おわりに

マラリア原虫やトキソプラズマを初めとする，多くのアピコンプレクス門原虫はアピコプラストと呼ばれる紅藻由来の共生器官を持っていることから，これらアピコンプレクス門原虫は葉緑体由来の多くの代謝経路を未だ保持している可能性が考えられている．事実，アピコンプレクス門原虫はイソプレノイドを合成するための経路(メバロチン経路)を消失しており，そのためイソプレノイド合成は，植物と同じDOXP-MEP経路を持つアピコプラストに依存していることが知られている[6]．高等植物において，アブシジン酸の生合成の多くのステップは葉緑体内で行われていることと，アブシジン酸生合成はイソプレノイドからβ－カロチンを合成することによって開始されることから，おそらくトキソプラズマにおいてもアブシジン酸生合成の大部分はアピコプラストにおいて行われている可能性が示唆できる．また，アピコンプレクス門原虫において既に光合成能を失ったアピコプラストが未だ原虫の生存に必須であるという理由の1つに，今回見出されたアブシジン酸生合成経路の存在があるのかもしれない．

参　考　文　献

1)Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. Emerg Infect Dis 1999 ; 5 : 607-25.

2)Kasper LH, Buzoni-Gatel D. Some opportunistic parasitic infections in AIDS: candidiasis, pneumocystosis, cryptosporidiosis, toxoplasmosis. Parasitol Today 1998 ; 14 : 150-6.

3)WHO. World Health Organization Report on Infectious Diseases: Removing obstacles to healthy development 1999.

4)Nagamune K, Hicks LM, Fux B, *et al.* Abscisic acid controls calcium-dependent egress and development in Toxoplasma gondii. Nature 2008 ; 451 : 207-10.

5)Chini E N, Nagamune K, Wetzel DM *et al.* Evidence that the cADPR signalling pathway controls calcium-mediated microneme secretion in Toxoplasma gondii. Biochem J 2005 ; 389 : 269-77.

6)Sibley LD. Intracellular parasite invasion strategies. Science 2004 ; 304 : 248-53.

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Basophils are potent antigen-presenting cells that selectively induce Th2 cells

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Basophils and mast cells are important effector cells in helminth-infected host and IgE-mediated allergic inflammation. Although they have the same progenitors, basophils and mast cells complete their terminal differentiation in the bone marrow and peripheral tissues, respectively, and only basophils circulate in the blood. Although it is recognized that basophils are important for Th2 responses, and it is also well established that IL-4 is required for Th2 differentiation from naïve CD4$^+$ T cells, the nature of the cells that produce "early" IL-4, remained elusive until recently. Three groups independently demonstrated that basophils are the predominant APC in inducing Th2 response against helminth parasites and allergens. Basophils express MHC class II and CD80/86, have the potential to take-up and process protein Ag (particularly Ag–IgE complex) and to present peptide in the context of MHC class II, and to produce IL-4. These Ag-pulsed basophils induce the development of Th2 cells both *in vitro* and *in vivo*. Thus, basophils contribute to Th2/IgE response by the production of IL-4 and presentation of MHC class II/peptide complex to naïve CD4$^+$ T cells, in contrast to the Th1-inducing action of DC. In this review, we summarize what is known regarding basophil function in allergy and parasite infection, examine the novel Ag-presenting function of basophils and discuss potential clinical implications of this finding.

Key words: Ag–IgE complex · Basophils · Helminth infection · Th2 response

Introduction

Mast cells, basophils and eosinophils are key effector cells in response to parasite infection and allergic inflammation [1–5]. Basophils and eosinophils are granulocytes, which mature in the bone marrow, circulate in the blood and are recruited to allergic inflammatory sites [3–5]. In contrast, progenitors of mast cells migrate from the bone marrow to the peripheral tissues and undergo their terminal differentiation *in situ*; mast cells that complete their differentiation in the skin or intestine develop into connective tissue mast cells and mucosal mast cells, respectively [1, 2]. Mast cells and basophils express the high-affinity receptor for IgE and, upon crosslinking of FcεR1-bound IgE with multivalent Ag, rapidly produce diverse preformed mediators, cytokines (*e.g.* IL-4 and IL-13) and lipid mediators, leading to the induction of immediate-type hypersensitivity [1–5]. Here, the author reviews the major functions of basophils as effector cells in the development of allergic inflammation and their novel function as Th2-inducing APC in helminth infection and allergy.

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Basophil development and its role in allergy

As mentioned, mast cells, basophils and eosinophils are the key innate effector cells involved in parasite-induced immune responses and allergic inflammation. Basophils are short-lived cells that account for less than 1% of circulating granulocytes in the blood. In contrast, mast cells are located in the tissue and mast cell progenitors have the potential to proliferate locally in the tissue in response to IL-3, IL-4 and IL-9, resulting in local mastocytosis.

A study by Arinobu *et al.* [6] has identified a common progenitor of basophils and mast cell precursor (BMCP), which arise from the granulocyte/monocyte progenitor (GMP). Eosinophil precursor also arises from GMP. The development from GMP to eosinophil precursor and BMCP, and from BMCP to basophil precursor or MCP, are regulated by the level and order of expression of transcription factors, C/EBPα and GATA-2 [6]. Morphologically, basophils and eosinophils have lobulated nucleus and secretory granules in the cytoplasm. Mast cells are round cells with a non-segmented nucleus and intracellular granules. Although basophils and mast cells are heterogeneous in their development and morphology, they are regarded to share a pathological role in allergic responses, as demonstrated by their potential to produce cytokines, vasoactive histamine and lipid mediators after FcεR1 crosslinkage [1–5]. Thus, individuals with atopy, after repeated exposure to a particular Ag such as pollen, exhibit immediate-type hypersensitivity. Furthermore, there is tight correlation between FcεR1 expression on basophils and IgE level in human peripheral blood [7], suggesting a positive feedback mechanism for the IgE-mediated immediate-type hypersensitivity reaction [8, 9]. Thus, once individuals with atopy start to produce IgE, they develop progressive allergic inflammation by increasing production of IgE and expression of FcεR1 on effector cells.

Basophil activation in parasitic infection and allergic inflammation: Role of IL-3

There appears to be at least two major pathways of basophil activation during allergic inflammation, one involving Ag/IgE signaling and the other that is mediated by PAMP and soluble mediators such as IL-18 and IL-33.

An important cytokine involved in both pathways of basophil activation is IL-3. IL-3 is not only an important growth factor for mast cells and basophils, IL-3 stimulation also induces basophil production of IL-4. Furthermore, basophils, when stimulated with a combination of IL-3 and crosslinking of FcεR1 by Ag, strongly produce IL-4 and IL-13, suggesting the importance of crosstalk between IL-3-mediated signaling and FcεR1-mediated signaling for IL-4 and IL-13 production. Furthermore, FcR common γ-chain (FcγR) may also be important in basophil activation. Recently, Hida *et al.* [10] demonstrated that basophils lacking FcγR could proliferate normally but failed to produce IL-4 in response to IL-3, suggesting that FcγR-mediated IL-3 signal is crucial in IL-4 production by basophils.

The effect of IL-3 can also be observed in IgE-independent basophil IL-4 production. We previously demonstrated that basophils express IL-18R and IL-33R and produce IL-4, IL-6, IL-13 and chemical mediators when stimulated with IL-3 plus IL-18/IL-33 *in vitro* (Fig. 1, left panel) [11, 12]. These results suggest the potential of basophils to induce allergic inflammation in an IgE-independent manner (innate-type allergic inflammation). Mouse basophils also express TLR1, TLR2, TLR4 and TLR6 and produce Th2 cytokines including IL-4 and IL-13 in response to stimulation with TLR ligands plus IL-3 [13].

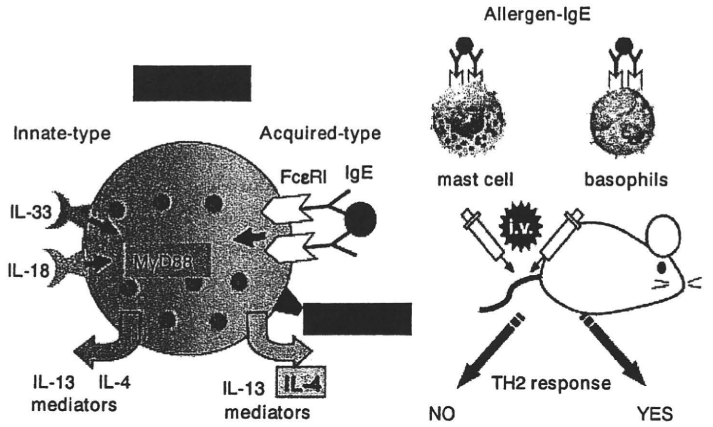
Thus, as stated, there are at least two major basophils activation pathways during allergic inflammation. One is an Ag/IgE-dependent pathway responsible for "acquired-type allergic inflammation" and the other is an IL-18, IL-33 or PAMP-dependent pathway responsible for "innate-type allergic inflammation."

IL-3 is also important for generation and peripheral accumulation of basophils during parasitic infections [14]. Infection of wild-type mice with *Strongyloides venezuelensis* or *Nippostrongylus brasiliensis* causes accumulation of basophils in the liver and spleen of the host [15]; however, this accumulation is not observed in IL-3-deficient mice [14]. Thus, IL-3 produced by Th2 cells is critically involved in generation, accumulation and activation of basophils.

In terms of the interactions between allergic inflammation and parasitic infection, we showed previously that nasal administration of IL-18 or IL-33 induces bronchial asthma entirely independently of allergen and IgE [12, 16]. As these cytokines are stored in the epithelial cells, infection with pathogens, including helminth parasites, bacteria, fungi and viruses or exposure to allergens, can induce the release of IL-18 and IL-33 from epithelial cells, causing IL-18 and/or IL-33-mediated allergic inflammation (Fig. 2), which is dependent on IL-18R and/or IL-33R and the adapter protein MyD88 pathway (Fig. 1). Basophils and mast cells also produce Th2 cytokines in response to parasite Ag (*e.g.* IPSE-α-1, a soluble glycoprotein Ag from eggs of *Schistosoma mansoni* and has been shown to stimulate basophils in an IgE-specific but Ag-nonspecific manner [17]). Basophils and mast cells may also respond to other parasite Ag, suggesting their role in defense against intestinal nematode such as *S. venezuelensis*, *N. brasiliensis* or *Trichuris muris*. Eosinophils are also effector cells of parasite infection – they defend against the tissue stage of helminth that is too large to be phagocytosed. IgE antibodies that bind to the surface of helminths activate eosinophils to produce granule content such as the major basic protein, which is highly toxic to helminths. Recruitment of eosinophils is also a well-known late hallmark of allergic inflammation and contributes to pathological processes in allergic diseases. Thus, basophils, mast cells and eosinophils are major effector granulocytes in parasitic infection and allergic inflammation.

Basophils in chronic allergic inflammation and systemic anaphylactic shock

Recent studies suggest that basophils also induce IgE-mediated chronic allergic inflammation and IgG1-mediated systemic anaphylactic shock [4, 18, 19]. Mukai *et al.* demonstrated that a single injection of multivalent Ag in the ear of mice passively sensitized with Ag-specific IgE induces immediate-phase, late-phase and delayed-onset of ear swelling characterized by infiltration with basophils and eosinophils [18]. Mast cell-deficient mice did not develop immediate- and late-phase ear swelling, suggesting mast cells are responsible for inducing these ear swellings. In contrast, depletion of basophils in wild-type mice diminished delayed-onset of ear swelling and eosinophilic infiltration. Moreover, transfer of basophils into FcεR1-deficient mouse showed that basophils are responsible for inducing delayed-onset ear swelling that is



Basophils are APC that can specifically induce Th2 cells.

Figure 1. Basophils are effectors cells and also inducers of Th2 response *in vivo*. Basophils, which produce IL-4, IL-13, and other mediators when stimulated with IL-3 plus Ag/IgE complex (acquired-type activation) also produce these cytokines and mediators, when stimulated with IL-3 plus IL-18 or IL-3 plus IL-33 (innate-type allergy). Furthermore, intravenous administration of basophils (but not mast cells) pulsed with allergen induce the development of Th2 cells in the peripheral lymphoid organs.

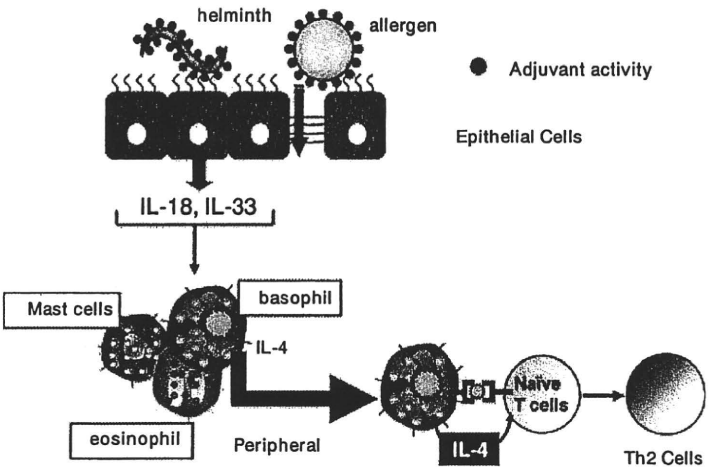


Figure 2. Interaction between epithelial cells and basophils. IL-18 or IL-33 derived from epithelial cells stimulated with helminth or allergen induces mast cells, basophils and eosinophils to produce Th2 cytokines, chemokines and chemical mediators. Among these granulocytes, basophils strongly produce IL-4 in response to IL-18 or IL-33. Basophils also uptake and process allergen and express allergen-derived peptide with MHC class II. These basophils prime Th2 responses in an MHC class II-dependent and IL-4-dependent manner.

associated with marked eosinophilic infiltration. Therefore, basophils seem to induce delayed-onset or chronic allergic inflammation by recruiting eosinophils [18].

It is well documented that mast cells and IgE are crucially involved in the development of systemic anaphylaxis. Interestingly, mice deficient for mast cells or IgE nevertheless develop systemic anaphylaxis, suggesting that an alternative pathway may be involved. Tsujimura *et al.* [19] clearly demonstrated that basophils and IgG1 induce mast cell-independent systemic anaphylaxis.

Role of Th cells in allergy and infection

Allergen-activated Th2 cells produce cytokines that induce allergen-specific IgE production by B cells and recruitment of mast cells, eosinophils and basophils to the site of allergic inflammation. Helminth infection also induces Th2 responses, resulting in high levels of IgE and recruitment of mast cells, eosinophils and basophils to the infected organ. Naïve $CD4^+$ T cells develop into Th1, Th2, Th17 cells and Treg upon activation by appropriate combination of antigenic signal, costimulation and cytokine signals by APC and accessory cells [20]. IFN-γ and IL-12 induce the development of Th1 cells, which are characterized by a high-level production of IFN-γ and are indispensable for eradication of intracellular pathogens [21]. IL-4 triggers the differentiation of Th2 cells [22]. Th2 cells are critically involved in clearing extracellular multi-cellular parasites such as helminths and in helping B cells to produce antibodies. Th2 cells are also involved in the pathogenesis of allergic inflammation. Th17 cells play an important role for the defense against extracellular pathogens and fungi [23]. Differentiation of Th17 cells is induced by TGF-β and IL-6 in the mouse and by TGF-β and IL-6 or IL-21 in the human [23] (see also review on IL-6 in this issue [24]). Treg can be induced by TGF-β and are involved in maintaining immune tolerance [25].

The initial source of the differentiation factors for both Th1 and Th2 cells are cells of the innate immune system responding to microbial Ag, parasitic Ag, or allergens. DC recognize bacteria through TLR and mature to express costimulatory molecules CD80/86 and to produce IL-12 and IL-18, favoring development of Th1 cells [26–28]. Thus, DC infected with intracellular bacteria induce Th1 cells.

Of particular importance to allergic inflammation and parasite infections is the nature of APC involved in polarizing Th2 responses. Ag-pulsed DC can also induce the development of Ag-specific naïve $CD4^+$ T cells into Th2 cells under the presence of IL-4 *in vitro* [20]; however, the APC involved in the development of Th2 response under physiological conditions remains uncertain. Several reports indicate that there are several pathways for the differentiation of naïve $CD4^+$ T cells into Th2 cells [29–33]. The Notch-ligand Jagged 1 and Jagged 2 on DC can trigger Th2 differentiation independently of IL-4 and STAT6 signaling [29]. Epithelial cells-derived cytokine, thymic stromal lymphopoetin (TSLP), activates DC to express OX40L, which induces the development of Th2 cells [30]. Aluminum adjuvant also induces Th2-cell differentiation, although exact mechanism remains uncertain [31]. In addition, M2 macrophages (also known as alternatively activated macrophages), eosinophils and mast cells are also important for the development of Th2 cells [32, 33].

Previous studies suggested that basophils may be critical in Th2 immunity. Min and colleagues [34] showed that naïve $CD4^+$ T cells stimulated with peptide-pulsed DC develop into Th2 cells when cultured with basophils from wild-type mice but not from IL-4-deficient mice. As both DC and basophils are added to the same culture, it was initially considered that DC deliver antigenic-specific signal and basophils promote the development of Th2 response by providing early IL-4 signal to Ag-activated $CD4^+$ T cells. It was also previously reported that helminth infection induces the development of Th2 cells and accumulation of basophils in the spleens and livers of host mice [15], suggesting that the relationship between Th2 cells and basophils may be more direct. *In vivo*, mice deficient in interferon-regulatory factor 2 show expansion of basophil and spontaneous Th2 differentiation [35], suggesting promotion of Th2 immune response by basophils. Furthermore, this Th2 differentiation is markedly reduced by the introduction of mutation in the gene-encoding c-Kit, because this mutation reduces the number of basophils [35]. Thus, although indirect evidence support the role of basophils in Th2 immunity, it is important to formally prove that basophils produce "early" IL-4, required for the development of naïve $CD4^+$ T cells into Th2 cells. Recently, three groups independently demonstrated that basophils and not DC, are the critical APC involved in Th2 differentiation *in vivo* [36–38].

Th2 development: Basophils as IL-4 provider

Medzhitov and colleagues [39] previously reported that basophils are important for the development of Th2 cells in response to papain. At day 3 after papain stimulation, basophils migrated into the T-cell zones of the draining lymph nodes, in which the basophils produce IL-4 and/or TSLP, which promote Th2 differentiation *in vivo*. Papain is a cysteine protease hydrolate enzyme from papaya that mimics the activity of proteases secreted by helminth parasites. Depletion of basophils with antibody against FcεR1 diminishes the development of Th2 cells, suggesting that basophils are involved in Th2 cell differentiation. This study [39] strongly indicates that basophils are critically involved in Th2 responses by their unique function to produce early IL-4 and TSLP in response to papain or bromelain. It remains uncertain, however, whether basophil-derived IL-4 is indeed involved in the development of Th2 cells in response to stimuli other than protease allergens.

Basophils as Th2-inducing APC

Data from our group also supported a role of basophils in Th2 responses – we reported that IL-18 and IL-33 synergize with IL-3 to strongly induce basophil, but not mast cell, production of IL-4 and IL-13 *in vitro*, respectively [11, 12] (Fig. 1, left panel), suggesting a role of basophils in promoting Th2 response by producing IL-4. Furthermore, basophils are shown to be an important regulator of Th2 responses *in vivo*, particularly in helminth-infected mice [3, 15]. As the size of helminths is too large to be phagocytosed directly by DC, it is more likely that DC take up Ag shed or secreted by parasites and present the Ag on MHC class II complex to naïves T cells in the context of IL-4 from parasite Ag-stimulated basophils. Although this is a persuasive hypothesis, the exact role of basophils in Th2 development remains to be formally demonstrated.

As noted, three groups independently demonstrated that contrary to our intuition, DC are not required for the development of Th2 responses to protease allergens, helminthic parasites or complexes of Ag and IgE [36–38]. All three groups demonstrated that basophils express MHC class II, CD80/86 and produce IL-4. Two groups showed that basophils induce Th2 cells in the absence of DC [36, 38]. Our group demonstrated that administration of Ag-pulsed basophils but not Ag-pulsed DC or mast cells selectively induces Th2 cells *in vivo* [37] (Fig. 1, right panel). Together, the three studies [36–38] suggest that basophils induce allergen or helminth-induced Th2 response by functioning as Th2-inducing APC. Artis and colleagues [36], using MHC II^{CD11c} transgenic mice, where MHC class II expression is restricted to $CD11c^+$ DC, demonstrated that these mice, when inoculated with *T. muris*, fail to develop Th2 response and to expel helminths. MHC II^{CD11c} transgenic mice do not secrete intestinal goblet-specific immune effector molecule resistin-like molecule β, which is induced by Th2 cells. Artis and colleagues [36] simultaneously demonstrated that this infection induced the development of Th1 cells, suggesting that $CD11c^+$ cells are required for the generation of Th1 cells; basophils, on the other hand, are dominant Th2-inducing APC that express IL-4 and MHC class II, as supported by depletion of basophils *in vivo*, which led to impaired protective Th2 immunity to *T. muris* in wild-type

mice. Contrary to these findings, however, Min and colleagues [14] demonstrated that basophil depletion in *N. brasiliensis*-infected mice did not affect the development of Th2 cells, suggesting that *N. brasiliensis* infection induces Th2 immunity even in the absence of basophils. We therefore need further studies to reconcile this apparent discrepancy.

Medzhitov and colleagues [38] demonstrated that skin DC are dispensable for mounting Th2 responses to papain. This group previously reported that, as with injection of the soluble Ag of *S. mannsoni* eggs, papain rapidly induces recruitment of basophils to the lymph node [39]. In the lymph nodes, basophils secrete IL-4 and TSLP, which are critically involved in the development of Ag-specific Th2 cells. Given that this treatment simultaneously induced recruitment of DC, Medzhitov and colleagues [39] initially considered that basophils function as accessory cells and DC present Ag in the presence of IL-4 from basophils. In the follow-up study, Medzhitov and colleagues [38] very clearly demonstrated that skin DC are not required for the development of Th2 cells in the draining lymph nodes. In this study [38], papain was injected into the ear, where skin DC capture Ag and present Ag-derived peptides to naïve T cells in the draining lymph nodes. If skin DC capture Ag and present it at the lymph node, rapid removal of this Ag-pulsed DC by prompt excision of the injection site should inhibit the Th2 response; however, this treatment failed to inhibit development of Th2 cells, suggesting that Ag capture by skin DC is not required for induction of papain-specific Th2 development. Instead, soluble papain can directly enter lymph nodes from injection site. Furthermore, selective depletion of $CD11c^+$ DC did not inhibit Th2 development to papain, although mice failed to develop Th1 responses. Artis's [36] and Medzhitov's [38] groups used the same strategy to deplete DC, using the CD11c-restricted diphteria toxin receptor mice, in which CD11c-expressing DC are efficiently depleted upon delivery of diphteria toxin, the two groups demonstrated that DC depletion only inhibited the development of Th1 cells without affecting the development of Th2 response. These results strongly indicated that other type/s of APC might be required for Th2 cytokine-dependent immune response. Medzhitov's group [38] demonstrated that OVA-pulsed basophils induce the development of OVA-specific naïve $CD4^+$ T cells into Th2 cells *in vitro*. They also show basophils can uptake, process and present soluble Ag. They further demonstrated that adoptive transfer of OVA-pulsed basophils induced Th2 response in MHC class II-deficient mice.

Basophils produce IL-4 and IL-13 upon stimulation with Ag/IgE complex. In addition, our *in vitro* studies demonstrated that, among mast cells and basophils, only basophils strongly produce IL-4 and IL-13 in response to IL-3 and IL-18 or IL-33 [11, 12]. These data suggest a role of basophils in the development of Th2 cells. These observations led us to examine the possibility whether basophils directly induce the development of Th2 cells, instead of functioning as accessory cells *in vitro* [37].

Splenic basophils from mice inoculated with *S. venezuelensis* produce large amounts of IL-4, IL-6 and IL-13 in the medium even in the absence of exogenous IL-3. In contrast, splenic basophils from naïve mice produce small amounts of IL-4, IL-6 and IL-13 only in IL-3-containing medium. Furthermore, basophils from infected mice express MHC class II and strongly induce the development of OVA-specific naïve $CD4^+$ T cells into Th2 cells *in vitro* in the presence of OVA peptide, IL-2 and IL-3 without IL-4 (neutral culture condition). Thus, we initially regarded only basophils from infected mice as potent APC; however, we soon found that splenic basophils from naïve mice also express comparable level of MHC class II and have the capacity to strongly induce the development of Th2 cells *in vitro* under neutral conditions [37]. We next examined bone marrow basophils and showed that these also have the potential to induce the development of Th2 cells. We purified basophils from bone marrow cells cultured with IL-3 for 10 days. Similar to splenic basophils, bone marrow basophils express MHC class II, CD80, CD86 and CD62L. Furthermore, bone marrow basophils can take-up and process protein Ag and express peptide in association with MHC class II. In particular, bone marrow basophils can efficiently uptake a low dose of Ag/IgE complex, and present Ag/MHC class II and produce IL-4, suggesting that they are potent Th2-inducing APC.

We also demonstrated that i.v. administration of OVA-pulsed basophils, which we prepared by culturing basophils with DNP–OVA and anti-DNP–IgE complexes, strongly induce OVA-specific Th2 cells in the spleen of naïve mouse (Fig. 1, right panel). We found that basophils' APC activity was enhanced when pulsed with DNP-OVA in the presence of anti-DNP IgE. In contrast, i.v. administration of OVA-pulsed DC failed to induce Th2 cells, although this treatment induced IFN-γ-producing Th1 cells. Thus, basophils are potent Th2-inducing APC *in vivo*. We transferred only $0.25–0.5 \times 10^6$ basophils and found dramatic induction of Th2 responses. We have also demonstrated that single i.v. administration of low-dose DNP–OVA/anti-DNP–IgE complex into naïve mice rapidly and preferentially induced OVA-specific Th2 cells in an endogenous basophil-dependent manner. Such sensitized mice promptly produced OVA-specific IgG1 antibody in response to i.v. administration of soluble OVA. Furthermore, IL-3 treatment prepares mice to be highly susceptible to Th2-inducing action of IgE complex by increasing the number of basophils.

Clinical implication: Basophils as a potential therapeutic target

Animals respond to allergen exposure by producing Ag-specific IgE. Such sensitized individuals, upon re-exposure to the same allergens, increase the production of IgE, which form allergen–IgE complexes by binding to allergens. These IgE complexes are captured by basophils that develop into Th2-inducing APC and present allergen-derived peptide with MHC class II and provide IL-4 to naïve $CD4^+$ T cells. Thus, basophils play a very important role in amplification of Th2-IgE responses, suggesting that they may be an important therapeutic target and depletion of basophils by antibody such as anti-FcεR1 might be an effective

therapeutic pathway. Published work has suggested that anti-IgE therapy is effective for Th2-IgE diseases [40]. The effect of anti-IgE therapy is believed to interfere with IgE-mediated activation of mast cells and basophils; however, on the basis of our research, another consequence of this antibody therapy might be the inhibition of basophil development into Th2-inducing APC, adding another rationale for anti-IgE therapy.

Concluding remarks

Basophils can uptake intact proteins and process them into peptides. Thus, basophils have the potential to induce primary Th2 response (Fig. 2). FcεR1 has a dominant effect during the memory phase of the Th2 response, because FcεR1 has the capacity to bind a very small amount of Ag–IgE complex and to present Ag-derived peptide with MHC class II. Denzel *et al.* [41, 42] reported that basophils bind large amounts of intact Ag *via* FcεR1-bound IgE. These basophils activate $CD4^+$ T cells to enhance Ag-specific B-cell memory responses (proliferation and Ig production) by presenting Ag and secretion of IL-4 and IL-6, suggesting that activated basophils induce and activate Th2-type cells, which help B cell proliferation and IgG1 production.

Atopic individuals are characterized by increased number of basophils at sites of allergic inflammation [43–45]. Although human mature basophils lack HLA-DR, we have demonstrated that some of them re-express HLA-DR when stimulated with IL-3 [37]. Given that IL-3 and other factors may be present at high concentrations at the site of allergic inflammation, accumulated basophils might re-express HLA-DR. Once the immune system of individuals with atopy start to produce Ag-specific IgE, they can steadily increase the amounts Ag and Ag-specific IgE complex. This allows basophils to take up Ag–IgE complex and become potent Th2 cell-inducing APC and induce progressive allergic inflammation in these individuals. Antibody therapy against IgE or basophils might be effective, because depletion of IgE or basophils could diminish basophil-dependent induction of Th2 cells.

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References

1 Galli, S. J., Grimbaldeston, M. and Tsai, M., Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. *Nat. Rev. Immunol.* 2008. 8: 478–486.

2 Galli, S. J., Kalesnikoff, J., Grimbaldeston, M. A., Piliponsky, A. M., Williams, C. M. and Tsai, M., Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol.* 2005. 23: 749–786.

3 Min, B., Basophils: what they 'can do' versus what they 'actually do'. *Nat. Immunol.* 2008. 9: 1333–1339.

4 Karasuyama, H., Mukai, K., Tsujimura, Y. and Obata, K., Newly discovered roles for basophils: a neglected minority gains new respect. *Nat. Rev. Immunol.* 2008. 9: 9–13.

5 Sullivan, B. M. and Locksley, R. M., Basophils: a nonredundant contributor to host immunity. *Immunity* 2009. 30: 12–20.

6 Arinobu, Y., Iwasaki, H., Gurish, M. F., Mizuno, S., Shigematsu, H., Ozawa, H., Tenen, D. G. *et al.*, Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl. Acad. Sci. USA* 2005. 102: 18105–18110.

7 Saini, S. S., Klion, A. D., Holland, S. M., Hamilton, R. G., Bochner, B. S. and Macglashan, D. W., Jr., The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils. *J. Allergy Clin. Immunol.* 2000. 106: 514–520.

8 Lantz, C. S., Yamaguchi, M., Oettgen, H. C., Katona, I. M., Miyajima, I., Kinet, J. P. and Galli, S. J., IgE regulates mouse basophil Fc epsilon RI expression *in vivo*. *J. Immunol.* 1997. 158: 2517–2521.

9 Yamaguchi, M., Lantz, C. S., Oettgen, H. C., Katona, I. M., Fleming, T., Miyajima, I., Kinet, J. P., Galli, S. J., IgE enhances mouse mast cell Fc(epsilon)RI expression *in vitro* and *in vivo*: evidence for a novel amplification mechanism in IgE-dependent reactions. *J. Exp. Med.* 1997. 185: 663–672.

10 Hida, S., Yamasaki, S., Sakamoto, Y., Takamoto, M., Obata, K., Takai, T., Karasuyama, H. *et al.*, Fc receptor gamma-chain, a constitutive component of the IL-3 receptor, is required for IL-3-induced IL-4 production in basophils. *Nat. Immunol.* 2009. 10: 214–222.

11 Yoshimoto, T., Tsutsui, H., Tominaga, K., Hoshino, K., Okamura, H., Akira, S., Paul, W. E. *et al.*, IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc. Natl. Acad. Sci. USA* 1999. 96: 13962–13966.

12 Kondo, Y., Kondo, Y., Yoshimoto, T., Yasuda, K., Futatsugi-Yumikura, S., Morimoto, M., Hayashi, N. *et al.*, Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system. *Int. Immunol.* 2008. 20: 791–800.

13 Yoshimoto, T. and Nakanishi, K., Roles of IL-18 in basophils and mast cells. *Allergol. Int.* 2006. 55: 105–113.

14 Kim, S., Prout, M., Ramshaw, H., Lopez, A. F., LeGros, G. and Min, B., Cutting edge: basophils are transiently recruited into the draining lymph nodes during helminth infection *via* IL-3, but infection-induced Th2 immunity can develop without basophil lymph node recruitment or IL-3. *J. Immunol.* 2010. 184: 1143–1147.

15 Min, B., Prout, M., Hu-Li, J., Zhu, J., Jankovic, D., Morgan, E. S., Urban, J. F., Jr. *et al.*, Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* 2004. 200: 507–517.

16 Ishikawa, Y., Yoshimoto, T. and Nakanishi, K., Contribution of IL-18-induced innate T cell activation to airway inflammation with mucus hypersecretion and airway hyperresponsiveness. *Int. Immunol.* 2006. 18: 847–855.

17 Schramm, G., Mohrs, K., Wodrich, M., Doenhoff, M. J., Pearce, E. J., Haas, H. and Mohrs, M., Cutting edge: IPSE/alpha-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent

IL-4 production by murine basophils *in vivo*. *J. Immunol.* 2007. 178: 6023–6027.

18 **Mukai, K., Matsuoka, K., Taya, C., Suzuki, H., Yokozeki, H., Nishioka, K., Hirokawa, K. *et al.***, Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells. *Immunity* 2005. 23: 191–202.

19 **Tsujimura, Y., Obata, K., Mukai, K., Shindou, H., Yoshida, M., Nishikado, H., Kawano, Y. *et al.***, Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* 2008. 28: 581–589.

20 **Zhu, J. and Paul, W. E.**, CD4 T cells: fates, functions, and faults. *Blood* 2008. 112: 1557–1569.

21 **Trinchieri, G.**, Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol.* 2003. 3: 133–146.

22 **Seder, R. A. and Paul, W. E.**, Acquisition of lymphokine-producing phenotype by CD4+T cells. *Annu. Rev. Immunol.* 1994. 12: 635–673.

23 **Korn, T., Bettelli, E., Oukka, M. and Kuchroo, K.**, IL-17 and Th17 cells. *Annu. Rev. Immunol.* 2009. 27: 485–518.

24 **Kimura, A. and Kishimoto, T.**, IL-6: Regulator of Treg/Th17 balance. *Eur. J. Immunol.* 2010. 40: 1830–1835.

25 **Sakaguchi, S., Yamaguchi, T., Nomura, T. and Ono, M.**, Regulatory T cells and immune tolerance. *Cell* 2008. 133: 775–787.

26 **Takeda, K., Kaisho, T. and Akira, S.**, Toll-like receptors. *Annu. Rev. Immunol.* 2003. 21: 335–376.

27 **Nakanishi, K., Yoshimoto, T., Tsutsui, H. and Okamura, H.**, Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. *Cytokine Growth Factor Rev.* 2001. 12: 53–72.

28 **Nakanishi, K., Yoshimoto, T., Tsutsui, H. and Okamura, H.**, Interleukin-18 regulates both Th1 and Th2 responses. *Annu. Rev. Immunol.* 2001. 19: 423–474.

29 **Amsen, D., Antov, A. and Flavell, R. A.**, The different faces of Notch in T-helper-cell differentiation. *Nat. Rev. Immunol.* 2009. 9: 116–124.

30 **Ito, T., Wang, Y. H., Duramad, O., Hori, T., Delespesse, G. J., Watanabe, N., Qin, F. X. *et al.***, TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J. Exp. Med.* 2005. 202: 1213–1223.

31 **Aimanianda, V., Haensler, J., Lacroix-Desmazes, S., Kaveri, S. V. and Bayry, J.**, Novel cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants. *Trends Pharmacol. Sci.* 2009. 30: 287–295.

32 **Anderson, C. F. and Mosser, D. M.**, A novel phenotype for an activated macrophage: the type 2 activated macrophage. *J. Leukoc. Biol.* 2002. 72: 101–106.

33 **Padigel, U. M., Hess, J. A., Lee, J. J., Lok, J. B., Nolan, T. J., Schad, G. A. and Abraham, D.**, Eosinophils act as antigen-presenting cells to induce immunity to *Strongyloides stercoralis* in mice. *J. Infect. Dis.* 2007. 196: 1844–1851.

34 **Oh, K., Shen, T., Le Gros, G. and Min, B.**, Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils. *Blood* 2007. 109: 2921–2927.

35 **Hida, S., Tadachi, M., Saito, T. and Taki, S.**, Negative control of basophil expansion by IRF-2 critical for the regulation of Th1/Th2 balance. *Blood* 2005. 106: 2011–2017.

36 **Perrigoue, J. G., Saenz, S. A., Siracusa, M. C., Allenspach, E. J., Taylor, B. C., Giacomin, P. R., Nair, M. G. *et al.***, MHC class II-dependent basophil-$CD4^+$ T cell interactions promote Th2 cytokine-dependent immunity. *Nat. Immunol.* 2009. 10: 697–705.

37 **Yoshimoto, T., Yasuda, K., Tanaka, H., Nakahira, M., Imai, Y., Fujimori, Y. and Nakanishi, K.**, Basophils contribute to Th2-IgE responses *in vivo* via IL-4 production and presentation of peptide-MHC class II complexes to $CD4^+$ T cells. *Nat. Immunol.* 2009. 10: 706–712.

38 **Sokol, C. L., Chu, N. Q., Yu, S., Nish, S. A., Laufer, T. M. and Medzhitov, R.**, Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat. Immunol.* 2009. 10: 713–720.

39 **Sokol, C. L., Barton, G. M., Farr, A. G. and Medzhitov, R.**, A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat. Immunol.* 2008. 9: 310–318.

40 **Adcock, I. M., Caramori, G. and Chung, K. F.**, New targets for drug development in asthma. *Lancet* 2008. 372: 1073–1087.

41 **Denzel, A. Maus, U. A., Rodriguez-Gomez, M., Moll, C., Nidermeier, M., Winter, C., Maus, R., *et al.***, Basophils enhance immunological memory responses. *Nat. Immunol.* 2008. 9: 733–742.

42 **Gauvreau, G. M., Lee, J. M., Watson, R. M., Irani, A. M., Schwartz, L. B., and O'Byrne, P. M.**, Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am. J. Respir. Crit. Care Med.* 2000. 161: 1473–1478.

43 **Irani, A. M., Huang, C., Xia, H. Z., Kepley, C., Nafie, A., Fouda, E. D., Craig, S., *et al.***, Immunohistochemical detection of human basophils in late-phase skin reactions. *J. Allergy Clin. Immunol.* 1998. 101: 354–362.

44 **Koshino, T., Arai, Y., Miyamoto, Y., Sano, Y., Itami, M., Teshima, S., Hirai, K., *et al.***, Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness. *J. Asthma* 1996. 33: 89–95.

45 **Macfarlane, A. J., Kon, O. M., Smith, S. J., Zeibecoglou, K., Khan, L. N., Barata, L. T., McEuen, A. R., *et al.***, Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J. Allergy Clin. Immunol.* 2000. 105: 99–107.

Abbreviations: **BMCP:** basophils and mast cell precursor · **FcγR:** FcR common γ-chain · **GMP:** granulocyte/monocyte progenitor · **TSLP:** thymic stromal lymphopoetin

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Basophils as APC in Th2 response in allergic inflammation and parasite infection

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Basophils are important effector cells, which contribute to protection against helminths and execute proinflammatory effector function during allergic inflammation. Basophils are also regulators of Th2 responses in helminth-infected hosts and in allergen-injected animals. Recently, three groups using different experimental systems have shown that basophils are antigen-presenting cells (APC), which induce Th2 cells both *in vitro* and *in vivo*. Basophils express MHC class II and CD80/86, have the potential to take-up and process protein antigen (Ag), particularly Ag-IgE complexes, and to present peptide with MHC class II and produce IL-4. However, relevance of basophils as Th2 cell-inducing APC *in vivo* has been challenged by several recent reports that favor the concept that basophils and DC cooperate or basophils merely amplify DC-driven Th2 cell differentiation. In this review, I summarize and discuss the data on the role of basophils as Th2 cell-inducing APC in allergy and parasite infection.

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Introduction

Mast cells, basophils, and eosinophils are important effector cells in helminth infection and allergic inflammation [1,2,3•,4•,5]. Basophils are rare circulating cells, accounting for less than 1% of total circulating granulocytes. Basophils, mast cells, and eosinophils arise from the same progenitor. Basophils and eosinophils complete their terminal differentiation in bone marrow. By contrast, mast cells migrate as immature cells from the bone marrow to the peripheral tissues, where they undergo their terminal differentiation [1,2]. Mast cells that complete their differentiation in the skin or in the intestine develop into connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs), respectively. Circulating basophils share several features with tissue-resident mast cells. Both cell types constitutively express FcεR1 and contain basophilic granules in the cytoplasm, and upon cross-linking of FcεR1-bound IgE with multivalent antigens, immediately release various kinds of effector molecules such as histamine and lipid mediators, and Th2-associated cytokines such as IL-4, IL-5, and IL-13, causing immediate type hypersensitivity [1,2,3•,4•,5]. Eosinophils also express FcεR1 and induce allergic inflammation by production of chemical mediators when stimulated with cross-linking of FcεR1-bound IgE with antigen. Owing to scarcity of cell number, paucity of specific basophil markers, and their functional similarity to mast cells, basophils have been simply regarded as effector cells of the Th2 immune response [3•,4•,5].

IL-3 is a growth factor for basophils. Furthermore, IL-3 is required for optimal basophil IL-4 and IL-13 production. FcR common γ-chain (FcRγ) is a widely expressed adapter, which bears an immunoreceptor tyrosine-based activation motif (ITAM) [6]. This adapter protein is known to associate with various FcR including FcεR1. Recent study by Hida and colleagues demonstrated that basophils lacking FcRγ could proliferate normally but failed to produce IL-4 in response to IL-3, suggesting that FcRγ-mediated IL-3 signal is crucially involved in induction of IL-4 production by basophils [7]. Now it is well recognized that basophils strongly produce IL-4 and IL-13 when stimulated with cross-linking of FcεR1 and IL-3.

The effect of IL-3 is also found in IgE-independent basophil IL-4 production [5,8,9•]. Basophils express IL-18R and IL-33R and very strongly produce IL-4 and IL-13 when stimulated with IL-18 or IL-33 in the presence of IL-3 *in vitro* [8,9•]. Basophils also express Toll-like receptor (TLR)1, TLR2, TLR4, and TLR6 and produce Th2 cytokines in response to IL-3 plus corresponding TLR ligands[10]. Thus, there are at least two major activation pathways for basophil IL-4 production. One is the Ag/IgE complex-induced activation pathway responsible for 'acquired-type allergic inflammation' and the other is the IL-18, IL-33, or pathogen associated molecular pattern (PAMP)-induced activation pathway responsible for 'innate type allergic inflammation'. Here, I review the major function of basophils as effectors cells in the development of allergic inflammation and the recently found novel function of basophils as Th2 cell-inducing APC in allergy and helminth infection.

Development of basophils, mast cells and eosinophils

Blood circulating basophils are mature cells with a life span of about 60 h. Injection of IL-3 increases basophil generation in bone marrow, resulting in an increase in the number of circulating basophils. By contrast, mast cells in the peripheral tissue have the potential to proliferate in response to IL-3 and IL-9 *in situ*. Thus, intestinal helminth infection induces intestinal mast cell hyperplasia in an IL-3-dependent manner. A study of the development of mouse eosinophils, basophils, and mast cells has identified that granulocyte/monocyte progenitor (GMP) is a common progenitor of basophils, mast cells, and eosinophils [11]. Both basophil/mast cell precursors (BMCP) and eosinophil precursor (EoP) arise from GMP. Development from GMP to EoP and BMCP, and from BMCP to basophil precursors (BaP) and mast cell precursors (MCP), are regulated by the level and order of expression of transcription factors, C/EBPα, and, GATA-2 [11]. However, in the case of development of human granulocytes, common myeloid progenitor (CMP) develops into GMP and EoP, which then develop into basophils/mast cells and eosinophils, respectively [11].

The major function of basophils as effector cells

Although basophils and mast cells are regarded as important effector cells in allergic response by their potential to promptly produce chemical mediators and cytokines after FcεR1 cross-linkage [1,2,3•,4•,5], recent studies suggest that basophils also induce IgE-mediated chronic allergic inflammation and IgG1-mediated systemic anaphylactic shock [4•,12,13•]. Karasuyama and colleagues demonstrated that a single injection of multivalent antigen in the ear of mice passively sensitized with antigen-specific IgE, elicits immediate-phase, late-phase, and delayed onset of ear swelling characterized by infiltration with basophils and eosinophils (chronic allergic inflammation) [12]. They showed that mast cell-deficient mice only developed chronic allergic inflammation, while basophil-depleted mice failed to develop it, suggesting that basophils are responsible for inducing chronic allergic inflammation. Very recently, a study by Voehringer and colleagues has confirmed this observation by using basophil-deficient mice (*Mcp8Cre*), which constitutively lack more than 90% of basophils [14••]. Furthermore, they demonstrated that reconstitution of *Mcpt8Cre* mice with bone marrow basophils restored this IgE-mediated chronic allergic inflammation response [14••]. These results strongly indicate that basophils are required and sufficient to induce IgE-mediated chronic allergic inflammation by recruiting eosinophils [12,14••].

It is well documented that mast cells and IgE are crucially involved in the development of systemic anaphylaxis. However, interestingly, mice deficient for mast cells, IgE, or FcεR1α chain still develop systemic anaphylaxis, indicating involvement of an alternative pathway. FcRγ-deficient mice that lack the expression of FcεR1 and stimulatory FcγR do not develop systemic anaphylaxis, suggesting that IgG also plays a crucial role in induction of systemic anaphylaxis. Karasuyama and colleagues demonstrated that basophils and IgG1 contribute to certain type of mast cell-independent systemic anaphylaxis [13•]. They demonstrated that basophils released a large amount of platelet-activating fator (PAF) when stimulated with allergen-IgG1 immune complexes. Based on this result, they speculated that basophils induce systemic anaphylaxis through the release of PAF that is 30,000 times more potent than histamine [13•]. However, basophil-ablated *Mcpt8Cre* mice are shown to normally develop IgE or IgG1-dependent systemic anaphylaxis, suggesting the possibility that basophils play a minor role in an induction of systemic anaphylaxis [14••]. In a previous study, Finkelman and colleagues reported that macrophages play a major role in IgG-mediated systemic anaphylaxis through the release of PAF [15]. These results suggest that both basophils and macrophage contribute to IgG1-mediated systemic anaphylaxis. However, further research is needed to determine which cell contributes most strongly to this IgG-mediated anaphylaxis.

Th subset

Naïve $CD4^+$ T cells develop into Th1, Th2, Th17, and Treg cells, when they are given antigenic signals, co-stimulatory signals, and appropriate cytokine signals by APC and accessory cells [16]. IFN-γ, IL-12, and T-bet control development of Th1 cells, which are highly effective in clearance of intracellular pathogens by the production of IFN-γ [16,17]. IL-4 and GATA-3 control development of Th2 cells [16,18], which produce IL-4, IL-5, IL-6, IL-9, and IL-13. These Th2 cytokines are important for the development of allergic inflammation and clearance of helminth infections via the induction of IgE production, activation of mast cells, basophils, and eosinophils. Th17 cell subset is important for the development of autoimmune diseases and for the clearance of extracellular pathogens and fungi by producing IL-17 [19]. Differentiation of Th17 cells is induced by TGFβ and IL-6 in the mouse and by TGFβ and IL-6/IL-21 in the human. Treg cells are induced by TGFβ and are essential for immune tolerance and regulation of allergy and autoimmunity [20].

It is well recognized that DCs play a central role in initiation of activation and differentiation of Th subsets. DCs sense microbes through TLRs and mature to express co-stimulatory molecules CD80/86 and to produce the cytokines that provide the appropiate instructive signal for the development of Th1, Th17, and Treg cells [19–21]. Antigen-pulsed DC also induce the development of Th2 cells under the influence of IL-4 *in vitro* [16]. Furthermore, several reports indicate the presence of

other pathways for the differentiation of naïve CD4+ T cells into Th2 cells [22–26]. DCs cannot produce IL-4, however DCs have the potential to induce Th2 cells via expression of the Notch ligand Jagged 1 and Jagged2 [22]. DCs also induce Th2 cells by expressing OX-40L after being stimulated with thymic stromal lymphopoetin (TSLP) [23]. Aluminum adjuvant induces Th2 cell differentiation, although the exact mechanism still remains uncertain [24]. In addition, M2 macrophages (also known as alternatively activated macrophages), as well as eosinophils and mast cells are also important for the development of Th2 cells [25,26]. Thus, it is important to determine which cell types help DCs to induce the development of Th2 cells.

Th2 development: basophils as accessory cells that produce early IL-4

Min *et al.* reported that naïve CD4+ T cells stimulated with peptide-pulsed DCs could develop into Th2 cells when co-cultured with basophils from wild type mice but not from IL-4-deficient mice [27••]. As DCs and basophils are added to the same culture, it was initially interpreted that DC deliver antigenic-specific signal, and basophils provide IL-4 for the development of Th2 cells. In *in vivo* studies, mice deficient in interferon-regulatory factor 2 (IRF2) or Lyn have increased numbers of basophils and exhibit spontaneous Th2 differentiation under steady state conditions [28,29]. However, introduction of mutation in the gene encoding c-Kit inhibits this spontaneous Th2 differentiation by reducing the number of basophils [28]. Thus, basophils might be required for the development of naïve CD4+ T cells into Th2 cells *in vivo*. Medzhitov and colleagues showed that basophils are important in initiation of the development of Th2 cells in response to the protease allergen, papain [30••,31]. At day 3 after subcutaneous papain injection, basophils enter and transiently reside in the T cell zones of the draining lymph nodes, where basophils are stimulated to produce IL-4 and/or TSLP, which promote Th2 differentiation *in vivo*. Basophils are also necessary for Th2 differentiation in the mice infected with *T. muris* [32]. Depletion of basophils with antibody against FcεR1 diminished the development of Th2 cells in both models of Th2 cell differentiation, suggesting that basophils are involved in Th2 cell differentiation by production of 'early IL-4' [31,32]. Furthermore, basophil production of IL-4 and IL-6 promotes the development of IL-10-producing CD8+ T cells *in vivo* [33], suggesting that basophils play important roles for the functional differentiation of CD4+ T cells and CD8+ T cells.

Induction of Th2 cells by basophils pulsed with Ag/IgE complex

Basophils promptly produce IL-4 and IL-13 when they are stimulated with Ag/IgE complex or with IL-18 and/or IL-33 in the presence of IL-3 [5,8,9•,10]. Thus, if basophils express MHC class II and CD80/86, we could speculate that basophils also have the potential to induce the development of Th2 cells. Three groups independently demonstrated that basophils constitutively express MHC class II, as well as co-stimulatory molecules such as CD40, CD80, and CD86 [31,32,34]. These groups further demonstrated that basophils are potent APCs [31,32,34]. We have reported that basophils have the capacity to induce Th2 differentiation both *in vitro* and *in vivo* [34].

We prepared splenic basophils from mice inoculated with *S. venezuelensis*, as helminth infection markedly induces an increase in the number of basophils in the spleen and liver [35]. Basophils from infected mice have the capacity to strongly produce IL-4, IL-6, and IL-13 in medium alone, even without IL-3 [34]. Furthermore, they express MHC class II and CD80/86 and induce the development of OVA-specific naïve CD4+ T cells into Th2 cells *in vitro* in the presence of OVA peptide, IL-2 and IL-3 without IL-4 (neutral culture condition) [34]. Thus, we initially regarded only those basophils derived from infected mice to be potent Th2 cell-inducing APC. However, we quickly learned that splenic basophils derived from naïve mice also produce IL-4, IL-6, and IL-13 in IL-3-containing medium [34]. Compared to the amount of cytokines produced by basophils from infected animals, the amount of cytokines produced by basophils from naïve mice is relatively low. Furthermore, they need the presence of IL-3 in the culture medium to produce these cytokine [7,34]. Nevertheless, splenic basophils from naïve mice express comparable levels of MHC class II and CD80/86 and have the capacity to induce the development of Th2 cells *in vitro* under neutral conditions. Thus, both types of basophils are potent APC that strongly induce Th2 cells *in vitro*.

We next tested whether bone marrow basophils also have the potential to induce the development of Th2 cells. We found that they express MHC class II, CD80, CD86, and CD62L, and take-up allergen such as OVA, and process them into small peptides [34]. Since they express FcεR1 abundantly, we speculated that they can take up a low dose of Ag/IgE complex, present Ag/MHC class II, and produce IL-4. Thus, we examined whether basophils become very potent Th2 cell-inducing APC, when Ag is provided as Ag/IgE complexes [34].

We prepared OVA-pulsed basophils by culturing basophils with DNP-OVA and anti-DNP IgE complexes. Then, we intravenously (iv) administered 0.25 or 0.5 million OVA-pulsed basophils to naïve mice. This treatment strongly induced OVA-specific Th2 cells in the spleen of naïve mice. By contrast, iv administration of OVA-pulsed DCs or mast cells failed to induce Th2 cells, although OVA-pulsed DCs induced IFN-γ producing Th1 cells. Thus, basophils are very potent Th2 cell-inducing APC even *in vivo*. We next tested whether

endogenous basophils are crucially involved in the development of Th2 cells *in vivo* by injecting DNP-OVA/anti-DNP IgE complexes into naïve mice. We found that a single iv administration of low dose DNP-OVA/anti-DNP IgE complexes into naïve mice rapidly and preferentially induced OVA-specific Th2 cells. This induction of OVA-specific Th2 by injection of Ag/IgE complex is strongly inhibited by depletion of basophils through injection of anti-FcεR1 antibody [34]. Furthermore, IL-3 treatment renders mice highly susceptible to Th2 cell-inducing action of IgE complex by increasing the number of basophils [34]. Taken together, these results strongly indicate that basophils are also potent Th2 cell-inducing APC *in vivo*.

Atopic individuals are characterized by increased numbers of basophils at sites of allergic inflammation [36–38]. Human mature basophils lack, or only weakly express, HLA-DR but have the potential to display increased HLA-DR expression when stimulated with IL-3 [34]. As levels of IL-3 and other factors may be increased at sites of allergic inflammation, we speculated that accumulated basophils might increase their HLA-DR expression and, thus, play a role as APC in peripheral tissues and augment Th2/IgE response.

To test our hypothesis, we injected Ag/IgE complexes into naïve mice, and, indeed, showed that this treatment efficiently induced Ag-specific Th2 cells *in vivo* [34]. Since naïve mice do not possess Ag-specific IgE, we speculate that this Ag/IgE complex-dependent induction of Ag-specific Th2 cell via basophils mainly account for the mechanism how repetitive exposure to allergen augment allergic inflammation. Once atopic individuals start to produce antigen-specific IgE, their concentration of Ag/Ag-specific IgE complexes may steadily increase. Subsequently, these complexes might be efficiently taken up by basophils via the FcεR1, resulting in these basophils becoming potent Th2 cell-inducing APC, which induce progressive allergic inflammation by enhancing Th2/IgE responses in these individuals. Furthermore, Ag/IgE stimulation upregulates the expression of FcεR1 on basophils [39,40]. Indeed, there is tight correlation between FcεR1 expression on basophils, and IgE levels in human peripheral blood [41], suggesting a positive feedback mechanism for IgE-mediated immediate type hypersensitivity. Furthermore, Denzel *et al.* reported that basophils could bind large amounts of antigens via FcεR1-bound IgE and produce IL-4 and IL-6, cytokines that are known to be required for antibody production in the spleen [42]. These basophils also activate Th2 type T cells, which help B cell proliferation and IgG1 production. Depletion of basophils decreased production of IgG1 [42]. Thus, Ag/IgE complexes and basophils play a crucial role in progression of allergic disease, and, therefore, antibody therapy against IgE or basophils might be an effective therapeutic means of treating persistent allergic disease.

Basophils as Th2 cell-inducing APC in allergy and helminth-infected hosts

Two groups revealed that basophils induce Th2 cells in the absence of DCs *in vivo* [31,32]. These studies suggest that basophils are involved in allergen-induced or helminth-induced Th2 responses by functioning as Th2 cell-inducing APC [31,32]. Artis and colleagues, by using MHC II^{CD11c} transgenic mice, where MHC class II expression is restricted to $CD11c^+$ DCs, demonstrated that these mice, when inoculated with *T. muris*, fail to develop Th2 responses or expel helminths [32]. They simultaneously demonstrated that this infection induced the development of Th1 cells, suggesting that $CD11c^+$ cells are required for the generation of Th1 cells. Then, they demonstrated that basophils are dominant Th2 cell-inducing APCs that express IL-4 and MHC class II. They proved this by the evidence that depletion of basophils *in vivo* reduced protective Th2 cell response to *T. muris* in wild type mice [32]. However, Min and colleagues have demonstrated that basophil depletion in *N. brasiliensis*-infected mice does not affect the development of Th2 cells, suggesting that basophils may not be essential for Th2 differentiation in mice infected with the murine hookworm, *N. brasiliensis* [43]. Nevertheless, this infection induces recruitment of basophils into the draining lymph nodes with similar kinetics as those in other models, suggesting that basophils are not required for protection against primary *N. brasiliensis* infection. However, interestingly, basophils are essential for protection against secondary *N. brasiliensis* infection, even in the absence of mast cells and $CD4^+$ T cells, which are both required for efficient worm expulsion during primary infection with *N. brasiliensis* [44]. A recent study by Voehringer and colleagues has demonstrated that *Mcpt8Cre* mice with constitutive and selective depletion of basophils, normally develop Th2 cells, a IgG1/IgE response, and eosinophilia upon infection with *N. brasiliensis* [14••]. However, they further demonstrated that basophils are essential for protection against secondary *N. brasiliensis* infection [14••,44]. These observations suggest a complex role for basophils in host defense against parasites, and also underline the complexity of regulation of acquired Th2 immunity by innate cells.

Medzhitov and colleagues also demonstrated that skin DCs are not required for mounting Th2 responses to papain. They previously reported that, like injection of the soluble antigens of *S. mansoni* eggs, papain injections rapidly induce recruitment of basophils to the lymph node [30••,31]. As this treatment simultaneously induces recruitment of DC, they initially considered that basophils function as accessory cells, and DC present Ag in the presence of basophil-derived IL-4. However, they

have demonstrated that skin DC are not required for the development of Th2 cells in draining lymph nodes [31]. They subcutaneously injected papain into the ear, where skin DCs capture antigens and present Ag-derived peptides to naïve T cells at draining lymph nodes. If skin DCs capture antigens and present this in the lymph node, rapid removal of these Ag-pulsed DCs by prompt excision of the injection site should inhibit Th2 response. However, this treatment failed to inhibit development of Th2 cells, suggesting that Ag captured by skin DC is not required for induction of papain-specific Th2 development. They demonstrated that soluble papain can directly enter lymph nodes from the injection site. Furthermore, selective depletion of $CD11c^+$ DCs did not inhibit Th2 development to papain, suggesting the presence of Th2-inducing APC other than DCs in the lymph nodes. Medzhitov and colleagues, as well as Artis and colleagues used CD11c-restricted diphteria toxin receptor mice, in which CD11c-expressing DC are efficiently depleted upon delivery of diphteria toxin [31,32]. They demonstrated that DC depletion only inhibited the development of Th1 cells without affecting the development of Th2 response. These results strongly indicate that other types of APC might be required for Th2 cytokine-dependent immune responses. Subsequently, they identified basophils as another type of Th2 cell-inducing APC, as this papain-induced Th2 cell differentiation could be blocked by the depletion of basophils by means of anti-FcεR1 antibody MAR-1 [31]. They have clearly shown that basophils can take up, process, and present soluble antigens. Furthermore, they demonstrated that basophils form immune synapses with T cells within 60 min of co-culture [31]. Finally, they demonstrated that adoptive transfer of OVA-pulsed basophils induced Th2 response in MHC class II-deficient mice.

The above-mentioned studies showed that basophils are potent APCs that preferentially induce Th2 cells both *in vitro* and *in vivo*. Mice deficient in the Src family tyrosine kinase, Lyn, have increased numbers of basophils, exhibit spontaneous Th2 differentiation, and produce IgE autoantibodies against various autoantigens, including dsDNA, that cause lupus-like nephritis in Lyn−/− mice [29]. Thus, basophils regulate not only allergic diseases and anti-helminth immunity but also autoimmune diseases.

Following studies

However, recent studies questioned the role of basophils as Th2 cell-inducing APCs. Tang *et al.* suggested that DCs and basophils cooperatively induce a Th2 cell response against papain [45]. Phythian-Adams *et al.* reported that depletion of $CD11c^+$ DCs during the priming stage of Th2 response against the helminth *Schistosoma mansoni* severely impaired Th2 induction, while basophil depletion using MAR-1 antibody had no measurable effect, underlining the important role of DCs in the development of Th2 response against helminth infection [46]. Furthermore, Hammad *et al.* demonstrated that Th2 responses against house dust mite are initiated by FcεR1-expressing inflammatory DCs, which is amplified by basophils [47]. Voehringer and colleagues examined basophil function using newly developed basophil-ablated mice. In this model they could not find the evidence that basophils are required for Th2 cell differentiation *in vivo* [14••]. They speculated that antibody-mediated depletion of basophils with MAR-1 (anti-FcεR1) may simultaneously deplete FcεR1 expressing inflammatory DCs [47]. They also suggested bystander effects of antibody-treatment, including activation of mast cells and macrophages, as well as the formation of immune complexes.

Conclusion

The data on the role of basophils as APCs are confusing but the issue is still interesting. As we reported previously [34], basophils pulsed with Ag/IgE complexes have potent Th2 cell-inducing APC activity both *in vitro* and *in vivo*. Basophils take-up intact OVA and present OVA-peptide in conjunction with MHC class II to OVA-peptide specific naïve $CD4^+$ T cells. Furthermore, when injected into naïve animal, these Ag-pulsed basophils also exhibited Th2 cell-inducing APC activity *in vivo*. However, their role as initiating cells of primary Th2 response might be minor. I suspect their important role for secondary Th2 response. Nevertheless, since basophils often show strong protective effect against helminth infection, they are still very interesting cells. To fully understand the role of basophils in Th2-mediated anti-helminth immunity, we definitively need further studies of these cells *in vivo*.

References and recommended reading

Papers of particular interest published within the period of review have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Galli SJ, Tsai M, Piliponsky AM: **The development of allergic inflammation.** *Naure* 2008, **454**:445-454.

2. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M: **Mast cells as "tunable" effector and immunoregulatory cells: recent advances.** *Annu Rev Immunol* 2005, **23**:749-786.

3. • Min B: **Basophils: what they 'can do' versus what they 'actually do'.** *Nat Immunol* 2008, **9**:1333-1339.
This is the comprehensive review of basophils and highlight the importance of basophils in immune response, parasite infection, and allergic inflammation. Potential involvement of basophils in clinical setting is also discussed.

4. • Karasuyama H, Mukai K, Tsujimura Y, Obata K: **Newly discovered roles for basophils: a neglected minority gains new respect.** *Nat Rev Immunol* 2008, **9**:9-13.
This review summarized newly discovered novel roles for basophils in allergic response and clearly showed that the basophils are functionally distinct from mast cells.

5. Sullivan BM, Locksley RM: **Basophils: a nonredundant contributor to host immunity.** *Immunity* 2009, **30**:12-20.

6. Malbec O, Daëron M: **The mast cell IgG receptors and their roles in tissue inflammation.** *Immunol Rev* 2007, **217**:206-221.

7. Hida S, Yamasaki S, Sakamoto Y, Takamoto M, Obata K, Takai T, Karasuyama H, Sugane K, Saito T, Taki S: **Fc receptor gamma-chain, a constitutive component of the IL-3 receptor, is required for IL-3-induced IL-4 production in basophils.** *Nat Immunol* 2009, **10**:214-222.
This paper showed the first time the requirement of cross-talk between FcRγ and IL-3R for basophil IL-4 production. FcRγ constititutively associates with the common β chain of IL-3R and transduce IL-3 activation signal by recruiting Syk.

8. Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, Paul WE, Nakanishi K: **IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils.** *Proc Natl Acad Sci USA* 1999, **96**:13962-13966.

9. • Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, Hoshino T, Fujimoto J, Nakanishi K: **Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system.** *Int Immunol* 2008, **20**:791-800.
This paper showed the first time that basophils simultaneously express IL-18R and IL-33R and strongly produce IL-4 and IL-13 when stimulated with IL-18 and/or IL-33 together with IL-3. IL-33 administration induces IL-13-dependent airway hyperresponsiveness and goblet cell hyperplasia in SCID mice, suggesting the presence and involvement of IL-33-responsive innate cells in allergic inflammation.

10. Yoshimoto T, Nakanishi K: **Roles of IL-18 in basophils and mast cells.** *Allergol Int* 2006, **55**:105-113.

11. Arinobu Y, Iwasaki H, Akashi K: **Origin of basophils and mast cells.** *Allergol Int* 2009, **58**:21-28.

12. Mukai K, Matsuoka K, Taya C, Suzuki H, Yokozeki H, Nishioka K, Hirokawa K, Etori M, Yamashita M, Kubota T *et al.*: **Basophils play a critical role in the development of IgE-mediated chronic allergic inflammation independently of T cells and mast cells.** *Immunity* 2005, **23**:191-202.

13. • Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, Kawano Y, Minegishi Y, Shimizu T, Karasuyama H: **Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis.** *Immunity* 2008, **28**:581-589.
Basophils are dispensable for IgE-mediated anaphylactic shock but are indispensable for IgG1-mediated systemic anaphylaxis. Thus, basophils play a crucial role in IgG1-mediated anaphylaxis.

14. •• Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D: **Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths.** *Immunity* 2010, **33**:364-374.
Newly discovered functions of basophils *in vivo* are mainly obtained from mice depleted of basophils by using monoclonal antibody specific for FcεR1 (MAR-1) or CD200R3(Ba103). The possibility that basophils and/or nonbasophils are activated by the Ab treatment has been concerned. Thus, establishement of basophil-deficient mice has been eagerly needed. These authors performed analysis of the functions of basophils *in vivo* and questioned the importance of basophils-induced systemic anaphylaxis and Th2 cell-inducing APC function of basophils *in vivo*.

15. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD: **Pathways of anaphylaxis in the mouse.** *J Allergy Clin Immunol* 2002, **109**:658-668.

16. Zhu J, Paul WE: **CD4 T cells: fates, functions, and faults.** *Blood* 2008, **112**:1557-1569.

17. Trinchieri G: **Interleukin-12 and the regulation of innate resistance and adaptive immunity.** *Nat Rev Immunol* 2003, **3**:133-146.

18. Seder RA, Paul WE: **Acquisition of lymphokine-producing phenotype by CD4+ T cells.** *Annu Rev Immunol* 1994, **12**:635-673.

19. Korn T, Bettelli E, Oukka M, Kuchroo K: **IL-17 and Th17 cells.** *Annu Rev Immunol* 2009, **27**:485-518.

20. Sakaguchi S, Yamaguchi T, Nomura T, Ono M: **Regulatory T cells and immune tolerance.** *Cell* 2008, **133**:775-787.

21. Takeda K, Kaisho T, Akira S: **Toll-like receptors.** *Annu Rev Immunol* 2003, **21**:335-376.

22. Amsen D, Antov A, Flavell RA: **The different faces of Notch in T-helper-cell differentiation.** *Nat Rev Immunol* 2009, **9**:116-124.

23. Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, Qin XF, Liu YJ, Gilliet M: **TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand.** *J Exp Med* 2005, **202**:1213-1223.

24. Aimanianda V, Haensler J, Lacroix-Desmazes S, Kaveri SV, Bayry J: **Novel cellular and molecular mechanisms of induction of immune responses by aluminum adjuvants.** *Trends Pharmacol Sci* 2009, **30**:287-295.

25. Anderson CF, Mosser DM: **A novel phenotype for an activated macrophage: the type 2 activated macrophage.** *J Leukoc Biol* 2002, **72**:101-106.

26. Padigel UM, Hess JA, Lee JJ, Lok JB, Nolan TJ, Schad GA, Abraham D: **Eosinophils act as antigen-presenting cells to induce immunity to Strongyloides stercoratis in mice.** *J Infect Dis* 2007, **196**:1844-1851.

27. •• Oh K, Shen T, Le Gros G, Min B: **Induction of Th2 type immunity in a mouse system reveals a novel immunoregulatory role of basophils.** *Blood* 2007, **109**:2921-2927.
In general, naïve CD4+ T cells stimulated with Ag-pulsed DCs develop into Th1 cells. However, in this report, authors showed that basophils have the capacity to induce the development of naïve CD4+ T cells stimulated with Ag-pulsed DCs into Th2 cells by production of IL-4, which simultaneously inhibits development of Th1 cells.

28. Hida S, Tadachi M, Saito T, Taki S: **Negative control of basophil expansion by IRF-2 critical for the regulation of Th1/Th2 balance.** *Blood* 2005, **106**:2011-2017.

29. Charles N, Hardwick D, Daugas E, Illei GG, Rivera J: **Basophils and the T helper 2 environment can promote the development of lupus nephritis.** *Nat Med* 2010, **16**:701-707.
This paper showed that activation of basophils by complexes of autoantigen/autoantigen-reactive IgE causes their homing to lymph nodes, where they promote Th2 cell differentiation and enhance the production of autoantibodies, causing lupus-like nephritis in Lyn−/− mice.

30. •• Sokol CL, Barton GM, Farr AG, Medzhitov R: **A mechanism for the initiation of allergen-induced T helper type 2 responses.** *Nat Immunol* 2008, **9**:310-318.
This paper showed the mechanisms how innate immune cells senses protein allergens and induce the development of Th2 cells *in vivo*. Basophils were recruited to lymph node in response to subcutaneous injection of protease allergens (e.g. papain) or schstosome soluble egg antigens. Then, basophils were directly activated to produce IL-4 and TSLP, which in combination induce Th2 cell differentiation *in vivo*.

31. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R: **Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response.** *Nat Immunol* 2009, **10**:713-720.

32. Perrigoue JG, Saenz SA, Siracusa MC, Allenspach EJ, Taylor BC, Giacomin PR, Nair MG, Du Y, Zaph C, van Rooijen N *et al.*: **MHC class II-dependent basophil-CD4$^+$ T cell interactions promote Th2 cytokine-dependent immunity.** *Nat Immunol* 2009, **10**:697-705.

33. Kim S, Shen T, Min B: **Basophils can directly present or cross-present antigen to CD8 lymphocytes and alter CD8 T cell differentiation into IL-10-producing phenotypes.** *J Immunol* 2009, **183**:3033-3039.

34. Yoshimoto T, Yasuda K, Tanaka H, Nakahira M, Imai Y, Fujimori Y, Nakanishi K: **Basophils contribute to Th2-IgE responses in vivo vai IL-4 production and presentation of peptide-MHC class II complexes to CD4$^+$ T cells.** *Nat Immunol* 2009, **10**:706-712.

35. Min B, Prout M, Hu-Li J, Zhu J, Jankovic D, Morgan ES, Urban JF Jr, Dvorak AM, Finkelman FD, LeGros G *et al.*: **Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite.** *J Exp Med* 2004, **200**:507-517.

36. Irani AM, Huang C, Xia HZ, Kepley C, Nafie A, Fouda ED, Craig S, Zweiman B, Schwartz LB: **Immunohistochemical detection of human basophils in late-phase skin reactions.** *J Allergy Clin Immunol* 1998, **101**:354-362.

37. Koshino T, Arai Y, Miyamoto Y, Sano Y, Itami M, Teshima S, Hirai K, Takaishi T, Ito K, Morita Y: **Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness.** *J Asthma* 1996, **33**:89-95.

38. Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, McEuen AR, Buckley MG, Walls AF, Meng Q *et al.*: **Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin.** *J Allergy Clin Immunol* 2000, **105**:99-107.

39. Lantz CS, Yamaguchi M, Oettgen HC, Katona IM, Miyajima I, Kinet JP, Galli SJ: **IgE regulates mouse basophil Fc epsilon RI expression in vivo.** *J Immunol* 1997, **158**:2517-2521.

40. Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, Kinet JP, Galli SJ: **IgE enhances mouse mast cell Fc(epsilon)RI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions.** *J Exp Med* 1997, **185**:663-672.

41. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, Macglashan DW Jr: **The relationship between serum IgE and surface levels of FcepsilonR on human leukocytes in various diseases: correlation of expression with FcepsilonRI on basophils but not on monocytes or eosinophils.** *J Allergy Clin Immunol* 2000, **106**:514-520.

42. Denzel A, Maus UA, Rodriguez Gomez M, Moll C, Niedermeier M, Winter C, Maus R, Hollingshead S, Briles DE, Kunz-Schughart LA *et al.*: **Basophils enhance immunological memory responses.** *Nat Immunol* 2008, **9**:733-742.

43. Kim S, Prout M, Ramshaw H, Lopez AF, LeGros G, Min B: **Cutting edge: basophils are transiently recruited into the draining lymph nodes during helminth infection via IL-3, but infection-induced Th2 immunity can develop without basophil lymph node recruitment or IL-3.** *J Immunol* 2010, **184**:1143-1147.

44. Ohnmacht C, Voehringer D: **Basophils protect against reinfection with hookworms independently of mast cells and memory Th2 cells.** *J Immunol* 2010, **184**:344-350.

45. Tang H, Cao W, Kasturi SP, Ravindran R, Nakaya HI, Kundu K, Murthy N, Kepler TB, Malissen B, Pulendran B: **The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling.** *Nat Immunol* 2010, **11**:608-617.

46. Phythian-Adams AT, Cook PC, Lundie RJ, Jones LH, Smith KA, Barr TA, Hochweller K, Anderton SM, Hämmerling GJ, Maizels RM *et al.*: **CD11c depletion severely disrupts Th2 induction and development in vivo.** *J Exp Med* 2010, **207**:2089-2096.

47. Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, Muskens F: **Lambrecht BN:Inflammatory dendritic cells – not basophils – are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen.** *J Exp Med* 2010, **207**:2097-2111.

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細菌性角膜炎からアカントアメーバ角膜炎に移行したと考えられる1例

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A Case of Acanthamoeba Keratitis following Bacterial Keratitis

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症例は35歳，男性で，2週間頻回交換ソフトコンタクトレンズを使用していた．左眼痛と視力低下に対し，近医眼科で抗菌薬，抗ウイルス薬を処方されたが軽快しないので鳥取大学眼科を紹介受診した．角膜中央に小円形の浸潤巣を認め，アカントアメーバ角膜炎と特定できる所見を認めず，まず細菌性角膜炎を疑い治療を開始したが，角膜擦過物のファンギフローラ$Y^{®}$染色でアカントアメーバcystを検出したため，アカントアメーバ角膜炎と診断し治療を変更した．角膜擦過物のreal-time PCR (polymerase chain reaction) でもアメーバDNAが検出され，後にアカントアメーバが分離培養された．抗真菌薬の点眼および内服，クロルヘキシジン点眼ならびに病巣掻爬にて病巣は軽快したが，治癒過程では病巣の中央が陥凹した．これはアカントアメーバ角膜炎の瘢痕期には通常認めず，細菌性角膜炎における瘢痕期の所見に一致すると考えられた．細菌感染がアカントアメーバ感染の温床となるといわれているが，本症例は角膜上でそれが生じていることを示唆する症例と考えられた．

The patient, a 35-year-old male who was a 2-week type frequent-replacement soft contact lens user, complained of pain and decreased visual acuity in his left eye. Since topical antibacterial and antiviral administration had resulted in no therapeutic response, he was referred to Tottori University Hospital. Initially, bacterial keratitis was suspected because of the presence of small, round infiltrates in the center of the cornea and no characteristic findings of acanthamoeba keratitis. The diagnosis, however, was subsequently changed to acanthamoeba keratitis, since acanthamoeba cysts were detected from the Fungiflora $Y^{®}$ staining of corneal scrapings. Later, acanthamoeba DNA was detected by real-time polymerase chain reaction of the corneal scrapings, and acanthamoeba was isolated by culturing. The lesion improved following the administration of topical and oral antifungals, topical chlorhexidine and epithelial debridement. The resultant scar formed a dent, which is characteristic of bacterial keratitis, but not of acanthamoeba keratitis. The findings in this case indicate that bacterial infection can be a base for acanthamoeba infection of the cornea.

〔Atarashii Ganka (Journal of the Eye) 27(6)：805～808，2010〕

Key words：アカントアメーバ角膜炎，細菌性角膜炎，ファンギフローラ$Y^{®}$染色．acanthamoeba keratitis, bacterial keratitis, Fungiflora $Y^{®}$ stainig.

はじめに

アカントアメーバは淡水や土壌に広く分布する原生動物であり，アカントアメーバがひき起こす角膜炎は1974年に英国[1]，1975年に米国[2]において相ついで報告され，わが国では1988年に石橋ら[3]によって初めて報告された．本来は外傷に伴い，非常にまれに認められる疾患であったが，近年コンタクトレンズ (CL) 装用者の重症角膜感染症として広く認められるようになり，特にここ数年わが国ではmultipur-

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pose solution（MPS）を使用した頻回交換CLの使用者での発症が急激に増加している[4]．石橋ら[3,5]は，その臨床経過を初期，移行期，完成期の3期に分類し，病期による臨床像の違いを明確にした．一方，塩田ら[6]もアカントアメーバ角膜炎の病期分類を行っており，臨床経過を1. 初期，2. 成長期，3. 完成期，4a. 消退期，4b. 穿孔期，5. 瘢痕期と5つに分類している．これは石橋ら[3,5]の分類に末期像を追加した分類となっている．

アカントアメーバ角膜炎の初期の臨床所見は非常に多彩で，特徴的な所見がみられないと的確な診断をするのは困難であると思われる．今回筆者らは細菌性角膜炎の所見を呈した病巣から早期にアカントアメーバを検出し，治療し得た症例を経験したので報告する．

I 症　　例

患者：35歳，男性．

主訴：左眼痛，視力低下

現病歴：2週間頻回交換ソフトコンタクトレンズを使用していた．2週間で交換するものを，期限を超えて3週間程度装用することが多かった．洗浄保存にはMPSを使用していたが，こすり洗いはほとんど行っていなかった．平成20年9月8日より左眼痛と視力低下を自覚し，9月11日に近医受診し，左眼角膜炎の診断でレボフロキサシン点眼，プラノプラフェン点眼，ヒアルロン酸点眼を処方された．9月22日には羞明と眼痛が悪化したため倉敷中央病院眼科へ紹介された．左眼に角膜混濁を認め，レボフロキサシン点眼継続にて経過をみられるも，軽快しなかった．9月24日よりヘルペス感染を疑い，アシクロビル眼軟膏を追加された．9月29日には混濁部に潰瘍を生じ，前房内に炎症細胞が出現した．アシクロビル眼軟膏は中止し，10月1日にアカントアメーバ感染疑いにて鳥取大学眼科（以下，当科）紹介受診となった．

初診時所見：視力は右眼0.1（1.2×sph−7.0D），左眼0.3（0.6×sph−6.5D）であった．左眼結膜にはほぼ全周に強い毛様充血を認めた．角膜は全体に軽度の浮腫があり，瞳孔領9時の位置に辺縁不明瞭な白色浸潤を認め，混濁の周辺から角膜中央にかけて淡いびまん性の表層混濁を呈していた（図1）．下方に強い輪部浮腫を伴っていたが，放射状角膜神経炎は認めなかった．角膜後面には多数の微細な角膜後面沈着物を認め，前房内には軽度の炎症細胞を認めた．

経過：白い円形の浸潤巣より，レボフロキサシン耐性菌による細菌感染を最も疑い，入院のうえ，モキシフロキサシン，ミクロノマイシンの頻回点眼，オフロキサシン眼軟膏，セファゾリン点滴を開始した．また，病巣の擦過を行い，細菌・真菌培養へ提出するとともにグラム染色，ファンギフローラ

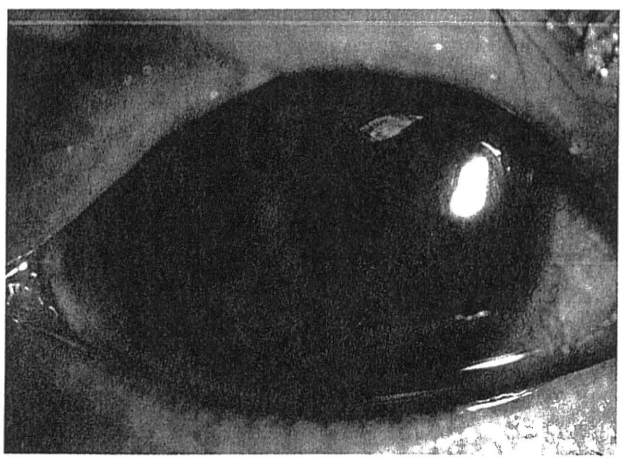

図1　初診時の前眼部写真
瞳孔領9時の位置に辺縁不明瞭な白色混濁を認め，混濁の周辺から角膜中央にかけてびまん性の表層混濁を呈していた．

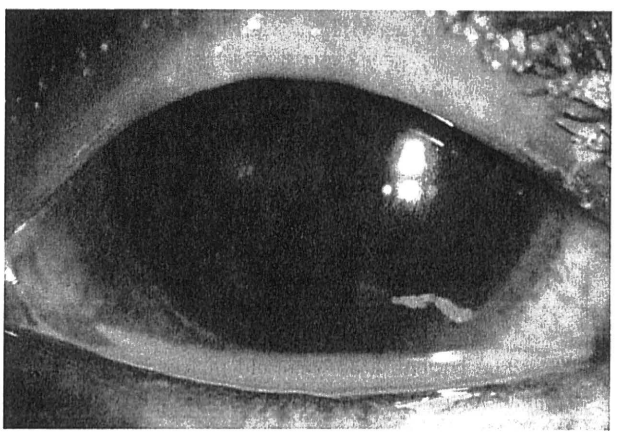

図2　入院翌日のフルオレセイン染色写真
9時の浸潤はやや拡大し，耳下側に向かって上皮の淡い混濁と不整が出現した．

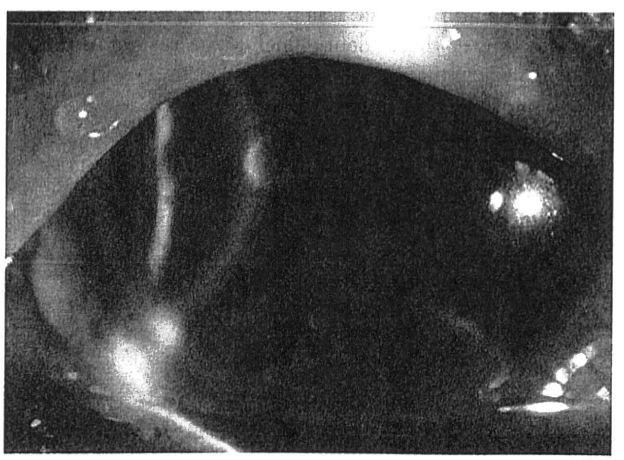

図3　治癒期の前眼部写真
病巣は全体に淡くなるとともに，中央が陥凹してきた．

Y® 染色を行い real-time PCR (polymerase chain reaction) で HSV (herpes simplex virus) と VZV (varicella-zoster virus) のスクリーニングを行った．入院翌日，初診時の角膜擦過物の検鏡を行ったところ，グラム染色ではグラム陽性球菌を検出した．ファンギフローラY® 染色ではアメーバ cyst と考えられる像が認められた．HSV，VZV の DNA は陰性であった．細菌に対する治療開始後，微細な角膜後面沈着物は著明に減少したが，毛様充血は依然強く，下方の輪部浮腫はむしろ増強していた．9時の浸潤はやや拡大し，病巣から耳下側へ向かって上皮の淡い混濁と不整が出現した（図2）．そこでアカントアメーバに対する治療に変更し，0.05% クロルヘキシジン液と 0.2% フルコナゾールの頻回点眼，イトラコナゾールの内服，週2回の病巣掻爬を開始した．抗菌薬の使用はモキシフロキサシン点眼とオフロキサシン眼軟膏のみとした．また，再度確認のため混濁部の擦過を行い，real-time PCR にてアカントアメーバ DNA の検索を行い，国立感染症研究所へアメーバの分離培養を依頼した．その結果，real-time PCR では 6.5×10^3 コピーのアカントアメーバ DNA が検出され，培養検査でも後にアカントアメーバが分離培養された．細菌，真菌培養は最終的に陰性であった．治療変更後，充血，輪部浮腫は徐々に軽快した．9時の病巣は全体に淡くなるとともに，中央が陥凹し，細菌性角膜炎における瘢痕期と矛盾しない所見を呈してきた（図3）．10月21日（治療変更後18日目）には毛様充血，輪部浮腫も大きく改善した．混濁はさらに淡くなり，この日の混濁部の角膜擦過物の PCR からはアメーバ DNA は検出されなかった．10月24日の擦過でもアメーバ DNA は検出されず，2回連続で陰性となったため，10月30日に当科退院となった．退院時視力は矯正 0.7 であった．退院後は紹介もとの倉敷中央病院にて通院加療中であり，発症約3カ月後の平成20年12月受診時の矯正視力は 1.2 と良好であった．

II 考　　按

アカントアメーバは広く土壌や淡水などに分布し，周囲の環境に応じて栄養型 (trophozoite) と嚢子型 (cyst) に変化するという特徴をもつ．栄養型は周囲の環境が好条件のときにみられ，細菌などの蛋白源を捕食し，増殖していく．嚢子型は周囲の環境が悪化したときにみられ，堅固なセルロース様構造をした二重壁に囲まれており，薬剤に抵抗性を示す[7]．アカントアメーバ角膜炎は外傷や CL 装用に伴う角膜障害からアカントアメーバが角膜内に侵入増殖して発症するといわれている．Jones ら[2]の予備実験では，動物モデルを使って傷害角膜にアカントアメーバを感染させても，単独ではなかなか感染が成立せず，アカントアメーバと細菌を同時に接種すると感染が成立するとしている．アカントアメーバ属の大半は他の細菌類を捕食して増殖することがよく知られているが，本症の患者のレンズケースからはアカントアメーバと同時に高頻度に細菌が分離培養されており[8]，レンズケース内でのアカントアメーバの増殖に細菌が関与し，さらには本症発症に関連していると推測される．

アカントアメーバ角膜炎の初期病変は非常に多彩で，上皮型角膜ヘルペスによく似た偽樹枝状病変，放射状角膜神経炎，点状・線状・斑状の角膜上皮下混濁，角膜輪部の充血および浮腫，強い結膜毛様充血，前房内の炎症細胞の出現などが特徴であるといわれている[5]．本症例においては，初診時から強い毛様充血と角膜輪部浮腫を認めていたが，アカントアメーバに特徴的とされる偽樹枝状病変，放射状角膜神経炎，斑状上皮下混濁は認めなかった．

一方，本症例では初診時より白い小円形の表層浸潤巣を呈しており，治癒過程においては浸潤巣の中央が陥凹してきた．これらの所見は細菌性角膜炎を示唆するものであり，特に瘢痕期に平坦化や陥凹を示すことはアカントアメーバではあまりなく，形状変化が少ないことがアカントアメーバ角膜炎の一つの特徴であるといわれている．

本症例では，誤った CL 使用法により角膜上皮が障害を受け，そこにケース内で増殖した細菌とアメーバが付着し，まず増殖しやすい細菌が増え，細菌性角膜炎を起こしたと推測された．この時点で抗菌薬が投与され細菌は死滅し，この死滅した細菌を捕食してアメーバが増殖して，アカントアメーバ角膜炎を続発してきたと思われた．細菌感染がアカントアメーバ感染の温床となるといわれているが，本症例は角膜上でそれが生じていることを示唆する症例であると考えられた．

アカントアメーバ角膜炎の確定診断には病変部にアカントアメーバの寄生を証明する必要があり，角膜の病巣部から得られた擦過標本もしくは生検材料を用いて直接検鏡，分離培養でアメーバの検出を行う必要がある．しかしながら，病巣擦過物の直接検鏡はサンプルの採取に技術を要し病初期には検出されにくく，分離培養においては検出までに時間を要し，量的に少ないとうまく検出できないという欠点がある．現在では confocal microscopy，HRA (Heidelberg Retina Angiograph) corneal module や PCR による補助診断の併用も早期診断に有用であると報告されている．PCR により培養検査でアカントアメーバが検出できなかった症例に対し，アカントアメーバ角膜炎の診断が可能であったとの報告[9,10]，培養検査より PCR のほうがアカントアメーバの検出感度が高いとの報告[11]がなされている．

本症例では病巣擦過物の real-time PCR を行い，初診時の診断の一助としただけでなく，入院中は治療効果判定の指標としても PCR を利用した．PCR は検体が微量でも検出可能であり，短時間で結果が得られることから，早期診断，早期治療が望まれるアカントアメーバ角膜炎において非常に有

用な検査であると考えられた.

文　献

1) Nagington J, Watson PG, Playfair TJ et al : Amoebic infection of the eye. *Lancet* **28** : 1537-1540, 1974
2) Jones DB, Visvesvara GS, Robinson NM et al : *Acanthamoeba polyphaga* keratitis and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. *Trans Ophthalmol Soc UK* **95** : 221-232, 1975
3) 石橋康久，松本雄二郎，渡辺亮子ほか：Acanthamoeba keratitis の一例—臨床像，病原体検査法および治療についての検討．日眼会誌 **92**：963-972, 1988
4) 福田昌彦：コンタクトレンズ関連角膜感染症の実体と疫学．日本の眼科 **80**：693-698, 2009
5) 石橋康久，本村幸子：アカントアメーバ角膜炎の診断と治療．眼科 **33**：1355-1361, 1991
6) 塩田　洋，矢野雅彦，鎌田泰夫ほか：アカントアメーバ角膜炎の臨床経過の病期分類．臨眼 **48**：1149-1154, 1994
7) 山浦　常，中川　尚，木全奈都子：アカントアメーバ．大橋裕一，望月　學編，眼微生物事典，p260-267，メジカルビュー社，1996
8) Jones DB：Acanthamoeba—The ultimate opportunist? *Am J Ophthalmol* **102** : 527-530, 1986
9) Zamfir O, Yera H, Bourcier T et al : Diagnosis of *Acanthamoeba* spp. keratitis with PCR. *J Fr Ophtalmol* **29** : 1034-1040, 2006
10) 並木美夏，増田洋一郎，浦島容子ほか：Polymerase chain reaction 法で診断されたアカントアメーバ角膜炎の1例．臨眼 **57**：777-780, 2003
11) Ordan J, Steve M, Nigel M et al : Polymerase chain reaction analysis of corneal epithelial and tear samples in the diagnosis of *Acanthamoeba* keratitis. *Invest Ophthalmol Vis Sci* **39** : 1261-1265, 1998

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重症コンタクトレンズ関連角膜感染症全国調査

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要 約

目 的：重症コンタクトレンズ(CL)関連角膜感染症の本邦における現状を把握する.

方 法：日本CL学会および日本眼感染症学会の主導による全国調査として，参加承諾を得た224施設に対し，2007年4月から2年間に入院加療を必要としたCL関連角膜感染症症例について臨床所見・細菌検査・CL装用管理の状況などを調査した.

結 果：350例が集積され，平均年齢28.0歳(9～90歳)であった．角膜擦過物からアカントアメーバが85例，緑膿菌が70例で検出されていた．2週間頻回交換ソフトCL装用者が196例(56.0%)を占めていた．終日装用CLを連続装用していたものが77例(22.0%)にのぼり，CLのこすり洗いを毎日実施していたものは67例にとどまるなど，CL装用およびそのケアについてずさんな実態が浮き彫りとなった.

結 論：重症のCL関連角膜感染症ではアカントアメーバや緑膿菌が起炎菌であった症例が多く含まれていた．CLに関する正しい使用法についての啓発と社会的管理体制の構築が望まれる.(日眼会誌 115：107—115, 2011)

キーワード：コンタクトレンズ，感染性角膜炎，アカントアメーバ，緑膿菌

Survey of Severe Contact Lens-associated Microbial Keratitis in Japan

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Abstract

Purpose：To understand the current state of severe contact lens(CL)-associated microbial keratitis in Japan.

Method：The survey was conducted by the Japan Contact Lens Society and the Japanese Association for Ocular Infection in 224 facilities from April 2007 to March 2009. Patients who were diagnosed with CL-associated microbial keratitis and hospitalized for treatment were enrolled. Clinical characteristics of the keratitis, microbiologic findings and the status of

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