Eosinophils and IgE Receptors: A Continuing Controversy

By Hirohito Kita and Gerald J. Gleich

DURING THE PAST 2 decades, considerable new information has been obtained about the functions of the eosinophil and its roles in human disease. Presently, the eosinophil is recognized as a proinflammatory granulocyte implicated in protection against parasitic infections and believed to play a major role in allergic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis. The eosinophil is an important source of cytotoxic cationic proteins, such as major basic protein (MBP), eosinophil peroxidase, and eosinophil cationic protein. These proteins are potentially two-edged swords; on the one hand, they protect the host against overwhelming helminth infections, but on the other hand, they damage the host’s tissues. Eosinophils also induce inflammation by releasing lipid mediators, oxygen metabolites, and cytokines. Numerous studies have shown the association of eosinophils and various human parasitic and allergic diseases. For example, at present, the most common worldwide cause of eosinophilia is probably infection with helminths, and high eosinophil counts correlate with lack of reinfection after treatment of Schistosoma haematobium infections. Analyses of patients infected with Onchocerca volvulus have shown striking deposition of the eosinophil granule MBP around degenerating microfilaria. In allergic asthma, eosinophilic and lymphocytic infiltration in the epithelium and lamina propria of the airways are consistently found even in mild and stable asthma. Indeed, correlations have been observed between the numbers of infiltrating eosinophils and asthma disease severity. Pulmonary segmental allergen challenge in allergic individuals causes eosinophil recruitment into the airways; this is associated with the release of eosinophil granule proteins and the increase in vascular permeability. Despite the strong associations among eosinophils, their cytotoxic granule proteins, and human diseases, the mechanism(s) responsible for eosinophil activation in vivo is largely unknown.

Helminth infections and allergic diseases are characteristically associated not only with peripheral blood and tissue eosinophilia, but also with high levels of both total and antigen-specific IgE antibodies. IgE antibodies may be involved in disease in three ways. First, the central feature in anaphylactic and immediate hypersensitivity reactions is IgE-dependent activation of mast cells and basophils leading to the release of histamine and other inflammatory mediators, such as prostaglandins and leukotrienes. Furthermore, upon activation through IgE receptors, human mast cells and basophils produce cytokines, such as interleukin-4 (IL-4) and IL-5, which are potentially important in the recruitment of eosinophils, thus causing chronic allergic inflammation. Second, IgE bound to receptors on antigen-presenting cells, such as CD23 on B cells and to high-affinity IgE receptors (FcεRI) on Langerhans’ cells and monocytes, can enhance antigen internalization and presentation to T cells, resulting in continuous activation of the immune system. Finally, IgE may mediate killing of the invading helminth and host cell damage by acting as a ligand for antibody-dependent cell-mediated cytotoxicity (ADCC) by macrophages and other immune cells. In fact, immunopathological studies showed a significant correlation between the production of antischistosome IgE antibodies and the acquisition of immunity against reinfection to S haematobium. In allergic diseases such as bronchial asthma, there is a close correlation between serum IgE levels and the prevalence and severity of these diseases. Thus, there is now converging evidence to support roles for IgE in resistance to helminthic infections and in the pathophysiology of allergic diseases in humans. Therefore, it is reasonable to speculate that IgE is involved in the activation of eosinophils in these diseases.

Early studies on the killing of schistosomula in vitro by human eosinophils used cells purified from normal or slightly eosinophilic individuals, together with heat-inactivated sera from individuals with schistosome infection. The results of these studies suggested that killing requires IgG and is independent of complement. IgG1 and IgG3 subclasses were effective in mediating ADCC by human eosinophils, whereas IgM, IgG2, and IgG4 were not only inactive, but blocked the effects of the active subclasses. A quite separate phenomenon was observed with low density, so-called hypodense eosinophils that can be isolated from individuals with very high eosinophil counts. Receptors for IgE were identified on both rat and human hypodense eosinophils, and hypodense human eosinophils were shown to kill schistosomula in the presence of IgE. Subsequently, this human eosinophil IgE receptor was shown to be similar,
but not identical, to the low-affinity IgE receptor (FcεRII) expressed on B cells (CD23). Eosinophils from patients with eosinophilia express another low-affinity IgE-binding molecule belonging to the S-type lectin family, called Mac-2. The cytotoxic function of eosinophils was abolished by the antibody against this molecule. More recently, in 1994, Gounni et al described FcεRI on eosinophils from patients with marked eosinophilia. The evidence to support this claim was comprehensive and included the following: inhibition of [125I] IgE binding to eosinophils by anti-FcεRI a-chain monoclonal antibody (clone 15.1); surface expression of FcεRI a-chain by flow cytomtery; immunostaining of tissue eosinophils with 15.1; the demonstration of FcεRI α-, β-, and γ-chain transcripts; release of eosinophil granule proteins after stimulation of eosinophils with 15.1; and inhibition of IgE-dependent eosinophil ADCC against schistosome targets by 15.1. Altogether, these findings suggest that human eosinophils express three receptors for IgE, namely FcεRI, FcεRII, and Mac-2, and that IgE induces eosinophil mediator release and ADCC through these receptors. Thus, on the basis of these reports, IgE-mediated activation of eosinophils was implicated as an important mechanism for host defense and in the pathophysiology of human disease.

However, the seemingly strong association between the eosinophil and disease becomes confusing and controversial in mice. For example, in helminth-infected animals, antibodies to IL-5 suppressed blood eosinophilia and eosinophil infiltration into the tissues. However, ablation of eosinophilia by anti–IL-5 was not associated with a diminution of resistance in mice infected with *S mansoni* or with *Trichinella spiralis*. Similarly, anti–IL-4 depletion of IgE responses failed to interfere with protective immunity to *S mansoni*. These findings suggest that neither eosinophils nor IgE are critical for immunity to these parasites in the mouse. In contrast, mice infected with *Trichuris muris* showed the exact opposite: their resistance to infection was associated with the production of Th2 cytokines, such as IL-4 and IL-5, tissue eosinophilia, and intestinal IgA production. In murine models of asthma using BALB/c mice sensitized and challenged with ovalbumin (OVA), one study showed that neither IL-5 nor eosinophils are required for airway hyperresponsiveness. In contrast, another study in which the *C57BL/6* mice rendered IL-5 deficient by homologous gene recombination were sensitized and exposed to OVA, the animals failed to develop eosinophil infiltration into the lungs, airway hyperresponsiveness, and lung damage; their littermate controls showed all these responses. Reconstitution of IL-5 production with recombinant vaccinia viruses completely restored antigen-induced eosinophilia and airway dysfunction in these IL-5–deficient mice, suggesting a central role for IL-5 and eosinophilia in the pathogenesis of allergic lung disease. The inconsistencies among these murine disease models, as well as the inconsistencies between the human and mice findings, allows recognition of potential difference(s) between human and mouse eosinophilic leukocytes.

Previously, Lopez et al have described the distribution of Fc receptors on eosinophils isolated from mice infected with the cestode, *Mesocestoides corti*. In these reports, the investigators determined that murine eosinophils, similarly to human eosinophils, expressed the type II Fc receptor for IgG (FcεRII), but they were unable to detect any IgE binding to mouse eosinophils. More recently, Jones et al thoroughly examined eosinophils obtained by bronchoalveolar lavage (BAL) from the lungs of CBA/J mice infected with *Toxocara canis* by flow cytomtery. They found that murine eosinophils are negative for surface IgM (sIgM), sIgA, sIgE, and FcεRI, but are positive for sIgG1 and FcεRII. Furthermore, they showed that culturing eosinophils for 24 or 48 hours with exogenous IgE and/or IL-4 did not induce IgE binding capacity or FcεRII expression. In this issue of *Blood*, de Andres et al expand these studies considerably by including FcεRI and Mac-2 and by examining mRNA transcripts and receptor-mediated cellular functions. de Andres et al examine murine eosinophils from two sources, namely eosinophils isolated from liver granuloma of CBA mice infected with *S mansoni* and bone marrow cells isolated from BALB/c mice and cultured with a combination of eosinophil growth factors. The results from these two different cell sources are virtually identical. Murine eosinophils lack IgE receptor expression; neither surface expression of FcεRII or Mac-2 nor binding of murine IgE to the cells could be detected. Reverse transcription polymerase chain reaction (RT-PCR) analyses did not detect mRNA transcripts for the α-chain of FcεRI or FcεRII, but did detect Mac-2 mRNA. In vitro culture of granuloma eosinophils did not induce IgE-binding or expression of IgE receptors. In contrast to the lack of IgE receptors, functioning IgG receptors, including FcεRIIb and FcεRIII, were detected on granuloma eosinophils, consistent with previous observations by others.

Studies of receptor expression have a number of potential pitfalls. For example, transcription of mRNA or even the presence of synthesized receptor protein within the cell does not necessarily indicate the expression of the receptor on cell surface. Another question concerns the potential discrepancies between receptor expression and actual functioning of the receptor. An antibody raised against an IgE receptor expressed on one cell type may not recognize the IgE receptor on another cell type, the best example being lack of binding of the antibody against human B-cell FcεRII (CD23) to human eosinophils. Finally, precautions are needed to minimize or eliminate contamination by other cell types, particularly in highly sensitive RT-PCR analyses. The careful and well-designed study by de Andres et al published in this issue of *Blood* examined transcription of mRNA, surface receptor expression, IgE-binding capacity, and receptor-mediated cellular function; thus, they seem to address all of these potential problems. Their observations, together with previous reports by others, provide convincing evidence that murine eosinophils, seemingly unlike human eosinophils, lack IgE receptors. Therefore, murine eosinophils may use the FcεRIII, complement receptors, and/or possibly FcεRI in their antigen-dependent cellular functions; these may explain the differences between mouse and human observations and the discrepancies among findings made in mice.

The issues regarding expression of IgE receptors on mouse
and human eosinophils deserve some comments and cautions. First, expression of IgE receptors on human eosinophils and their IgE-dependent functions are not phenomena commonly seen in eosinophils from all sources. Almost all the studies that showed the presence of IgE receptors on human eosinophils have used eosinophils from patients with marked eosinophilia, including the hypereosinophilic syndrome and diseases associated with skin disorders and lymphomas.18,19,24,25 There are no data to support expression of IgE receptors on eosinophils from healthy donors and/or subjects with mild to moderate eosinophilia due to more common conditions, such as allergy and helminth infection. Three studies that sought IgE receptors in these conditions did not detect Fc,RII on peripheral blood eosinophils,36,37 and detected only minimal levels of Fc,RI expression on eosinophils infiltrating into the bronchial tissues of patients with asthma38 and on blood eosinophils from patients with allergic rhinitis.37 Furthermore, IgE-dependent functions of eosinophils were not observed in blood eosinophils from normal individuals, whereas these eosinophils did respond vigorously to IgG1 and IgG3 through Fc,RII.16,39,40 Another level of complexity is added by the discrepancy between receptor expression and IgE-mediated cellular functions of eosinophils. Normal eosinophils, which fail to mediate IgE-dependent ADCC, do mediate such killing after activation with platelet-activating factor (PAF) even in the absence of the expression of IgE receptors.31

Second, in association with the activation status issue described above, expression of IgE receptors on eosinophils may be tightly regulated by various environmental factors. For example, in humans the expression of Fc,RII was limited to eosinophils from persons with marked eosinophilia and, among them, to a unique cell population of hypodense eosinophils.19,42 Expression of the mRNA transcript for Fc,RI α-chain was detected in peripheral blood eosinophils from patients with allergic rhinitis, but not in those from normal individuals.35 In addition, transcription of mRNA for Fc,RI α-chain was enhanced by IL-4 in these human eosinophils,35 similar to results with human mast cells.43 The expression of Fc,RII is tightly regulated in a tissue-specific manner, and IL-4 again is the most potent inducer of Fc,RII expression for various types of cells.44 Therefore, those blood or tissue conditions with abundant IL-4 may favor the expression of IgE receptors on eosinophils. Because they used eosinophils isolated from an almost ideal source, namely liver granuloma of mice infected with S. mansoni, the findings by de Andres et al34 are particularly informative. In addition to tissue and disease specificity, mice may also display another level of complexity. Because strains of mice differ greatly in their capacities to produce IL-4 and high levels of serum IgE,45 expression of IgE receptors on eosinophils may differ, depending on the strain. By using mice selected for heightened production of IgE, Eum et al46 concluded that the recruitment of eosinophils to the airways and high IgE titers are both required for lung pathology of allergic BP2 mice, and they speculated that IgE activation of eosinophils is important in this mouse model. It would be interesting if eosinophils from these animals were subjected to the rigorous analyses used by de Andres et al.34 Furthermore, van der Vorst et al47 reported the in situ localization of IgE cytophilic antibodies on murine eosinophils in the gut tissues of Swiss albino mice infected with Hymenolepis diminuta, although this study needs to be interpreted with caution due to the ubiquitous expression of IgE binding proteins in murine tissues.48 Thus, as summarized in Table 1, IgE receptor expression may be disease, tissue, species, and/or strain specific, and the observations obtained in a certain condition should not be generalized.

Finally, several published articles from investigators who studied human eosinophil IgE receptors need to be reconciled. In 1988, the eosinophil IgE receptor was originally found to have a low affinity (Kd of 10⁻⁷ mol/L) and to

<table>
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<th>Authors</th>
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Abbreviations: BM, bone marrow; (+/−), minimal surface expression of receptor or presence of mRNA without surface expression of receptor protein.

* IgE-dependent function after stimulation with PAF.
possess a molecular weight corresponding to that of FcRII.\textsuperscript{20,24} IgE-binding and IgE-dependent ADCC to schistosomula were completely inhibited by antibody to either FcRII\textsuperscript{19} or Mac-2.\textsuperscript{24} Hence, earlier studies showed evidence for the expression of the low-affinity IgE receptor without any evidence for the high-affinity IgE receptor, FcRI. However, later in 1994, the same investigators reported the presence of FcRI on human eosinophils, and all of the IgE-dependent functions of human eosinophils were explained by this receptor.\textsuperscript{25} Again, IgE-dependent ADCC for schistosomula was essentially completely inhibited by antibody to FcRI, raising an obvious question as to how the IgE binding and ADCC can be totally inhibited by antibodies against three distinct IgE receptors. Thus, more confirmatory work is needed to finally resolve the presence or absence of IgE receptors on human eosinophils, as well as on mouse eosinophils.

In conclusion, with the recent development of techniques to manipulate genes and with the increased availability of a wide variety of immunologic reagents for mice, the numbers of murine models of human immunity and disease have strikingly increased. The studies by de Andres et al.\textsuperscript{16} in this issue of Blood warn us of potential cellular differences between mouse and human immunologic responses and encourage us to reexamine the suitability of mouse models for human diseases. At the same time, their report raises unanswered questions regarding the expression of IgE receptors on human eosinophils. Further studies on IgE receptors on mouse and human eosinophils may solve the existing controversies and help to interpret and to understand the pathophysiologic mechanisms of human eosinophilic disorders, the roles of eosinophils in human immunity, and their murine models.

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