

Innate Th2-type immune responses and the natural helper cell, a newly identified lymphocyte population

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Purpose of review

We discuss recent progress in the understanding of Th2 cytokines derived from innate cells involved in the pathophysiology of various allergic diseases.

Recent findings

Recent studies have identified a previously unrecognized lymphocyte population capable of producing Th2 cytokines in response to IL-25 and/or IL-33 independently of T or B cells.

Summary

Newly identified cells responsive to IL-25 and/or IL-33 include natural helper cells, MPP^{type2}, nuocytes and innate helper type 2 cells. These cells produce large amounts of Th2 cytokines, most notably IL-5 and IL-13, leading to eosinophilia and goblet cell hyperplasia and are critical for antihelminth reactions. IL-5 and IL-13 also play a role in pathophysiology of allergic diseases such as asthma and allergic diarrhea. There are similarities and differences between these newly identified cell populations. MPP^{type2} cells can potentially differentiate to other myeloid cells, making this cell type distinct from the others. Natural helper cells constitutively produce Th2 cytokines, support the self-renewal of B1 cells and enhance IgA production. Unlike other innate Th2 producers, natural helper cells are present in fat-associated lymphoid clusters and likely play additional roles in the regulation of homeostasis in adipose tissues.

Keywords

adipose tissue, eosinophilia, goblet cell hyperplasia, IL-25, IL-33

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Introduction

Inflammatory responses induced by Th2 cytokines are characterized by the mobilization and activation of mast cells, basophils and eosinophils and are distinct from inflammatory responses induced by Th1 or Th17 reactions [1,2]. Th2-mediated inflammatory responses also induce goblet cell hyperplasia in airway and intestinal epithelia. Although the antigen-specific Th2 response plays a central role, accumulating evidence indicates the involvement of innate immune cells in the onset and pathophysiology of Th2 responses.

Studies [3,4] on the relatively new cytokines, IL-25 and IL-33, revealed that epithelial cells play important roles in inducing Th2 immune responses against allergens and helminth infections. Epithelial cells produce IL-25 and IL-33 in response to allergens and helminthes [5,6]. For example, house dust mites stimulate lung epithelial cells through TLR4 [7]. Administration of IL-25 and IL-33 induces rapid production of Th2 cytokines such as IL-5 and IL-13 in mice independently of T or B cells, and such innate production of Th2 cytokines plays important roles

in the pathophysiology of allergic reactions as well as in protection against helminthes [8–10]. However, which cells are responsible for the production of Th2 cytokines in response to IL-25 and IL-33 has remained an intriguing question.

Recent studies from our group as well as others identified a previously unrecognized cell population(s) capable of producing large amounts of IL-5 and IL-13 in response to IL-25 and/or IL-33. This review summarizes the characteristics and functions of such innate cells in the production of Th2 cytokines. In particular, we focus on natural helper cells found in visceral adipose tissue [11•].

IL-25 and IL-33, Th2-inducing cytokines derived from epithelial cells

IL-25, also called IL-17E, is a member of the IL-17 family [8] and induces Th2 immune responses [9] unlike other family members that induce neutrophil-mediated inflammatory responses. The receptor for IL-25 is composed of IL-17RA and IL-17RB while the receptor for other IL-17 family members is a heterodimer of IL-17RA

and IL-17RC [12]. While originally reported to be produced by Th2 cells [8], it was later shown that IL-25 is also produced by epithelial cells: lung epithelial cells infected with *Aspergillus fumigatus* [9] or sensitized with OVA [13] as well as gut epithelial cells infected with *Nippostrongylus brasiliensis* [14] produce IL-25. Mast cells activated by cross-linking of FcεRI also produce IL-25 [15].

Administration of IL-25 induced eosinophilia and IgE production in mice [8]. Repeated nasal administration of IL-25 resulted in the expression of IL-5 and IL-13 in the lung and induced airway hypersensitivity [9]. Similarly, a transgenic mouse line expressing either mouse or human IL-25 exhibited increased levels of serum IL-5 and IL-13 and eosinophilia [16,17]. *In vitro*, IL-25 induces the production of IL-5 and IL-13 from a CD4⁺CD8[−]MHC-II^{high}CD11c^{dull}F4/80^{low} non-T and non-B (NTNB) population [8]. Intriguingly, IL-25 induces the production of IL-5 and IL-13 but not IL-4 [8]. IL-5 and IL-13 are produced in Rag2^{−/−} mice upon IL-25 administration, confirming the production of these cytokines by NTNB cells [9]. Mice lacking the cytokine receptor common γ chain (γ_c) are incapable of producing IL-5 and IL-13 in response to IL-25 [9].

IL-25-deficient mice were unable to elicit a Th2 response upon *Trichuris muris* infection and are thus unable to control the infection [18]. Similarly, the expulsion of *N. brasiliensis* was significantly delayed in IL-25-deficient mice but administration of recombinant IL-25 leads to the induction of IL-5 and IL-13 from c-Kit⁺ NTNB cells and expulsion of *N. brasiliensis* [19].

IL-33 is a member of the IL-1 family [20] expressed in a variety of cells including fibroblasts, epithelial cells, endothelial cells, and adipocytes [21–23]. Intriguingly, IL-33 is localized in the nucleus and functions as a heterochromatin-associated transcriptional repressor like IL-1α [24,25]. IL-33 can be cleaved by caspase 1 *in vitro* [26] but caspases 3 and 7 cleave IL-33 at the IL-1-like domain and inactivate IL-33 during apoptosis [27,28]. In contrast, full-length IL-33 is released from cells upon necrotic cell death and functions as an alarmin to stimulate a variety of cells [27]. Similar alarmins that are also present in the nucleus and are released upon cellular damage include HMGB-1 [29], IL-1α [30] and SAP130 [31].

IL-33R is composed of ST2 and IL-RAcP [22] and is expressed on various types of cells including Th2 cells [32], mast cells [33] and basophils [34–36]. IL-33R is also expressed on CD34⁺ hematopoietic progenitor cells and IL-33 induces the differentiation of mast cells from CD34⁺ cells [37]. Interestingly, a truncated form of ST2 named soluble ST2 (sST2) is secreted and acts as a negative regulator of IL-33 [38].

Key points

- IL-25 and IL-33 derived from epithelial cells play important roles in the induction of Th2 responses involved in antihelminth responses and pathophysiology as well as allergic diseases.
- IL-25 and IL-33 induce Th2 cytokines such as IL-5 and IL-13 from recently identified innate Th2 cytokine-producing cells, leading to eosinophilia and goblet cell hyperplasia.
- There are similarities and differences in recently identified innate Th2 cytokine producers.
- Natural helper cells support the production of IgA from B cells and self-renewal of B1 cells.
- Natural helper cells are present in fat-associated lymphoid clusters localized in visceral adipose tissue and likely play additional roles in adipose tissues.

IL-33 induces the production of IL-5 and IL-13 from mast cells [39,40] and CD34⁺ cells [37] *in vitro*. Basophils also respond to IL-33 by producing IL-4, IL-5, IL-6, and IL-13 [34]. Administration of IL-33 induces Th2 cytokine production and associated physiological changes in mice including IgE production, eosinophilia and goblet cell hyperplasia [10]. IL-33 is also involved in various Th2-type inflammatory responses induced by *Pseudomonas aeruginosa* and RSV [41,42]. Mice deficient for IL-33 are resistant to allergen-induced airway hypersensitivity [43•]. Administration of sST2 blocks the allergic response in the allergic airway inflammation model [38,44]. These results collectively indicate the importance of IL-33 in Th2-type immune responses.

Accumulating evidence shows that IL-33 is involved in allergic diseases in humans. The expression levels of IL-33 in epithelial cells are higher in asthmatic patients than healthy individuals [45•] and even higher in treatment-resistant patients [46•]. IL-33 levels were higher in patients with allergic conjunctivitis [47•], rhinitis [48] and atopic dermatitis [49]. Polymorphisms of IL-33 and ST2 are associated with allergic diseases including asthma in humans and asthmatic patients have a lower level of sST2 than healthy controls [50•].

Although IL-25 and IL-33 induce Th2 cytokines *in vivo*, the identity of the cell(s) responsible for the production of Th2 cytokines has been obscure. As mentioned, mast cells, basophils and CD34⁺ hematopoietic progenitor cells produce Th2 cytokines. A fraction of NKT cells express IL-25R and respond to IL-25 to produce IL-13 [51]. However, compared with the amounts of IL-5 and IL-13 produced *in vivo*, production levels of Th2 cytokines by these cells were low, suggesting the presence of yet to be discovered cell type(s). We have recently identified a previously unrecognized lymphocyte population in the

visceral adipose tissue that we named natural helper cells [11^{••}]. Cells sharing similar characteristics with natural helper cells have also been reported by others [52^{••}–54^{••}].

Natural helper cells producing Th2 cytokines

We noted a previously unrecognized γ_c -dependent lymphoid structure located along the blood vessels in the mouse and human mesentery, an adipose tissue in the peritoneal cavity [11^{••}]. We named these lymphoid clusters ‘fat-associated lymphoid clusters’ or FALC. Cells in FALC are in direct contact with ambient adipocytes and no fibrous capsule is present around FALC. FALC are also present in visceral adipose tissues around the kidney and genitalia but very few are found in the subcutaneous fat tissue or the omentum. FALC are structurally similar to the ‘milky spot’ in the omentum, which is considered a gateway of cells between the circulation and the peritoneal cavity [55]. However, unlike the milky spot [56], T and B cell zones or germinal center structures are not observed in FALC. FALC are present in ROR γ -deficient and *aly/aly* mice, suggesting that the differentiation pathway of FALC is distinct from those of lymph nodes or Peyer’s patches involving lymphoid tissue inducer (LTi) cells [57].

T and B cells, macrophages and dendritic cells are present in FALC. In addition, a novel cell population expressing c-Kit, Sca-1, IL-2R, IL-7R and IL-33R but no lineage (Lin) markers makes up 20–40% of total cells in the lymphocyte gate [11^{••}]. A cell population expressing c-Kit and IL-7R α is also present in human FALC. Giemsa staining and electron microscopy showed that these cells have characteristics of lymphoid cells. These cells were present in Rag2^{-/-} and *nu/nu* mice but absent from γ_c ^{-/-} and IL-7^{-/-} mice, indicating that these cells are likely of lymphoid lineage with differentiation dependent on IL-7. Microarray analysis also showed similarity between natural helper cells and T and NK cells. SCF and IL-7 supported the survival of and IL-2 induced the proliferation of these cells without changing their surface phenotype. Based on the observations that these lymphocytes proliferate in response to IL-2 and exhibit innate type helper functions by producing Th2 cytokines (see below), we named these cells natural helper cells [11^{••}].

Natural helper cells constitutively produce IL-5 and IL-6, which are involved in antibody production [58,59]. IL-5 is a critical growth factor for B1 cells, which are abundant in the peritoneal cavity and play an important role in innate-type immune responses by producing natural antibodies [60,61]. Indeed, natural helper cells support the production of IgA from B cells and self-renewal of B1 cells, indicating that natural helper

cells have helper functions under steady-state conditions.

In addition, natural helper cells are triggered to produce even larger amounts of IL-5, IL-6 and IL-13 in response to IL-33 or a combination of IL-2 and IL-25. It should be noted that natural helper cells do not respond to IL-25 without IL-2. Five thousand natural helper cells are able to produce microgram amounts of IL-5 and IL-13 in response to IL-33 during a 5-day culture period and this is much higher than the amounts produced by mast cells [33], basophils [34,35] or polarized Th2 cells [32]. Unlike basophils, natural helper cells do not produce IL-4 in response to IL-33 or a combination of IL-2 and IL-25. Notably, production of IL-4 can be induced by a combination of 12-O-tetradecanoylphorbol-13-acetate and ionomycin but, so far, this is the only stimulation that results in the production of IL-4 by natural helper cells.

Administration of IL-33 or *N. brasiliensis* infection induced the production of IL-5 and IL-13 as well as goblet cell hyperplasia in the intestine of Rag2^{-/-} but not γ_c ^{-/-}Rag2^{-/-} mice. Adoptive transfer of natural helper cells into γ_c ^{-/-}Rag2^{-/-} mice restored the production of IL-5 and IL-13 and goblet cell hyperplasia [11^{••}]. Natural helper cells thus provide IL-5 and IL-13 to limit the helminth invasion before helminth-specific T cells are induced.

Multiple types of innate type Th2 cytokine-producing cells

After our description of natural helper cells [11^{••}], others have reported cells sharing characteristics similar to those of natural helper cells [52^{••}–54^{••}].

Using knock-in mice in which GFP is inserted into the IL-4 allele, termed ‘4get’ mice [62], Saenz *et al.* [52^{••}] identified IL-4-producing NTNB cells (GFP⁺ cells) in gut-associated lymphoid tissues upon IL-25 administration. Adoptive transfer of GFP⁺ cells into IL-25-deficient mice resulted in the production of IL-4, IL-5 and IL-13 by lymphocytes in mesenteric lymph nodes, mucin secretion and protective immunity against *T. muris*. Cells induced by IL-25 administration shared cell surface markers with hematopoietic stem cells or multipotent progenitor cells and were named ‘MPP^{type2}’. This population seems to be heterogeneous and c-Kit⁺GFP⁺ cells differentiate into mast cells while c-Kit⁺GFP⁻ cells have the potential to differentiate into basophils and macrophages.

Using another knock-in mouse in which GFP was inserted into the IL-13 allele, Neill *et al.* [53^{••}] reported that NTNB cells producing IL-5 and IL-13 (GFP⁺ cells) were induced in the mesenteric lymph nodes, spleen and

small intestine after administration of IL-25 or IL-33. They named these GFP⁺ cells 'nuocytes', nu being the 13th letter of the Greek alphabet although these cells also produce IL-5. As observed for NTNB cells in an earlier report [8], nuocytes express MHC class II and respond to IL-25 alone, which are characteristics distinct from those of natural helper cells (Table 1). In addition, microarray analysis showed that nuocytes are more similar to myeloid cells than lymphoid cells. Mice lacking IL-17RB are unable to respond to IL-25 or control *N. brasiliensis* infection. Adoptive transfer of wild-type nuocytes into IL-17RB-deficient mice restored their ability to expel *N. brasiliensis*.

In naïve 4get mice, eosinophils, basophils, mast cells, NKT cells and Th2 cells are GFP⁺ [63–65]. Price *et al.* [54^{••}] recognized an additional Lin[−]c-Kit⁺GFP⁺ population in naïve 4get mice. They also employed IL-13 reporter mice and observed that such c-Kit⁺NTNB cells produce IL-5 and IL-13 in response to IL-25 or IL-33 administration and *N. brasiliensis* infection. Intriguingly, IL-4-GFP⁺ cells do not produce IL-4 at the protein level. Although mRNAs for IL-5 and IL-13 are strongly induced, it is unknown whether these cells produce IL-5 and IL-13 proteins. The authors named these cells innate helper type 2 (Ih2) cells. Ih2 cells are absent in $\gamma_c^{-/-}$ Rag2^{-/-} mice incapable of controlling *N. brasiliensis* infection but adoptive transfer of Ih2 cells followed by administration of IL-25 induced eosinophilia and antihelminth activity. It is unknown whether Ih2 cells are able to produce cytokines in response to IL-25 alone.

At the moment, the relationship between natural helper cells, nuocytes, MPP^{type2} and Ih2 cells is unclear. There are similarities and differences as summarized in Table 1. One important difference between natural helper cells and the other cells is that natural helper cells do not

respond to IL-25 alone [11^{••},52^{••}]. It is intriguing that there is no evidence of the production of IL-4 as a protein from these cells even though the *il-4* gene is active as determined by microarray analyses and knock-in approaches.

Perspectives

Given that IL-5 and IL-13 are critical factors for the induction of eosinophilia and goblet cell hyperplasia, respectively, and given that these two processes are involved in the pathogenesis of allergic diseases such as asthma and allergic diarrhea, it stands to reason that the innate Th2 cytokine producers described here play important roles in these allergic diseases.

Natural helper cells do not respond to TLR ligands such as LPS or lectins such as concanavalin A ([11^{••}], our unpublished observations). It is unknown at the moment whether the innate Th2 cytokine producers discussed here directly respond to allergens or microbial/parasitic antigens.

In addition to their roles in allergic diseases and protection against helminth infection, natural helper cells may have additional roles in adipose tissues. Accumulating evidence demonstrates the importance of interaction between adipocytes and immune cells in the regulation of homeostasis in adipose tissues. Impaired regulation under pathogenic conditions such as obesity results in inflammatory responses in adipose tissues leading to insulin resistance [66]. One subset of macrophages, the M1 macrophages, is involved in the inflammation in adipose tissues [66,67]. IL-33 induces the M2 subset of macrophages but high levels of Th2 cytokines induce M1 macrophages [36,68,69]. Because such phase transition in macrophages plays a critical role in the course of inflammation in adipose tissues, it will be of interest and of importance to elucidate the functions of natural helper cells in adipose tissues.

Table 1 Characteristics of innate Th2 cytokine-producing cells as examined by surface phenotypes

Markers	Cells			
	Nuocyte	MPP ^{type2}	Ih2 cell	
Natural helper cell				
c-Kit	+	+/-	+	+/-
CD45	+	+	+	+
IL-7R α	+	lo	-/lo	?
Sca-1	+	+	+	-
Thy-1	+	+	?	+
CD34	-	-	-/lo	?
CD25	+	-*	?	+ ^a
CD44	+	+	?	+
CD69	+	?	-	+ ^a
CD62L	-	?	-/lo	?
Fc ϵ RI	-	-	-	?
T1/ST2	+	+/-	-/lo	?
MHC class II	-	+	? ^b	?

Ih2, innate helper type 2.

^a On the basis of microarray analysis.

^b GFP[−] cells expressed MHC class II after cultivation with a combination of IL-3 and SCF.

Conclusion

Recently identified natural helper cells, MPP^{type2}, nuocytes and innate helper type 2 (Ih2) cells produce Th2 cytokines in response to IL-25 and/or IL-33. There are similarities and differences between these cell populations. IL-5 and IL-13 produced by these cells lead to eosinophilia and goblet cell hyperplasia and are critical for antihelminth reactions. These cells likely play a role in pathophysiology of allergic diseases such as asthma and allergic diarrhea.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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