

Basophils: what they ‘can do’ versus what they ‘actually do’

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Basophils, the least abundant granulocytes, have poorly understood functions. They have been linked to the development of T helper type 2 immunity during parasite infection and allergic inflammation. Emerging evidence has not only shown the critical involvement of basophils in the development of T helper type 2 immunity but also provided useful animal models with which basophil functions can be further examined. However, distinctions must be made between what basophils ‘can do’ after *in vitro* manipulation and what they ‘actually do’ during *in vivo* immune responses; these may be very different. In this review, the functions of basophils determined on the basis of analysis of *in vitro* and *in vivo* systems and their potential involvement in clinical settings are discussed.

Basophils are rare circulating granulocytes that originate from CD34⁺ hematopoietic progenitors in the bone marrow. They arise from basophil progenitors (lineage-negative CD34⁺FcεRIα^{hi}c-Kit[−]) in which expression of the key transcription factors C/EBPα and GATA-2 seems to be key in lineage determination¹. Although mast cells complete their maturation processes in peripheral tissues such as skin and intestine, basophils are believed to complete their maturation in the bone marrow and thus exit the bone marrow fully matured. A study has identified bipotent basophil–mast cell progenitors in the mouse spleen that are able to differentiate into either basophils or mast cells². However, the contribution of such progenitors to basophil development *in vivo*, as well as the relationship between basophil–mast cell progenitors and basophil progenitors, remain to be determined. Moreover, whereas mast cells are believed to persist in tissues, basophils are short-lived cells with an expected half-life of approximately a few days. Nonetheless, basophils resemble mast cells in many ways; both cells express the high-affinity immunoglobulin E (IgE) receptor FcεRI and both rapidly produce cytokines, histamine and lipid mediators after crosslinkage of their Fc receptors, which suggests their involvement in immune responses. Indeed, the potential function of basophils as immune cells has been reviewed^{3–5}.

Basophils have been suggested to be the main effector cells in response to parasite infection and allergic inflammation. However, the lack of distinct phenotypic markers that allow the identification of basophils as well as the lack of animal models have hampered understanding of these cells in the context of immunity. Studies aiming to characterize basophil biology in mouse models have been developed by several independent laboratories^{6–11}. Although skepticism has been

raised about the identity of mouse basophils¹², firm evidence indicating their existence and importance in the immune system now seems convincing.

Phenotypically, mouse basophils are less-granular cells than human basophils are, a difference evident by flow cytometry, as mouse basophils typically are in the low-forward-scatter, low-side-scatter (FSC^{lo}SSC^{lo}) gate (Fig. 1). They have high expression of FcεRI and FcγRII and III, as well as CD49b, Thy-1, 2B4 and CD200R3 (Fig. 1). One notable feature of basophils is that in contrast to other myeloid lineage cells, which are CD45^{hi}, basophils are CD45^{int} (ref. 7; Fig. 1b); CD45 is a particularly useful marker, because FcεRI expression can change during immune responses^{13–15}. Phenotypically, basophils differ from mast cells and eosinophils in their lack of expression of the cell surface marker c-Kit (CD117) and the chemokine receptor CCR3, respectively^{6,9,16,17}.

What basophils ‘can do’

The data used to determine basophil functions have been provided by *in vitro* studies in which basophils isolated from peripheral blood or bone marrow culture are stimulated with various factors and their products are examined. The most notable and well characterized products of activated basophils include the cytokines interleukin 4 (IL-4) and IL-13, vasoactive histamine and lipid mediators such as leukotrienes^{18,19} (Fig. 2).

Basophils may be activated *in vitro* by both immunoglobulin-dependent and immunoglobulin-independent stimulation. Crosslinking of surface Fc receptors of basophils results in the immediate release of cytokines and histamine^{20,21}. IL-3 is crucial in ‘conditioning’ basophils, particularly by enhancing the production of cytokines in response to immunoglobulin crosslinkage. Thus, crosslinkage of immunoglobulin receptors in the absence of IL-3 induces modest production of IL-4, whereas adding IL-3 during stimulation or IL-3 pretreatment considerably enhances the immunoglobulin-mediated production of these cytokines^{20,22,23}. For immunoglobulin-independent mechanisms, the anaphylatoxin C5a²¹,

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Published online 14 November 2008; doi:10.1038/ni.f.217

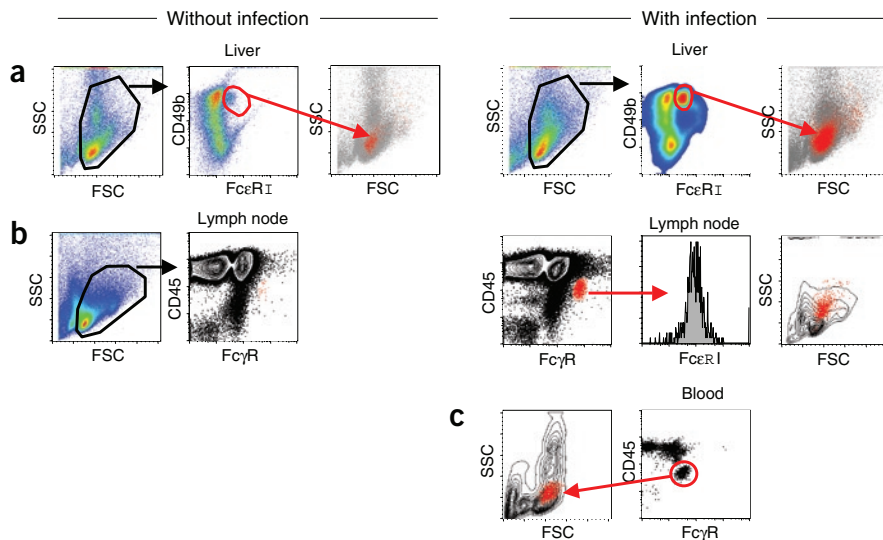


Figure 1 Gating strategy for mouse basophils. The phenotypic features of mouse basophils have been reported. Markers expressed on basophils include FcεRI, FcγRII and III, Thy-1, CD45, CD49b and 2B4. By flow analysis, basophils have been defined as FcεRI⁺CD49b⁺ (ref. 8), FcεRI⁺B220⁺ (ref. 14), FcεRI⁺Thy-1.2⁺ (ref. 6), FcγR⁺CD45^{int} (ref. 7) or CD49b⁺CD45^{int} (ref. 10). Typical gating strategies of basophils from the liver (**a**), lymph nodes (**b**) and blood (**c**) are presented here. Basophils are defined as FcεRI⁺CD49b⁺ and as FcγR⁺CD45^{int} cells. Basophils (red outlined gates) belong in the FSC^{lo}SSC^{lo} group (red dots in FSC-SSC dot plots) and differ from highly granulated SSC^{hi} cells such as eosinophils. Basophils defined as FcγR⁺CD45^{int} cells have high expression of FcεRI. Basophil numbers increase considerably in these tissues after infection with *N. brasiliensis* (right (**a–c**) versus left (**a,b**)).

the neutrophil-activating peptide NAF-NAP-1 (ref. 24), proteases²⁵ and parasite antigens²⁶ have all been shown to stimulate basophils to produce cytokines and to release histamine. The effect of IL-3 is also found in immunoglobulin-independent stimulation of basophils; thus, IL-3 is a general enhancer of the degree of basophil activation.

Innate components involved in basophil activation have been examined. Human basophils express Toll-like receptor 2 (TLR2) and TLR4 yet are selectively activated by TLR2 ligands, which induce nuclear localization of the transcription factor NF-κB as well as cytokine secretion^{27,28}. Despite their TLR4 expression, basophils fail to respond functionally to the TLR4 ligand lipopolysaccharide, as shown by their failure to activate NF-κB or to secrete cytokines; this might be due to the lack of CD14 expression by basophils²⁸. Unlike their human counterparts, mouse basophils express many TLRs, including TLR1, TLR2, TLR4 and TLR6 (ref. 29). Stimulation of mouse basophils with the TLR2 ligand peptidoglycan or with the TLR4 ligand lipopolysaccharide induces the production of T helper type 2 (T_H2) cytokines from basophils²⁹. However, the functions of TLRs *in vivo* or of other innate components in basophil activation are poorly understood.

In vitro, basophils are able to alter lymphocyte responses. Naive CD4⁺ T cells stimulated with peptide-pulsed dendritic cells in the presence of basophils differentiate into effector T cells of the T_H2 phenotype that produce IL-4, IL-10 and IL-13 (refs. 10,30); without the addition of basophils, T cells produce little or no T_H2 cytokines. Such results suggest that basophils may promote the development of T_H2 immune responses by providing the initial IL-4 to activated naive CD4⁺ T cells. That possibility is indirectly supported *in vivo* by a report of mice deficient in interferon-regulatory factor 2 (IRF2), which have more basophils and spontaneous T_H2 immunity³¹. The large numbers of basophil in IRF2-deficient mice are diminished to those of normal mice by the introduction into IRF2-deficient mice of a mutation in the gene encoding c-Kit that has been shown to result in fewer basophils

and mast cells^{31,32}. After this manipulation, spontaneous T_H2 differentiation is no longer prominent, which suggests that accelerated basophil expansion seems to be responsible for the T_H2 polarization of IRF2-deficient mice. Basophil-mediated T_H2 differentiation is mediated mainly by IL-4 produced by basophils, as little T_H2 differentiation is induced when IL-4-deficient basophils are used in coculture³⁰. The importance of basophil-derived IL-4 during *in vivo* T_H2 immunity remains to be determined, as other factors that promote T_H2 immunity, such as thymic stromal lymphopoietin, could be produced by basophils⁹. Notably, the T_H2 differentiation promoted by basophils *in vitro* is partially impaired in Transwell culture experiments, which suggests the possible involvement of a component of cell-to-cell contact³⁰. Although the nature of such a contact-dependent mechanism is unclear, these are intriguing data that await further investigation. The finding that basophils make close contact with CD4⁺ T cells in draining lymph nodes supports this possibility⁹.

It has been shown that human basophils, after IgE crosslinkage, secrete IL-25 (IL-17E), a newly identified IL-17 family cytokine that is important in allergic inflammation³³.

Injection of IL-25 induces considerable production of T_H2 cytokines (IL-4, IL-5 and IL-13) and eosinophilia in bronchoalveolar lavage fluid and lung tissue^{34,35}. Notably, basophils from atopic subjects secrete more IL-25 after activation, which indicates that basophils may augment the activation of T_H2 memory T cells that have high expression of the IL-25 receptor³³. Similarly, higher IL-25 expression is also found in the lung tissue of mice infected with the helminth *nippostrongylus*³⁴. *In vivo* production of IL-25 by activated basophils, the targets of IL-25 and the contribution to T_H2 immunity remain to be determined.

Basophils have also been shown to induce switching of the B cell isotype to IgE *in vitro*^{36–38}. Cells of a human basophilic line or basophils generated from umbilical cord blood cells are able to induce IgE synthesis in B cells after activation. IL-4 produced by activated basophils is critical for the switching, as IL-4 neutralization abrogates the IgE synthesis^{37,38}. A notable result is that activated basophils upregulate surface expression of CD40 ligand, which interacts with CD40 on B cells to provide help^{37,38}. Blockade of CD40 ligand is sufficient to abrogate IgE synthesis, which further supports the idea of critical involvement of the CD40 ligand–CD40 interaction in this process³⁷. Whether basophil-mediated synthesis of IgE occurs *in vivo* by a similar mechanism remains unclear.

What basophils ‘actually do’

The measurement of *in vivo* cytokine responses has usually depended on indirect methods, as cytokine production is rapidly terminated after activation. In general, cytokine production is determined after *in vitro* restimulation, which is equivalent to determining the potential of the cells to produce cytokines when they are ‘maximally’ activated. Because of the critical importance of IL-4 in T_H2 immunity and the close correlation between basophils and T_H2 immunity, the hypothesis that basophils drive the development of T_H2 immunity *in vivo* has been proposed^{4,39,40}. However, despite the considerable evidence of basophil

functions obtained with *in vitro* studies, evidence of *in vivo* basophil function in T_H2 differentiation is relatively limited.

Approaches with transgenic mice expressing green fluorescent protein (GFP) under control of the *Il4* promoter-enhancer have provided important insights into *in vivo* IL-4 expression^{41,42}. Indeed, the first evidence of *in vivo* basophil functions emerged from IL-4 reporter animal studies^{8,16} showing that basophil IL-4 expression is constitutively acquired during lineage differentiation in the bone marrow, which thus allows the basophils to rapidly produce IL-4 after stimulation⁴³. IL-4 is not required for this process, as basophil *Il4*-GFP expression is not altered in IL-4-deficient mice⁸. The finding that stimulation of basophils in the presence of actinomycin D induces IL-4 production further supports the hypothesis that basophils are important regulators of immune responses through their early IL-4 production^{43–45}.

However, a discrepancy between GFP expression and IL-4 secretion has been noted in one strain of GFP reporter mice. In IL-4-GFP 'enhanced-transcript' mice ('4get' mice), in which the gene encoding GFP is downstream of an internal ribosome entry site and *Il4*, GFP expression is not always a direct indicator of IL-4 secretion but instead may represent basal transcription of *Il4*, as may occur in nonactivated T_H2 cells, natural killer T cells and basophils⁴⁶. This is in contrast to 'G4' mice, in which the gene encoding GFP replaces the first exon of *Il4* and in which GFP expression is a faithful surrogate of IL-4 expression⁷. Therefore, evidence from studies of 4get mice indicates that basophils are spontaneously 'programmed' to be able to express IL-4 mRNA, but their actual production of IL-4 *in vivo* in such studies is a matter of uncertainty¹⁶. In contrast, studies of G4 mice have shown that unstimulated basophils express little GFP⁷. That finding has been confirmed by the development of a reporter mouse in which the human gene encoding CD2 is inserted into the *Il4* locus. When these mice are crossed with 4get mice, it is possible to analyze both the potential to produce IL-4 and actual IL-4 production⁴⁷. Such studies have shown, in confirmation of the work with G4 mice⁷, that unstimulated GFP⁺ basophils isolated from reporter mice infected with the nematode *Heligmosomoides polygyrus* fail to express human CD2 or to produce detectable IL-4 *ex vivo* unless stimulated, which indicates a lack of IL-4 protein production⁴⁷.

Despite the uncertainties with the various reporter mice described above, evidence supporting basophil IL-4 production *in vivo* has been demonstrated indirectly in recombination-activating gene 2–deficient mice that receive IL-4-deficient CD4⁺ T cells and are then infected with *Nippostrongylus brasiliensis*. These mice have high serum concentrations of IL-4, which suggests *in vivo* production of IL-4 by basophils⁷.

Basophils have been shown to be critically involved in mounting a robust cutaneous hypersensitivity reactions that lead to tick rejection⁴⁸. Both basophil and eosinophils migrate to sites of inflammation. Depletion of basophils with anti-basophil serum (raised in rabbits immunized intradermally with guinea pig basophils) eliminates basophils, diminishes eosinophil recruitment and abolishes the protective immunity⁴⁸. It is possible that basophils recruited to sites of tick infestation release anti-tick effector molecules such as histamine that dislodge ticks from their attachment sites⁴⁹. Alternatively, basophils may secrete chemotactic factors that recruit eosinophils that mediate anti-tick immunity^{50,51}.

Similar immunoregulatory functions of basophils have been shown in IgE-mediated chronic allergic dermatitis characterized by infiltration of both basophils and eosinophils^{6,11,52}. Depletion of basophils with an antibody to CD200R3 that recognizes a basophil surface molecule results in the elimination of basophils as well as the skin lesion⁵³. More notably, basophil depletion results in many fewer infiltrating

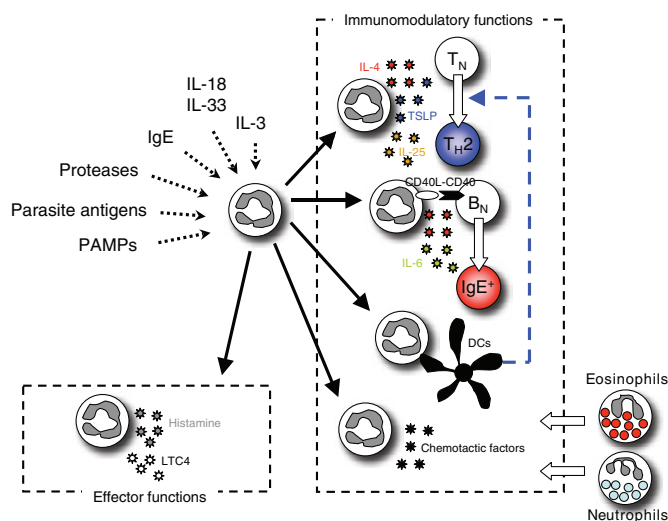


Figure 2 Functions of basophils. Basophils can be activated by many signals, including cytokines, immunoglobulins, proteases and parasite-associated antigens. Activated basophils secrete cytokines that support the development of IL-4-producing CD4⁺ T cells and of IgE-secreting B cells associated with T_H2 immunity; produce chemotactic factors that recruit other inflammatory cells such as eosinophils and neutrophils into inflammatory sites; and release effector molecules such as histamine and leukotrienes. Basophils are also able to modulate the functions of antigen-presenting cells through which T_H2 immunity is favored. PAMPs, pathogen-associated molecular patterns; T_N , naive T cell; B_N , naive B cell; CD40L, CD40 ligand; DC, dendritic cell; LTC4, leukotriene C4.

inflammatory cells, such as eosinophils and neutrophils⁶. Therefore, basophils seem to serve a critical function in the development of IgE-mediated chronic allergic inflammation by recruiting other inflammatory cells and not necessarily by acting as effector cells.

Basophils have also been shown to be important in the development of T_H2 differentiation in response to cysteine protease allergens *in vivo*⁹. Immunization of mice with papain or bromelain induces strong T_H2 immunity characterized by effector CD4⁺ T cells that produce T_H2 type cytokines as well as by IgE-producing B cells⁹. A notable finding is that at day 3 of the response, basophils are recruited into the draining lymph nodes, where they are believed to localize in close proximity to activated T cells and to produce factors that promote T_H2 differentiation (Fig. 3). Depletion of basophils with antibody to FcεRI (MAR1) or neutralization of either IL-4 or thymic stromal lymphopoietin diminishes T_H2 differentiation, which suggests that both IL-4 and thymic stromal lymphopoietin produced by basophils are involved in promoting T_H2 differentiation.

A report has indicated that basophils also enhance memory B cell responses¹⁰. This study has shown that basophils can capture large amounts of antigen through surface FcR-bound immunoglobulin and enhance humoral memory responses through the production of IL-4 and IL-6 (ref. 10). Depletion of basophils increases the susceptibility of immunized mice to sepsis induced by bacteria challenge¹⁰. Finally, basophils are the main IL-4-producing cells during primary and secondary antigen immunization^{54,55}. Therefore, basophils seem to control adaptive immunity in the context of infection and of allergic inflammation.

Activation of basophils *in vivo*

What triggers basophil activation *in vivo*? Although IgE crosslinkage stimulates basophils to produce IL-4, the relative contribution of

IgE-mediated basophil activation *in vivo* remains to be determined. It has been reported that antigens with protease activity, such as papain or bromelain, induce *in vivo* T_H2 immunity that can be abolished by heat inactivation of the proteases⁹. *In vitro* stimulation of basophils with active papain induces cytokine production by basophils⁹. In fact, proteases from helminths and house dust mites are also known to induce basophil IL-4 production²⁵ (Fig. 3). It is possible that proteases directly activate cells through protease-activated receptors. Although human basophils do not express such receptors⁵⁶, the expression of protease-activated receptors on mouse basophils remains to be determined. Alternatively, proteases may cleave as-yet-unidentified factors that contribute to basophil activation. Notably, papain induces basophil IL-4 production in serum-free conditions, which suggests that the unknown substrate of protease is expressed on the basophil membrane⁹. The house dust mite protease Der p1 cleaves CD23 and CD25 from activated B cells and T cells, respectively^{57,58}. Although basophils express CD25 but not CD23 (refs. 8,59), the protease targets involved in basophil activation remain to be determined. Der p1 is also able to cleave the dendritic cell surface molecules DC-SIGN and DC-SIGNR, both of which are involved in T_H1 immunity through interaction with intercellular adhesion molecule 3 expressed on naive T cells⁶⁰. Given that proteases are potent T_H2 adjuvants, it will be use-

ful to determine whether protease-mediated basophil activation and subsequent IL-4 production underlies T_H2 immunity.

N. brasiliensis excretory-secretory proteins from adult parasites are known to induce T_H2 immunity *in vivo*⁶¹. In support of that fact, these proteins have been shown to cause 'alternative' maturation of dendritic cells to further promote T_H2 differentiation (Fig. 3); their enzymatic activity seems critical in mediating T_H2 differentiation, as heat inactivation abolishes their effects⁶². However, enzymatic activity is not always associated with basophil activation capacity and/or T_H2 adjuvanticity. For example, IPSE- α -1, a glycoprotein from *Schistosoma mansoni* eggs, activates IL-4 production by basophils⁶³. Notably, IgE is required for IPSE antigen to 'deliver' activation, although the antigen specificity of the IgE molecule is not involved.

In addition to the 'priming' effects of IL-3 on basophils described above, IL-3 is critically involved in enhancing the generation and/or differentiation of basophils *in vivo* after parasite infection³². Basophil production and peripheral accumulation are much higher in wild-type mice infected with *Strongyloides venezuelensis* or *N. brasiliensis*; this is not found in IL-3-deficient mice infected with the same parasites³². IL-3 produced by activated parasite-specific CD4⁺ T cells enhances the differentiation of basophils from precursors in the bone marrow⁶⁴. Particularly notable is that the defect in IL-3-deficient mice is found only after parasite infection; IL-3 deficiency does not affect the basal maintenance of basophils in the absence of infection³². IL-3 produced in local draining lymph nodes may circulate and stimulate basophil precursors in the bone marrow and increase the differentiation of the precursor cells (Fig. 3). However, all activated T cells, including CD8⁺ T cells, are able to produce IL-3 after activation, although T_H2 CD4⁺ T cells tend to produce more IL-3 (ref. 65). Therefore, it is difficult to link T cell production of IL-3 to basophil T_H2 responses. These results suggest that there may be additional factors involved in this process.

IL-33, a newly identified member of the IL-1 family, is the ligand of the receptor ST2 and has been linked to T_H2 immunity⁶⁶. Basophils have high expression of IL-33 receptor and produce IL-4 and IL-13 in response to IL-33, which is dependent on ST2 and the adaptor protein MyD88 pathway⁶⁷. *In vivo* administration of IL-33 induces airway hyper-responsiveness and goblet cell hyperplasia⁶⁷. The basophil functions *in vivo* in response to these cytokines need further investigation.

Basophils in helminthic and HIV-1 infection

Although there is evidence of parasite infection-induced basophilia in mice, whether this also occurs in humans is unclear. Can basophilia be used as an indicator of parasite infection in humans? A field study from Papua New Guinea has demonstrated that peripheral blood basophils are much less abundant after anthelmintic treatment⁶⁸. Basophils from patients infected with pathogens of the genus *Toxocara*, *Ascaris*, *Onchocerca*, *Wuchereria* or *Strongyloides* release histamine when stimulated with parasite antigens^{69–71}. In addition, basophils from patients with active filarial infection secrete IL-4 after exposure to parasite antigens; IL-4 production in response to the antigen is much greater by such basophils than by CD4⁺ T cells on a 'per-cell' basis⁷². As IL-4 production is not detected in antigen-exposed basophils from uninfected people, the IL-4 induction seems antigen specific and is probably mediated by surface-bound parasite-specific immunoglobulin. Indeed, the amount of histamine released from the basophils is proportional to the serum concentration of antigen-specific IgE⁶⁹. However, it is notable that immunoglobulin-independent stimulation of blood basophils by schistosoma egg antigens also induces activation^{26,73}. A disappointing finding has been obtained in a retrospective study screening for basophilia in patients with a single or multiple parasitic infection(s)

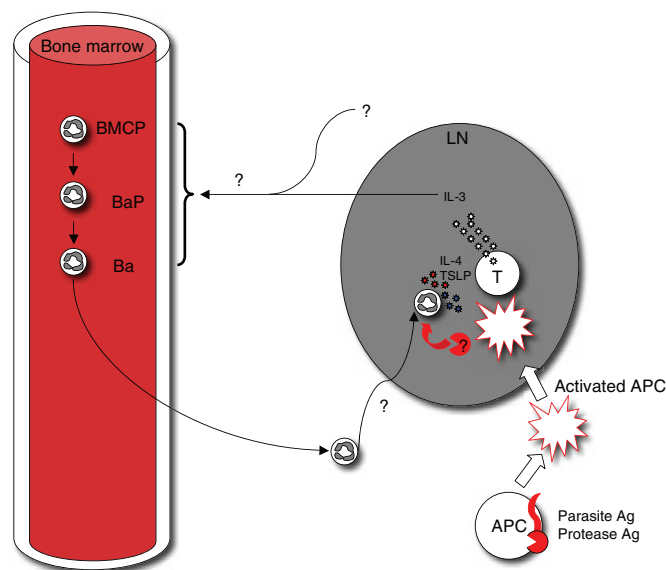


Figure 3 Proposed models of basophil function during parasite infection. Parasite-associated antigen (Ag) is taken up by tissue-resident antigen-presenting cells (APC), through which the antigen-presenting cells undergo maturation and migration into the draining lymph node (LN), where priming of antigen-specific CD4⁺ T cells (T) occurs. Circulating basophils enter the draining lymph nodes and are further activated to produce factors that promote T_H2 differentiation. Mechanisms leading to basophil recruitment and activation are unclear, although parasite-associated antigens with (or without) protease activity may be involved in this process. IL-4 or thymic stromal lymphopoietin (TSLP) produced by activated basophils in the lymph nodes may mediate the process of T_H2 differentiation of activated naive CD4⁺ T cells. Activated T cells then produce IL-3, which is critical for enhancing basophil production in the bone marrow. The cellular mechanism as well as targets of IL-3-mediated basophil production are poorly understood. Given that IL-3 can be produced by all activated T cells, these results suggest that IL-3 is not the only factor involved in basophil production in response to parasite infection and suggest the existence of other factor(s) associated with parasites or allergens. BMCP, basophil–mast cell progenitor; BaP, basophil progenitor; Ba, basophil.

with a cutoff of 290 basophils per milliliter of blood⁷⁴. Only four patients had more blood basophils, which indicates that basophilia is not a reliable marker for parasite infection in humans. However, this negative result does not necessarily indicate that basophils are not important effector cells in parasite infection. Instead, the possibility that basophils may increase their sensitivity and allow a better response to parasite infection has been proposed⁶⁸. Alternatively, it is also possible that parasite infection recruits basophils to other tissues; therefore, screening for blood basophils may not be a valid test.

Although higher concentrations of IgE and a disturbed balance of T_H1-T_H2 immunity are often found in patients infected with human immunodeficiency virus type 1 (HIV-1), the underlying cellular mechanisms are poorly understood. It is speculated that FcεRI⁺ basophils are important in these processes^{75,76}. The HIV glycoprotein gp120, a chief protein that mediates viral entry by binding to CD4, is a member of the immunoglobulin superantigen family. It interacts with products of the variably heavy-chain region V_H3 gene, which has the largest representation in the human immunoglobulin repertoire⁷⁷. Binding of gp120 to the V_H3 region of IgE increases T_H2 cytokine production by basophils^{76,78}. In addition, Tat protein, a viral product critically involved in immunodeficiency that is secreted by HIV-1-infected cells⁷⁹, induces chemotaxis of basophils through interaction with CCR3 (ref. 80). Tat can also induce the production of IL-4 and IL-13 by human basophils⁷⁶.

A particularly notable finding is that such viral products, after interacting with basophils, induce the upregulation of chemokine receptors such as CCR3 (ref. 80) and CXCR4 (ref. 81), both of which can serve as coreceptors for HIV⁸². Therefore, it is possible that FcεRI⁺ cells, including basophils and mast cells, may be infected with HIV-1 and may be a reservoir of HIV infection. Human basophils can be infected by M-tropic HIV-1 virus *in vitro*⁸³. Indeed, intact viral particles have been found in blood basophils of HIV-1-infected patients by electron microscopy⁸³. Overall, although the biological importance of HIV-1 infection in basophils needs further investigation, basophils stimulated by virus products of HIV-1 may provide a critical impetus to drive a T_H1-T_H2 shift in infected people by providing key T_H2 cytokines.

Basophils in allergy

Basophils are rarely found in normal tissues⁸⁴. Their numbers increase considerably in sites of allergic inflammation seen in the airways of asthmatic patients^{85–87} or in the skin of patients with atopic dermatitis⁸⁸. Notably, the expression of IL-4 mRNA by basophils identified with monoclonal antibody 2D7⁸⁹ increases after allergen provocation in sensitive patients⁹⁰. In addition to IL-4, basophil products, including leukotriene C4 and histamine, also cause the symptoms of acute and chronic allergic inflammation⁸⁴. Indeed, basophils are the main cells that produce IL-4 and IL-13 in the peripheral blood of asthmatic patients after antigen activation⁹¹. However, the contribution of basophils to the pathogenesis of asthma remains to be determined.

Basophilopenia

Although the low normal concentration of basophils (20–80 cells per microliter) makes it difficult to diagnose abnormally low or absence of basophils in normal people, patients who consistently lack eosinophils and basophils have been reported^{92,93}. These patients develop recurrent infections and many clinical symptoms, including vasomotor rhinitis, alopecia totalis and widespread scabies⁹². However, it remains unclear whether the lack of basophils is responsible for the disorders found in such patients.

Perspectives

Identifying the early source of IL-4 responsible for T_H2 immunity has been the central question in this area since the discovery of the involvement of IL-4 in *in vitro* T_H2 differentiation. After countless reports proposing cell types able to produce IL-4 in certain circumstances, subsequent advances have suggested that basophils might be a source of IL-4 that may drive *in vivo* T_H2 immunity.

Where should research move from here? First, pathways leading to basophil lineage differentiation need to be defined. Studies defining the common precursors that become either basophils and mast cells and the molecular switch determining lineage fate are a promising beginning². Furthermore, more detailed and rigorous gene profiles of basophils need to be analyzed. Microarray analyses seeking basophil-specific genes should be the next step. However, it is difficult to determine the appropriate cell population with which basophil gene profiles should be compared. Although microarray analysis of gene expression in basophils and eosinophils isolated from parasite-infected mice and in human basophils that release histamine ('releaser') and those that do not degranulate after anti-IgE challenge ('nonreleaser') has already generated useful results^{16,94}, more experiments are needed to better define basophil functions, especially those comparing resting and activated basophils. In humans, CD203c and CD63 are thought to indicate basophil activation status^{95–97}, yet there are no reliable markers for activated mouse basophils. Finally, elucidating basophil IL-4 production should be particularly interesting, given that IL-4 production by T cells requires epigenetic modification associated with *Il4* chromatin remodeling and transcriptional activation⁹⁸. A report showing that regulation of *Il4* in basophils is independently controlled by conserved noncoding sequence 2 and DNase I-hypersensitive site 4, located in distal and proximal 3' enhancers, respectively, further supports the idea of a unique mechanism that controls IL-4 expression in basophils⁹⁹.

In conclusion, if early studies focusing on what basophils 'can do' after *in vitro* manipulation were the prelude, now is the time to start a new chapter to focus what basophils 'really do' *in vivo*. More exciting studies that will solve the mystery of these rare yet powerful cells lie ahead. The show has already begun for all to enjoy.

ACKNOWLEDGMENTS

I thank W.E. Paul for review of the manuscript.

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1. Iwasaki, H. & Akashi, K. Myeloid lineage commitment from the hematopoietic stem cell. *Immunity* **26**, 726–740 (2007).
2. Arinobu, Y. *et al.* Developmental checkpoints of the basophil/mast cell lineages in adult murine hematopoiesis. *Proc. Natl. Acad. Sci. USA* **102**, 18105–18110 (2005).
3. Mitre, E. & Nutman, T.B. Basophils, basophilia and helminth infections. *Chem. Immunol. Allergy* **90**, 141–156 (2006).
4. Min, B. & Paul, W.E. Basophils and type 2 immunity. *Curr. Opin. Hematol.* **15**, 59–63 (2008).
5. Falcone, F.H., Zillikens, D. & Gibbs, B.F. The 21st century renaissance of the basophil? Current insights into its role in allergic responses and innate immunity. *Exp. Dermatol.* **15**, 855–864 (2006).
6. Mukai, 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* **23**, 191–202 (2005).
7. Mack, M. *et al.* Identification of antigen-capturing cells as basophils. *J. Immunol.* **174**, 735–741 (2005).
8. Min, B. *et al.* Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite. *J. Exp. Med.* **200**, 507–517 (2004).
9. Sokol, C.L., Barton, G.M., Farr, A.G. & Medzhitov, R. A mechanism for the initiation of allergic inflammation. *Nature* **435**, 67–71 (2005).

An elegant study showing that basophils are critical mediators of chronic allergic inflammation.

The first study that identified mouse basophils as the main IL-4-producing cells during parasite infection.

- of allergen-induced T helper type 2 responses. *Nat. Immunol.* **9**, 310–318 (2008).
- Study demonstrating basophil production of T_H2 -inducing cytokines such as IL-4 and thymic stromal lymphopoietin and their involvement in *in vivo* T_H2 differentiation.**
10. Denzel, A. *et al.* Basophils enhance immunological memory responses. *Nat. Immunol.* **9**, 733–742 (2008).
11. Tsujimura, Y. *et al.* Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. *Immunity* **28**, 581–589 (2008).
12. Lee, J.J. & McGarry, M.P. When is a mouse basophil not a basophil? *Blood* **109**, 859–861 (2007).
13. Kennedy Norton, S. *et al.* IL-10 suppresses mast cell IgE receptor expression and signaling in vitro and in vivo. *J. Immunol.* **180**, 2848–2854 (2008).
14. Lantz, C.S. *et al.* IgE regulates mouse basophil FcεRI expression in vivo. *J. Immunol.* **158**, 2517–2521 (1997).
15. Yamaguchi, M. *et al.* Regulation of mouse mast cell surface FcεRI expression by dexamethasone. *Int. Immunol.* **13**, 843–851 (2001).
16. Voehringer, D., Shinkai, K. & Locksley, R.M. Type 2 immunity reflects orchestrated recruitment of cells committed to IL-4 production. *Immunity* **20**, 267–277 (2004).
17. Voehringer, D., van Rooijen, N. & Locksley, R.M. Eosinophils develop in distinct stages and are recruited to peripheral sites by alternatively activated macrophages. *J. Leukoc. Biol.* **81**, 1434–1444 (2007).
18. Schroeder, J.T., MacGlashan, D.W., Jr & Lichtenstein, L.M. Human basophils: mediator release and cytokine production. *Adv. Immunol.* **77**, 93–122 (2001).
19. Paul, W.E. Interleukin-4 production by FcεR⁺ cells. *Skin Pharmacol.* **4** Suppl 1, 8–14 (1991).
20. Le Gros, G. *et al.* IL-3 promotes production of IL-4 by splenic non-B, non-T cells in response to Fc receptor cross-linkage. *J. Immunol.* **145**, 2500–2506 (1990).
21. Kurimoto, Y., de Weck, A.L. & Dahinden, C.A. Interleukin 3-dependent mediator release in basophils triggered by C5a. *J. Exp. Med.* **170**, 467–479 (1989).
22. MacDonald, S.M., Schleimer, R.P., Kagey-Sobotka, A., Gillis, S. & Lichtenstein, L.M. Recombinant IL-3 induces histamine release from human basophils. *J. Immunol.* **142**, 3527–3532 (1989).
23. Brunner, T., Heusser, C.H. & Dahinden, C.A. Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J. Exp. Med.* **177**, 605–611 (1993).
24. Dahinden, C.A. *et al.* The neutrophil-activating peptide NAF/NAP-1 induces histamine and leukotriene release by interleukin 3-primed basophils. *J. Exp. Med.* **170**, 1787–1792 (1989).
25. Phillips, C., Coward, W.R., Pritchard, D.I. & Hewitt, C.R. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J. Leukoc. Biol.* **73**, 165–171 (2003).
26. Falcone, F.H. *et al.* Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen. *Eur. J. Immunol.* **26**, 1147–1155 (1996).
27. Bieneman, A.P., Chichester, K.L., Chen, Y.H. & Schroeder, J.T. Toll-like receptor 2 ligands activate human basophils for both IgE-dependent and IgE-independent secretion. *J. Allergy Clin. Immunol.* **115**, 295–301 (2005).
28. Sabroe, I., Jones, E.C., Usher, L.R., Whyte, M.K. & Dower, S.K. Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: a critical role for monocytes in leukocyte lipopolysaccharide responses. *J. Immunol.* **168**, 4701–4710 (2002).
29. Yoshimoto, T. & Nakanishi, K. Roles of IL-18 in basophils and mast cells. *Allergol. Int.* **55**, 105–113 (2006).
30. 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* **109**, 2921–2927 (2007).
31. 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* **106**, 2011–2017 (2005).
32. Lantz, C.S. *et al.* Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* **392**, 90–93 (1998).
- Study identifying critical functions for IL-3 in increasing basophil production in response to parasite infection and demonstrating that IL-3 is paradoxically dispensable for maintaining homeostatic basophil numbers in the absence of parasite infection.**
33. Wang, Y.H. *et al.* IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J. Exp. Med.* **204**, 1837–1847 (2007).
34. Hurst, S.D. *et al.* New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J. Immunol.* **169**, 443–453 (2002).
35. Tamachi, T., Maezawa, Y., Ikeda, K., Iwamoto, I. & Nakajima, H. Interleukin 25 in allergic airway inflammation. *Int. Arch. Allergy Immunol.* **140** Suppl 1, 59–62 (2006).
36. Yanagihara, Y. *et al.* Induction of human IgE synthesis in B cells by a basophilic cell line, KU812. *Clin. Exp. Immunol.* **108**, 295–301 (1997).
37. Yanagihara, Y. *et al.* Cultured basophils but not cultured mast cells induce human IgE synthesis in B cells after immunologic stimulation. *Clin. Exp. Immunol.* **111**, 136–143 (1998).
38. Gauchat, J.F. *et al.* Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* **365**, 340–343 (1993).
39. Galli, S.J. & Franco, C.B. Basophils are back! *Immunity* **28**, 495–497 (2008).
40. Min, B. & Paul, W.E. Basophils: in the spotlight at last. *Nat. Immunol.* **9**, 223–225 (2008).
41. Shinkai, K., Mohrs, M. & Locksley, R.M. Helper T cells regulate type-2 innate immunity *in vivo*. *Nature* **420**, 825–829 (2002).
42. Hu-Li, J. *et al.* Regulation of expression of IL-4 alleles: analysis using a chimeric GFP/IL-4 gene. *Immunity* **14**, 1–11 (2001).
43. Gessner, A., Mohrs, K. & Mohrs, M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J. Immunol.* **174**, 1063–1072 (2005).
44. Gibbs, B.F. *et al.* Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur. J. Immunol.* **26**, 2493–2498 (1996).
45. Haas, H. *et al.* Early interleukin-4: its role in the switch towards a Th2 response and IgE-mediated allergy. *Int. Arch. Allergy Immunol.* **119**, 86–94 (1999).
46. Stetson, D.B. *et al.* Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J. Exp. Med.* **198**, 1069–1076 (2003).
47. Mohrs, K., Wakil, A.E., Killeen, N., Locksley, R.M. & Mohrs, M. A two-step process for cytokine production revealed by IL-4 dual-reporter mice. *Immunity* **23**, 419–429 (2005).
48. Brown, S.J., Galli, S.J., Gleich, G.J. & Askenase, P.W. Ablation of immunity to *Amblyomma americanum* by anti-basophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J. Immunol.* **129**, 790–796 (1982).
49. Kemp, D.H. & Bourne, A. *Boophilus microplus*: the effect of histamine on the attachment of cattle-tick larvae—studies in vivo and in vitro. *Parasitology* **80**, 487–496 (1980).
50. Kierszenbaum, F., Ackerman, S.J. & Gleich, G.J. Destruction of bloodstream forms of *Trypanosoma cruzi* by eosinophil granule major basic protein. *Am. J. Trop. Med. Hyg.* **30**, 775–779 (1981).
51. Butterworth, A.E., Wassom, D.L., Gleich, G.J., Loegering, D.A. & David, J.R. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* **122**, 221–229 (1979).
52. Obata, K. *et al.* Basophils are essential initiators of a novel type of chronic allergic inflammation. *Blood* **110**, 913–920 (2007).
53. Kojima, T. *et al.* Mast cells and basophils are selectively activated in vitro and in vivo through CD200R3 in an IgE-independent manner. *J. Immunol.* **179**, 7093–7100 (2007).
54. Aoki, I., Kinzer, C., Shirai, A., Paul, W.E. & Klinman, D.M. IgE receptor-positive non-B/non-T cells dominate the production of interleukin 4 and interleukin 6 in immunized mice. *Proc. Natl. Acad. Sci. USA* **92**, 2534–2538 (1995).
55. Khodoun, M.V., Orekhova, T., Potter, C., Morris, S. & Finkelman, F.D. Basophils initiate IL-4 production during a memory T-dependent response. *J. Exp. Med.* **200**, 857–870 (2004).
56. Falcone, F.H., Morroll, S. & Gibbs, B.F. Lack of protease activated receptor (PAR) expression in purified human basophils. *Inflamm. Res.* **54** Suppl 1, S13–S14 (2005).
57. Schulz, O., Sewell, H.F. & Shakib, F. Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J. Exp. Med.* **187**, 271–275 (1998).
58. Shakib, F., Schulz, O. & Sewell, H. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. *Immunol. Today* **19**, 313–316 (1998).
59. Agis, H. *et al.* Comparative immunophenotypic analysis of human mast cells, blood basophils and monocytes. *Immunology* **87**, 535–543 (1996).
60. Furmonaciene, R. *et al.* The protease allergen Der p 1 cleaves cell surface DC-SIGN and DC-SIGNR: experimental analysis of in silico substrate identification and implications in allergic responses. *Clin. Exp. Allergy* **37**, 231–242 (2007).
61. Marsland, B.J., Camberis, M. & Le Gros, G. Secretory products from infective forms of *Nippostrongylus brasiliensis* induce a rapid allergic airway inflammatory response. *Immunol. Cell Biol.* **83**, 40–47 (2005).
62. Balic, A., Harcus, Y., Holland, M.J. & Maizels, R.M. Selective maturation of dendritic cells by *Nippostrongylus brasiliensis*-secreted proteins drives Th2 immune responses. *Eur. J. Immunol.* **34**, 3047–3059 (2004).
63. Schramm, G. *et al.* Cutting edge: IPSE/α-1, a glycoprotein from *Schistosoma mansoni* eggs, induces IgE-dependent, antigen-independent IL-4 production by murine basophils in vivo. *J. Immunol.* **178**, 6023–6027 (2007).
64. Shen, T. *et al.* T cell-derived IL-3 plays key role in parasite infection-induced basophil production but is dispensable for in vivo basophil survival. *Int. Immunol.* **20**, 1201–1209 (2008).
65. Else, K.J., Entwistle, G.M. & Grencis, R.K. Correlations between worm burden and markers of Th1 and Th2 cell subset induction in an inbred strain of mouse infected with *Trichuris muris*. *Parasite Immunol.* **15**, 595–600 (1993).
66. Schmitz, J. *et al.* IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* **23**, 479–490 (2005).
67. Kondo, Y. *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.* **20**, 791–800 (2008).
68. Gibbs, B.F., Papenfuss, K. & Falcone, F.H. A rapid two-step procedure for the purification of human peripheral blood basophils to near homogeneity. *Clin. Exp. Allergy* **38**, 480–485 (2008).
69. Nielsen, B.W. *et al.* Immune responses to nematode exoantigens: sensitizing antibodies and basophil histamine release. *Allergy* **49**, 427–435 (1994).
70. Gonzalez-Munoz, M., Garate, T., Puente, S., Subirats, M. & Moneo, I. Induction of histamine release in parasitized individuals by somatic and cuticular antigens from *Onchocerca volvulus*. *Am. J. Trop. Med. Hyg.* **60**, 974–979 (1999).

71. Genta, R.M. *et al.* Specific allergic sensitization to Strongyloides antigens in human strongyloidiasis. *Lab. Invest.* **48**, 633–638 (1983).
72. Mitre, E., Taylor, R.T., Kubofcik, J. & Nutman, T.B. Parasite antigen-driven basophils are a major source of IL-4 in human filarial infections. *J. Immunol.* **172**, 2439–2445 (2004).
73. Haisch, K. *et al.* A glycoprotein from *Schistosoma mansoni* eggs binds non-antigen-specific immunoglobulin E and releases interleukin-4 from human basophils. *Parasite Immunol.* **23**, 427–434 (2001).
74. Mitre, E. & Nutman, T.B. Lack of basophilia in human parasitic infections. *Am. J. Trop. Med. Hyg.* **69**, 87–91 (2003).
75. Marone, G., Florio, G., Petraroli, A., Triggiani, M. & de Paulis, A. Role of human FcεRI⁺ cells in HIV-1 infection. *Immunol. Rev.* **179**, 128–138 (2001).
- An excellent review proposing a new function for basophils during HIV-1 infection in human.**
76. Marone, G., Florio, G., Petraroli, A., Triggiani, M. & de Paulis, A. Human mast cells and basophils in HIV-1 infection. *Trends Immunol.* **22**, 229–232 (2001).
77. Karray, S. & Zouali, M. Identification of the B cell superantigen-binding site of HIV-1 gp120. *Proc. Natl. Acad. Sci. USA* **94**, 1356–1360 (1997).
78. Florio, G., Petraroli, A., Patella, V., Triggiani, M. & Marone, G. The immunoglobulin superantigen-binding site of HIV-1 gp120 activates human basophils. *Aids* **14**, 931–938 (2000).
79. Ensoli, B., Barillari, G., Salahuddin, S.Z., Gallo, R.C. & Wong-Staal, F. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* **345**, 84–86 (1990).
80. de Paulis, A. *et al.* Tat protein is an HIV-1-encoded beta-chemokine homolog that promotes migration and up-regulates CCR3 expression on human FcεRI⁺ cells. *J. Immunol.* **165**, 7171–7179 (2000).
81. Jinquan, T. *et al.* Chemokine stromal cell-derived factor 1α activates basophils by means of CXCR4. *J. Allergy Clin. Immunol.* **106**, 313–320 (2000).
82. Moore, J.P. Coreceptors: implications for HIV pathogenesis and therapy. *Science* **276**, 51–52 (1997).
83. Li, Y. *et al.* Mast cells/basophils in the peripheral blood of allergic individuals who are HIV-1 susceptible due to their surface expression of CD4 and the chemokine receptors CCR3, CCR5, and CXCR4. *Blood* **97**, 3484–3490 (2001).
84. Marone, G., Triggiani, M. & de Paulis, A. Mast cells and basophils: friends as well as foes in bronchial asthma? *Trends Immunol.* **26**, 25–31 (2005).
85. Macfarlane, A.J. *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.* **105**, 99–107 (2000).
86. Gauvreau, G.M. *et al.* Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am. J. Respir. Crit. Care Med.* **161**, 1473–1478 (2000).
87. Koshino, T. *et al.* Airway basophil and mast cell density in patients with bronchial asthma: relationship to bronchial hyperresponsiveness. *J. Asthma* **33**, 89–95 (1996).
88. Irani, A.M. *et al.* Immunohistochemical detection of human basophils in late-phase skin reactions. *J. Allergy Clin. Immunol.* **101**, 354–362 (1998).
89. Kepley, C.L., Craig, S.S. & Schwartz, L.B. Identification and partial characterization of a unique marker for human basophils. *J. Immunol.* **154**, 6548–6555 (1995).
90. Nouri-Aria, K.T. *et al.* Basophil recruitment and IL-4 production during human allergen-induced late asthma. *J. Allergy Clin. Immunol.* **108**, 205–211 (2001).
91. Devouassoux, G., Foster, B., Scott, L.M., Metcalfe, D.D. & Prussin, C. Frequency and characterization of antigen-specific IL-4- and IL-13- producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J. Allergy Clin. Immunol.* **104**, 811–819 (1999).
92. Juhlin, L. & Michaelsson, G. A new syndrome characterised by absence of eosinophils and basophils. *Lancet* **1**, 1233–1235 (1977).
93. Tracey, R. & Smith, H. An inherited anomaly of human eosinophils and basophils. *Blood Cells* **4**, 291–300 (1978).
94. Youssef, L.A. *et al.* Histamine release from the basophils of control and asthmatic subjects and a comparison of gene expression between “releaser” and “nonreleaser” basophils. *J. Immunol.* **178**, 4584–4594 (2007).
95. Bühring, H.J., Streble, A. & Valent, P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int. Arch. Allergy Immunol.* **133**, 317–329 (2004).
96. Eberlein-König, B. *et al.* Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* **61**, 1084–1085 (2006).
97. Boumiza, R. *et al.* Marked improvement of the basophil activation test by detecting CD203c instead of CD63. *Clin. Exp. Allergy* **33**, 259–265 (2003).
98. Ansel, K.M., Djuretic, I., Tanasa, B. & Rao, A. Regulation of Th2 differentiation and IL4 locus accessibility. *Annu. Rev. Immunol.* **24**, 607–656 (2006).
99. Yagi, R., Tanaka, S., Motomura, Y. & Kubo, M. Regulation of the IL4 gene is independently controlled by proximal and distal 3′ enhancers in mast cells and basophils. *Mol. Cell. Biol.* **27**, 8087–8097 (2007).