

Basophils as T_H2-inducing antigen-presenting cells

Tomohiro Yoshimoto

Laboratory of Allergic Diseases, Institute for Advanced Medical Sciences, Hyogo College of Medicine, Nishinomiya, Hyogo 663-8501, Japan

Correspondence to: T. Yoshimoto; E-mail: tomo@hyo-med.ac.jp

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Abstract

Recent studies have revealed a role for basophils as antigen-presenting cells that preferentially induce T_H2 cells in response to complexes of antigen plus antigen-specific IgE, to protease allergens or to helminthic parasites *in vitro* and *in vivo* through the production of 'early IL-4' and the presentation to CD4⁺ T cells of complexes of peptide plus MHC class II. These findings have revealed previously unknown functions of basophils and will potentially aid the design of novel therapeutic strategies for T_H2–IgE-mediated diseases such as bronchial asthma.

Keywords: allergy, antigen presentation, dendritic cells, IgE, L-4

Introduction

In 1986, Mosmann *et al.* (1) proposed the T_H1/T_H2 dichotomy of CD4⁺ T-cell lineage commitment in which they described the presence of two distinct T_H cell subsets, each expressing a definite cytokine profile. T_H1 cells develop in the presence of IL-12, produce primarily IFN- γ and IL-2 and are involved in cell-mediated immunity. IFN- γ elicits delayed-type hypersensitivity responses, activates macrophages and is highly effective in clearance of intracellular pathogens. T_H2 cells differentiate in the presence of IL-4, produce IL-4, IL-5, IL-6, IL-9 and IL-13 and are critical for humoral immunity (2). These T_H2 cytokines are important for the development of allergic diseases and the clearance of helminth infections by induction of IgE production, activation of mast cells and basophils and eosinophilic inflammation. IFN- γ , IL-12 and the transcription factor T-bet control T_H1 development, whereas IL-4 and the transcription factor GATA-3 control T_H2 development (3) (Fig. 1A). The concept of a T_H1/T_H2 balance provided the basis for understanding the molecular mechanisms of immune responses and/or diseases and has been widely accepted as a paradigm of the immune system for more than two decades.

Differentiation of naive CD4⁺ T cells into T_H1 or T_H2 cells requires three signals: (i) TCR triggering through peptide-antigen recognition in the context of MHC class II molecules, (ii) amplification of TCR signalling via CD80 and/or CD86 and CD28 co-stimulatory molecules and (iii) the appropriate cytokine, i.e. IL-12 for T_H1-cell differentiation and IL-4 for T_H2-cell differentiation. In the case of T_H1 cells, dendritic cells (DCs) function as antigen-presenting cells (APCs) and provide all three signals. DCs use pattern-recognition receptors, such as Toll-like receptors (TLRs), to recognize pathogen-associated molecular patterns (PAMPs). This results in increasing levels of MHC class II and co-stimulatory molecules and production

of the appropriate cytokine, IL-12, for T_H1-cell differentiation (4–7).

For T_H2-cell differentiation, however, DCs cannot provide all three required signals because DCs cannot produce IL-4 (8), the cytokine essential for T_H2-cell differentiation (9, 10). Nevertheless, there are several reports indicating that DCs do play a functional role of T_H2-cell differentiation. These DC-mediated T_H2 responses may use several pathways, including: (i) the expression of notch ligands (jagged1 and jagged2) on DCs induces naive CD4⁺ T cells to differentiate into T_H2 cells (11); (ii) an epithelial cell-derived cytokine, thymic stromal lymphopoietin (TSLP), activates DCs to induce T_H2-cell differentiation (12, 13) and (iii) a tumour necrosis factor super-family member (OX40 ligand)-dependent differentiation of T_H2 cells is mediated by DCs (14).

DCs still cannot produce IL-4, the cytokine indispensable for T_H2-cell differentiation. We designate this type of IL-4 as 'early IL-4' to distinguish it from IL-4 produced by T_H2 cells. The nature of early IL-4-producing cells remains unclear (15). Other APCs, in addition to DCs, may therefore contribute to T_H2-cell differentiation (Fig. 1A).

Basophils are rare circulating granulocytes that originate from progenitor cells in the bone marrow and are important effector cells in IgE-mediated allergic inflammation (16–18). Basophils constitute <1% of blood leucocytes and are not usually present in tissues. They may, however, be recruited to inflammatory sites where antigen is present and contribute to immediate hypersensitivity reactions (19–24). Studies also suggest that basophils induce IgE-mediated chronic allergic inflammation and IgG1-mediated systemic anaphylactic shock (25–27). Basophils are thus primary effector cells in allergic disorders.

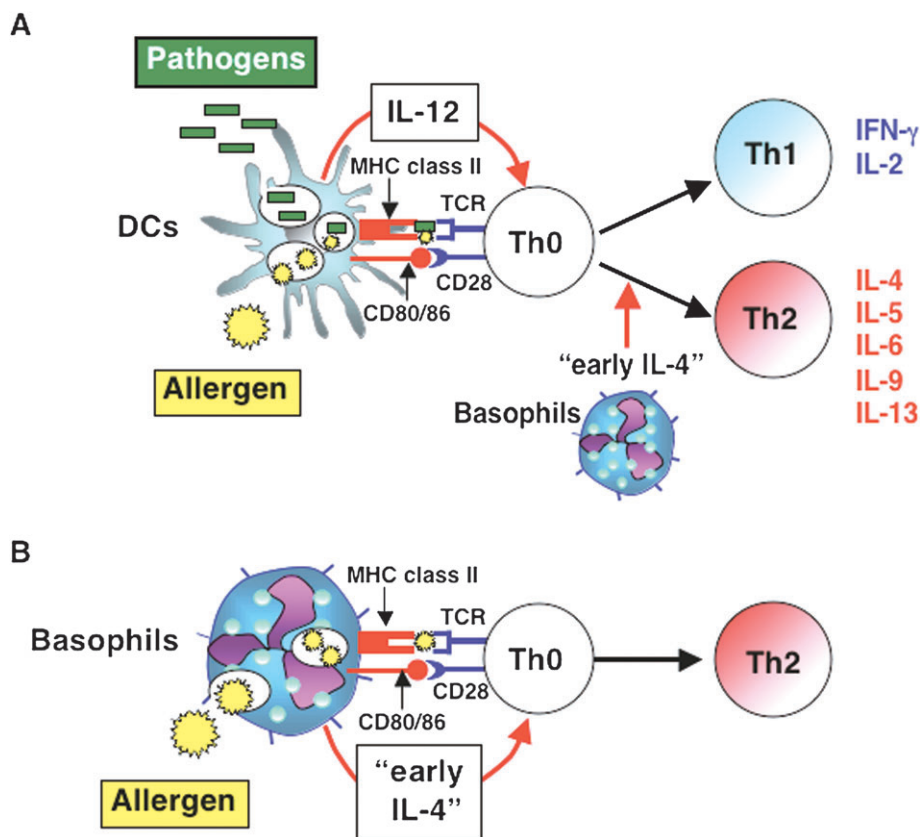


Fig. 1. T_H2 -cell differentiation by basophils. **(A)** Differentiation of naive helper T cells into T_H1 or T_H2 cells requires three signals: (i) TCR triggering through peptide-antigen recognition in the context of MHC class II molecules, (ii) amplification of TCR signalling via CD80 and/or CD86 and CD28 co-stimulatory molecules and (iii) an appropriate cytokine, IL-12 for T_H1 -cell differentiation and IL-4 for T_H2 -cell differentiation. For T_H1 -cell differentiation, which develops in response to viral and bacterial pathogens, DCs function as APCs and provide all three signals. For T_H2 -cell differentiation, which develops in response to an allergen, DCs cannot provide all three required signals because of the lack of ‘early IL-4’, the cytokine essential for T_H2 -cell differentiation. Cells, such as basophils, that produce early IL-4 are indispensable in this model. **(B)** Basophils meet all the criteria for APCs in T_H2 -cell differentiation. Upon exposure to allergens, basophils process the allergic protein into peptide fragments, express MHC class II and CD80/CD86 co-stimulatory molecules and preferentially induce naive helper T cells into T_H2 cells by basophil-derived early IL-4. DCs are not required for T_H2 -cell differentiation in this model.

Recent studies have revealed a role for basophils not only as effector cells but also as APCs that preferentially induce T_H2 cells in response to complexes of antigen plus antigen-specific IgE (28), in response to protease allergens (29) and in response to helminthic parasites (30). Basophils produce IL-4 and present complexes of peptide plus MHC class II to $CD4^+$ T cells both *in vitro* and *in vivo* (Fig. 1B). Here, we begin by describing the nature of basophils as early IL-4-producing cells and then detail the role of basophils as APCs in the initiation and augmentation of T_H2 -IgE responses.

‘Acquired-type allergy’ and ‘innate-type allergy’

IL-18- and IL-33-mediated basophil activation

It is well documented that basophils and mast cells release large amounts of IL-4 and IL-13 after cross-linkage of their IgE receptor, Fc ϵ receptor I (Fc ϵ RI), by complexes of antigen plus antigen-specific IgE (16–18) (Fig. 2A). IL-4 initiates and promotes T_H2 responses and additionally activates these effector cells (9, 10), while IL-13 contributes to the development of allergic responses by inducing mucus formation and eosinophilic infiltration via the production of eotaxin

(31). Upon linkage of their Fc ϵ RI, basophils and mast cells produce various other bioactive chemical mediators such as histamine and lipid metabolites (16). Thus, cross-linkage of Fc ϵ RI is associated with the development of acquired-type allergy, and allergen and IgE antibody are therefore essential molecules in this process.

In contrast, we have revealed a type of allergy that does not require allergen and IgE antibody. Cultured bone marrow-derived murine basophils (Fc ϵ RI $^+$ c-Kit $^-$) and mast cells (Fc ϵ RI $^+$ c-Kit $^+$) express IL-18R α chain and produce T_H2 cytokines (IL-4, IL-6, IL-9 and IL-13) and histamine in response to stimulation with IL-3 and IL-18, even without Fc ϵ RI cross-linkage (32) (Fig. 2B). In addition, bone marrow-derived basophils and mast cells express ST2 (IL-33R α chain), recently identified as the receptor for a member of the IL-1 family, IL-33 (33). Like IL-18, IL-33 can stimulate these cells to produce T_H2 cytokines without cross-linkage of Fc ϵ RI (33). Importantly, when comparing basophils and mast cells, only basophils can produce IL-4 and IL-9 in response to IL-18 or IL-33 in the presence of IL-3 (33). From these results, we concluded that there are two types of allergy, one of which requires allergen and IgE antibody and

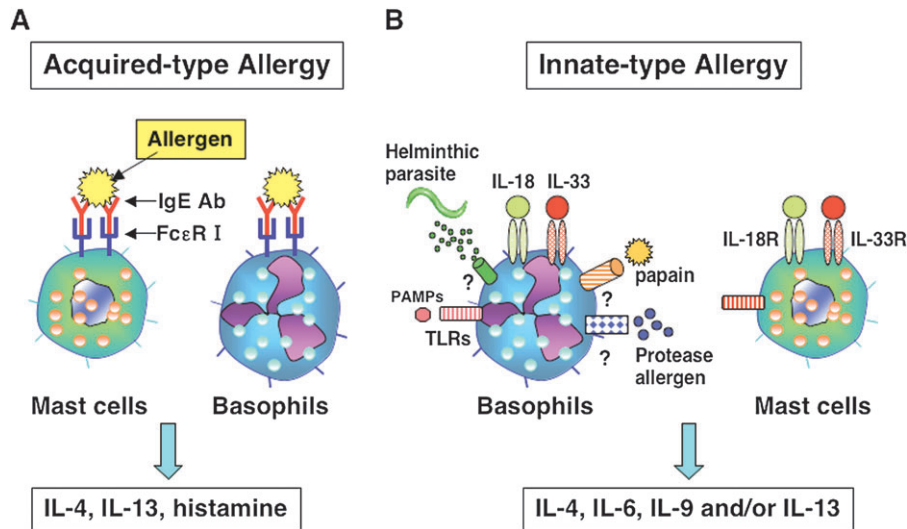


Fig. 2. IL-18/IL-33-, protease- and TLR-mediated basophil activation. **(A)** Allergy is believed to occur after mast cells or basophils are stimulated via FcεR1 by complexes of antigen plus antigen-specific IgE and produce T_H2 cytokines and histamine. In this case, the allergen and the IgE antibody are essential molecules. **(B)** In contrast, IL-18/IL-33-induced allergic disorders do not require T_H2 cells or IgE. IL-18/IL-33 stimulates basophils to produce IL-4, IL-6, IL-9 and IL-13, even without FcεR1 cross-linkage. In addition, basophils can produce T_H2 cytokines in an IgE-independent manner after exposure to components of allergens and helminths (e.g. proteases such as papain) via unknown receptors or after stimulation by PAMPs via TLRs. Because of the relative involvement of the acquired/innate immune systems, we propose that the former type of allergic response be termed 'acquired-type allergy' and the latter 'innate-type allergy'.

another that does not require these molecules. We have designated these two types of allergy: acquired-type allergy and innate-type allergy (34). IL-18 and IL-33 are essential regulators for innate-type allergy (Fig. 2B).

Protease-mediated basophil activation

Basophils can produce T_H2 cytokines on exposure to proteases from allergens and helminths in an IgE-independent manner. Protease allergens from dust mite (Der p 1) and protease secreted from the hookworm *Necator americanus* can induce IL-4, IL-5 and IL-13 production from the human basophil cell line KU812 and from human basophils purified from peripheral blood (35). Production of these T_H2 cytokines was blocked by protease inhibitors (35), indicating that proteolytic antigens can directly activate basophils. Furthermore, papain, the cysteine protease allergen, can strongly induce the expression of transcripts encoding IL-2, T_H2 cytokines (IL-4, IL-6 and IL-13), IL-31, TSLP and the CCL1 chemokine, which have all been associated with T_H2 -cell differentiation and recruitment (2, 12, 13, 36), in purified bone marrow-derived basophils without cross-linkage of FcεR1 (37).

In addition, it has been reported that basophils transiently enter draining lymph nodes ~3 days after *in vivo* injection with papain (37). Most strikingly, it was reported that basophils in the lymph nodes of mice immunized with papain produced IL-4 and TSLP (37). Protease-activated receptors are possible targets for proteases from allergens and helminths; however, human basophils lack expression of protease-activated receptors (38), although other myeloid cells including mast cells, eosinophils, neutrophils, macrophages and DCs have been reported to express protease-activated receptors. The receptor that recognizes proteases on basophils thus remains unknown. Proteases from allergens and

helminths could nevertheless induce innate-type allergy via activating basophils to produce T_H2 cytokines (Fig. 2B).

Toll-like-receptor-mediated basophil activation

The cytoplasmic portion of IL-18R and IL-33R is homologous to that of TLRs, which have been identified as signalling receptors of the innate immune system and recognize corresponding PAMPs (39, 40). Myeloid differentiation factor 88 (MyD88) is an adapter molecule essential for signalling through IL-18R, IL-33R or TLRs (6, 39, 40).

It has been reported that human basophils express high levels of TLR2 and TLR4 (41) and produce T_H2 cytokines when stimulated with ligands for TLR2 but not TLR4 (42). We have recently reported that bone marrow-derived murine basophils selectively express TLR1, 2, 4 and 6 and produce significant amounts of T_H2 cytokines (IL-4, IL-6 and IL-13) in response to IL-3 plus peptidoglycan (PGN) or IL-3 plus LPS via TLR2 or TLR4, respectively, even without FcεR1 cross-linkage (28) (Fig. 2B). It is well known that allergic inflammatory responses are also induced under some infectious conditions.

The findings of our study thus suggest that basophils stimulated by bacterial components may play a key role in induction of innate-type allergy, providing a clue to understanding the mechanisms of allergic diseases triggered by bacterial infection.

IL-3-mediated basophil activation

Stimulation of IL-18, IL-33, PGN or LPS alone can directly induce small amounts of IL-4 production in bone marrow-derived murine basophils; however, in the presence of IL-3, IL-4 production was significantly increased (28, 32, 33). IL-3 is thus a critical factor to induce/enhance IL-4 production from basophils stimulated with IL-18/IL-33 and ligands for TLRs.

IL-3 is a critical cytokine for the proliferation of basophils from bone marrow cells (16–18). IL-3 stimulation alone can also directly induce IL-4 production in basophils (28, 32). Hida *et al.* (43) reported that this IL-3-induced IL-4 production depended on the presence of the Fc receptor common γ -chain (FcR γ) because FcR γ -deficient mice could not produce IL-4 in response to IL-3 stimulation. They also revealed that FcR γ associated constitutively with the common β -chain of IL-3R, indicating that IL-3R signalling through FcR γ induced IL-4; however, basophils lacking FcR γ developed normally and proliferated efficiently in response to IL-3. There are thus different pathways for basophil proliferation and basophil-induced IL-4 production.

Basophils as a source of early IL-4 for T_H2 differentiation

Basophils can rapidly produce large amounts of IL-4 in response to several stimuli, including IL-18/IL-33, proteases and ligands for TLRs, making them a possible candidate for a source of early IL-4, which is critical for T_H2 -cell differentiation. It has been reported by several groups that basophils may initiate T_H2 -cell differentiation *in vitro* in the presence of DCs and antigen (44, 45). Min *et al.* reported that stimulation of naive CD4⁺ T cells with antigen in the presence of DCs and basophils is sufficient to promote the development of IL-4-producing T_H2 cells without exogenous IL-4 (45). Basophil-mediated T_H2 -cell differentiation was mainly mediated by the IL-4 produced by basophils because T_H2 -cell differentiation was not detected when IL-4-deficient basophils were used.

Notably, Min *et al.* also showed that, at least *in vitro*, the T_H2 -promoting capacity of basophils was in part due to direct cell–cell contact with CD4⁺ T cells (45). This led to the recent finding that basophils express MHC class II and function as APCs, as described below. These studies by Min *et al.* nevertheless clearly provided a proof of principle that basophils can promote T_H2 -cell differentiation in the presence of DCs and antigen via basophil-derived early IL-4.

Min *et al.* further demonstrated that, *in vivo*, CD4⁺ T cells stimulated in a basophil-enriched environment, induced by injection of IL-3 into mice, preferentially differentiated into IL-4-producing T_H2 cells (45) in response to hapten immunization. In line with this study, mice lacking the transcription factor, IFN regulatory factor 2 (IRF2), which increased the number of basophils (2- to 3-fold in the spleen), showed spontaneous T_H2 polarization of CD4⁺ T cells *in vivo* (44). The genetic reduction of basophil numbers by mutation of the *kit* gene in IRF2-deficient (IRF2^{−/−}) mice abolished such a T_H2 polarization (44). Whether basophils are involved in T_H2 differentiation *in vivo* via early IL-4 produced by basophils during primary exposure to an antigen remains unclear, however.

Sokol *et al.* (37) clearly demonstrated that basophils are critical in the development of T_H2 differentiation in response to the cysteine protease papain *in vivo*. Immunization of mice with papain subcutaneously without any additional adjuvants induced a strong T_H2 response characterized by T_H2 cytokine (IL-4, IL-5 and IL-13) production and the presence of papain-specific IgE in sera. Notably, basophils are transiently recruited into the T-cell zones of the draining

lymph nodes 3 days after immunization with papain, and these cells produce IL-4 (37). Basophils are normally found in the circulation and few, if any, are detected in secondary lymphoid tissues under normal conditions. At present, the mechanism by which basophils are recruited into draining lymph nodes remains unknown. Nevertheless, depletion of basophils by treatment with anti-Fc ϵ RI α antibody (MAR-1), blockage of the IL-4R α chain or neutralization of TSLP diminished T_H2 -cell responses induced by papain *in vivo* (37). Taken together, these results clearly indicate that basophil-derived early IL-4 and TSLP are critical factors in promoting T_H2 -cell differentiation (Fig. 1A).

Basophils as APCs *in vitro*

Very recently, three independent groups have published work that has led to a new paradigm for the induction of T_H2 cells (28–30). In addition to the capacity of basophils to produce early IL-4, basophils have antigen-presenting capabilities to preferentially promote T_H2 responses both *in vitro* and *in vivo*. All three groups showed that murine basophils express MHC class II and co-stimulatory molecules such as CD40, CD80 and CD86 on their surface (28–30). Basophils thus have all the characteristics required of T_H2 -inducing APCs, i.e. expression of MHC class II, co-stimulatory molecules and early IL-4. Sokol *et al.* (29) reported that after papain immunization, peripheral blood basophils and lymph node basophils expressed CD40 and CD86 but not CD80. While analysing expression of co-stimulatory molecules on bone marrow-derived basophils, we identified expression of CD80 and low levels of CD86 but not CD40 (28). Such differences are presumably due to differences in the maturation status of basophils. Basophils also expressed CD62L, which allows cells to home to lymph nodes, indicating their potential to enter lymph node tissues to initiate T-cell responses *in vivo* (28). Most importantly, a fraction of human cord blood-derived immature basophils (CD203c⁺c-Kit[−]) expressed HLA-DR. In addition, mature peripheral blood basophils expressed HLA-DR after being cultured for 24 h with IL-3 (28). MHC class II expression on basophils is thus not unique to mice.

Bone marrow-derived basophils, in the absence of any other APCs, showed the capacity to strongly induce naive ovalbumin (OVA)-specific CD4⁺ T cells to develop into T_H2 cells *in vitro* in the presence of OVA peptide (amino acids 323–339; OVA_{323–339}) without exogenous IL-4 (neutral conditions), whereas splenic DCs could induce T_H2 cells only under T_H2 conditions (28). Additional IL-4 stimulation (T_H2 conditions) only modestly enhanced the capacity of basophils to induce T_H2 -cell development, suggesting that bone marrow basophils produced sufficient IL-4 for maximal development of T_H2 cells. Indeed, basophils from IL-4-deficient mice could not induce the development of T_H2 cells under neutral conditions (28). But such IL-4-deficient basophils could strongly induce T_H2 cells under T_H2 conditions, allowing us to conclude that endogenous IL-4 from basophils is essential for the development of naive CD4⁺ T cells into T_H2 cells.

Similar to our results, Sokol *et al.* (29) and Perrigoue *et al.* (30) demonstrated that IL-4-producing basophils were MHC

class II positive and could promote MHC class II-dependent differentiation of antigen-specific T_H2 cells *in vitro* under neutral conditions. Sokol *et al.* (29) showed that this T_H2 differentiation was augmented in the presence of papain, which increased basophil expression of MHC class II, IL-4 and TSLP. These results indicate that basophils activated by a protease allergen can augment direct presentation of allergen to $CD4^+$ T cells.

To provide definitive proof that basophils can function as APCs, it was necessary to demonstrate the ability of basophils to take up and process whole-OVA protein into OVA peptide fragments. Our data demonstrated that naive $CD4^+$ T cells cultured with whole-OVA protein in the presence of basophils differentiated into T_H2 cells without IL-4, under neutral condition (28). Basophils are thus able to process whole-OVA protein, in fact 2,4 dinitrophenyl (DNP)-conjugated OVA, into OVA peptides and display these peptide fragments in association with MHC class II and produce IL-4.

Basophils have a unique potential to increase their capacity to induce IL-4 when they are cross-linked with antigen plus antigen-specific IgE (16–18). It is thus crucial to demonstrate the potential of basophils to increase APC activity when pulsed with antigen–IgE complexes. Basophils pulsed with a low dose of DNP–OVA protein ($6.2 \mu\text{g ml}^{-1}$) modestly induced T_H2 cells, whereas treatment with a high dose of DNP–OVA protein ($100 \mu\text{g ml}^{-1}$) strongly induced T_H2 cells. The addition of anti-DNP IgE to the culture, representing cross-linkage of Fc ϵ RI, induced higher numbers of T_H2 cells even with a low dose of OVA protein ($6.2 \mu\text{g ml}^{-1}$) (28). These results suggested that basophils, taking advantage of their expression of Fc ϵ RI, might efficiently take up a low dose of antigen and induce antigen-specific T_H2 responses in an IgE-dependent manner.

Basophils as APCs *in vivo*

In the absence of DCs, basophils are able to generate T_H2 -cell responses *in vivo* in response to complexes of antigen plus antigen-specific IgE, in response to the protease allergen papain or in response to helminthic parasites (28–30). Using mice in which MHC class II expression is restricted to $CD11c^+$ DCs (MHC class II ^{CD11c}) as well as mice in which $CD11c^+$ DCs can be selectively depleted by injection of diphtheria toxin (CD11c-DTR), Perrigoue *et al.* (30) demonstrated that DCs are not required for the generation of protective T_H2 responses to the gastrointestinal nematode, *Trichuris muris*. Depletion of basophils by treatment with anti-Fc ϵ R1 α antibody (MAR-1) resulted in lower expression of *il4* messenger RNA and impaired protective immunity to *T. muris* (increased parasite burden) (30). Using CD11c-DTR mice, Sokol *et al.* (29) also demonstrated that $CD11c^+$ DCs were dispensable for the development of T_H2 responses after papain immunization.

To confirm directly that basophils can act as T_H2 -promoting functional APCs *in vivo*, we cultured bone marrow-derived basophils, mast cells or splenic DCs with DNP–OVA and anti-DNP IgE for 16 h *in vitro* and then transferred them into normal mice through the tail vein (28). Four days after transfer, we challenged the mice intravenously with OVA protein,

and 2 days after challenge, we prepared splenic $CD4^+$ T cells, stimulated them with OVA *in vitro* and measured T_H2 cytokines in the culture supernatants. Only OVA-pulsed basophils promptly and strongly induced T_H2 cells in the spleen. Furthermore, mice immunized with OVA-pulsed basophils promptly produced OVA-specific IgG1 and IgE in sera, when intravenously challenged with OVA protein (28), whereas mice immunized with OVA-pulsed basophils purified from MHC class II-deficient mice failed to induce such T_H2 responses *in vivo* (T. Yoshimoto, unpublished data).

In a complementary study, Sokol *et al.* carried out another basophil-adoptive transfer experiment (29). Because basophils have short life spans (~ 60 h) (46) and show poor survival after transfer, they used bone marrow basophils derived from mice transgenic for the anti-apoptotic protein Bcl-2 (47) to improve survival after transfer. The transfer of antigen-loaded basophils into wild-type or MHC class II-deficient mice induced T_H2 -cell differentiation *in vivo*. These two independent basophil-adoptive transfer studies (28, 29) confirmed that MHC class II-positive basophils are sufficient for the initiation of T_H2 responses *in vivo*.

Furthermore, to demonstrate the contribution of basophils in the induction and up-regulation of T_H2 –IgE responses *in vivo*, we intravenously injected IgE complexes composed of DNP–OVA plus anti-DNP IgE into naive mice or mice depleted of basophils by treatment with anti-Fc ϵ RI antibody (28). The OVA–IgE complex ‘preferentially’ induced OVA-specific T_H2 cells in the spleen of naive mice and OVA-specific IgG1 in sera. Depletion of basophils significantly diminished these T_H2 responses. These results indicated that basophils are responsible for inducing OVA-specific T_H2 cells by taking up complexes of DNP–OVA plus anti-DNP IgE, presenting OVA peptide along with MHC class II and producing sufficient amounts of IL-4 to initiate and promote T_H2 responses *in vivo* (Fig. 3A).

Controversies in this field

Three independent groups have clearly demonstrated that basophils have the antigen-presenting capacity to promote T_H2 immunity both *in vitro* and *in vivo* (28–30). As mentioned above, however, DCs have been clearly shown to induce T_H2 -cell differentiation *in vitro* and *in vivo* via notch ligands and OX40L (11, 14). In addition, a potential role for alternative activated macrophages (M2a) in T_H2 induction has been noted (48).

Recently, Kim *et al.* reported that basophils are dispensable for the development of T_H2 differentiation following infection with the gastrointestinal nematode *Nippostrongylus brasiliensis* (49). Similar to papain immunization (29), circulating basophils were transiently recruited into draining lymph nodes following *N. brasiliensis* infection; however, depletion of basophils by treatment with MAR-1 did not abolish infection-induced development of T_H2 cells (49). Similarly, we demonstrated that *N. brasiliensis*-infected mice showed increased T_H2 cytokine production in mesenteric lymph nodes cells, increased IgE production in sera and complete parasite expulsion, even when basophils were depleted by treatment with MAR-1 (T. Yoshimoto, unpublished data). Taken together, these data suggest that

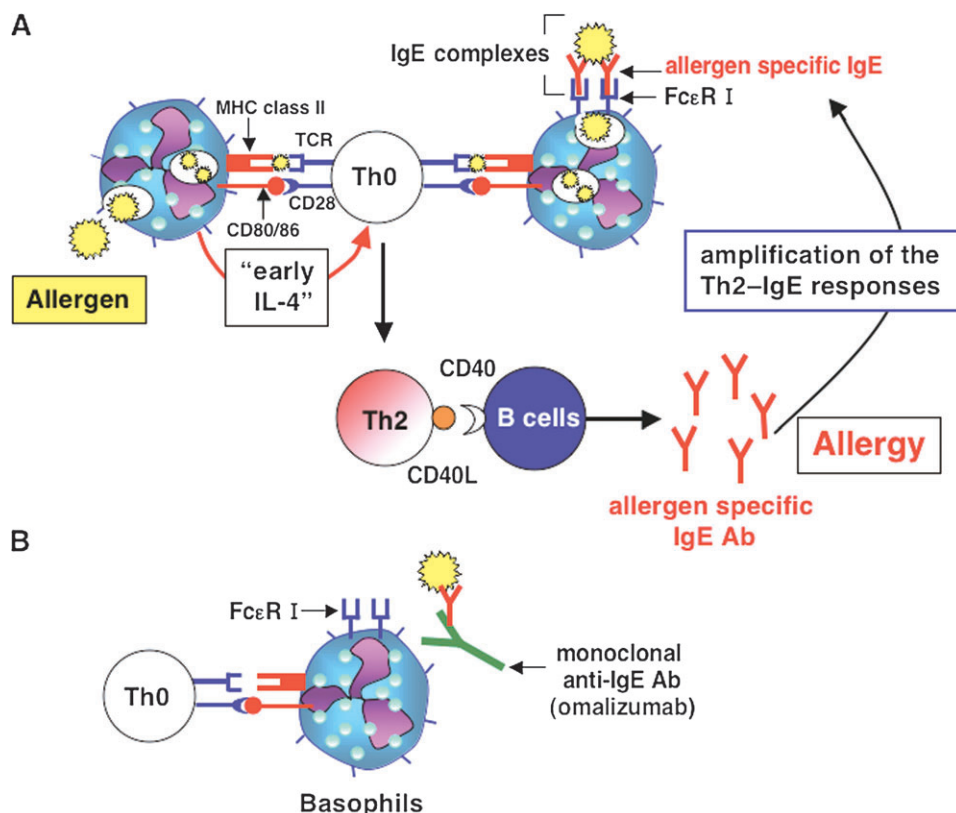


Fig. 3. Role of basophils in amplification of the T_H2 -IgE response. **(A)** Basophils initially induce T_H2 cells *in vivo* via IL-4 production and via the presentation of complexes of allergen peptide plus MHC class II to $CD4^+$ T cells. These activated T_H2 cells produce IL-4 and express CD40L that, in combination, induce B cells to proliferate and produce allergen-specific IgE *in vivo*. Immune complexes of allergen and allergen-specific IgE 'preferentially' augment the development of allergen-specific T_H2 responses in an endogenous basophil-dependent manner. **(B)** Anti-IgE therapy (omalizumab) may interfere with the generation of allergen-pulsed basophils and therefore inhibit endogenous basophil-dependent amplification of T_H2 responses *in vivo*.

there are multiple pathways leading to T_H2 -cell differentiation *in vivo*.

Concluding remarks

Atopic individuals are characterized by having more basophils at the sites of allergic inflammation (19–24). Once such individuals begin to produce antigen-specific IgE, the amount of complexes of antigen plus antigen-specific IgE can steadily increase, allowing basophils to augment their uptake of IgE complexes. Although mature human basophils lack HLA-DR, we have shown that some can re-express HLA-DR when stimulated with IL-3 (28). It is thus possible that mature basophils at allergic inflammation sites, characterized by abundant production of IL-3 and other factors, do express HLA-DR. This would mean that basophils could become potent APCs and induce progressive allergic inflammation in these individuals.

Our results indicate that basophils initially induce T_H2 cells *in vivo* via IL-4 production and presentation complexes of allergen peptide plus MHC class II to $CD4^+$ T cells. These activated T_H2 cells produce IL-4 and express CD40L, which, in combination, induce B cells to proliferate and produce allergen-specific IgE *in vivo*. Furthermore, immune complexes of allergen and the resultant allergen-specific IgE preferentially

augment the development of allergen-specific T_H2 responses in an endogenous basophil-dependent manner. Studies have shown that basophils can also capture antigen by binding to surface antigen-specific IgE-FcεRI (50). It is also reported that activated human basophils produce IL-4 and IL-13 and express functional CD40L, which in combination induce B cells to produce IgE (51). Thus, Basophils are important in the amplification of T_H2 -IgE responses (Fig. 3A). It has been reported that anti-IgE therapy (omalizumab) is effective for T_H2 -IgE-mediated diseases such as bronchial asthma (52). The rationale for such therapy is that anti-IgE antibodies are believed to interfere with IgE-mediated activation of mast cells and basophils. On the basis of our results, there is another rationale for such therapy; namely, inhibition of the generation of allergen-pulsed basophils (Fig. 3B). Basophils might thus represent an important therapeutic target cell.

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