

Roles of IL-18 in Basophils and Mast Cells

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ABSTRACT

Basophils and mast cells are effector cells in allergen/IgE-mediated immune responses. They induce type 1 immediate immune response in airway or other organ, resulting in bronchial asthma and other allergic diseases. However, they also play a critical role in host defense against infection with helminthes. Upon linkage of FcεRI with a complex of allergen and IgE, basophils and mast cells release a large amount of Th2 cytokines and chemical mediators. Therefore these responses are “acquired allergic responses” and induce allergic diseases, such as bronchial asthma. However, basophils and mast cells derived from cultured bone marrow cells with IL-3 for 10 days express IL-18Rα chain and produce Th2 cytokines in response to the stimulation with IL-3 and IL-18 without FcεRI cross-linkage. Furthermore, they produce Th2 cytokines upon stimulation with several TLR ligands, such as LPS. This finding may suggest the presence of allergen/IgE-independent allergic responses, which we would like to designate as “innate allergic response”. However, *in vivo* treatment with IL-18 and IL-2 protects against gastrointestinal nematode infection by activating intestinal mucosal mast cells in STAT6-independent manner, suggesting the importance of innate allergic response against helminth infection. Here we discuss the functional role of IL-18-induced “innate allergic response” in disease and host defense.

KEY WORDS

basophils, IL-18, mast cells, nematode infection, Th2 cytokines

INTRODUCTION

IL-18 was discovered as a potent IFN-γ-inducing factor that is produced by macrophages and dendritic cells upon stimulation with microbes or microbe products.¹ Thus, IL-18 has been categorized in an innate immune cytokine. There are several pathways to induce IFN-γ, a crucial factor for inflammatory responses. Th1 cells produce abundant IFN-γ in response to the appropriate antigen. We now know that a large amount of IFN-γ is also produced by a wide variety of cell types in response to the innate immune cytokines, such as IL-12, IL-15 and IL-18.²⁻⁸ IL-18 is the first cytokines demonstrated to activate T cells to produce plentiful IFN-γ without T cell receptor (TCR) engagement.^{3,4} Resultant IFN-γ then activates macrophages to produce nitric oxide,⁹ leading to eradication of intracellular pathogens,¹⁰⁻¹² or tissue injuries (Fig. 1).¹³ However, our recent studies also clarified that IL-18 induce Th2 cytokines production from T cells or mast cells/basophils.^{14,15} Without TCR en-

gagement, IL-18 with IL-2 induces T cells to produce IL-4 and IL-13 and to express CD40 ligand, which in combination induce B cell IgE response (Fig. 1). Indeed, administration of IL-18 or IL-18 plus IL-2 into naïve mice induces IgE in a CD4⁺ T cell-, IL-4- and STAT6-dependent manner.^{15,16} Moreover, transgenic mice over-expressing IL-18 in their keratinocytes, spontaneously produce IgE and develop atopic dermatitis.¹⁵⁻¹⁷ Intriguingly, this dermatitis develops even in the absence of STAT6 activation, which is required for Th2 cell development and IgE responses.¹⁸ Thus, IL-18 induces dermatitis in an IgE-independent manner. Antigen plus IgE induces allergic response by activation of basophils and mast cells.¹⁹ However, IL-18 induces allergic inflammation without Th2/IgE. Therefore, we proposed to designate the former type as “acquired allergic response” and the latter as “innate allergic response” (Fig. 2).⁸

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Received 13 January 2006.

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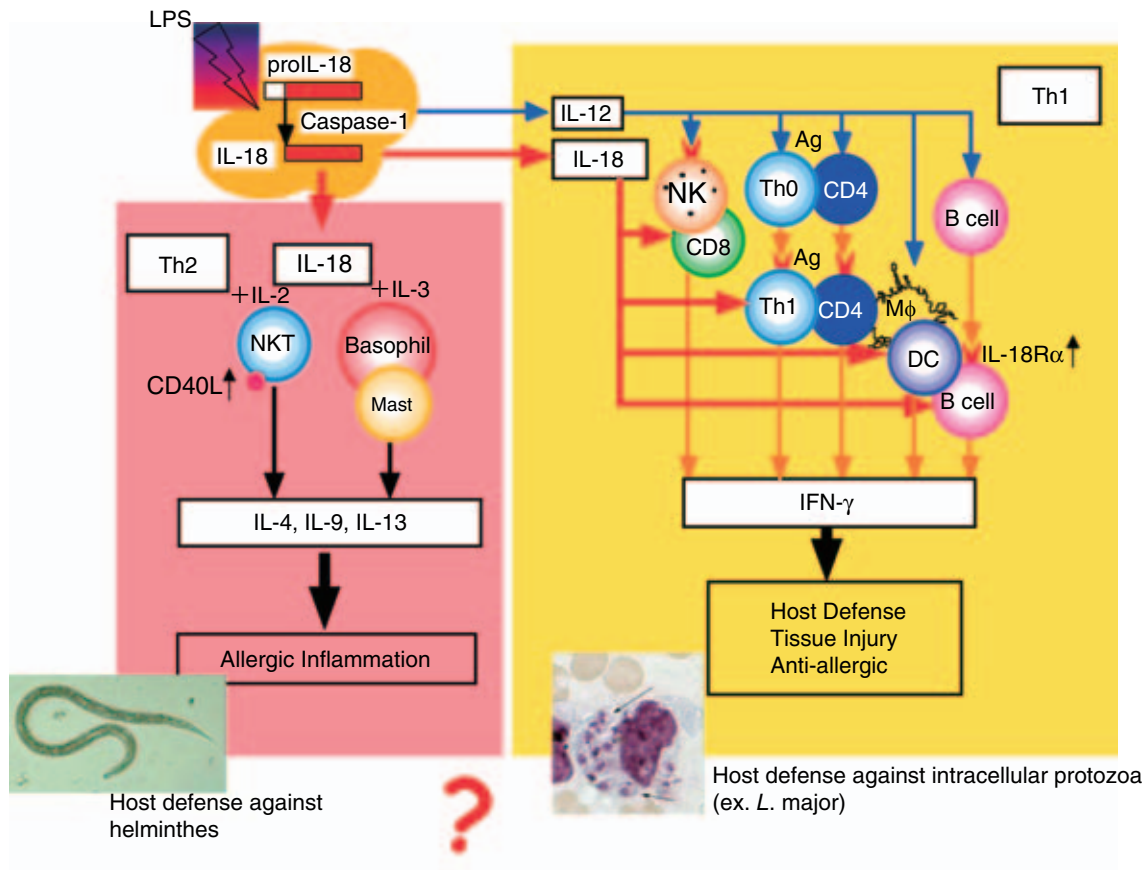


Fig. 1 Pleiotropic action of IL-18. IL-18 together with IL-12 stimulates various cells to produce significant amounts of IFN- γ , which activates macrophages to produce nitric oxide, leading to eradication of intracellular pathogens, like *Leishmania major*. However, without IL-12, IL-18 promotes Th2 cytokines productions from T cells and basophils/mast cells. Thus, IL-18 regulates both Th1 and Th2 responses depending on its cytokine milieu. The expulsion of some types of gastrointestinal nematodes depends on the action of Th2 responses, leading to the speculation of the protective roles of IL-18 against helminth infection.

IL-18-INDUCED ACTIVATION OF BASOPHILS AND MAST CELLS

It is well documented that basophils and mast cells release a large amount of Th2 cytokines (IL-4, IL-5, IL-9 and IL-13) after cross-linkage of their Fc ϵ RI by antigen and antigen-specific IgE.¹⁹ IL-4 initiates and promotes Th2 responses and is the most important determinant of Ig class switching to IgE.^{20,21} IL-5 induces maturation and activation of eosinophils.²² IL-13 induce mucus production by causing goblet cell hyperplasia.^{23,24} Furthermore, IL-13 induces airway hyperresponsiveness (AHR).²⁵⁻²⁸ Basophils and mast cell also produce various bioactive chemical mediators such as histamine and lipid metabolites.¹⁹ Thus, cross-linkage of Fc ϵ RI induces the development of "acquired type allergic response". However, we found that cultured bone marrow-derived basophils and mast cells express IL-18R α chain and produce IL-4/IL-13 and histamine in response to the stimulation

with IL-3 and IL-18 even without Fc ϵ RI cross-linkage.¹⁴ Thus, IL-3 and IL-18 induce "innate allergic response" by direct activation of basophils and mast cells (Fig. 2).

TOLL-LIKE RECEPTORS-MEDIATED ACTIVATION OF BASOPHILS AND MAST CELLS

The cytoplasmic portion of IL-18R is homologous to that of Toll-like receptors (TLRs), which have been identified as signaling receptor of the innate immune system and recognize corresponding pathogen-associated molecular patterns (PAMP).^{7,29} Myeloid differentiation factor 88 (MyD88) is an adapter molecule essential for signaling through either IL-18R or TLRs.^{30,31} Eleven mammalian TLRs have been described so far and microbial ligands corresponding each member have been identified.²⁹ Upon entry of invading pathogens, DCs recognize them through TLRs and mature to express co-stimulatory molecules CD80 and CD86 and to produce IL-12.²⁹ It is

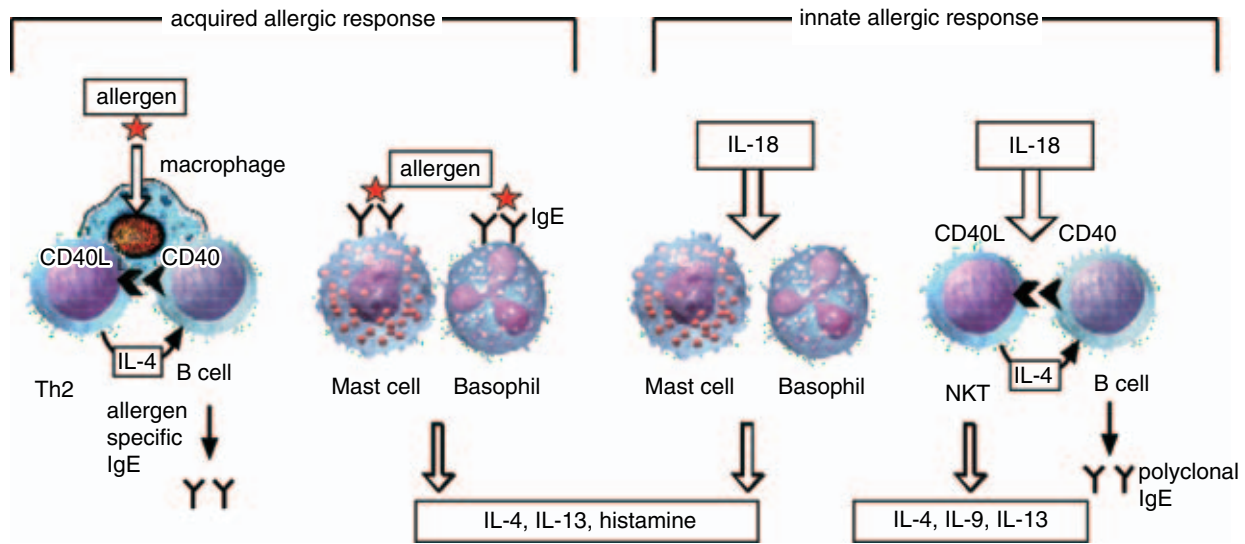


Fig. 2 IL-18-induced innate allergic response. Allergen induces allergen-specific Th2 cells and the resultant allergen-specific IgE, which can activate effector cells, such as basophils and mast cells, to induce allergic disorders. In contrast, IL-18-induced allergic disorders do not require Th2 cells or IgE. IL-18 stimulates basophils and mast cells to produce IL-4 and IL-13 even without FcεRI cross-linkage. CD4⁺ T cells (NKT cells), stimulated with IL-2 and IL-18 without TCR engagement express CD40 ligand and produce IL-4 and induce B cells to secrete IgE both *in vitro* and *in vivo*. Thus, we propose the former type be named “acquired allergic response” and the latter named “innate allergic response”.

widely accepted that IL-12 induces Th1 responses but inhibits Th2 responses to achieve the efficient inflammatory responses.

IL-18 is secreted from various cells via activation of TLRs after stimulation with microorganisms and their products in a caspase-1-dependent manner.³² Notably, upon stimulation via TLRs, IL-18 as well as other proinflammatory cytokines including IL-12 are released.⁷ However, IL-12 does not block the activity of IL-18-stimulated basophils or mast cells to produce IL-4/IL-13.¹⁴ Thus, basophils and mast cells produce Th2 cytokines even under Th1-inducing condition.

Basophils and mast cells also produce Th2 cytokines via activation of TLRs on their cell surface with PAMP. Mast cells produce Th2 cytokines when stimulated with ligands for TLR2 or TLR4, suggesting their role in induction of Th2 response.³³⁻³⁶ TLR2 recognizes Gram-positive bacterial components, including peptidoglycan (PGN) and lipoprotein, and TLR4 recognizes lipopolysaccharide (LPS), a component of Gram-negative bacteria.^{29,37,38} Human basophils were recently found to express high levels of TLR2 and TLR4.³⁹ More recently, it has been reported that human basophils produce Th2 cytokines when stimulated with ligands for TLR2 but not TLR4, suggesting that basophils can play an important role in promoting and amplifying the Th2 responses.⁴⁰ Although roles of TLRs on mast cells and human basophils have been fully examined, expression and functional roles of TLRs on murine basophils remain unclear.

We have recently revealed that bone marrow-derived murine basophils selectively express TLR1, 2, 4 and 6 and produce significant amounts of Th2 cytokines (IL-4, IL-6 and IL-13) in response to IL-3 plus PGN or to IL-3 plus LPS via TLR2 or TLR4, respectively, even without FcεRI cross-linkage (un-published observation). Consistent with the previous reports,³⁵ PGN- or LPS-stimulated mast cells produce small amounts of IL-6 and IL-13, which are significantly increased when additionally stimulated with IL-3. However, notably, compared with basophils, mast cells are poor producers of IL-4 even when they were stimulated with IL-3 and TLR ligands. Co-stimulation with IL-12 fails to attenuate these responses, substantiating further that basophils favor induction of Th2 response. It is known that allergic inflammatory responses are also induced under some infectious condition.^{41,42} Thus, our study suggests that bacterial components-stimulated basophils may play a key role for induction of “innate allergic responses”, providing a clue to understanding the mechanisms of allergic diseases triggered by bacterial infection (Fig. 3)

HELMINTH-INDUCED INTESTINAL MUCOSAL MAST CELLS

It is well known the expulsion of some types of gastrointestinal nematodes depends on the action of Th2 responses. There are two types of Th2-mediated host protective immunity against gastrointestinal nematode infections. One is worm expulsion by activated

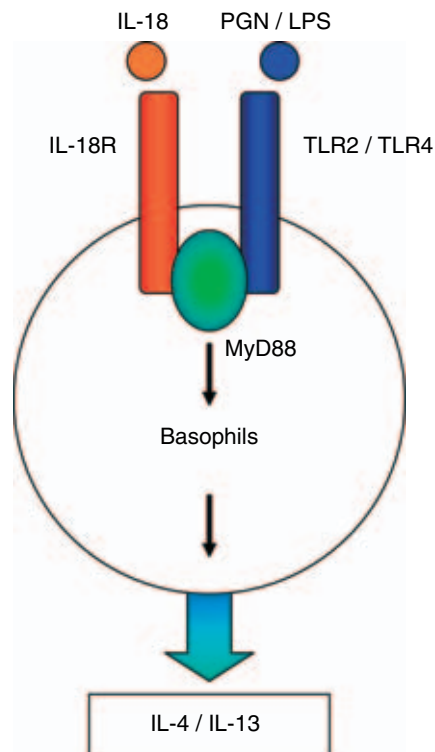


Fig. 3 IL-18R- and TLR2/TLR-4 mediated Th2 cytokines production from basophils. Basophils express both IL-18R α chain and TLRs. IL-18 and TLR ligand(s) stimulate basophils through corresponding receptor and signaling through adaptor molecule, MyD88.

intestinal mast cells.⁴³ *Strongyloides venezuelensis* is expelled by activated intestinal mast cells. The other types of worm expulsion is mediated by mucus derived from activated goblet cells stimulated by IL-13.⁴⁴ *Nippostrongylus brasiliensis* is expelled by this mucous product (Fig. 4).

The role of intestinal mucosal mast cells (MMC) in worm expulsion has been studied extensively in various experimental host-parasite systems. In the case of infection with *Strongyloides venezuelensis* (*S. venezuelensis*) third-stage larvae (L3), host mice complete parasite expulsion within 2 weeks, which is tightly associated with level of intestinal mastocytosis.^{45,46} Therefore, mast cell-deficient *W/W^v* mice infected with *S. venezuelensis* L3 show a significant delay in parasite expulsion.⁴⁵ Furthermore, parasite expulsion is more severely impaired in *W/W^v* mice that are deficient for IL-3 gene expression. In these mice, MMC responses are almost completely absent and *S. venezuelensis* continue to parasitize in the intestine for >50 days.⁴⁵ In the case of infection of mice with *Trichinella spiralis* (*T. spiralis*) or *Trichuris muris* (*T. muris*), IL-9 expression correlates well with the resistant phenotype and its elevation *in vivo* results in the

enhancement of intestinal mastocytosis and parasite expulsion.^{47,48} Furthermore, IL-9 transgenic mice that display increased intestinal MMC more rapidly expel *T. muris* or *T. spiralis* than wild-type mice.^{47,49} Therefore, both IL-3 and IL-9 are deeply involved in recruitment and activation of MMC in mice infected with gastrointestinal nematode.

It is well-established evidence that mouse mast cell protease-1 (mMCP-1), selectively expressed by intestinal MMC, participates in the effector phase response to intestinal nematodes expulsion.^{43,50-53} Indeed, mMCP-1-deficient mice fail to expel gastrointestinal nematode.⁵⁰ Miller *et al.* reported that mMCP-1 is not detectable in the culture of bone marrow-derived mast cells stimulated with IL-3 alone.⁹ However, mast cells begin to produce mMCP-1 when additionally stimulated with IL-9, SCF and TGF- β .⁹ Thus, it has been speculated that IL-3 and IL-9 from Th2 cells in mice infected with gastrointestinal nematode induce precursor cells to develop into mMCP-1+MMC together with SCF and TGF- β from gut epithelium.

IL-18 PLUS IL-2-INDUCED INTESTINAL mMCP-1+ MMC

As mentioned above, without TCR engagement, IL-18 with IL-2 can induce IL-4 and IL-13 production by CD4⁺ T cells *in vitro* (Fig. 1).¹⁵ In addition to IL-4/IL-13, IL-18 plus IL-2 stimulates CD4⁺ T cells to produce IL-3 and IL-9.¹⁶ Furthermore, administration of IL-18 plus IL-2 into naïve mice induces increases in serum levels of IL-3, IL-4, IL-9 and IL-13.¹⁶ As, IL-3, IL-4 and IL-9 are well-known potent mast cell growth factors⁵⁴⁻⁵⁶ and essential to induce intestinal mMCP-1+ MMC,⁹ we tested whether daily administration of IL-2 and/or IL-18 induces accumulation of MMC in intestines of the mice, and found that IL-18 and IL-2 dose-dependently increase the number of intestinal MMC.⁵⁷ Furthermore, these intestinal MMC express mMCP-1, suggesting that they are activated.⁵⁷ Taken together, these results clearly indicate that treatment with IL-18 and IL-2 induces accumulation, maturation and activation of intestinal MMC, namely intestinal mastocytosis (Fig. 5).

The cellular and molecular bases for IL-18 plus IL-2-induced mMCP-1+MMC have been examined. Wild-type mice depleted of CD4⁺ T cells by the pretreatment with anti-CD 4 antibody or RAG-2 deficient (RAG-2^{-/-}) mice, lacking both T cells and B cells, exhibit poor accumulation of MMC in response to administration of IL-18 plus IL-2, indicating a requirement of CD4⁺ T cells.⁵⁷ Interestingly, STAT6^{-/-} mice display intestinal mastocytosis when injected with IL-2 and IL-18, suggesting STAT6-independent mastocytosis.⁵⁷ IL-18 plus IL-2-stimulated CD4⁺ T cells produce IL-3 and IL-9, well-known potent mast cell growth factors.^{54,55} Indeed, administration of IL-3 and IL-9 into naïve mice for 2 weeks dose-dependently in-

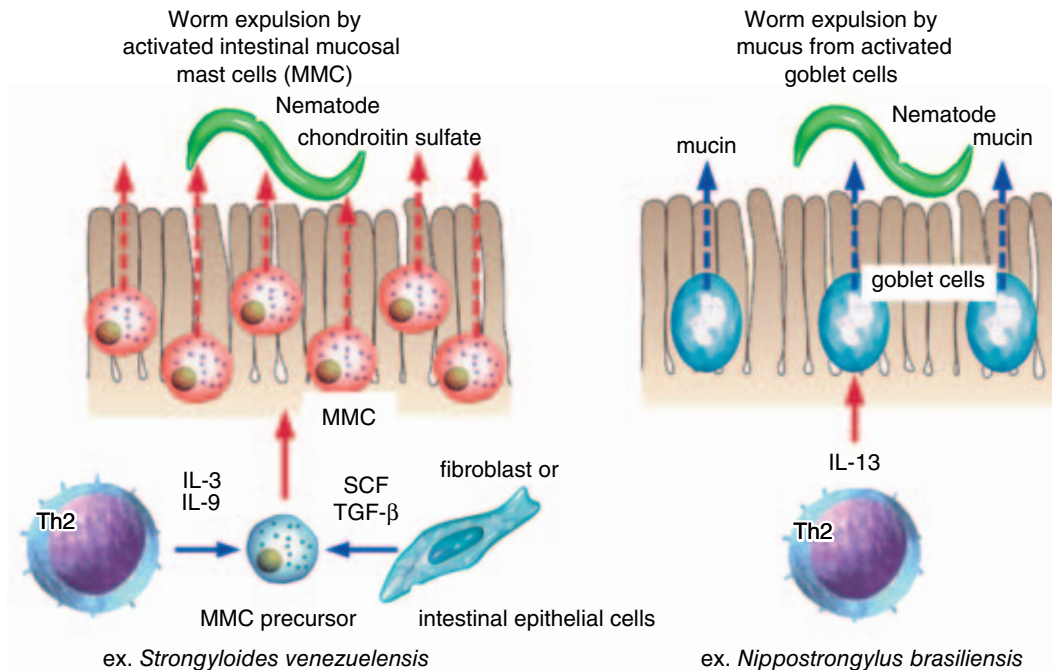


Fig. 4 Th2-mediated expulsion of gastrointestinal nematode. There are two types of Th2-mediated host protective immunity against gastrointestinal nematode infections. One is worm expulsion by activated intestinal mast cells, which are developed from precursor cells stimulated with IL-3, IL-9 from Th2 cells and SCF and TGF- β from intestinal epithelial cells. *Strongyloides venezuelensis* is expelled by these activated mast cells. The other is worm expulsion by mucus from IL-13-stimulated goblet cells. *Nippostrongylus brasiliensis* is expelled by this mucous product.

duce mMCP-1⁺MMC in the intestine.⁵⁷ Thus, IL-2 plus IL-18 treatment seems to induce mMCP-1⁺MMC by virtue of IL-3 and IL-9 from CD4⁺ T cells and IL-3 seems to be most critical for mMCP-1⁺MMC induction (Fig. 5)

IL-18 PLUS IL-2-INDUCED INTESTINAL MMC-DEPENDENT EXPULSION OF *S. VENEZUELENSIS*

To examine the functional role of intestinal MMC induced by IL-18 plus IL-2, we surgically implanted adult worms in the duodenum of mice pretreated with IL-2 and/or IL-18 for 13 days and recovered invading parasites at 16 h after implantation.⁴⁶ IL-18 plus IL-2-treated wild-type mice reject implanted worms almost completely, while mice that received PBS, IL-2 or IL-18 alone are heavily parasitized with implanted worms (Fig. 5). However, mast cell-deficient W/W^v mice even treated with IL-2 and IL-18 fail to reject them, indicating the rapid expulsion of implanted adult worms is mediated by the function of activated intestinal MMC.⁵⁷ Importantly and as expected, IL-2 and IL-18-pretreated STAT6^{-/-} mice also gain the capacity to rapidly reject implanted parasites. These results taken together suggest that IL-18 with IL-2 protects against gastrointestinal nematode infec-

tion by activating MMC-dependent innate type 2 immunity (Fig. 5)

ROLE OF ENDOGENOUS IL-18 FOR INDUCTION OF INTESTINAL MMC

Wild-type mice inoculated with *S. venezuelensis* L3 show a significant increase in serum levels of IL-18 (days 4 to 14), and complete worm expulsion within 12 days. Thus, to address the role of endogenous IL-18 in the induction of intestinal MMC for the host defense against *S. venezuelensis* L3 infection, the capacity of IL-18^{-/-} mice or IL-18R α ^{-/-} mice to expel *S. venezuelensis* was examined. Comparing to infected wild-type mice, IL-18^{-/-} or IL-18R α ^{-/-} mice infected with *S. venezuelensis* L3 exhibit significantly delayed worm expulsion.⁵⁷ Wild-type mice completed worm expulsion by day 12, while IL-18^{-/-} or IL-18R α ^{-/-} mice requested 16 days. However, they eventually expelled infected parasites, suggesting the contribution of Th2 cells that were generated by parasite infection in IL-18^{-/-} or IL-18R α ^{-/-} mice. Thus, we assume the possible contribution of Th2 cells to this late-phase induction of mMCP-1⁺MMC, namely worm expulsion. These results taken together indicate involvement of two types of intestinal MMC activation, IL-18-dependent (innate type-2) MMC activation and Th2

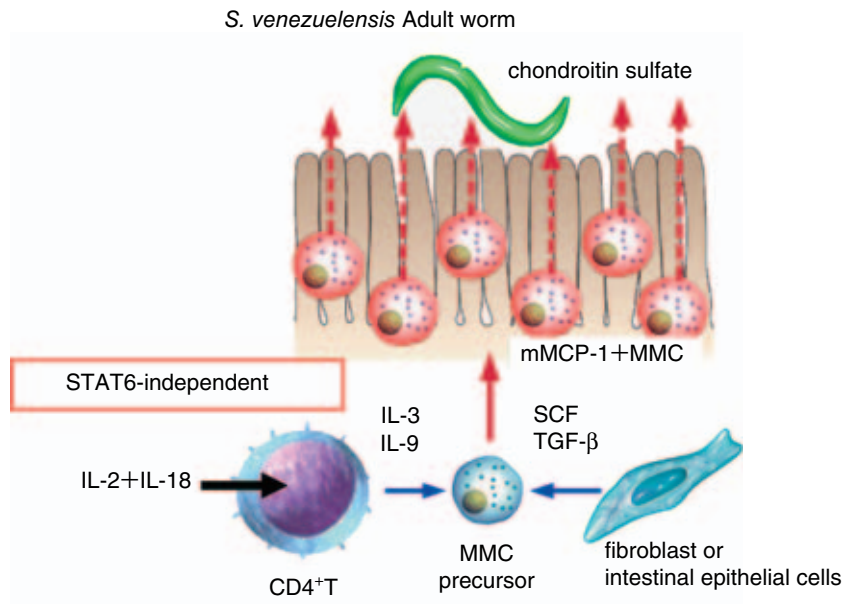


Fig. 5 IL-18 -induced intestinal MMC-dependent expulsion of *S. venezuelensis*. *In vivo* treatment with IL-18 and IL-2 stimulates CD4⁺ T cells to produce IL-3/IL-9, which develop precursor cells into mMCP-1⁺ MMC together with SCF and TGF-β from gut epithelium. These activated mast cells promptly expel implanted adult *S. venezuelensis* by producing chondroitin sulfate, which interfere *S. venezuelensis*'s anchoring.

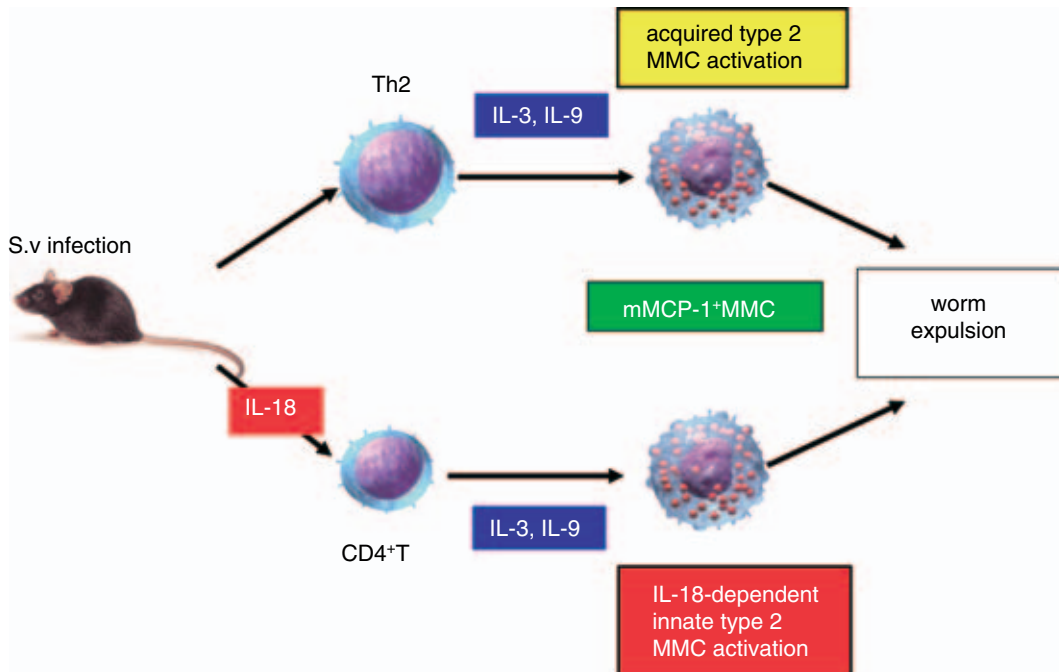


Fig. 6 Collaboration between Th2 cell-dependent and IL-18-dependent mastocytosis for parasite expulsion. *S. Venezuelensis* expulsion might be induced by two types of intestinal mMCP-1 + MMC activation, Th2 cells-dependent (acquired type-2) MMC activation and IL-2 and IL-18-dependent (innate type-2) MMC activation.

cells-dependent (acquired type-2) MMC activation for *S. venezuelensis* expulsion (Fig. 6).

ROLE OF IL-18-DRIVEN IL-3 FOR INDUCTION OF BASOPHILS AND INTESTINAL MMC

Although it is well known that IL-4 is critical to polarization of CD4⁺ T cells to a Th2 phenotype, mainly *in vivo* system, initial IL-4 producing cells *in vivo* are poorly understood. Several cell types have been reported to produce IL-4, including conventional CD4⁺ T cells,^{58,59} NKT cells⁶⁰ and mast cells.⁶¹ It has been reported that infection with gastrointestinal nematode, *Nippostrongylus brasiliensis* (Nb) resulted in an increase in the number of splenic FcεRI⁺, non-B, non-T cells.⁶² Most of these FcεRI⁺ cells were basophils morphologically and produced IL-4 in response to FcεRI cross-linkage, suggesting that basophil-derived IL-4 may play a physiologically important role in IgE production.⁶³ Recently, Paul and his colleagues have more clearly demonstrated that Nb infection induces substantial IL-4⁺ basophils in the lung, liver and spleen in a STAT6 independent manner.⁶⁴ Recruitment of basophils into these tissues is dependent on CD4⁺ T cells.⁶⁴ We have also observed that *S. venezuelensis* infection induces substantial IL-4⁺ basophils in the liver and spleen in a STAT6 independent manner (unpublished data).

Nb-induced basophil recruitment and IL-4 production has been partially inhibited by the treatment with anti-IL-3 Ab.⁶⁴ Glli and his colleagues have demonstrated that IL-3 does enhance basophil accumulation during *S. venezuelensis* infection.⁴⁵ We suggested *in vivo* IL-2 plus IL-18 treatment induces mMCP-1⁺ MMC via IL-3 and IL-9 production from CD4⁺ T cells.⁵⁷ Thus, infection with a parasite that induces a "Th2-type response" resulted in accumulation of tissue basophils in the tissues, where basophils may act as major IL-4-producing cells and protect host against various pathogens by augmenting Th2 response (Fig. 5).

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