

use high-resolution microscopy to determine the interaction frequencies and to describe the allelic-exclusion mechanism more precisely in statistical terms.

It is important to emphasize that the data provided in the Schlimgen *et al.* study cannot necessarily be generalized to all antigen-receptor loci. For example, *Igk* locus allelic exclusion is established by a predetermined mechanism².

Thus, although the data from Schlimgen *et al.* move forward understanding of the mechanics of *Tcrb* allelic exclusion, they also raise new questions ripe for future study.

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Basophils now enhance memory

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Additional immune functions of basophils have been identified in recent years. Mack and colleagues add to this growing list by showing that basophils enhance humoral memory responses by producing interleukins 4 and 6 in response to specific antigen.

Immunological memory takes the form of either existing circulating antibodies or expanded populations of antigen-specific B cells and T cells with potential effector functions¹. Among such functions is the recognition of previously encountered pathogens and reactivity toward allergens and other (noninfectious) antigens. After reencountering any antigen, circulating antibodies bind the antigen in the first step toward its elimination by phagocytic cells. Alternatively, reencounter of antigen by certain types of immune cells can lead to antigen presentation and/or cytokine secretion that then leads to 'reactivation' and population expansion of antigen-specific memory T cells and B cells. Two functionally different memory B cell types exist, two functionally different B cell types exist, (quiescent) memory B cells and long-lived antibody-secreting plasma cells, both of which are generated in germinal centers as the products of antigen selection, affinity maturation and differentiation. Long-lived plasma cells continuously secrete voluminous quantities of antibodies that circulate in the blood², whereas quiescent memory B cells wait for reexposure to antigen to reactivate them, at which time they expand their populations and differentiate into additional antibody-secreting cells^{3,4}. B220⁺CD19⁺ cells (non-B cells) have been shown to be the main antigen-binding cell population in the bone marrow, spleen and peripheral blood

after protein immunization. These 'antigen-capturing cells' have been subsequently characterized as basophils⁵, and their ability to capture antigens has been shown to be dependent on basophil-expressed high-affinity immunoglobulin E (IgE) receptors (FcεRI) and antigen-specific IgE molecules generated by B cells after immunization. Given the ability of basophils to also produce large amounts of interleukin 4 (IL-4) and IL-6, factors known to promote humoral B cell responses, Mack and colleagues, in this issue of *Nature Immunology*, evaluate the potential function of these 'antigen-capturing' basophils in amplifying antigen-specific memory responses in mice⁶.

Basophils are the least prevalent granulocytes in the circulation, representing less than 1% of blood leukocytes in healthy humans and mice. Basophils originate and mature in the bone marrow before entering the circulation. As basophils are the main source of IL-4 in allergen- and helminth parasite-activated peripheral blood mononuclear cells, they have been studied mainly in the context of allergy and parasite infection⁷. Similar to mast cells, basophils express FcεRI and secrete histamine, cytokines and other inflammatory mediators. But unlike the relatively fecund mast cell research, basophil research has been hampered in its progress because of the lack of basophil-deficient mouse models and limitations in the number of human basophils available. However, the tide now seems to have changed. Important findings have been made, notably the identification of basophil progenitors⁸ and the functions of basophils in IgE-mediated chronic allergic inflamma-

tion⁹, IgG1-mediated systemic anaphylaxis¹⁰ and protease allergen-induced differentiation of T helper type 2 (T_H2) cells¹¹.

Using as an antigen the fluorescent protein allophycocyanin (APC), which makes specific antibody easily detectable by flow cytometry, Mack and colleagues first show the long-term presence of APC-binding basophils after the first immunization as well as after restimulation⁶. They find that these basophils are activated by antigen and are the main sources of IL-4 and IL-6 in cultures of splenocytes and bone marrow cells from immunized mice. They show this by *in vitro* and *in vivo* depletion of basophils. Production of IL-4 and IL-6 by antigen-exposed basophils requires the Fc receptor γ-chain (Fcγ), which is the common signaling component of FcεRI, FcγRI and FcγRIII. Consistent with that, basophils lacking only FcεRI can still be activated by antigen, and residual activity seems to be due to FcγRIII (FcγRI is not expressed in basophils). Depletion of basophils 2 days before restimulation of APC-immunized mice leads to about 50% less APC-specific IgG1 and 60–80% less APC-specific IgG2a. Similar lowering of the frequency of APC-specific B cells and APC-specific plasma cells occurs in basophil-depleted mice after restimulation. The function of basophils in amplifying humoral memory response is also shown with two other antigens, phycoerythrin and pneumococcal surface protein A, with or without adjuvant. Notably, mice have less protection from sepsis after *Streptococcus pneumoniae* infection when they are vaccinated a second time with pneumococcal surface protein A in the absence of basophils⁶, thus demonstrating the clinical importance

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of basophil-mediated amplification of the memory response.

Adoptive transfer of splenocytes and bone marrow cells (depleted of plasma cells) from APC-immunized mice allows the transfer APC-reactive basophils to naive mice. Such recipient mice produce APC-specific IgG after being immunized with APC, but mice that had received basophil-depleted cells (depleted of plasma cells as well) produce much less APC-specific IgG1. These experiments emphasize the specific function of basophils in the memory but not the primary response.

Mack and colleagues have done additional *in vitro* studies that provide insight into how activated basophils enhance the humoral immune response (Fig. 1). Basophils activated by IL-3 or antibody to FcεRI induce B cell proliferation and the production of IgM and IgG1 in the presence of activated CD4⁺ T cells; this B cell proliferation and immunoglobulin production requires IL-6, IL-4 and cell contact. The IL-6 is derived from activated basophils, and interactions between CD40 and its ligand (CD40L) are also involved in these functions (CD40L is expressed on basophils). The *in vitro* results collectively indicate that activated basophils enhance the humoral memory response by secreting IL-6 and by altering the phenotype of CD4⁺ T cells so that they are better B cell 'helpers' (that is, by inducing CD4⁺ T cell upregulation of IL-4, IL-5, IL-10, IL-13 and the transcription factor GATA-3 and down-regulation of interferon-γ and IL-2)⁶.

The key to the success of this study seems to be the development by Mack and colleagues of a protocol to deplete mice of basophils *in vivo*: intraperitoneal injection of 5 μg of the monoclonal antibody MAR-1, which recognizes the FcεRI α-chain (FcεRIα), administered twice daily for 3 days keeps basophils undetectable for 12 days or more in most treated mice. This treatment does not seem affect CD4⁺ T cells, B cells, monocytes or dendritic cells, although it does result in a mild diminution in mast cells; to control for this additional affect of MAR-1, Mack and colleagues also demonstrate the importance of basophils but not mast cells in enhancing memory response by using mast cell-deficient mice. As no basophil-deficient mice are available yet, the new basophil-depletion protocol, as well as a basophil-specific monoclonal antibody⁹, will be useful

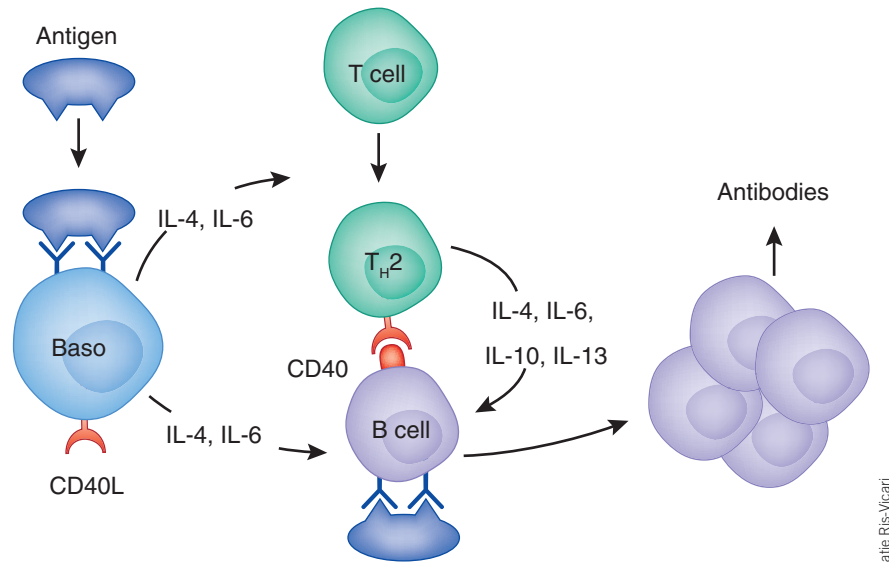


Figure 1 Mechanisms for basophil-mediated amplification of the humoral memory response. After secondary immunization, basophils (Baso) expressing FcεRI capture antigens through antigen-specific IgE previously produced by B cells after primary immunization. Activated basophils then secrete a panel of mediators, including IL-4 and IL-6, and express cell surface receptors, including CD40L. T cells exposed to activated basophils adopt a TH2 phenotype and induce exaggerated B cell proliferation and antibody production in the presence of basophils. Basophil-derived IL-6 is critical and IL-4 and cell contact (through CD40L-CD40 interactions) contribute to this process. It is possible that basophils may make a direct contact with B cells through CD40L-CD40 interactions (not presented).

in future investigations of basophil-specific functions.

As with most breakthroughs in biology, this study also raises important issues that need to be addressed in the future. First, it is not clear whether the observed enhancing effects of basophils on humoral memory response are specific for the TH2 response. Here, basophils are shown to amplify not only TH2-associated IgG1 but also TH1-associated IgG2a responses when mice are immunized with APC in the presence of heat-killed *Bordetella pertussis* (TH2-skewing adjuvant) or with pneumococcal surface protein A without adjuvant. Although these results suggest that basophil-mediated amplification of the memory response is not specific for TH2 'help' for B cells, it will be useful to test immunization together with TH1-skewing adjuvants, such as Freund's complete adjuvant, with which very few IgE molecules are produced. Second, it is not clear whether there are functional differences between basophil-bound IgE and IgG. IgE seems to have considerable involvement in amplifying memory response in the conditions studied. However, supposedly residual functions for basophil-bound IgG via FcγRIII might be

more important in other immunization settings. Third, it will be useful to determine where basophil-mediated regulation of T cells and B cells takes place. For example, although a published study has shown that B220⁺ IgE⁺ cells, assumed to be B cells, are not localized to the germinal center after primary immunization¹², it has not been formally tested whether such cells actually are basophils. Finally, it will be very important to study whether basophils have the same function in human immune responses.

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