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Review Article

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Innate Type-2 Cytokines: From Immune Regulation to Therapeutic Targets

Hye Young Kim [1,2,3,†], **Dongjin Jeong** [4,5,†], **Ji Hyung Kim** [6], **Doo Hyun Chung** [4,5,7,*]

[1]Laboratory of Mucosal Immunology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 03080, Korea
[2]Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul 03080, Korea
[3]Multitasking Macrophage Research Center, Ewha Womans University, Seoul 03760, Korea
[4]Laboratory of Immune Regulation, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 03080, Korea
[5]Ischemic/Hypoxic Disease Institute, Seoul National University Medical Research Center, Seoul 03080, Korea
[6]Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea
[7]Department of Pathology, Seoul National University College of Medicine, Seoul 03080, Korea

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***Correspondence to**
Doo Hyun Chung
Department of Pathology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea.
Email: doohyun@snu.ac.kr

[†]Hye Young Kim and Dongjin Jeong contributed equally to this work.

ORCID iDs
Hye Young Kim
https://orcid.org/0000-0001-5978-512X
Dongjin Jeong
https://orcid.org/0000-0001-7122-1220
Ji Hyung Kim
https://orcid.org/0000-0001-6785-2345
Doo Hyun Chung
https://orcid.org/0000-0002-9948-8485

ABSTRACT

The intricate role of innate type-2 cytokines in immune responses is increasingly acknowledged for its dual nature, encompassing both protective and pathogenic dimensions. Ranging from defense against parasitic infections to contributing to inflammatory diseases like asthma, fibrosis, and obesity, these cytokines intricately engage with various innate immune cells. This review meticulously explores the cellular origins of innate type-2 cytokines and their intricate interactions, shedding light on factors that amplify the innate type-2 response, including TSLP, IL-25, and IL-33. Recent advancements in therapeutic strategies, specifically the utilization of biologics targeting pivotal cytokines (IL-4, IL-5, and IL-13), are discussed, offering insights into both challenges and opportunities. Acknowledging the pivotal role of innate type-2 cytokines in orchestrating immune responses positions them as promising therapeutic targets. The evolving landscape of research and development in this field not only propels immunological knowledge forward but also holds the promise of more effective treatments in the future.

Keywords: Type-2 cytokines; Natural killer T-cells; ILC2; Interleukin-33; Interleukin-25; TSLP

INTRODUCTION

The immune system, a sophisticated network of cells, serves as a formidable guardian, defending the host organism against a diverse range of threats, encompassing pathogens, toxins, and damaged cells (1). Beyond its defensive functions, the immune system also plays pivotal roles in regulating tissue repair and maintaining homeostasis across metabolic, endocrine, and neural systems. Cytokines, released by immune cells into the extracellular space and bloodstream, act as key mediators for these intricate processes. Among these cytokines, the classical type-2 cytokines, including IL-4, IL-5, and IL-13, hold particular significance. Their orchestration of beneficial immune responses is critical for the host's defense against parasitic infections and the promotion of multisystem homeostasis (2).

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AHR, airway hyperreactivity; APC, Ag presenting cell; DC, dendritic cell; EPO, eosinophil peroxidase; FDA, Food and Drug Administration; GATA3, GATA-binding protein-3; ILC, innate lymphoid cell; ILC2, type-2 innate lymphoid cell; IL-1RAcP, IL-1 receptor accessory protein; iNKT, invariant NKT; lfTSLP, long-form TSLP; MBP, major basic protein; RORα, RAR-related orphan receptor-alpha; sfTSLP, short-form TSLP; ST2L, transmembrane isoform of ST2; WAT, white adipose tissue.

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However, the regulatory ability of type-2 cytokines also implies the potential for destruction when dysregulated. Indeed, these cytokines play substantial pathogenic roles in various inflammatory diseases, such as asthma, allergies, and autoimmune disorders (3).

The immune system is a complex network, with adaptive and innate immune cells collectively safeguarding the host organism against diverse threats. While both cell types contribute to the production of type-2 cytokines, innate immune cells, such as NKT cells, innate-lymphoid cells (ILCs), and granulocytes (mast cells, eosinophils, and basophils), emerge as key sources of these pivotal signaling molecules. In contrast to adaptive immune cells, which require prior Ag exposure, innate immune cells exhibit an immediate response, releasing large amounts of type-2 cytokines upon activation by danger signals, such as the presence of microbial components (4).

Strategically positioned as sentinels, particularly in barrier tissues, innate immune cells play a crucial role in initiating immune cascades that protect the host from pathogens and other challenges. This review embarks on an exploration of the multifaceted roles of innate type-2 cytokines in immune regulation. We will delve into the cellular origins of these cytokines, exploring their intricate interactions and shedding light on factors amplifying the innate type-2 response, including TSLP, IL-25, and IL-33. Our discussion will encompass the production of classical innate type-2 cytokines by various immune cells and the resulting complex web of cellular and molecular interactions. To illustrate these roles, we will focus on scenarios such as helminth infections, allergies, fibrosis, and adipose-tissue metabolism/obesity. Furthermore, we will examine recent advancements in therapeutic strategies, particularly the use of biologics targeting critical cytokines (IL-4, IL-5, and IL-13), providing insights into both challenges and opportunities.

ROLE OF EPITHELIAL CELL-DERIVED ALARMINS IN TYPE-2 IMMUNITY

Epithelial-derived cytokines are increasingly recognized for their pivotal roles in the regulation of type 2 immune responses. Primarily produced by epithelial cells, these cytokines have emerged as central players in the context of type 2 immunity, orchestrating immune responses against parasitic infections, allergies, and tissue repair (5). In the upcoming sections, we will explore how these cytokines play central roles in orchestrating type-2 immune responses, influencing anti-parasite reactions, allergies, fibrosis, and anti-obesity adipose-tissue metabolism (**Fig. 1**).

IL-25

IL-25, also known as IL-17E, holds a unique position within the IL-17 family as it has the distinctive capability to directly induce type-2 immunity (6). IL-25 is secreted by various cell types, including lung epithelial cells and synovial fibroblasts, in response to external stimuli such as helminth infections or allergens. Subsequently, IL-25 binds to the heterodimeric IL-25R receptor, composed of IL-17RA and IL-17RB, elucidating the nuanced relationship between IL-25 release and its binding to the receptor following diverse stimuli (7-9). Upon secretion, IL-25 initially exists in an inactive disulfide-linked homodimeric form, undergoing post-translational maturation processes to become bioactive (**Fig. 1**). Notably, matrix metalloproteinase-7-mediated cleavage of IL-25 enhances the pathogenic production of type-2 cytokines in allergen-challenged lungs (10).

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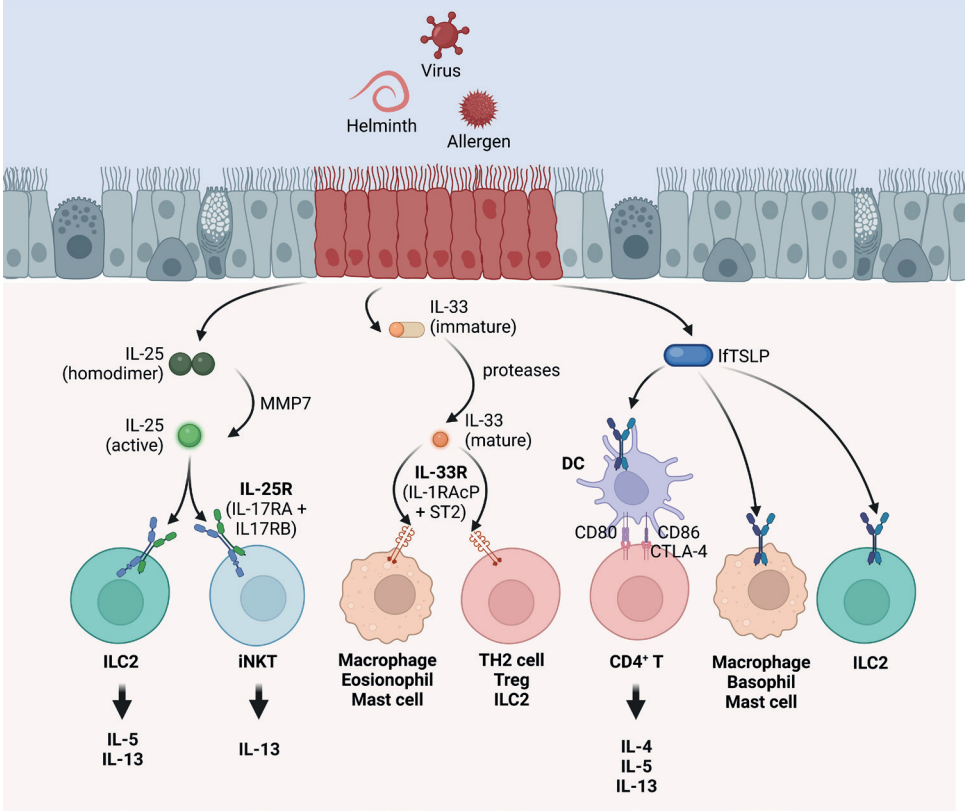

Figure 1. Type-2 immunity is initiated by epithelial cell-derived alarmins that then provoke the release of other type-2 cytokines by innate cells.
When epithelial cells are damaged or triggered by allergens, viruses, or parasites, they produce the alarmins IL-25, IL-33, and/or TSLP. These cytokines can be considered to be a subset of innate type-2 cytokines. IL-25 is released in a disulfide-linked homodimeric form that is inactive until it is cleaved by MMP7. The cleaved IL-25 then binds to the IL-25R heterodimer (IL-17RA and IL-17RB), which is constitutively expressed on ILC2s and *i*NKT cells. This binding event induces these cells to secrete IL-13. The ILC2s also produce IL-5. IL-5 and IL-13 are classical innate type-2 cytokines. With regard to IL-33, it is normally sequestered in the epithelial-cell nucleus in its immature full-length form. When the epithelial cells are activated, they release the immature protein into the extracellular space, where it undergoes proteolytic cleavage into mature IL-33 fragments. These fragments bind to the heterodimeric ST2 receptor (IL-1RAcP and ST2L) on macrophages, ILCs, mast cells, basophils, eosinophils, and Th2 cells. These cells then secrete classical type-2 cytokines. With regard to TSLP, it is expressed as long-form (lfTSLP) and short-form (sfTSLP) isoforms. Both bind to the TSLP heterocomplex (IL-7Rα and TSLPR), which is expressed by mast cells, ILC2s, macrophages, basophils, and DCs. sfTSLP is thought to act as a homeostatic immune decoy. lfTSLP promotes type-2 cytokine production by directly activating innate-immune cells. In the case of DCs, lfTSLP increases their expression of costimulatory molecules and promotes DC activation of type-2 cytokine-producing $CD4^+$ T cells.

The crucial role of IL-25 in asthma is underscored by observations in mouse models, where intratracheal administration of IL-25 induces lung eosinophilia, IgE production, and airway hyperreactivity (AHR) (11,12). These responses involve, in part, type-2 ILCs (ILC2s), which secrete IL-5 and IL-13 in response to IL-25. Notably, IL-25 cannot induce these cells to produce IL-4 (12). Additionally, pathogenic $CD4^+$ invariant NKT (*i*NKT) cells expressing the IL-17RB component of IL-25R produce significant amounts of type-2 cytokines, such as IL-13, upon stimulation with IL-25 *in vivo* and *in vitro* (**Fig. 1**) (13,14).

IL-25 also plays a crucial role in anti-parasite defense; mice deficient in IL-25 cannot mount Th2 responses against parasites such as *Trichuris muris* (15). Conversely, intraperitoneal administration of recombinant IL-25 promotes the expulsion of *Nippostrongylus brasiliensis* from the gut (11).

IL-33

IL-33, a member of the IL-1 family, is primarily expressed by various tissue cell types, including epithelial cells, fibroblasts, and endothelial cells. Additionally, dendritic cells (DCs) and mast cells can release IL-33 in inflammatory conditions (16). This cytokine binds to a

receptor complex consisting of the signaling transmembrane isoform of ST2 (ST2L) and the promiscuous accessory protein IL-1 receptor accessory protein (IL-1RAcP). Constitutively expressed by macrophages, ILCs, mast cells, basophils, and eosinophils, this receptor complex initiates signaling cascades, such as NF-κB and AP-1, leading to the expression of inflammatory molecules, including type-2 cytokines (17). Under homeostatic conditions, the full-length 270 amino-acid IL-33 protein is sequestered in the nucleus of epithelial and endothelial cells. Upon exposure to allergens, bacteria, or viruses, immature IL-33 is released into the extracellular space. Proteases released by innate effector cells cleave the immature IL-33, generating mature IL-33 fragments (18-20). These mature forms interact with immune cells bearing the ST2L receptor (21-24), displaying markedly increased activity in generating type-2 immune responses compared to the immature form (**Fig. 1**).

IL-33 plays a crucial role in allergic inflammation. IL-33-deficient mice exhibit resistance to allergen-induced AHR (25). Additionally, intranasal delivery of soluble ST2, functioning as a decoy receptor for IL-33, using adenovirus effectively blocks allergic airway inflammation in animal models (26). Notably, individuals with allergic conjunctivitis, rhinitis, and atopic dermatitis display elevated serum levels of IL-33 compared to healthy counterparts (27).

TSLP

TSLP, a member of the IL-2 cytokine family, is primarily produced by epithelial and stromal cells in various tissues, including the skin, intestine, and lungs (28). TSLP exhibits a dual regulatory role in immune responses, mediated by its two distinct isoforms: short-form TSLP (sfTSLP) and long-form TSLP (lfTSLP) (29). Under homeostatic conditions, sfTSLP is constitutively expressed, acting as a sentinel for tissue integrity and contributing to immune tolerance and barrier function. This prevents unwarranted inflammatory responses. However, in inflammatory environments, lfTSLP is expressed, serving as a potent alarm signal that alerts the immune system to the presence of danger, particularly during infections or tissue damage (29). These isoforms influence various immune cells expressing the TSLP receptor, a heterodimer of the IL-7 receptor alpha chain and the TSLPR chain. DCs, for instance, undergo phenotypic changes and upregulate costimulatory molecules such as OX40L, CD80, and CD86 when stimulated with TSLP. Consequently, TSLP plays a pivotal role in DC Ag presentation to CD4+ T cells, activating them into cells that produce type-2 cytokines (30). TSLP also directly activates innate immune cells, including mast cells, ILC2s, NKT cells, epithelial cells, macrophages, and basophils, prompting the production of type-2 cytokines (5) (**Fig. 1**). These cytokines, in turn, initiate type-2 immune responses combating parasitic infections, mediating allergies and fibrosis, and inducing anti-obesity adipocyte metabolism (**Fig. 2**). Thus, TSLP serves as a bridge between the innate and adaptive arms of the immune system, orchestrating the emergence of a complex web of type-2 immune responses.

KEY INNATE TYPE-2 CYTOKINE-PRODUCING CELLS

Here, we explore more extensively the discussion of key innate-immune cell types responsible for the production of type-2 cytokines, building upon the brief mentions provided earlier. In this section, we scrutinize the intricacies of several pivotal players to gain a deeper understanding of their roles in orchestrating type-2 immune responses.

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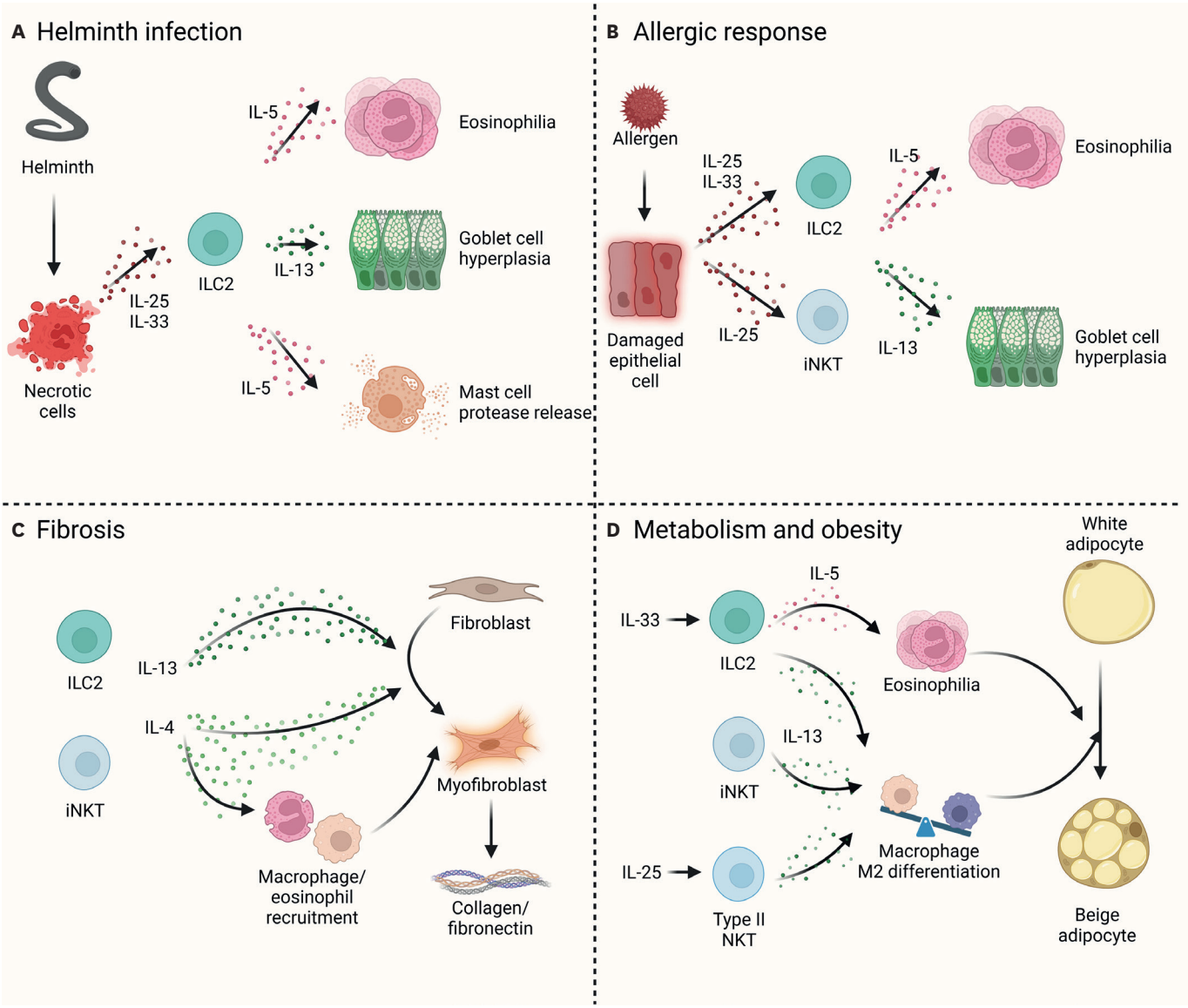

Figure 2. Effects of innate type-2 cytokines in protective and pathogenic immune responses.
(A) Helminth infections often lead to tissue damage and the release of alarmins such as IL-25 and IL-33 by the necrotic cells. The alarmins bind to local innate-immune cells such as ILC2s, which prompts them to rapidly produce classical type-2 cytokines such as IL-5 and IL-13. IL-5 induces mast cells to degranulate; the released proteases then damage the helminths. IL-5 also induces eosinophilia, which is crucial for controlling parasitic infections, while IL-13 promotes eosinophil recruitment to the infected site. IL-33 also initiates goblet-cell hyperplasia in the lung and intestine, thereby creating an unfavorable environment for helminths. (B) Allergens damage epithelial cells and induce them to release the alarmins IL-25 and IL-33. These directly activate ILC2s and *i*NKT cells, which release type-2 cytokines and thereby trigger allergic reactions such as asthma and atopic dermatitis. For example, IL-5 and IL-13 induce eosinophilia and local eosinophil recruitment, respectively. IL-13 also generates goblet-cell hyperplasia in the lung, thereby inducing the mucus overproduction that characterizes allergic diseases. (C) Fibrosis is mediated by IL-13/IL-4-secreting ILC2s and *i*NKT cells. These cytokines directly induce fibroblasts to differentiate into myofibroblasts, which produce vast amounts of collagen and fibronectin. They also potently contract the extracellular matrix. Both activities result in stiffly remodeled tissue that is a hallmark of fibrosis and interferes with normal tissue functions. IL-4 from ILC2s/*i*NKT cells also activates macrophages and induces eosinophil recruitment. These effects further promote myofibroblast differentiation. (D) Adipose-tissue metabolism is driven by macrophages. Alternatively-activated (M2) macrophages promote adipocyte browning, which releases energy in the form of heat. The differentiation of adipose-tissue M2 macrophages is initiated by hormones, exercise, and environmental stimuli such as cold, which induce the release of the alarmins IL-25 and IL-33 in adipose tissue. These alarmins activate local ILC2s, *i*NKT cells, and type-II NKT cells, which secrete IL-5 and IL-13. IL-5 induces eosinophilia and IL-13 recruits these cells into the adipose tissue. The eosinophils produce IL-4, which converts the resident macrophages into M2 macrophages that release mediators that promote adipocyte browning. When the innate immune cell-eosinophil-M2 macrophage axis is disrupted, classically-activated macrophages (M1) predominate and the adipocytes accumulate fat and induce obesity.

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NKT cells

NKT cells represent a unique cell type with both the surface markers and functional characteristics of conventional T cells and NK cells. Crucially, NKT cells express TCRs capable of recognizing glycolipid Ags presented by CD1d molecules, which are non-polymorphic MHC class I-like proteins (31). Additionally, they constitutively express markers associated with activated or memory T cells, endowing NKT cells with the remarkable ability to rapidly produce type-2 cytokines, including IL-4 and IL-13, as well as type-1/type-17 cytokines within minutes to hours of TCR activation (31-34).

NKT cells can be broadly categorized into type-I and type-II NKT cells based on their TCR-repertoire diversity (31). Type-I NKT cells, also known as *i*NKT cells, express semi-invariant TCRβ chains paired with Vα14 chains in mice and Vα24 chains in humans. They primarily recognize glycolipid Ags such as α-galactosyl ceramide, derived from marine sponges. In contrast, type-II NKT cells exhibit more diverse TCRs and respond to various lipid Ags (e.g., sulfatide) (35).

Understanding the role of NKT cells in type-2 immunity requires an examination of their development and differentiation, predominantly occurring within the thymus. Two developmental models, namely the linear maturation (36) and lineage-differentiation models (37-39), provide insight. The latter categorizes *i*NKT cells into distinct subsets based on their expression of transcription factors and cytokine production. *i*NKT1 cells express T-bet and produce Th1 cytokines (IFN-γ and TNF-α), *i*NKT2 cells express GATA3 and type-2 (Th2) cytokines (IL-4 and IL-13), and *i*NKT17 cells express RAR-related orphan receptor-alpha (RORα) and produce Th17 cytokines (IL-17A and IL-22) (37-39). Recent single-cell RNA sequencing analyses further categorized iNKT2 cells into two subsets based on their expression of *Icos* and the Promyelocytic Leukemia Zinc Finger-encoding gene *Zbtb16* (40).

Notably, the production of type-2 cytokines by *i*NKT2 cells depends not only on TCR stimulation by CD1d-expressing Ag-presenting cells (APCs) but also on their expression of β-catenin (41). However, the underlying mechanisms remain poorly understood.

ILCs

ILCs emerged in the intricate landscape of the immune system in 2010, adding a layer of complexity to our understanding (42). These cells, predominantly found in barrier tissues, distinguish themselves from other immune cells by lacking markers characteristic of the myeloid and lymphoid lineages and, notably, lacking Ag-specific receptors (43). Unlike T cells, ILCs are promptly activated by engaging their constitutively expressed surface receptors with signals from the tissue microenvironment, including cytokines, microbial products, nutrient components, lipid mediators, and neuronal transmitters (43). This direct activation mechanism allows ILCs to respond swiftly to environmental cues, primarily through cytokine production.

ILCs can be categorized into three subsets (ILC1, ILC2, and ILC3) based on the transcription factors governing their activities and the cytokines they produce. This subdivision closely mirrors the differentiation of Th cells into Th1, Th2, and Th17/22 cells, positioning ILCs as innate counterparts to these T-cell subsets (43). ILC1s, akin to Th1 cells, constitutively express T-bet, which is encoded by *Tbx21* and plays a crucial role in the early development of ILC1s (44). Consequently, ILC1s bear receptors for key cytokines such as IL-12 and IL-18 that induce them to produce IFN-γ and TNF-α (45). ILC2s, the innate equivalent of Th2 cells, are prevalent in mucosal tissues, particularly the lungs. Their development and expansion are dependent

on specific transcription factors, including RORα and GATA3 (46). They bear the ST2 receptor and are exquisitely sensitive to IL-33: once triggered in this fashion, ILC2s release copious amounts of type-2 cytokines, including IL-5, IL-9, and IL-13 (47). ILC3s, paralleling Th17/22 cells, are governed by the RORγt and aryl hydrocarbon receptor transcription factors. They express receptors for IL-1 and IL-23 and secrete IL-17 and IL-22 (48).

Multiple studies, including those on mouse models, suggest the crucial role of ILC2s in the pathophysiology of asthma and allergic inflammation. For instance, patients with severe eosinophilic asthma show an accumulation of IL-5- and IL-13-producing ILC2s in their sputum after an allergen challenge (49). ILC2s contribute to tissue damage in the lung and skin, primarily through an IL-13-dependent mechanism, and promote allergic inflammation through intricate communications with local DCs and Th2 cells (50). In homeostatic tissues, ILC2s modulate the amplitude of type-2 responses, ensuring a calibrated reaction to environmental threats (51). Understanding the triggers leading to excessive or deranged ILC2 responses holds promise for the development of targeted therapies for type 2-associated diseases.

Granulocytes (mast cells, eosinophils, and basophils)

Granulocytes, encompassing mast cells, eosinophils, and basophils, swiftly respond to triggers by producing substantial amounts of type-2 cytokines. Beyond this, they reciprocate to these cytokines and other stimuli by releasing preformed mediators, including cytokines, proteases, bioactive lipids, and histamine, thereby playing integral roles in fostering airway inflammation (23,52,53).

Mast cells, enduring participants in immune activation, engage not only in the immediate response to microbial threats but also contribute to later infection stages. Their functions extend to promoting helminth clearance through protease release and acting as effectors in protective memory immune responses upon secondary infection (54). Well-recognized for their involvement in asthma, allergy, and anaphylaxis, mast cells, when activated, release cytokines such as IL-4 and IL-13, modulating both innate and adaptive immune responses. Additionally, they release prostaglandin D2, activating ILC2s and inducing various mechanisms to enhance type-2 immune responses (55).

Basophils, which share some functional features with tissue-resident mast cells, respond to various stimuli, including cytokines such as IL-3, IL-18, IL-33, and TSLP. Notably, they display the classical features of APCs, including the expression of peptide-MHC-II complexes, which they acquire from DCs in draining lymph nodes *via* trogocytosis (56). Since basophils also produce relatively high levels of IL-4, they are major promoters of Th2-cell differentiation (29,53). These cells play such an important pathogenic role in allergies that they are the subject of the Basophil Activation Test, which is a widely employed *in vitro* assay for characterizing and diagnosing allergic responses in patients (56).

Eosinophils, integral in allergic inflammation and airway injury/repair processes alongside mast cells and basophils, release granule proteins like major basic protein (MBP) and eosinophil peroxidase (EPO) upon activation. This release contributes to airway injury, and the subsequent release of growth factors and cytokines, including IL-5, further amplifies inflammation (57). The migration of eosinophils from the blood to the airways, a critical step influencing asthma severity, is orchestrated by type-2 cytokines (58). These cytokines include IL-4, which upregulates VCAM-1 on eosinophils: this helps the eosinophils adhere to blood vessels, thereby facilitating their migration to the lung and airways (58,59). Moreover, IL-13 promotes eosinophil

migration by inducing epithelial cells to release chemokines such as eotaxin. Furthermore, IL-5, a potent eosinophilopoietic cytokine, supports eosinophil survival (56,60).

ROLE OF INNATE TYPE-2 CYTOKINES IN HEALTH AND DISEASE

Innate type-2 cytokines in host defense from helminths

Innate type-2 immunity stands as a critical defender against helminth infections, suggesting its evolution as a specialized response tailored to counter parasitic threats (61). The defense mechanism starts when helminths breach the skin or mucosal barriers, inducing tissue damage that prompts the release of alarmins, notably IL-25 and IL-33 (5). These alarmins initiate the innate immune response by triggering local innate immune cells, especially ILC2s, to release key cytokines, such as IL-5 and IL-13 (62). IL-5, in turn, promotes eosinophilia by inducing eosinophil proliferation in the bloodstream and activating these cells. IL-13 recruits eosinophils to the infection site, where activated eosinophils release granule proteins like MBP and EPO, exhibiting direct cytotoxicity against the parasite (63).

This eosinophilic response is pivotal for controlling helminth infections, such as the lung stage of *N. brasiliensis* (11). Within the infection site, activated eosinophils not only unleash granule proteins with direct parasite toxicity but also sustain their own inflammation and induce inflammation in other innate-immune cells by releasing IL-5. Furthermore, IL-13 derived from eosinophils and other innate-immune cells triggers goblet-cell hyperplasia in the intestine and lung, effectively impeding the early attachment of the parasite to the intestinal/lung wall. IL-13 also stimulates mast cells to release proteases that actively expel parasites from the intestine (64,65) (**Fig. 2**).

While innate type-2 cytokines predominantly govern the protective response against helminths, murine schistosomiasis is associated with activated *i*NKT cells producing both IL-4 and the type-1 cytokine IFN-γ. This dual cytokine production may result from signaling by DCs presenting specific lipid Ags on CD1d molecules. When DCs are sensitized with schistosome eggs *in vitro*, they can induce *i*NKT cells to secrete both IFN-γ and IL-4 (66). The exact role of IFN-γ in schistosomiasis, typically associated with downregulation of Th2 responses, remains unclear.

Innate type-2 cytokines in allergic responses

Allergic reactions, manifested in various forms such as hay fever, asthma, and skin rashes, share a common underlying mechanism—a heightened immune response to typically harmless environmental substances (67,68). This intricate process involves complex interactions among various cellular players and molecules, with crucial roles assigned to the alarmins IL-33 and IL-25. Released by epithelial or other structural/immune cells upon encountering triggering allergens, these cytokines initiate downstream allergic responses (15,27). Notably, both alarmins strongly activate ILC2s, which inherently express receptors for these molecules and rapidly produce type-2 cytokines, setting off the cascade of events characteristic of allergic reactions (62).

Specifically, IL-5 induces eosinophilia, while IL-13 recruits eosinophils to the lungs. IL-13 also induces goblet-cell hyperplasia in the lungs, leading to mucus hypersecretion (69) (**Fig. 2**). Given the integral roles of eosinophilia and mucus hypersecretion in the pathogenesis of

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allergic diseases like asthma and atopic dermatitis, alarmins, ILC2s, and their innate type-2 cytokines emerge as pivotal players in allergic diseases, supported by numerous lines of evidence. For instance, a genome-wide association study has revealed that polymorphisms in genes encoding IL-33 and the IL-33 receptor (ST2) are associated with allergic asthma (70). Additionally, patients with asthma exhibit higher IL-33 levels and ILC2 frequencies in their peripheral blood and sputum compared to control subjects (71).

*i*NKT cells and their type-2 cytokines may also participate in allergic disease. Terashima et al. (13) showed that IL-25 activates a specific lung-resident *i*NKT-cell subset expressing both CD4 and IL-17RB. These cells then produce type-2 cytokines and chemokines, leading to AHR. The significance of these IL-17RB$^+$ CD4$^+$ *i*NKT cells was underscored by the observation that their specific depletion in mice attenuated IL-25-induced AHR (13). There is also evidence that IL-33 can induce murine and human *i*NKTs to secrete type-2 cytokines (72,73). Further studies, including the characterization of ST2$^+$ *i*NKT cells and their role in type-2 immune responses, are warranted.

Innate type-2 cytokines in fibrosis

Fibrosis, characterized by the excessive accumulation of extracellular matrix components in tissues, profoundly affects organ function in various organs, including the lungs, liver, and skin (74). A pivotal contributor to these fibrotic disorders is IL-13, originating from ILC2s, *i*NKT cells, and other immune cells. This potent cytokine activates fibroblasts, the primary producers of extracellular matrix (2), prompting their differentiation into myofibroblasts. Myofibroblasts, known for producing abundant collagen and fibronectin, are highly contractile, driving tissue contraction, stiffening, and remodeling—a hallmark of fibrosis that ultimately impairs normal tissue function (2,75). Another type-2 cytokine, IL-4, also contributes to fibrotic responses (76) by inducing myofibroblast differentiation. Additionally, IL-4 promotes fibrosis by recruiting inflammatory cells like macrophages and eosinophils, which release profibrotic mediators (e.g., TGF-β), exacerbating the fibrotic process (77). In the lungs, chronic injuries and insults can lead to conditions such as asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis, all resulting in airway remodeling and eventual fibrotic scarring (78). IL-13 and IL-4 are implicated in driving these fibrotic responses, often elevated in pulmonary diseases and correlating with fibrosis severity (79,80).

Similarly, innate type-2 cytokines may contribute to liver fibrosis induced by chronic infections like schistosomiasis and liver flukes. These infections trigger local production of IL-4, IL-5, and IL-13, associated with the formation of fibroproliferative lesions in the liver (81). Type-2 cytokine-driven liver fibrosis also plays a role in the pathology of hepatitis C and hereditary or infectious liver diseases, including primary sclerosing cholangitis, primary biliary cirrhosis, autoimmune hepatitis, biliary atresia, and non-alcoholic steatohepatitis (82).

Innate type-2 cytokines in metabolism and obesity

Obesity, characterized by abnormal fat accumulation in white adipose tissue (WAT), is a multifaceted metabolic disorder influenced by genetic and environmental factors (83). WAT's primary function is to store excess energy in the form of fat, but it also exhibits adaptive responses to various stimuli, including hormones, exercise, and prolonged exposure to cold temperatures. A crucial adaptation is the beigeing or browning of adipocytes, primarily occurring in subcutaneous WAT. This process involves the uncoupling of the mitochondrial electron transport chain, facilitating thermogenesis (83). Obesity associates with the loss of this adaptive energy-regulating mechanism.

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Remarkably, alarmin IL-33, ILC2s, and type-2 cytokines play beneficial roles in countering obesity by promoting WAT browning. Although the mechanisms are not fully elucidated, they involve stromal cells in WAT producing IL-33 (84). This activates local ILC2s, inducing them to release IL-5 and IL-13. These innate type-2 cytokines promote the accumulation of eosinophils, which, in turn, produce IL-4. IL-4 converts WAT-resident macrophages into alternatively activated (M2) PPARγ$^+$ macrophages. These M2 macrophages release molecules, possibly catecholamines, and/or anti-inflammatory molecules, inducing WAT browning (85). Disruptions in this delicate balance, such as the deletion of PPARγ in macrophages, are associated with insulin resistance and weight gain (85,86) (**Fig. 2**).

*i*NKT cells contribute to WAT homeostasis and browning by producing IL-4 and IL-10, inducing the alternative activation of macrophages. Conversely, *i*NKT cells can also produce IFN-γ, and IFN-γ-expressing *i*NKT cells have been implicated in local WAT inflammation. This inflammation induces the local accumulation of proinflammatory M1 macrophages, neutrophils, mast cells, and T cells, associated with insulin resistance (87). Additionally, treating obese mice with IL-25 has been shown to induce weight loss and elevate the numbers of IL-13-expressing type-II NKT cells, the other category of NKT cells. Treating obese mice with IL-25 induced weight loss, elevated the numbers of IL-13-expressing type-II NKT cells in WAT, and adoptively transferring splenic type-II NKT cells from IL-25-treated normal mice into obese mice induced more effective weight loss and improved glucose tolerance compared to transferring ILC2 and iNKT cells (88). This finding suggests a potential hierarchy of action, highlighting the crucial role of type-II NKT cells.

In summary, innate type-2 cytokines and their downstream immune responses play crucial roles in both defense against helminths and maintaining metabolic homeostasis in adipose tissue. However, dysregulation can lead to severe pathogenic effects, contributing to common and devastating diseases such as allergies and fibrosis. Understanding the mechanisms that induce or disturb innate type-2 cytokine production holds the potential for valuable insights into developing therapeutic strategies.

ADVANCES IN THERAPEUTIC STRATEGIES FOR TYPE-2 IMMUNE DISEASES

Targeting TSLP, IL-25, and IL-33

Given their position at the top of the immune cascades that lead to type-2 immune diseases, particularly allergies, targeting the TSLP, IL-25, and IL-33 alarmins may be a promising way to manage these diseases (5). With regard to TSLP, this approach would neutralize its ability to activate mast cells, basophils, and other cells that contribute to type-2 immune diseases, including DCs, monocytes, NKT cells, and, in some settings, CD4$^+$ Th2 cells (28). Similarly, blocking IL-25 and IL-33 would reduce their ability to induce type-2 immune responses by ILCs and other innate-immune cells (5,12,27). The potential of this alarmin-targeting strategy is supported by studies demonstrating that mice lacking adaptive type-2 immune responses (B and T cells) still efficiently develop type-2 inflammation in response to triggers such as allergens (47,62). Moreover, treatment with alarmins directly induces type-2 immune responses: for example, IL-25 administration induces lung eosinophilia, IgE production, and AHR in mice (11,12). Similarly, intravenous IL-25 administration along with a suboptimal dose of allergen generates *i*NKT cell-mediated AHR (13). Conversely, the development of oxazolone-induced colitis by mice is inhibited by neutralizing Abs to IL-25 or

its receptor; this effect associates with reduced intestinal infiltration and IL-13 production of iNKT cells (89). Therefore, targeting alarmin signaling holds promise for alleviating type-2 inflammation-related diseases, especially those affecting mucosal barrier tissues.

To date, clinical trials have primarily examined the ability of TSLP, IL-25, and IL-33 blockers to improve allergic diseases. These drugs include the IL-33/ST2-pathway inhibitors astegolimab, itepekimab, and tozorakimab. The trials on these drugs are currently in phase-3. There are also the TSLP-pathway inhibitors tezepelumab, which has received Food and Drug Administration (FDA)-approval for asthma, and ecleralimab, an inhaled drug, is in phase-2 clinical trials (5). Moreover, Abs that specifically target TSLP have shown promise in reducing allergen-induced bronchoconstriction and airway eosinophilia in individuals with allergies, both before and after allergen exposure (90).

Despite these promising developments, the clinical value of blocking IL-25, IL-33, or TSLP remains uncertain. Thorough investigations into specific mechanisms, disease relevance, and translational research are essential to establish the full therapeutic potential and safety of these interventions in allergic diseases.

Targeting IL-4 and IL-13 signaling

Targeting IL-4 and IL-13 signaling emerges as a promising therapeutic approach, considering their pivotal roles in driving key pathogenic features in type-2 immune diseases (2). A spectrum of interventions has been developed, including antagonists, antisense oligonucleotides, microRNAs, and humanized monoclonal Abs against IL-4Rα, IL-13Rα1, GATA3, and the cytokines themselves (91). However, the clinical trials targeting IL-13 or IL-4 have yielded mixed outcomes. While initial results with anti-IL-13 Abs like lebrikizumab and tralokinumab were promising, phase-3 trials showed poor outcomes (92,93). This discrepancy may arise from the functional overlap of IL-4 and IL-13, as they can signal through the same receptor (the IL-4Rα/IL-13Rα1 heterodimer). Despite this, IL-4 can also signal through the IL-4Rα/common gamma chain heterodimer, contributing to distinctive functions in type-2 immune diseases. Specifically, IL-13 acts as a primary disease-inducing effector cytokine, while IL-4 amplifies type-2 immunity by facilitating the expansion of the $CD4^+$ Th2 cell population in secondary lymphoid organs (94). Consequently, blocking both IL-4 and IL-13 may yield more promising outcomes. Dupilumab, which binds to IL-4Rα and disrupts signaling by both cytokines, has demonstrated efficacy in reducing asthma exacerbations and received FDA approval for moderate-severe uncontrolled asthma (95). Dupilumab also shows effectiveness in other type-2 immune diseases, including allergic rhinitis (96), nasal polyposis (97), and atopic eczema (98).

However, caution is warranted, as targeting both IL-13 and IL-4 may not always be safe. Depleting both cytokines in chronic schistosomiasis leads to a skewed and lethal type-1-driven inflammatory response in the liver and intestines (99). Selectively blocking IL-13 signaling preserves protective features of the type-2 immune response while significantly reducing pathological fibrosis, resulting in less morbidity and mortality during chronic infection (100). These observations underscore the need for tailored therapeutic strategies aligned with specific disease contexts and their immunological dynamics.

Targeting IL-5 and eosinophils

Another promising therapeutic strategy for diseases associated with type-2 immunity involves targeting IL-5 and eosinophils. IL-5, produced by alarmin-activated innate-

immune cells, predominantly ILC2s, strongly stimulates the activation and proliferation of eosinophils. As these cells are rapidly recruited to sites of inflammation and collectively produce substantial amounts of IL-4 and IL-13, they play critical roles in the pathophysiology of various type 2-driven diseases, including asthma, eosinophilic esophagitis, and hyper-eosinophilic disorders (3,63). This eosinophilic response is often amplified by IL-5 that is produced by $CD4^+$ Th2 cells in the affected tissue (3).

Several drugs regulating the development, recruitment, and survival of eosinophils are currently under investigation. These include humanized monoclonal Abs blocking the binding of IL-5 to IL-5Rα, which have advanced to phase-2 and phase-3 clinical trials (101). Another intervention, benralizumab, an IL-5Rα-specific monoclonal Ab, eliminates eosinophils through Ab-dependent cellular cytotoxicity. Combining IL-5-neutralizing Abs like mepolizumab and reslizumab with benralizumab effectively reduces eosinophil numbers, leading to the approval of this intervention for severe eosinophilic asthma (102,103).

While preliminary findings appear promising, it remains imperative to ascertain whether other cell types, including ILC2s, *i*NKT cells, basophils, and mast cells might compensate for the reduction in eosinophils, as observed in select preclinical studies (104,105). Exploring the potential for sustained protection achieved through the targeting of IL-5 and eosinophils is an area warranting further investigation.

CONCLUDING REMARKS

In this review, we have extensively delved into the pivotal and potent regulatory roles of innate type-2 cytokines in both safeguarding against parasitic infections and addressing obesity, while also contributing to the onset of inflammatory diseases such as asthma and fibrosis. It is evident that these cytokines intricately connect a network of cells that perceive danger signals, engage in crosstalk via various mediators, and subsequently set in motion downstream immune responses, often mediated by the same type-2 signals. The proximity of these cytokines to the initiation of immune cascades underscores their potential as promising therapeutic targets, particularly in the context of allergic diseases. Notably, certain biologics targeting these cytokines or their key cellular sources have demonstrated efficacy, although the field grapples with complexities and challenges, with continuous evolution.

Further research into the immunological dynamics of innate type-2 cytokines is imperative to forge innovative therapeutic strategies tailored to specific type-2 immunity-related diseases. These efforts hold the promise of providing relief to individuals grappling with these conditions, marking a crucial stride in the ongoing advancement of immunological knowledge and the pursuit of effective therapeutic targets.

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