collagen is a key component of wound healing and several types of collagen-infused wound dressings are available on the market for this purpose [64]. COL3a1 mutations in humans are often associated with Ehlers–Danlos syndrome, a connective tissue disorder resulting in fragile blood vessels and skin [65]. Reduced COL3a1 expression in mice resulted in defective wound repair associated with increased scar tissue [66]. This suggests that in addition to its structural role, type 3 collagen (COL3a1) also contributes to optimal tissue repair with minimal fibrosis. To regulate and remodel the collagen-rich ECM, fibroblasts also secrete MMPs, which breakdown collagen, and TIMPs, which in turn regulate MMP activity [67, 68].

For muscle injuries, IL-4 and IL-13 responsiveness of the recently identified stem cell population—the fibro/adipogenic progenitors (FAP)—is critical for muscle regeneration [60]. Following treatment with cardiotoxin to induce muscle necrosis, IL-4-producing eosinophils were recruited that stimulated proliferation and differentiation of FAPs into myofibroblasts, allowing new muscle formation. IL-4 also acted directly on FAPs to induce clearance of cellular debris, a function that is normally attributed to AAMs. In addition to forming mature myofibers, the structural cells of the muscle, myofibroblasts also express α-smooth muscle actin and collagen, which act to initiate wound contraction and faster tissue recovery [45, 69]. Nevertheless, excessive myofibroblast activation can result in pathological fibrosis; following treatment with carbon tetrachloride to induce liver fibrosis, mice treated with blocking antibodies to myofibroblasts exhibited reduced disease severity [70].

Reepithelialization of the wound, the final step in this process involves proliferation, differentiation, and migration of epithelial cells and fibroblasts over the wound edge. Although proliferation and migration of epithelial cells is mainly directed by growth factors such as EGF, bronchial and intestinal epithelial cells also express IL-4Rα [71, 72], and will proliferate and migrate in response to IL-4 and IL-13 for the final stage of wound healing.

Clinical Implications of Th2 Immune Responses in Wound Healing and Fibrosis

Defective or aberrant wound healing are debilitating conditions that occur in several diseases, and the annual worldwide market for improved wound care products exceeds $5 billion [73]. A better understanding of this complex process may provide new therapies to improve wound care. As summarized above, Th2 cytokines induce an array of pathways that are effective at coordinating several stages of the wound healing process. However, studies on the therapeutic manipulation of Th2 cytokines and Th2 cyokine-activated pathways to improve wound healing are limited and in their infancy. Employing a rat model of glomerulonephritis where injection of a nephrotoxin induces severe kidney injury and inflammation, adenovirally transfected macrophages that overexpressed IL-4 could ameliorate disease [74]. In another study of mouse colitis induced by chemical injury with dinitrobenzene sulfonic acid, the transfer of AAMs, but not CAMs, reduced disease severity [75]. These studies suggest that treatment with Th2 cytokines or Th2 cytokine-activated cells can improve the disease outcome in some organ injury models. However, whether these treatments ameliorated disease severity through downregulating inflammation and/or acted further downstream to improve tissue remodeling or repair is unclear. Recent preclinical studies of skin wound models have shown promising results of tissue regeneration using mesenchymal stem cells (MSC) [73]. MSCs are multipotent progenitor cells that are easily available from a variety of adult tissue, including the bone marrow and muscle tissue. Due to their regenerative nature and ability to differentiate into several tissue cell-types, MSC are a potential new therapy for improved wound healing. It is unclear if Th2 cytokines promote MSC function. However, recent studies demonstrating the importance of IL-4 in mediating muscle progenitor cell-induced tissue regeneration suggests that the therapeutic effect of Th2 cytokines on stem cell-mediated wound healing warrants further investigation [60].

While the Th2 immune pathway can promote wound healing, excessive Th2 cytokine response occurs in several debilitating clinical conditions. For this reason, many therapies employ inhibitors of Th2 cytokine associated factors to improve wound healing. In particular, overactive Th2 immune responses result in aberrant wound healing, notably the formation of scar tissue (fibrosis) instead of regenerated tissue that is identical to the tissue prior injury. In cases of skin wounds, scar tissue is undesirable due to its appearance and reduced tensile strength compared to normal skin.

However, fibrosis of organs such as the lung (e.g., idiopathic pulmonary fibrosis) or the liver (e.g., schistosomiasis) is even more debilitating as the fibrotic tissue cannot perform essential organ functions. Indeed, an estimated 45 % of deaths in the USA are linked to fibrotic disorders [2]. The formation of scar tissue can be due to a variety of factors, from excess ECM deposition, failed vascularization of new tissue, or even an abundance of inflammatory cells. An exaggerated inflammatory response is one of the most common reasons for scar formation. Inflammatory cells secrete an abnormal concentration of pro-fibrotic cytokines such as TGF-β, PDGF, and IL-4 that stimulate fibroblasts to secrete excess type 3 collagen and granulation tissue [62]. Although an overactive Th2 response causes several debilitating diseases, the generation of such a strong Th2 immune response may have evolved as an important survival mechanism. Indeed, suffering from fibrosis is a far more suitable alternative to fatal blood loss or secondary infection in the situation where tissue remodeling does not occur and there is no protective barrier from the external environment.

Nevertheless, understanding the delicate balance between the various T helper cytokine responses (including Th1 and Th2) is essential to devise new therapies for optimized wound healing. Recent studies have focused on inhibiting TGF-β or its downstream signaling pathway. In systemic sclerosis, where excessive collagen accumulation occurs in the skin and organs [76], clinical trials with anti-TGF-β1 are underway [77]. Additionally, recent therapies include targeting the activation site of latent TGF-β, or disrupting the downstream TGF-β signaling components such as tyrosine kinase or SMAD [78]. However, a new therapy to reduce scar formation involves treatment with Avotermin (human recombinant TGF-β3) [79], suggesting that there is still much complexity in the wound healing process that must be resolved for the design of more efficient and specific therapies for wound healing.

Conclusion

The Th2 immune pathway is not only a dominant cytokine response in helminth infection, chronic infection, and allergy but also an integral component of the wound healing program (Fig. 1). Th2 cytokines IL-4 and IL-13 instruct tissue repair in part through activation of AAMs, but can also act directly on non-immune cells such as fibroblasts and tissue progenitor cells to orchestrate the later stages of wound healing. AAMs express a battery of cytokines, growth factors, and enzymes that act to dampen inflammation while initiating tissue remodeling and tissue regeneration. Finally, in response to Th2 cytokines and AAM-derived factors, non-immune cells, including fibroblasts and epithelial cells, migrate and proliferate to generate newly formed functional tissue and replace the wound. Although beneficial in promoting wound healing, the Th2 response must be tightly regulated to prevent detrimental fibrosis that is observed in several clinical diseases. It is ultimately the interplay between initiator, responder, and effector cells that leads to an optimal wound healing process. Whereas deep injuries, infections, or inflammatory diseases can cause malfunctions in the wound healing process, we can take advantage of various targets in the Th2 pathway

to improve wound resolution. Employing models of injury including infection, burns, mechanical or chemical injuries, researchers can develop new therapeutic targets to promote wound healing and tissue repair while limiting fibrosis.

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Immune Response to Helminth Infections and Its Role in Treatment for Autoimmune Disorders

Rowann Bowcutt, Martin J. Wolff, and P'ng Loke

Introduction

The term "helminth" originates from the Greek word helmins, for parasitic worms. This is a descriptive rather than a phylogenetic term and covers all multicellular animals that have adopted parasitic lifestyles, consisting of platyhelminths that include the trematodes (Schistosomes) and cestodes (tape worms), as well as the nematodes (roundworms). Together helminths affect over one billion of the world's population and have a severe impact on the quality of life, with disease burdens estimated to be in the range of 4.5–39 million disability adjusted life years (DALYs) for the developing world [1, 2]. Although the impact of helminthiases on poor regions of the world continues to cause a cycle of reduced productivity and poverty, in this chapter we mainly discuss the immune-regulatory effects of helminths. Helminths have a remarkable variety of complex life cycles, from direct fecal-oral transmission (e.g., *Trichuris* and *Ascaris*) to development through free-living stages (e.g., hookworm larvae) or dependence on vectors (e.g., schistosome and filarial worms). Helminths invade through many different routes (e.g., orally, through the skin by mosquito bite) and live in different locations of the body (e.g., the gastrointestinal tract, the blood vessels, lymphatics, and tissues). Because humans and other mammalian hosts have coevolved to tolerate these parasites and minimize their virulence [3], the absence of helminths in the developed world may partly influence the rise of autoimmune disorders over the last few decades, as the immune system is no longer being tuned by the presence of helminths [4]. There are currently efforts to determine if the reintroduction of helminths, or their biological products [5, 6], can be used therapeutically for the treatment of autoimmune diseases [7].

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The Coevolution of Helminths with Their Mammalian Hosts

Hosts and parasites are constantly engaged in evolutionary interactions that alter the selection of genes for both parties [8]. The mammalian immune system has coevolved with helminth parasites over time and genes have been selected on both sides to optimize what is essentially a biotic partnership. During the course of human evolution, the majority of *Homo sapiens* were likely colonized by helminths, as are most mammals in the wild [9]. The fact that such a large number of people even today harbor chronic helminth infection without serious adverse effects is likely due to the years of coevolution to optimize the relationship between the parasite and human host [10]. Children that grow up in regions where helminths are endemic carry parasites for much of their lives because they are repeatedly exposed to infection and it takes a long time to develop protective immunity [11, 12].

As noted by the classical paper from May and Anderson [13], helminths are unlike "microparasites" such as viruses, bacteria, and protozoa because they generally do not replicate within their host (with the exception of *Strongyloides stercoralis*). Hence, the worm that persists in infected people is the same organism, with the same genetic makeup, that infected the person originally [13, 14]. While many microparasites cause acute infections with long-lived protective immunity, helminths induce chronic infections, with the same individual organism living in the host for long periods of time [14]. This difference has important effects on the relationship between the host and the parasite, which in turn affects the ecology and evolution of the transmission process [13]. Helminths that are directly transmitted from host to host tend to establish persistent infections with continual reinfections [14]. Since the generation time of these helminth parasites within the host is much greater than "microparasites", the strategy of antigenic variation is unlikely to be available for immune evasion. Hence, helminths have evolved distinct mechanisms to coexist with their host through the activation of an immune regulatory network instead [8, 15, 16]. How helminths can survive in their hosts for years or even decades despite immune recognition by the hosts has long fascinated immunologists.

Helminths have much more complex genomes than "microparasites" and carry similar number of genes as their mammalian hosts (up to 20,000 genes) [17]. The complete reference genomes of *Schistosoma mansoni* [18], *Schistosoma japonicum* [19], *Brugia malayi* [20], *Trichinella spiralis* [21] have now been published. Most recently, the genomes of four tapeworms [22] and *Loa loa* [23] were also published. Most of the earlier genomes were sequenced using traditional Sanger technology. With next generation sequencing approaches cutting the costs of sequencing down dramatically, helminth genomics will be revolutionized and the pace of discovery of helminth derived biological active products will surely accelerate. Since helminths do not replicate within the hosts, their immune evasion mechanisms are all hard wired into their genome by the process of coevolution with their hosts. Their genomes could be considered to be treasure troves of potent biologically active molecules that could be developed for therapeutic use. A better understanding of these host–parasite interactions, through a combination of field studies, clinical trials and laboratory studies with animal models, may enable us to develop novel therapeutic

strategies to regulate the immune response, either positively or negatively. We may also learn how the immune system has evolved over time to minimize the virulence of different types of pathogens.

Parasite Virulence, Host Resistance and Disease Tolerance

While helminth parasites have evolved mechanisms to maximize their goals of increasing transmission, the mammalian hosts have evolved mechanisms to minimize "virulence" caused by the parasites [24, 25]. Virulence here is defined as the reduction of fitness for the host as a result of the burden of carrying infection by the parasite [24, 25]. Two of the main mechanisms that can reduce virulence of the parasites are to reduce parasite colonization of the host (or "resistance") or to minimize the negative impact of carrying the parasites without necessarily reducing parasite numbers (or "tolerance") [3, 26, 27] (Fig. 1). A symbiotic relationship would be one where there is essentially an absence of any negative impact on host fitness from the presence of helminths. While such relationships are possible, some helminths are much more virulent than others, and these have a tremendous impact on the health of the world's population.

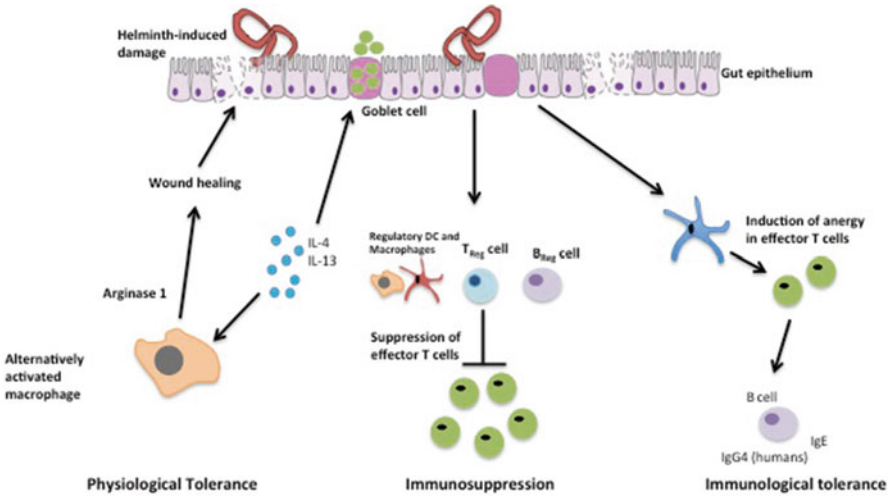

Fig. 1 There are various, albeit interlinked, ways in which a host immune system may develop tolerance to helminth infection. Physiological tolerance involves innate cells such as alternatively activated macrophages that act to minimize damage caused by infection. Regulatory lymphocytes such as Tregs, regulatory DCs and macrophages and B regulatory cells release suppressive cytokines that dampen down effector T cell responses in a process called immunosuppression. Finally effector T cells can also enter a state of anergy whereby they are not fully activated by DCs and thus confer a state of immunological tolerance

Resistance mechanisms that reduce worm burden in individuals are essential because morbidity is often directly associated with worm burden [2]. Both the innate and the adaptive arms of the immune system play an important role in resistance. However, it is also clear that there is a "cost" for being able to mount very strong effector responses that lead to resistance. This cost could be the bystander destruction of the host tissue as a result of immunopathology. Therefore there is a tradeoff between resistance and collateral tissue damage [3, 8]. This was elegantly demonstrated in a study of Soay sheep in Scotland, where clear evidence was collected that resistance to helminth infection could improve survivability during harsh conditions [28]. However, the cost of this resistance was increased autoimmune susceptibility and reduced reproductive ability [28].

In addition to resistance, the mammalian host also employs the strategy of "tolerating" the presence of helminths, for example by reducing the amount of tissue damage that is caused by the parasite [3, 8]. While this strategy does not reduce the number of parasites that reside within the host, it reduces the virulence of the parasite in reducing host fitness. Based on the natural distribution of worm burden among infected individuals in an endemic region (discussed below), tolerating the presence of a small number of parasites (but resisting heavy worm burdens) appears to be the most innocuous strategy for the host, when it is in an environment where it is constantly being challenged and reinfected by helminths.

Heterogeneity of Infection

The number of helminth parasites that are typically carried by each infected individual is often described by a negative binomial probability distribution [13, 14]. This model shows an aggregated distribution whereby there is a greater variance in worm numbers than the mean number of worms per person. This means that most people are infected with relatively few worms, but a few people carry large numbers of parasites. 70 % of the worm burden in a population may occur in only 15 % of the infected individuals [2]. This heterogeneity in worm burden is likely to be a result of variation in immune responses against the parasites, since as people age and become more resistant to infections through building immunity against the parasites over time, there is less aggregation of the distribution of parasites among the hosts [14]. For epidemiology assessments that affect control strategies, "worm burden" or the number of worms that infects an individual is an important measurement [1, 2, 29]. Individuals can be classified into categories of light, moderate and heavy infections by the WHO [1, 29].

However, depending on whether the helminths are tissue dwelling or located in the gastrointestinal tract, worm burden may not always correlate with pathology. The tissue dwelling helminths that cause lymphatic filariasis, onchocerciasis, and schistosomiasis do not always cause greater pathology with greater intensity of infection [11]. For these parasites, individuals with high parasite numbers may be asymptomatic, while individuals with lower-level infections may suffer more chronic pathology because of higher immune reactivity causing more damaging immunopathology [12, 16]. This is in contrast with the gastrointestinal nematodes, whereby symptoms such

as diarrhea, weakness and physical, nutritional and cognitive impairment of children especially are more directly related to the number of parasites in each individual.

This heterogeneity in worm burden and immune responses against helminths, bears important consequences for virulence as well as the potential use of helminths therapeutically. Genetic variability in the parasite population as well as the human population could certainly contribute to this heterogeneity, but there is still a limited understanding of the nature of these genetic differences in the two populations. With the cost of genomic analyses decreasing with the advent of next generation sequencing technology, greater effort should be underway to obtain data characterizing the genetic variability of host and helminths, in relation to disease pathogenesis as well as drug resistance.

Coinfections and the Microbiota

The widespread prevalence of helminths in the developing world, where many other infectious diseases carry important burdens on the health of the population, indicates that coinfection is the norm rather than the exception in many parts of the world. Any researcher that has conducted field studies in developing countries will appreciate that single infections, while predominant among mouse experiments, is uncommon within a human population. Coinfections by helminths may therefore exert powerful regulatory effects on the immune response against other pathogens (e.g., malaria, TB, and HIV/AIDS), as well as on the priming of the immune response by vaccination. More recently, there has been a growing appreciation for the impact of the commensal microbiota on immune responses [30] and helminths may have a substantial impact on the intestinal microbiota in particular [31].

Helminth infections primarily affect the same developing world niche as HIV [32, 33], TB [33, 34] and malaria [35]. A recent series of reviews provides a comprehensive picture of our current knowledge for some of these interactions and provides some interesting hypotheses [36]. Whereas there is considerable experimental evidence in mice that helminth coinfected hosts are less resistant to TB infection as a result of diminished Th1 immunity [34] and increased alternatively activated macrophages [37], the evidence for a detrimental effect of helminth infection on the pathogenesis of human tuberculosis is much less clear cut [33, 34]. There is also a surprisingly neutral outcome for many studies on interactions between helminths and HIV [36]. Hence, the epidemiological evidence that helminth infections may have a detrimental effect on human susceptibility or pathogenesis to other infectious diseases remains rather inconclusive [36].

As vaccines are being developed for HIV and malaria, the development process should take into consideration the helminth infection status of individuals, since specific helminths may suppress vaccine-specific immune responses. It will also be important to test candidate vaccines in animal models that resemble the intended target human population. Hence, there may be a need to develop vaccines capable of overcoming the suppressive effects of certain helminth infection [38], or else administration of anti-helminthics to the target population may be necessary prior to

vaccination. Very few studies to date have addressed whether anti-helminthic treatment may reduce pathogenesis of other infections or improve responses to vaccines. One exception is schistosomiasis, which has been shown to have a particularly detrimental effect on HIV transmission and progression [32], probably because of the strong inflammatory response driven by the schistosome egg. Anti-helminthic treatment against soil-transmitted nematodes with albendazole during pregnancy did not improve responses to BCG, tetanus, or measles, or reduce malaria, diarrhea, and pneumonia in infancy [39]. One possibility to explain these surprisingly neutral epidemiological results is that the immune regulatory effects of helminths have been selected to avoid disrupting protective responses against other dangerous pathogens [36].

In addition to the burden of infections, the intestinal microbiota communities of residents of developing countries are also very different to residents from the developed world [40, 41]. For example, the genus *Prevotella* is more abundant in the fecal microbiome of children in developing countries compared to Europe and USA [41]. While it has not yet been investigated, helminth infections may have a substantial impact on the microbiota of residents from developing countries. In animal models, the nematode *Heligmosomoides polygyrus* was shown to alter the gut microbiota of healthy mice [42] and members of the Lactobacillaceae family was increased after infection [42]. *Trichuris suis* infection of pigs also alters the microbiota [43, 44]. Since *Trichuris suis* is under investigation as a therapeutic agent for inflammatory bowel diseases (IBD) and the microbiota is also altered in IBD patients, helminth infection may influence IBD symptoms either directly or indirectly through alterations to the microbiota. Additional evidence that helminths may reverse dysbiosis of the microbiota comes from macaques suffering from chronic colitis that have been treated with *Trichuris trichiura* [45]. An exciting area of future research would be to understand the interaction between helminths, the microbiota and the host immune responses.

Immune Responses to Helminths

Helminth infection in humans is characterized by two key features; (1) A predominantly type 2 immune response [46], driven by the increased production of the cytokines such IL-4 and IL-13 and (2) downregulation of the immune response as depicted by elevated levels of IL-10, TGF-b and frequency of regulatory T cells.

Type 2 Immune Responses to Helminths

Elevated levels of type 2 cytokines (IL-4, IL-5, IL-9, and IL-13) and CD4+ T helper (T_H) 2 cells are found in helminth infected individuals in endemic regions [14]. IL-4 is a key cytokine that also plays a critical role in promoting B cell responses

secretion of Immunoglobulin E (IgE) [46]. IgE can engage a positive feedback loop to amplify the type 2 response by activating basophils, eosinophils, and mast cells through Fc receptors to produce even more IL-4. This type 2 (or T_H2) immune response likely evolved to protect mammalian hosts from helminth infections [3]. Since helminths are large multicellular organisms, they can cause considerable amounts of tissue damage to their mammalian hosts and the type 2 response may have evolved to contain the tissue damage that is cause and to tolerate the presence of these organisms [3, 47]. In addition to disease tolerance, the type 2 response is also important for mediating resistance against the helminths, especially in the gastrointestinal tract [48]. IL-4 and IL-13 will signal through IL-4Ra and STAT6 in intestinal epithelial cells (IECs) to increase goblet cell differentiation and mucus production (Fig. 1). Additionally, these cytokines will also increase proliferation and turnover of the IECs, which may sweep parasites embedded in the epithelial layer into the lumen for expulsion [49]. At the same time, these signaling events will act on intestinal muscle cells to increase contraction to help flush the worms out of the gut [48]. Indeed human epidemiological studies showed that elevated levels of IL-9, IL-10, and IL-13 negatively correlated with *Ascaris lumbricoides* (*A. lumbricoides*) infection [50], and a negative correlation with IgE was observed with *T. trichiura* infection intensity [51]. Additional epidemiological data from studies with people infected by hookworm [52], roundworm and whipworms [50, 53, 54] have been consistent with the model that increased type 2 responses is associated with increased parasite resistance (Fig. 2).

The type 2 responses described above that may mediate parasite resistance may also enhance the intestinal mucosal barrier against gut bacteria. The host response has the dual aims of parasite expulsion as well as mucosal healing [48]. In addition to T_H2 cytokines, IL-22 is also induced by *T. trichiura* [55] and *Necator americanus* [56] in the intestinal tract. IL-22 has similar effects as TH2 cytokines on colonic epithelial cell function [57, 58], including the stimulation of goblet cell and Paneth cell differentiation, as well as increased mucus production and antimicrobial peptide expression, and the activation of anti-apoptotic pathways. Other cells activated by type-2 cytokines such as alternatively activated macrophages can also promote mucosal healing [59]. Hence, the mechanisms activated by type 2 responses to mediate parasite resistance also enhance the epithelial barrier and these responses have been demonstrated to have protective effects in murine models of colitis [59–61].

Immune Regulation During Helminth Infections

In an endemic region where human populations have a high prevalence of helminth colonization, a large proportion of individuals have asymptomatic infections, which is associated with suppressed reactivity against the parasites [12, 14]. Peripheral T cells from these infected patients are unresponsive to stimulation with parasite

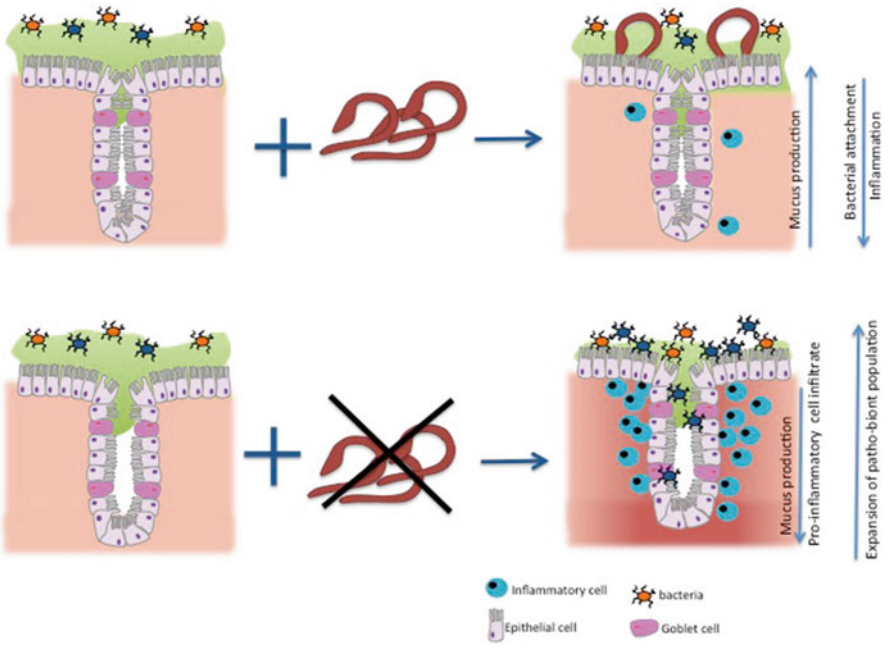

Fig. 2 The presence or absence of helminth infection in the gut can have a dramatic influence on the physiological conditions. Helminth infection promotes mucus production from goblet cells. Increased mucous barrier helps protect the underlying epithelium from pathogenic attack. In addition, helminth infection can affect the host microbiome. In the presence of helminth infection a balance between particular microbial populations is maintained, whereas, in the absence of helminth, an otherwise symbiotic bacteria, due to exacerbated proliferation, can become pathogenic and thus cause increased inflammation and pathology

antigens and responses to other antigens are also reduced [12]. These original immuno-epidemiological observations for many different types of helminths [15, 16] eventually led to efforts to identify various immune regulatory mechanisms that may be induced during helminth infection [8], in addition to the type 2 responses already described above. Since these suppressed responses can be reversed by anti-helminthic treatment [15, 16], the assumption is that active suppression by living parasites is required.

Since regulatory T cells (Tregs) have been extensively studied as one of the most potent regulators of the immune response, they have also been investigated during helminth infections [62]. In an endemic population of long-term filarial exposed individuals, the large proportion of asymptomatic cases with patent infections (microfilaremic, Mf+, with bloodstream microfilariae) have increased levels of the suppressive cytokine interleukin-10 (IL-10) [63] and increased number of T cells expressing CTLA-4 (cytotoxic T lymphocyte antigen 4) [64]. In schistosomiasis,

individuals with less pathology but are chronically infected have higher frequencies of CD4+ CD25 high Tregs [65]. In gastrointestinal nematode infections such as Ascaris, hookworm and *T. trichiura*, patients also have a higher frequency of circulating CD4+ CTLA-4+ T cells [66] and increased levels of the cytokines IL-10 and TGF-β are significantly linked with hyporesponsiveness and susceptibility [67].

In mouse models of helminth infections, treatment with neutralizing antibodies against CTLA-4 can enhance cytokine responses [68–71] and in some cases can promote parasite clearance [69, 72]. Treatment of helminth infected mice with depleting antibodies against CD25 have also demonstrated the importance of CD25+ Tregs in reducing CD4+ T cell effector responses and subverting parasite clearance [62]. Mice with FoxP3-expressing Tregs expressing the diphtheria toxin receptor (or DEREG mice) have more specifically targeted FoxP3-expressing Tregs in regulating effector responses that mediate parasite killing [71] and inflammatory pathology [73]. In summary, Treg populations that are induced by helminths appear to benefit both parasite and host by limiting pathology and reducing the intensity of parasite resistance mechanisms by the host.

IL-10 producing regulatory B cells are a more recent discovery that have been shown to play an important role in limiting disease severity during autoimmune diseases [74] and then later found to be induced by helminths [75]. In these mouse studies whereby helminths was first shown to suppress allergic inflammation, it was then shown that suppression could be reversed by depleting these B cells or transferred to a recipient animal by transferring B cells. In a male worm only infection model (without inflammatory schistosome eggs), *S. mansoni* could protect mice from anaphylaxis and allergic airway inflammation through an IL-10 dependent mechanism, which was found to be produced by regulatory B cells [76]. In *H. polygyrus* infected mice, adoptive transfer of mesenteric lymph node B cells could suppress DerP1 induced airway inflammation, but this was independent of IL-10 [77].

Alternatively activated (or M2) macrophages [78] have also been shown to induce by helminth infections and may be a key part of immune regulation during helminth infections [8]. These cells may directly suppress effector CD4+ T cell responses by upregulating arginase 1. This enzyme plays an important role in both immune regulation as well as tissue repair, by competing for L-arginine and generating proline [8, 78]. Alternatively activated macrophages that are expanded during helminth infections have also been shown to promote Foxp3+ Treg differentiation through the production of retinoic acid [79] and hence mediate immune regulation through an indirect mechanism by promoting Treg differentiation. Increased abundance of alternatively activated macrophages has been noted in several study cohorts [11, 80] and has been suggested to be responsible for the bystander suppression of autoimmune diseases.

While the type 2 responses are important in limiting parasite numbers and reducing tissue damage caused by the parasites, the immune regulatory network triggered by the helminths benefits the parasite as well as the host by reducing the effectiveness of the resistance mechanisms of the host while also limiting tissue damage from immune pathology [12] (Fig. 3).

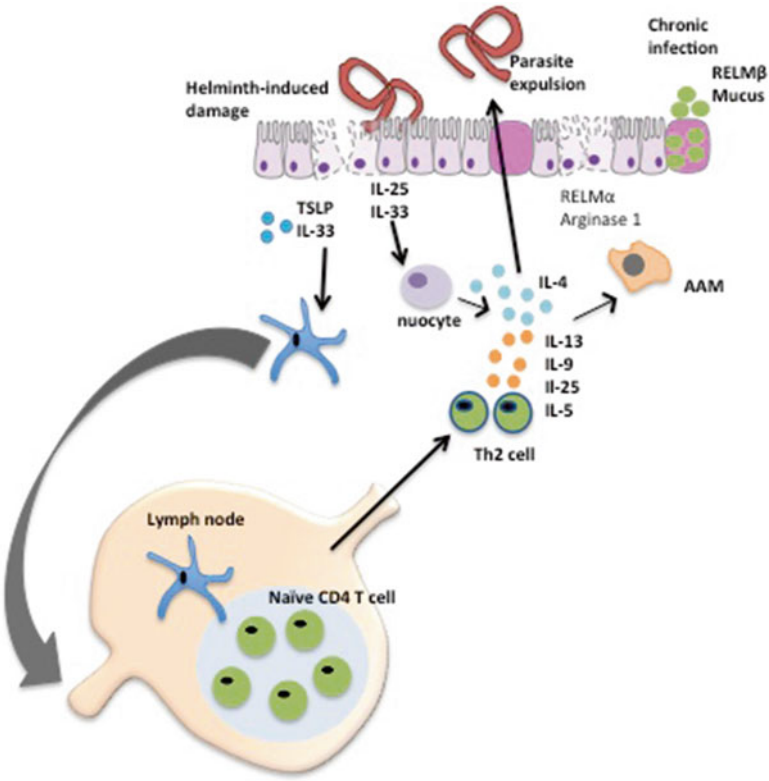

Fig. 3 Intestinal helminth infection causes the intestinal epithelial cells to express a variety of alarmins and immunoregulatory cytokines such as IL-33 and TSLP. These have an ability to influence intestinal DC responses, guiding them towards a Th2 response. Conditioned DCs then migrate to the draining lymph node where they prime naïve CD4 cells into Th2 effector T cells. These cells then promote parasite expulsion through the release of Th2 cytokines such as IL-4, Il-13, IL-5, and IL-9. In addition, IL-25 and IL-23 release from epithelial cells can promote IL-4 production from innate cells such as nuocytes which can then cause the alternative activation of macrophages which are involved in tissue repair

Helminths and Autoimmune Diseases

As discussed above, there is an intimate relationship between host and parasites that profoundly influence the immune system. The epidemiology of autoimmune diseases and helminth infections has led to the hypothesis that helminth infection could improve inflammatory diseases (Fig. 4). Animal models as well as clinical studies have been supportive of these concepts, although it is important to note that the very different properties of different helminth life cycles influence the host in profoundly different ways and there are certainly examples of detrimental effects of helminth infections in the context of inflammatory diseases [81, 82]. Nonetheless, there is an increasing interest in the therapeutic use of these parasites for treatment of autoimmune diseases [7].

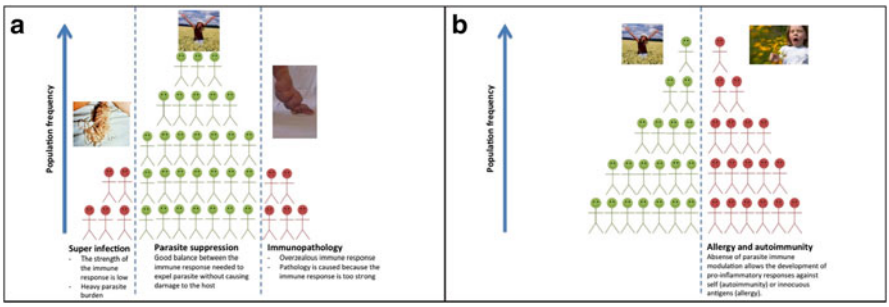

Fig. 4 The immune response must strike a balance between being strong enough to eliminate infection without causing detrimental damage to the host. The coevolution of parasite and host will have allowed the positive selection of genes that have a mild suppressive effect on parasite survival, thus promoting low level parasite infection with little morbidity for the host. The majority of the human population living in parasite endemic areas will fall in to this category. However, in a small fraction of people the immune system will mount a response that is either too weak resulting in high parasite burden, or too strong resulting in excessive inflammation and pathology (**a**). In the developed world, where childhood exposure to parasites is absent, the immune system has become more pro-inflammatory and developed sensitivity to either innocuous or host antigens. This has allowed the development of pathology such as allergy and autoimmunity (**b**). Images are modified and sourced from: http://neglected-tropical-diseases.wikispaces.com/Lymphatic+filariasis

Increasing Inflammatory Diseases of the Modern Developed World

Autoimmunity occurs through an aberrant immune response triggered by interactions between host genetics and environmental factors in multiple different ways that are gradually being elucidated [83]. During the past 50 years the developed world has seen a dramatic rise in inflammatory diseases such as autoimmunity and allergy [84]. The time scale for these increases has been too short to be accounted for by genetic factors, thus environmental changes must have a role. Among the hypotheses proposed is the hygiene [85] and "old friends" hypothesis [86], which suggests that the improvement in living conditions and vaccination strategies has reduced exposure to certain infectious agents during childhood, leading to the development of unregulated immune responses and the onset of inflammatory disorders. In the 1960s, it was first noticed that the incidence of rheumatoid arthritis in western Nigeria was uncommon [87] and the authors proposed that malaria and other parasitic infections may alter the immune system of African natives such that they are protected from the onset of such diseases [87]. Later on Strachan observed an inverse correlation between children suffering from hay fever and the number of older siblings [88] and proposed that this could be a relationship between hygiene and immunity. Indeed, the distribution of allergic and autoimmune diseases is a mirror image of the distribution of many common infectious diseases.

Even within developed countries (e.g., in Europe), growing up in a rural environment results in exposure to various allergens and microbes that may decrease the chances of contracting allergic disease [89]. In developing countries also, Gabonese school children infected with *Schistosoma haematobium* showed less allergic reactivity to the house dust mite allergen than their non-infected classmates [90], Later studies by the same group showed that long term treatment with anti-helminthic drugs to cause clearance of the parasites *A. lumbricoides* or *T. trichiura* resulted in an increase in skin sensitivity to house dust mite allergens. When there is movement of people from developing to developed countries, migration studies have shown that offspring of immigrants coming from a country with low prevalence for type-1 diabetes [91] or multiple sclerosis [92] acquire the same disease incidence as the host country within the first generation. Similar observations have since been made for various inflammatory disorders such as inflammatory bowel disease. As populations in developing countries, such as Brazil [93] and India [94], become more socioeconomically advanced a rise in cases of IBD has been observed.

Other factors are certainly involved in the observed increase in allergy and autoimmunity in the developed world, beyond helminth infection. Other investigators have performed similar anti-helminthic allergy trials to those described above but failed to find any increase in atopy or clinical allergy upon deworming of school children [95]. In a large study of children in Denmark, *Enterobius vermicularis* infection did not reduce risk for any chronic inflammatory diseases [96].

We are at an early stage of understanding how host genetics, such as susceptibility genes that have been identified for diseases such as IBD [97], multiple sclerosis [98] and type 2 diabetes [99], interact with changing environmental factors to cause disease. Individuals may have genetic predispositions to certain autoimmune diseases and when carrying a helminth infection (or with specific microbiota) the development of disease is somehow controlled, but upon removal of the parasite (or treatment with antibiotics and dietary changes) the predisposition is unmasked and disease develops. These complex interactions between the host and the environment resulting in heterogeneity of outcomes are difficult to model with inbred strains of mice and additional work with human populations is necessary for a better understanding.

Clinical Trials of Helminth on Autoimmune Diseases

The observation that helminths can regulate inflammatory responses has led to a number of completed and active clinical trials exploring the use of helminths to treat autoimmunity and allergic diseases [100]. The clinical trials to date have used either the pig whipworm, *Trichuris suis*, or the human hook worm, *N. americanus* [101].

T. suis is closely related to the human whipworm *T. trichiura*; however, *T. suis* differs on a morphological and molecular level [102–105]. TSO, or *Trichuris suis* ova is now being produced under pathogen-free conditions and has a self life of approximately 2 years making it a viable therapeutic product. *T. suis* can transiently colonize the human colon but does not cause disease or multiply inside their host,

hence direct transmission from one person to another is unlikely. Pig farmers are readily exposed to this helminth and have not ever reported symptoms. The first therapeutic treatment with *T. suis* was in 2003 by Summers et al. on seven patients with inflammatory bowel disease [106]. In this trial and subsequent trials by the same group TSO treatment significant improved symptoms for both ulcerative colitis and Crohn's disease without any side effects [107–110]. In a randomized placebo-controlled double-blind study for 54 subjects with moderate to severe ulcerative colitis, after 12 weeks of therapy, 43.3 % of the individuals treated with TSO had improved symptoms compared to 16.7 % in the placebo group. An open label study of TSO in 29 patients with active Crohn's disease showed a remission rate of 72.4 % [110]. Larger phase II dose-escalation trials of TSO in Crohn's disease are ongoing in Europe currently (Dr. Falk Pharma, GmbH; NCT01279577) and the USA (Coronado Biosciences and OvaMed GmbH; NCT01434693). We are also recruiting moderate to severe ulcerative colitis patients to conduct an exploratory mechanistic trial of TSO in order to better characterize the mucosal immune response at NYU School of Medicine (NCT01433471). Unfortunately, the phase II studies of TSO in Crohn's disease have since failed to show significant benefit over placebo, partly the result of a strong placebo effect. However, a NIAID funded Phase II study is still ongoing for ulcerative colitis (NCT01953354).

Because of the systemic immune regulatory effects of helminths, helminths have also been tested for other diseases [111, 112]. In a study of 12 patients with relapsing-remitting multiple sclerosis who happened to be infected with various helminths (*Hymenolepis nana*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides sterocolaris*, and *Enterobius vermicularis*), Correale et al. reported that helminth infected patients had a significantly lower number of exacerbations and fewer magnetic resonance imaging changes compared with uninfected patients [113]. This was associated with increased regulatory cytokine production (e.g., IL-10 and TGF β) and CD4+CD25+FoxP3+ T cells and regulatory B cells in the infected cohort [113, 114]. Fleming et al. then treated five subjects with treatment-naïve relapsing-remitting multiple sclerosis with TSO [115] and observed reduced lesions in treated individuals at the end of 12 weeks of TSO treatment. Lesion incidence increased again after the completion of the treatment phase, indicating that any protective effects were transient. There is an active study for MS and clinically isolated syndrome (TRIOMS) in Germany (NCT01413243).

Although TSO appears safe so far the possibility of symptomatic infection cannot be entirely ruled out [116]. Diarrhea and abdominal pain were reported in 30 % of patients receiving TSO for treatment for allergic rhinitis [111]. However, these problems were transient, peaking at about day 40, after which the incidence of adverse effects lessened to levels similar with placebo. TSO has been studied in IBD patients on concomitant prednisone, thiopurines, and other immunosuppressants, suggesting relative safety in immunocompromised hosts [117]. Thus far, every study has shown that TSO is very well tolerated; although biological activity in terms of objective measures of beneficial effect remain elusive. The species barrier between the pig parasite and the human host makes appropriate dosing regimens to induce substantial biological activity to be particularly difficult. It is also difficult to establish the equivalent of pharmacokinetics for these live organisms.

The hookworm *N. americanus* has also been used in clinical trials but with less reported success than TSO. While hookworm can upregulate immunoregulatory molecules such as IL-10 and TGF-β [85, 118–120], infection can also cause serious adverse effects, most notably gastrointestinal symptoms and iron deficiency anemia secondary to chronic blood loss [118]. Dose-ranging studies of *N. americanus* in humans have shown that doses higher than ten larvae correlate with more frequent adverse events [118, 121]. This is a very small number compared with the 2500 TSO being used in Phase II trials, hence having a much narrower therapeutic window before having adverse effects. Thus far randomized double-blinded studies of *N. americanus* as treatment for asthma [122] and celiac disease [123] has not demonstrated statistically significant benefits. The relatively low inoculation dose of hookworm (10–15 worms) used in these trials may have been insufficient to induce an immunosuppressive phenotype in this patient population. Remarkably, when infection with 20 *N. americanus* was combined with a 12 week microchallenge regimen (10–50 mg), subjects were now able to tolerate a large gluten challenge (3 g daily) for 2 weeks [124]. Hence, we are just beginning to understand the nuances of different potential therapeutic strategies.

In addition to these clinical trials, some individuals have opted to self-treat with helminths and online forums exist where patients can share their experiences with each other. One individual who infected himself with the helminth *T. trichiura* for his symptoms of ulcerative colitis provided us with longitudinal data on changes to his mucosal responses [55]. Before taking *T. trichiura* the patient had severe disease with extensive ulceration of the mucosal epithelium and the development of crypt abscesses. The patient ingested 500 embryonated *T. trichiura* eggs with an additional 1000 eggs 3 months later. The months following this he observed an improvement in his symptoms until he was eventually symptom free with no need for additional UC medication [55]. Additional personal success stories have been noted through use of hookworm to treat allergy and Crohn's disease [125]. While intriguing and informative in certain cases, these case reports are no alternative to large and rigorous clinical trials that are ongoing.

Mechanisms of Action for Helminth Treatment

Data from many different mouse models have also demonstrated that helminth infection may be beneficial to the host under specific conditions [4]. One of the first studies was with the nonobese diabetic (NOD) mice that spontaneously develop type1 diabetes; however, when infected with *S. mansoni* the incidence of diabetes dramatically reduced [126]. Furthermore, diabetes could be prevented in NOD mice by administration of the parasite egg alone [126] and soluble egg or worm extracts [127]. Type-1 diabetes has since been shown to be prevented by *Trichinella spiralis* [128], *Litmosoides sigmodontis* [129], and *Heligmosomoides polygyrus* [116] infections. In these particular studies, the induced Th2 response during helminth infection was the leading cause for the prevention of inflammatory autoimmune disease.

However, many of the mechanism described in the earlier section on immune responses to helminths may likely act in concert to suppress inflammatory diseases. For example, the filarial parasite *Fasciola hepatica* prevents experimental autoimmune encephalomyelitis in a TGF-beta dependent manner [130]. Whereas, *H. polygyrus* infection protected mice lacking the regulatory cytokine IL-10 from the development of spontaneous colitis, however, disease resolution was caused by an increase in IL-13 rather than IL-4 or IL-5 [131]. Hence, the different helminths could be suppressing different diseases through different mechanisms.

Tregs were an obvious candidate and have been studied extensively in mouse models of helminth treatment. However, these functional studies of Treg populations have shown that there is considerable heterogeneity in the role of Treg subsets among helminth infections, when used to suppress inflammatory diseases. There are examples of where they are important, as well as irrelevant.

Suppression of allergic airway disease by infection with the intestinal nematode *H. polygyrus* could be reversed by depleting CD25+ cells with a depleting antibody, and adoptive transfer of CD4+CD25+ T cells from infected mice (of both wild-type and IL-$10^{-/-}$ genetic backgrounds) can mediate the therapeutic benefits of infection [132]. Similarly, reduced airway inflammation in response to *S. mansoni* eggs was dependent on CD25+ cells, but not IL-10 receptor signaling [133]. However, neither the depletion of CD25+ cells nor TGFβ neutralization affected the suppression of airway inflammation mediated by the tissue dwelling filarial nematode *Litomosoides sigmodontis* [134]. In contrast to the asthma model, *H. polygyrus*-mediated inhibition of diabetes onset, in NOD mice as well as cyclophosphamide-induced diabetes, was not reversed by CD25 depletion or IL-10 neutralization [135]. The antidiabetic effect of schistosomal egg antigens (SEA) was transferable by adoptive transfer of unfractionated, but not CD25-depleted splenocytes from SEA-exposed mice [136]. In contrast, while the suppression of diabetes by *L. sigmodontis* infection did not appear to be dependent on CD25+, FoxP3+ Tregs, or IL-10 signaling, neutralization of TGFβ reversed the therapeutic effect [137]. TGFβ was also shown to be critical in *Fasciola hepaticus*-mediated protection against experimental autoimmune encephalomyelitis, a model of multiple sclerosis [130]. In a study of DSS-induced colitis, CD25 depletion also did not reverse the protective effect of *S. mansoni* exposure [138].

Therefore, distinct Treg subsets induced by the same helminth infection may mediate protection against different inflammatory diseases (for example, TGFβ is essential for *L. sigmodontis*-mediated suppression of diabetes but not allergic airway disease). Finally, for some models of helminthic therapy (for example, chemically induced colitis) a role for helminth-elicited Tregs remains to be demonstrated. In summary, the different variety of helminths, living in different tissues and with different genomes is suppressing different inflammatory diseases through different mechanisms. There is probably not a “common mechanism” by which helminths are eliciting bystander suppression against inflammatory conditions. Individual mechanisms will have to be established for every different disease and helminth combination. Furthermore, these are all studies conducted with inbred strains of mice, thus in human conditions where different genotypes and environment leads to heterogeneity of responses, the situation is likely to be even more complex.

In addition to the Treg compartment, which could be considered part of the adaptive immune response, innate immune cells such as dendritic cells (DCs) and macrophages (MFs) are also important for regulating the immune response during helminth infection. Since the phenotypic markers and tools for characterizing these cells are not as advanced as for T cells, there is even greater gaps in understanding how they function to suppress disease. For example, it was demonstrated that Balb/c mice infected with male worms of *S. mansoni* are protected from DSS-induced colitis through a macrophage dependent pathway and not through IL-10, TGFβ or Tregs [138]. But the type of macrophage responsible was not determined. Extracts from the tapeworm *H. diminuta* can reduce inflammation caused by DNBS-induced colitis, perhaps because it suppresses macrophage activation [139]. But the mechanism by which this may occur is still unknown. Intestinal DCs and MFs are clearly important in regulating mucosal homeostasis [140], but we are still at early stages of understanding their regulation under homeostatic as well as inflammatory conditions. It should not be surprising that their phenotype will be altered by the presence of helminths in the intestinal tract. As described above, alternatively activated macrophages (or M2 cells) are induced by helminths to repair tissue damage [3] and have also been shown to suppress colitis [141], so these could well be important in suppressing colitis during helminth infection.

For inflammatory bowel diseases, ulcerative colitis (UC) and Crohn's disease (CD) have different pathophysiologies [142]. For a substantial subset of UC patients the intestinal mucus gel layer has been found to be abnormal in both quantity and quality [143]. Muc2, which is the most important mucin in the intestine [144], is reduced in rectal mucus samples from UC patients [145] and displays altered glycosylation [146] and reduced sulfation [147]. Genetic deficiency [148, 149] or terminal misfolding [150] of Muc2 can cause severe and spontaneous colitis in mice without experimental perturbation. Impaired glycosylation of mucins due to specific glycosyltransferase deficiencies also increases susceptibility to colitis [151].

In addition to mucins, phospholipids such as Phosphatidylcholine (PC) contribute towards the mucus gel in the intestine. Abnormalities in phospholipid species have also been described in UC patients, with a significant decrease in PC [152–154]. Indeed, clinical trials by oral intake of delayed-release PC have shown promising results as treatment for some UC patients [154]. Since helminth infections, especially of the intestinal tract are also associated with quantitative and qualitative changes in the mucus gel, we have hypothesized that this may be an additional mechanism of action for helminths to suppress colitis [101].

From the case study described above in which an individual self infected with the human whipworm *T. trichiura*, IL-22 producing CD4+ cells (or TH22 cells) was found to be induced in parts of the colon that were colonized by the worms [55]. When larger number of UC patients were examines, TH22 cells was found to be depleted in regions of the colon with active inflammation and this depletion was associated with changes to the microbiota [155]. However, "mono" TH17 cells not producing other cytokines (that were examined) were enriched in regions with active inflammation in an inverse relationship with the TH22 cells [55, 155]. TGF-b could inhibit the differentiation of TH22 cells from human lamina propria CD4+ cells [155], just as was demonstrated in mice [156].

Further evidence for IL-22 induction in the human intestinal mucosa during helminth infection comes from trials with patients suffering from celiac disease [56]. Patients given the hookworm, *Necator americanus* (*N. americanus*), and in vitro analysis of intestinal biopsies restimulated with *N. americanus* excretory/secretory proteins showed an upregulation of IL-22 mRNA whereas biopsies taken before *N. americanus* infection did not [157]. Hence, the induction of TH22 cells or increased production of IL-22, in addition to TH2 cells by Trichuris infection may improve the mucosal barrier function by increasing mucus production by goblet cells and proliferation of intestinal epithelial cells. IL-22 is a member of the IL-10 cytokine family [158] and downstream signaling events can induce genes involved in antimicrobial defense, epithelial repair (wound healing) and mucin production [159].

A consequence of this response may be to reduce the quantity of attached bacteria to the intestinal mucosa. Evidence to support this comes from a study of macaques suffering from chronic colitis [45]. A previous study had demonstrated dysbiosis in these macaques [160]. When the macaques were treated with *T. trichiura*, alterations to the mucosal microbiota in intestinal tissues during colitis and after helminth treatment was examined and found to restore bacterial diversity and reversed the dysbiosis of mucosal microbiota communities to more closely resemble healthy control animals [45]. In a field study of indigenous communities in Malaysia, helminth colonization was associated with increased diversity of the gut microbiota [161]. As described in a section above, the changes to the mucus gel of the intestine during helminth infection will have a major impact on the gut microbial environment. Some of the protective effects of helminth infection may be a result of indirect effects downstream of gut microbiota alterations rather than direct effects of helminth infection.

Conclusion and Future Perspectives

Helminths and humans have coevolved to minimize virulence during infection while enabling parasite transmission. Usually, helminths infect without causing too much damage to the host, but the heterogeneity in responses to helminths leads to severe morbidity in a proportion of infected individuals. While there is an increasing interest in the therapeutic use of these parasites for treatment of autoimmune diseases, there is still a limited understanding of the heterogeneity of responses to different helminths. This could be a complex relationship between host genotype, parasite genotype and the intestinal microbiota of infected individuals. Helminths may act through different mechanisms for intestinal versus extra-intestinal inflammatory diseases (e.g., multiple sclerosis). While regulatory cells and cytokines probably play an important role in the mechanism of action, stimulation by the helminths of type 2 immunity may have direct effects on mucosal barrier function to play an equally important role in inflammatory bowel diseases. Increased mucus production, changes to the composition of mucus, and increased epithelial cell turnover, may reduce inflammatory responses triggered by gut bacteria. Alterations to the gut bacteria by the presence of helminths may also be beneficial against

inflammatory diseases, since there is preliminary evidence that microbial diversity is increased by helminth colonization. Through the combination of mechanistic clinical trials and animal models of helminth infection and inflammatory diseases, novel pathways that mediate the coexistence of helminths and humans may be identified and targeted as new strategies for controlling inflammatory diseases.

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Type 2 Immunity and Metabolism

Priya Prahalad, Justin I. Odegaard, and Ajay Chawla

Introduction

Human physiology is the product of a millennia-long evolutionary balancing act of mammals caught between the many and varied threats of a brutish world. Starvation, infection, predation, and exposure all loomed as ever-present threats, pulling in different directions and demanding a portion of generally meager resources. Even today, in a significantly less brutish world of hygiene and caloric plenty, we must still balance the demands of remaining infectious threats and our own increasingly unpredictable behavior (including an evolutionarily eclectic diet). Our great evolutionary talent then is perhaps our remarkable ability to adapt to these pressures and find a balance through which limited resources can meet numerous, and often conflicting, demands.

Given their millennia of cohabitation and co-regulation, it is unsurprising that humans' various physiologic systems have evolutionarily converged around common regulatory axes able to coordinately control disparate processes in pursuit of

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shared biological goals. While examples of inter-system coordination are far from rare, much of this concept's recent acceptance has been driven by the wealth of observations concerning immunity and metabolism, two evolutionarily disparate and physiologically crucial systems [1, 2]. In this chapter, we discuss immunity's participation in core metabolic processes at the cellular level and in specific tissues. More specifically, we highlight the contribution of type 2 immune responses to metabolic function in leukocytes, white and brown adipose tissues, and the liver in the contexts of both health and disease.

General Principles of Energy Metabolism

Much of mammalian homeostasis is organized around the central rhythms of feeding [3, 4]. As such, metabolism, in a very basic sense, can be divided into periods of feeding (Fig. 1a), during which anabolic, nutrient-storing processes dominate, and periods of fasting (Fig. 1b), during which catabolic processes mobilize nutrient stores to meet continued energy demands. Each of these basic metabolic states is, in turn, regulated by a central axis (or axes) of control. During feeding, for example, overall metabolic function focuses on nutrient processing and storage and is coordinated primarily through the insulin regulatory axis [3, 5]. Here, in response to elevated glucose levels (such as occur postprandially), insulin directs its target tissues—primarily the liver, adipose tissue, and skeletal muscle—to take up glucose from the blood and store it as either glycogen or lipid while simultaneously inhibiting the release of such stores. Without continued feeding, these processes rapidly reestablish baseline nutrient levels, removing the stimulus for insulin secretion, effectively terminating insulin-stimulated nutrient uptake and storage, and, importantly, relieving insulin's inhibitory effect on catabolic, nutrient-liberating processes. Even with the termination of postprandial insulin-stimulated uptake, however, metabolic consumption continues to remove nutrients from the circulation, lowering concentrations below baseline. In the absence of nutrient intake (fasting), declining nutrient levels activate a broadly catabolic metabolic program, largely mediated by the glucagon control axis, with the overall purpose of mobilizing stored nutrients to support continued metabolic activity [6].

These descriptions, of course, are vastly simplified. In reality, metabolism spans a far greater variety of conditions than simply "fed" and "fasted"; however for the purposes of this review, understanding the basic concepts governing storage and release of energy provides a framework for understanding the interconnectedness between metabolic homeostasis and immune balance.

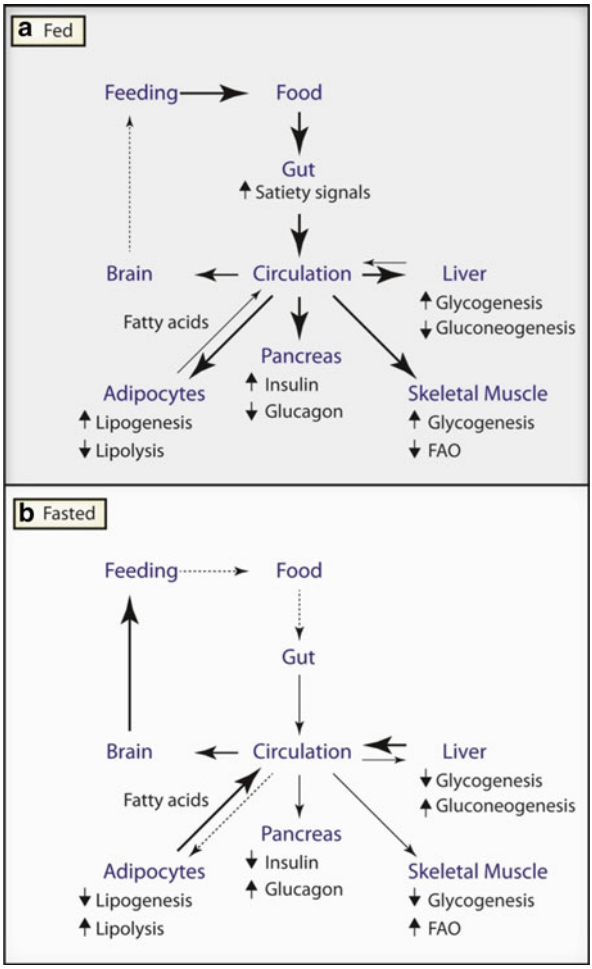

Fig. 1 Nutrient handling in the fed and fasted states. In the fed state (**a**), high circulating nutrient levels stimulate the release of insulin, which simultaneously drives nutrient uptake and storage in peripheral tissues such as skeletal muscle, adipose tissue, and liver and inhibits their release of stored nutrients. Without continued feeding, these actions decrease circulating nutrient levels, abolishing the incident stimulus for nutrient storage. In fasted mammals (**b**), nutrient levels fall below baseline, triggering catabolic mediators such as glucagon that drive mobilization of stored nutrients into circulation while inhibiting their uptake. These catabolic mediators also drive a parallel effector response of increased hunger and, thus, feeding behavior, which eventually similarly increases the circulating concentration of nutrients

Metabolism Fuels Immune Responses

In addition to organism-wide regulation (e.g., insulin), metabolism is also tightly controlled at the cellular level, where cells autonomously and flexibly regulate energy balance by matching substrate availability to physiologic demand and function [7]. For example, under conditions of relatively low bioenergetic needs, cells catabolize glucose and fatty acids via oxidative metabolism, a process by which these substrates are converted first to two-carbon intermediates that are then oxidized

more completely to carbon dioxide and water. This process is highly efficient, producing more ATP per substrate molecule than any other (36 molecules of ATP per molecule of glucose); however, it requires both time (oxidative metabolism being relatively slow) and oxygen and produces no carbon molecules that may be used biosynthetically (at least in mammals). Under conditions of intense cellular energy or synthetic demand, these requirements can limit oxidative metabolism's ability to fully support cellular function. In this context, cells switch from this aerobic program to an anaerobic that relies instead on glycolysis, the process by which glucose is broken down into pyruvate. In oxidative metabolism, this pyruvate is then further oxidized, as discussed above; however, when glycolytic flux outstrips the capacity of the TCA cycle to accept further input (such as occurs when oxygen is limiting), it is converted to lactate and discharged from the cell. While this partial oxidation only yields two molecules of ATP per molecule glucose (1/18th the yield of full oxidation) and, furthermore, is unable to burn fatty acids at all, its relative rapidity and independence from oxygen allows the flux through this pathway to be increased to tremendously high levels, producing much more ATP per unit time than the more efficient oxidative pathway. Moreover, glycolytic metabolism also produces biosynthetically useful carbon intermediates that can be incorporated into macromolecule synthesis [8, 9]. While they are common, kinetics and oxygen supply limitations are not the only reasons that cells employ a glycolysis-based metabolic program; in fact, aerobic glycolysis, also known as the Warburg effect, is often seen in the context of intense biosynthetic requirements, where glycolysis- and TCA cycle-derived carbon intermediates are required as precursors molecules [10]. Indeed, the consumption of intermediate metabolic substrates is such that glucose is often supplemented by other carbon sources such as glutamine.

Like many other cell types, leukocytes employ these regulatory mechanisms to support their own tissue-specific functions [11]. For example, long-term, low-intensity states, such as the antiparasitic response and type 2 immune programs in general, require the sustained energy provision of oxidative metabolism, while short-lived, high-intensity states, such as the antibacterial response and type 1 programs in general, demand the ATP- and biosynthetic precursor-generating capacity of the glycolytic pathway. Indeed, these bioenergetic/biosynthetic programs are so central to immune functions that they are cued directly by the same stimuli that activate the immunologic effector responses themselves. For example, TLR-4 ligation by LPS (Fig. 2a) initiates both classic type 1 NFκB-/JNK-/IRF-dependent inflammatory effector responses as well as a HIF1α-dependent glycolytic program anchored by increased GLUT1 and phosphofructo-2-kinase/fructose-2,6,bisphosphatase (PFKF) B3 expression [12–16]. Type 2 stimuli pathways [17]. IL-4 stimulation of macrophages (Fig. 2b), for example, activates STAT6, which, in turn, promotes expression of both type 2 immune effectors as well as metabolic effectors such as PPAR-γ and -δ, PGC-1β, and PFKFB1 that drive a broad program of oxidative metabolism [18–22]. Importantly, the metabolic programs that associate with either type 1 or type 2 responses are not only required for proper immune function but are indeed sufficient to drive these programs even in the absence of canonical immune stimuli. For example, forcing expression of the oxidative metabolic program in macrophages

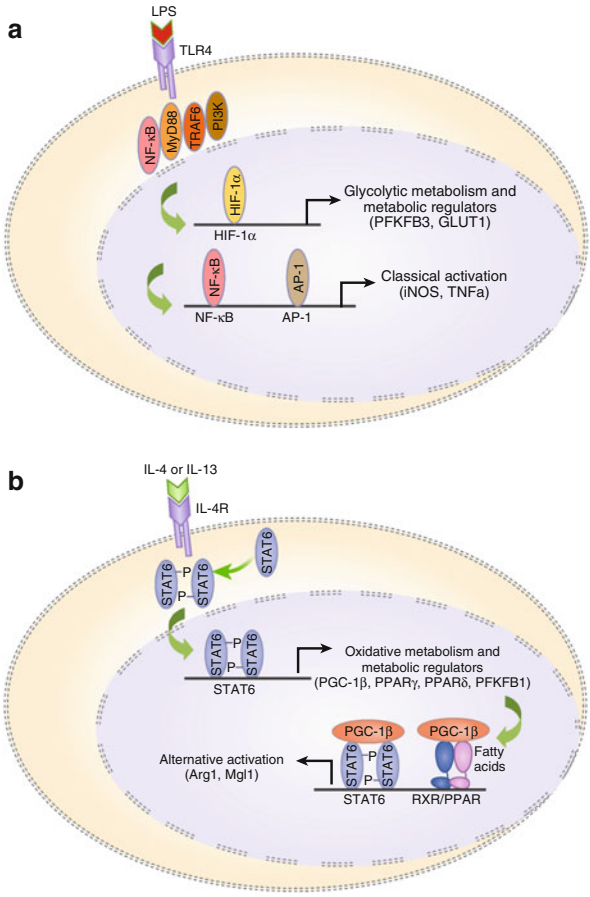

Fig. 2 Metabolic programs of type 1 and type 2 macrophage responses. Type 1 stimuli such as LPS (**a**) trigger an intracellular signaling cascade that activates both a HIF-1α-dependent glycolytic program involving PFKFB3 and GLUT1 that supports type 1 immune responses. Type 2 stimuli such as IL-4 (**b**), in contrast, activate a STAT6-dependent program of oxidative metabolism involving PPARs, PGC-1β, and PFKFB1, which, in turn, supports the type 2 immune response. Induction of either metabolic program alone is capable of biasing the macrophage immune response towards the associated type of immune response

not only enhances type 2 immune responses but also abrogates the ability of the cell to deploy type 1 responses, indicating that the cell's metabolic state alone is sufficient to instruct immune effector responses [21]. This basic phenomenon has been further observed throughout the innate and adaptive immune system in such varied contexts as dendritic cell maturation and antigen presentation, T lymphocyte activation biasing and memory function, and, though the details are yet unclear, B cell function, demonstrating that the integration of metabolic and immune responses is a conserved coordination strategy [11].

Immunity Regulates White Adipose Tissue Function

The integration of metabolic and immune responses at the cellular level is critical to proper immune function; however, this cooperative paradigm applies also to broader metabolic processes as well. Indeed, it is now clear that immunity constitutes one of

the major local and systemic metabolic control axes, acting to support and coordinate the functions of individual cells, tissues, and processes throughout the body while metabolic state [1, 4, 23]. White adipose tissue, the primary site of long-term energy storage in mammals, is the physiologic context in which immunity's influence has been most extensively mapped [24, 25]. Here, adipocyte homeostasis and white adipose tissue function as a whole are both carefully regulated by a complex immune regulatory circuit [4]. Under conditions of metabolic normalcy (Fig. 3a), adipose tissue endothelial cell-derived IL-33 drives resident type 2 innate lymphoid cell (ILC2) accumulation and production of IL-5 and IL-13 [26, 27]. ILC2-derived IL-5, in turn, recruits and maintains a substantial resident population of eosinophils that produce IL-4, which in collaboration with ILC2-derived IL-13, supports a large population of alternatively activated resident macrophages [19, 26, 28]. These macrophages form the nexus of a type 2 immune microenvironment that comprises regulatory T cells, invariant natural killer T cells, and other leukocytes with whom macrophages collaborate to actively promote adipocyte insulin sensitivity both directly and indirectly through suppression of insulin-antagonizing type 1 immune activity [4]. Indeed, congruent with these findings, helminth infection or instillation of helminth-derived glycans, potent inducers of type 2 responses, enhances glycemic control and insulin action in obese mice [28, 29]. In this manner, adipocytes remain primed for maximal insulin-stimulated nutrient uptake and storage.

Under conditions of sustained nutrient excess (Fig. 3b), however, the storage capacity of adipocytes is eventually exhausted, leading to super-physiologic levels of metabolic substrates overflowing into cellular compartments ill-suited for substrate handling [1, 23, 30]. Such nutrient spillover can lead to both cell-intrinsic and -extrinsic dysfunction (such as diacylglycerol and ceramide accumulation, abnormal protein stress) and, with continued overnutrition, adipocyte cell death [5, 31–33]. While these mechanisms each comprise unique pathways and effects, nearly all converge upon and inhibit the insulin signaling pathway, generally through serine inhibition of insulin receptor substrate (IRS) proteins, blunting insulin action in stressed tissues and stemming nutrient influx [1, 23]. Moreover, these pathways also activate IKKβ and JNK signaling to initiate and support an ongoing type 1 immune response within overfed adipose tissue [34–38]. This inflammatory response disrupts the type 2 milieu of lean adipose tissue directly through production of inflammatory mediators such as IL-1β and TNF-α as well as indirectly through the recruitment of large numbers of Ly6c^{Hi} monocytes that differentiate into moderately inflammatory macrophages [39–42]. Despite the absence of marked differences in the inflammatory potential of these macrophages when compared to residents, their large number is sufficient to skew the adipose tissue microenvironment, transforming it into one dominated by type 1 responses. This shift is mirrored in the T lymphocyte populations where regulatory T cell and invariant NKT cell dominance is diluted by ingressing T_h1 cells, CD8$^+$ T cells, and NKT cell populations with rearranged T cell receptor loci [43–45]. Moreover, B cells and neutrophils also immigrate into inflamed adipose tissue, supporting the type 1 milieu and exacerbating metabolic dysfunction and inflammation through auto-reactive antibodies and elastase production, respectively [46–48].

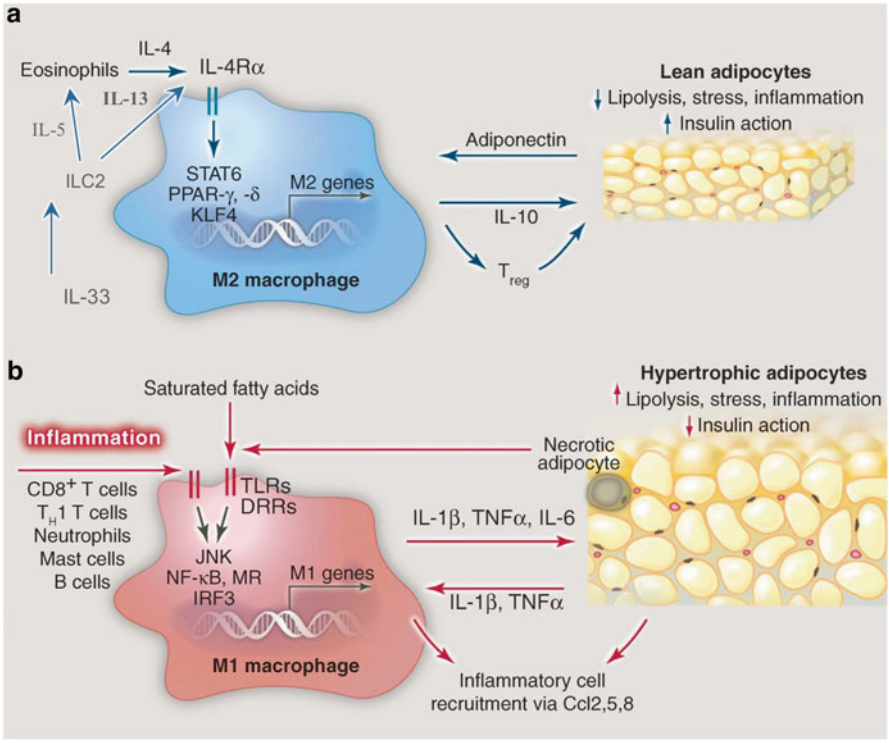

Fig. 3 Immune programs influence adipose tissue function. In lean adipose tissue (**a**), IL-33 supports a population resident ILC2 cells that recruit and maintain eosinophils within the adipose tissue. In conjunction with ILC2-derived IL-13, eosinophil-derived IL-4 maintains adipose tissue resident alternatively activated macrophages that, in collaboration with regulatory T cells and other leukocytes, maintain a tolerogenic type 2 immune microenvironment. This immunologic milieu actively promotes adipocyte insulin sensitivity and function as well as adiponectin secretion that supports type 2 macrophage activation. In obese adipose tissue (**b**), in contrast, the physiologic stigmata of overnutrition activate tissue resident macrophages and other leukocytes to produce type 1 inflammatory cytokines that exacerbate adipocyte insulin resistance, enhance cellular stress responses, and recruit additional leukocytes. Stressed and necrotic adipocytes, in turn, reinforce the inflammatory microenvironment. *STAT* signal transducer and activator of transcription, *KLF* Kruppel-like factor, *TLRs* Toll-like receptors, *DRRs* danger recognition receptors, *MR* mineralocorticoid receptor, *IRF* interferon regulatory factor

Immunity Regulates Liver Function

While the white adipose tissue serves as the body's primary site of long-term energy storage, the liver is the primary short-term energy-handling depot [6]. Here, glucose is stored as and released from glycogen as well as synthesized via gluconeogenesis, while lipids are synthesized, exported, imported, and burned. Despite this increased metabolic complexity, insulin's actions remain similar to other tissues in their promotion of nutrient uptake and storage and inhibition of their release. Unlike in other

tissues, however, the liver's ability to liberate stored or synthesized glucose in addition to lipids means that insulin resistance manifests as an elevated glucose secretion rate in addition to the more traditional metrics of decreased glucose uptake and elevated fatty acid release.

As in white adipose tissue, metabolic normalcy in the liver is characterized by a type 2-biased immune microenvironment in which resident macrophages (Kupffer cells) promote insulin sensitivity and regulate lipid metabolism in hepatocytes and maintain the tolerogenic milieu [4]. Unlike the adipose tissue, however, relatively little is known of how this microenvironment is maintained, how its effects are exerted, or what roles the various leukocyte lineages play therein. What is clear is that Kupffer cell alternative activation is critical for proper tissue function as selective abrogation of this phenotype via macrophage-specific PPAR-δ deletion leads to hepatocyte insulin resistance and disrupted lipid metabolism [18, 20], a phenotype that can be recapitulated in lean rodents by selective Kupffer cell depopulation using gadolinium salts [49, 50]. In addition to promoting insulin sensitivity, some observations suggest that type 2 responses in the liver have the ability to exert insulin-like influences directly on hepatocytes. For example, a recent report described a novel metabolic regulatory circuit in which feeding triggers a marked increase in the intrahepatic expression of IL-13, which acts directly on hepatocytes to inhibit gluconeogenesis [51]. While the signaling pathway identified is noncanonical and the proximate components remain unclear, the data suggest that type 2 responses contribute to the liver's postprandial anabolic shift.

Similar to the adipose tissue, overfeeding in the liver leads to exhaustion of nutrient storage capacity and tissue dysfunction via mechanisms similar to those identified in adipocytes (most notably IKKβ and JNK pathway activation). Unlike the adipose tissue, however, cellular stress and inflammatory activation in the liver do not result in the large leukocyte population shifts seen in adipose tissue. Instead, leukocyte numbers change only incrementally, while phenotypes and activation biases shift dramatically. For example, Kupffer cell numbers remain stable throughout obesity (a small decrease in resident macrophages is offset by new monocyte recruitment); however, the overall activation profile shifts sharply from a type 2 to a type 1 bias, suggesting either conversion of individual cells or population replacement [18, 20, 52]. Similarly, the NKT cell population shifts from invariant NKT cells to those with rearranged T cell receptors with a concurrent shift from type 2 to type 1 cytokine profiles. As in the adipose tissue, neutrophils also ingress, solidifying the type 1 environment and exacerbating metabolic dysfunction.

Immunity Regulates Brown Adipose Tissue Function

The weight of the ongoing obesity epidemic has, with good reason, skewed interest disproportionately towards tissues with obvious roles in obesity-related metabolic dysfunction, such as the liver and white adipose tissue. More recently, however, interest has begun to shift to other, less well-studied tissues with the potential for therapeutic intervention. Brown adipose tissue, with its remarkable capacity to burn

fat, is of particular interest in this avenue of research. Despite being the primary site of non-shivering thermogenesis [53, 54], brown adipose tissue has traditionally been largely dismissed in adults as its metabolic contributions were believed to be restricted to the neonatal and infant periods, after which they rapidly declined to insignificance. A spate of recent studies, however, have debunked this view by demonstrating both the existence and functional activity of substantial amounts of brown adipose tissue in healthy adults [55–58]. Indeed, one estimate places the average adult's brown adipose depots at ~63 g, which burn ~4.1 kg of fat each year [58].

In non-shivering thermogenesis, cold exposure elicits an increase in sympathetic nervous system tone that directs brown adipocytes to dramatically increase fatty acid oxidation while simultaneously increasing the expression of respiratory chain uncoupling protein-1 [53, 54, 59]. The uncoupled dissipation of such a large proton gradient liberates large amounts of heat, combating heat loss in cold environments. Until recently, this pathway was thought to be mediated by direct sympathetic innervation of brown adipocytes; however, recent work has demonstrated an unexpected requirement for alternatively activated macrophages [60]. Interestingly, these cells comprise a critical component of the sympathetic efferent limb, producing ~50 % of all catecholamines present within cold-stimulated brown adipose tissue. Given the magnitude of their contribution, macrophage-derived catecholamines are unsurprisingly necessary for the activation of brown adipocyte responses; however, they also appear to mediate a second, parallel pathway by which white adipocytes are directed to release the fatty acids necessary to fuel continued thermogenic activity in brown adipocytes. Congruent with these findings, abrogation of macrophage alternative activation or type 2 immune responses in general results in thermogenic responses that are not only effete but also relatively short-lived. Relatedly, metabolic regulators that are critical to and induced by type 2 immune responses (such as PPAR-γ) have been shown to promote the acquisition of thermogenic capacity by white adipocytes in a so-called "browning" response [61].

As discussed above, the thermogenic response has become of great interest in obesity research for its potential ability to burn away excess calories [59]; however, some have additionally suggested that metabolic derangements within brown adipose tissue contribute directly to obesity itself. While the data supporting this hypothesis are currently limited to observational studies describing diminished brown adipose depots in the obese, the antagonism between type 1 immune programs (such as are prevalent in obese white adipose tissue) and type 2 programs (such as are necessary for brown adipose tissue function) is well established, providing a plausible mechanism by which obesity might impair brown adipose tissue function and, thus, therapeutic potential.

Therapeutic Implications

The comingling of immunity and metabolism provides not only important insight into basic physiology but also a unique opportunity to intervene therapeutically therein, opening a substantial formulary of well-characterized immune- and

metabolism-targeted pharmaceuticals to possible use in new clinical contexts. Indeed, even the basic pharmacologic classifications of immune- and metabolism-targeted interventions are being rethought as some well-established "metabolic" pharmaceuticals (such as thiazolidinediones, a widely used class of synthetic PPAR-γ agonists) have been shown to rely upon immunomodulation for their therapeutic efficacy just as some "immune" agents (such as rapamycin) are now known to act through metabolic pathways [62]. One of the oldest and best-studied examples of repurposed therapeutics is that of the salicylates, the most commonly used pharmacologic family in the world, whose efficacy in diabetes is documented in the medical literature as far back as 1901 [63–66]. While the adverse effect profile of many of these compounds precludes their routine use in this manner, recent trials with salsalate have returned encouraging results [67, 68]. Similarly, other immune agents targeting type 1-associated cytokines (e.g., canakinumab, infliximab, and entanercept), cellular populations (e.g., CD3- and CD20-targeting antibodies), and signaling pathways (e.g., amlexanox) have demonstrated potential for combatting metabolic dysfunction [45, 46, 69, 70].

While strategies utilizing traditional immune agents have largely relied on inhibiting type 1 responses, metabolic agents with immune activity more often act by actively skewing the immune timbre towards type 2 responses. For example, polyunsaturated fatty acids, AMP-activated protein kinase agonists, PPAR agonists, DPP-4 inhibitors, and some statins (e.g., pravastatin, rosuvastatin, and fluvastatin) are all metabolic agents that exercise their therapeutic effects at least in part by promoting type 2 immune responses [71, 72]. While this does indirectly inhibit type 1 activity, the active promotion of type 2 responses appears to be important, congruent with the importance of type 2 responses in healthy metabolic function. Even the efficacy of surgical (e.g., gastric bypass) and behavioral (e.g., exercise) interventions has been suggested to involve an ability to skew the immune milieu towards type 2 response [73–76].

The potential of type 2-promoting interventions has even lead some to propose purposeful parasite infection or administration of parasite-derived material to treat obesity-related metabolic disease. The literature clearly supports this approach's efficacy in inhibiting type 1-associated pathology, including classical inflammatory diseases such as Crohn's disease, ulcerative colitis, and multiple sclerosis, and even suggests potential in type 1-associated metabolic disease; however, this approach has understandably failed to attract widespread interest [77–79]. Microbial manipulation, however, also includes approaches targeted at noninfectious agents as well, which have gained much broader acceptance. Indeed, the gut microbiome is now accepted as a powerful determinant of a variety of systemic pathology including obesity-related metabolic disease, and gut flora-directed interventions are being hotly pursued [75, 80].

Conclusion

The research enterprise has long deconstructed physiology into discipline-bounded silos with each, at times arbitrarily defined, system studied independently from the others. While this approach has produced much of our extant knowledge, one of the broad trends in biological research over the past two decades has been interdisciplinary investigation in which these divisions are actively bridged. The returns of such efforts have been varied; however, as we discuss above, this movement has fundamentally changed our understanding of both metabolism and immunity by demonstrating just how inextricably the two are entwined. Indeed, metabolism is now understood to both support and direct immune activation, with oxidative metabolism driving type 2 responses while glycolysis drives type 1, just as immunity is understood to both support and direct metabolic processes, with type 2 immunity actively directing and maintaining metabolic health while type 1 responses skew metabolism away from baseline. Appreciation of this relationship has opened an entirely new regulatory axis to therapeutic intervention in both immune and metabolic diseases that, hopefully, will provide new tools to improve clinical outcomes.

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