

Murine models of inflammation: role of CD23

The role of IgE in eosinophil recruitment and bronchial hyperresponsiveness has been extensively studied with murine models of inflammation. Many investigators using various knockout models have clearly shown that both IgE-dependent and -independent pathways play a role in eosinophil recruitment and bronchial hyperresponsiveness after allergen challenge, illustrating the complexity of airways inflammation. The expression of this response is likely to involve many interacting pathways, and it will be a considerable challenge to determine key points within these pathways that will yield novel targets for future therapeutic strategies.

Y. Riffo-Vasquez, S. Pitchford

Sackler Institute of Pulmonary Pharmacology, Division of Pharmacology and Therapeutics, GKT School of Biomedical Sciences, London

D. Spina

Sackler Institute of Pulmonary Pharmacology, Department of Respiratory Medicine and Allergy, GKT School of Medicine, King's College London, London, UK

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D. Spina

Sackler Institute of Pulmonary Pharmacology
Department of Respiratory Medicine and Allergy
GKT School of Medicine
King's College London
Bessemer Rd
London SE5 9PJ
UK
Tel. (0171) 346 3169
Fax: (0171) 346 3589
Email: domenico.spina@kcl.ac.uk

Allergy is caused by overproduction of the antibody immunoglobulin E (IgE) in response to common environmental antigens such as those present in pollen, foods, house-dust mites, animal danders, fungal spores, and insect venoms. A number of studies have found a strong correlation between total serum IgE levels and bronchial hyperresponsiveness in asthma (1–3), and this trait appears to be specific for chromosome 5 (4) and chromosome 11q13 (5). IgE exerts its action via specific receptors to which it binds, the “high-affinity” IgE receptor Fc ϵ RI (receptor for the Fc region of the ϵ -chain of IgE) and a “low-affinity” IgE receptor, Fc ϵ R2 (CD23). Both the Fc ϵ RI and the Fc ϵ R2 are expressed on eosinophils, monocytes, platelets, macrophages, and Langerhans' cells, and binding of IgE to these receptors can alter the function of these cells (6). The Fc ϵ RI, which is located on mast cells and basophils, is responsible for immediate hypersensitivity reactions. By virtue of its ability to associate with a number of different ligands, CD23 can have both stimulatory and negative influences on IgE production and inflammatory responses (7, 8). This review will summarize the role of IgE and CD23 in inflammation and also discuss the role of IgE-independent pathways in this response.

CD23

CD23 is thought to mediate a number of effects including regulation of IgE synthesis, antigen presentation, proliferation and differentiation of B cells, and activation of monocytes, effects which can be ascribed to the membrane and soluble forms of CD23 (9). Soluble CD23 (sCD23) augments spontaneous production of IgE (10), while antibodies directed against sCD23 have been shown to inhibit the synthesis of IgE by mononuclear cells from atopic subjects (11) and rats (12). Similarly, monoclonal antibodies directed against CD23 produced a small decrease in IgE production in mice (13). In contrast, CD23 efficiently focused protein antigens bound to IgE for antigen presentation to T cells and antigen focusing to B cells in the mouse *in vitro* (14, 15). Studies in man have shown that this process of antigen focusing by CD23 (16, 17) involves activation of CD21 on B cells (9). In contrast, cross-linking of membrane-bound CD23 by antigen results in inhibition of B-cell growth and differentiation in man (11, 18–20). Interestingly, monoclonal antibodies directed against CD23 inhibited IL-2-R expression in CD8-positive, but not CD4-positive, cells and produced modest inhibition of B-cell activation in mice (13).

Studies using CD23 knockout mice reveal that CD23-deficient mice produce higher levels of IgE than their wild-type counterparts (8, 13, 21, 22) without any significant effect on B- or T-cell growth and differentiation (8, 23, 24). Conversely, mice overexpressing membrane, but not soluble, CD23 exhibit a weaker IgE response (25). In contrast, CD23 knockout mice do not present IgE-mediated enhancement of IgG1 production, a fact which suggests that CD23 can play a role in antigen focusing (23). Thus, it is clear that the overwhelming evidence supports the notion that the major action of CD23 *in vivo* is to impart a negative signal for IgE production in murine models of inflammation.

IgE-dependent mechanisms

A number of studies have investigated the role of IgE-dependent pathways on the recruitment of eosinophils and bronchial hyperresponsiveness in murine models of inflammation. Thus, the passive transfer of ovalbumin-specific IgE (26) or ovalbumin-specific B cells (27) to naive animals, and subsequent challenge with antigen, resulted in pulmonary recruitment of eosinophils. In contrast, antibodies directed against IgE can inhibit eosinophil recruitment after antigen challenge in mice (28, 29). A similar observation has been made after intravenous administration of rhuMAB-E25, a monoclonal antibody directed against the Fc ϵ R1-binding domain of human IgE. Thus, both the late asthmatic response and attendant eosinophilia in bronchoalveolar lavage fluid after antigen challenge were significantly reduced in allergic asthmatics after treatment with rhuMAB-E25 (30). These studies support the view that IgE-dependent mechanisms can induce the recruitment of eosinophils to the airways. However, it was of interest that the ability of IgE to recruit eosinophils to the airways in passively immunized mice was significantly less than that observed in actively immunized mice challenged with antigen (26), suggesting that factors additional to IgE are required for the recruitment of eosinophils to the airways.

Therefore, it is of interest that mice which have a disruption in the expression of the μ gene, and are thereby unable to produce immunoglobulins, the so-called B-cell-deficient mice (31–33), and mice with a disrupted gene encoding the heavy chain of IgE (34), or CD40-deficient mice (35), retain the capacity to recruit eosinophils after antigen challenge of immunized animals. Taken together, these studies suggest that both IgE-dependent and -independent mechanisms can induce the recruitment of eosinophils to the airways. In support of this view, we have demonstrated that acute antigen challenge results in significantly greater recruitment of eosinophils in CD23 knockout mice than wild-type counterparts, a fact which may be a consequence of

the significantly higher levels of IgE observed in CD23 knockout mice (22). However, after chronic challenge protocols, the recruitment of eosinophils to the airways is not different between CD23 knockout and wild-type mice, suggesting that IgE- and CD23-independent mechanisms play a greater role in the recruitment of eosinophils to the airways after chronic antigen exposure (22). Moreover, a study utilizing a relatively short period of exposure to antigen (36) compared with other studies (28, 37, 38) demonstrated a significant impairment in the recruitment of eosinophils to the lung after antigen challenge in mast-cell-deficient mice.

It would appear that IgE-dependent and -independent mechanisms may also account for bronchial hyperresponsiveness in murine models of inflammation. Thus, passive transfer of IgE or IgG1 (26), or ovalbumin-specific B cells (27), to naive animals and subsequent challenge with antigen resulted in a significant increase in tracheal smooth-muscle sensitivity to electrical field stimulation. It is unclear what the exact relationship is between increased tracheal smooth-muscle sensitivity to endogenously released acetylcholine and bronchial hyperresponsiveness *in vivo*. However, administration of a monoclonal antibody against IgE before antigen challenge suppressed bronchial hyperresponsiveness in immunized mice (29).

Similarly, under conditions in which serum levels of ovalbumin-specific IgE are raised, as in CD23-deficient mice, bronchial hyperresponsiveness is significantly increased after antigen challenge compared with wild-type mice (13, 21, 22). From these studies, we conclude that CD23 may negatively regulate bronchial hyperresponsiveness via a pathway that remains to be established. Alternatively, there may exist a possible role for CD8 T cells in modulating the bronchial hyperresponsiveness observed in CD23 knockout mice. We have demonstrated a tendency for an increase in the recruitment of CD8 T cells in CD23 knockout mice (22). Conversely, IL-2-receptor expression is reduced in CD8-positive T cells in mice treated with a monoclonal antibody to CD23, implying that CD8 T-cell activation is reduced, and this treatment also resulted in a reduction in bronchial hyperresponsiveness in a murine model of inflammation (13). Clinical studies of late-phase reactions in asthma have provided compelling evidence that CD8 T cells can moderate antigen-induced airway inflammation (39). In an animal model of airway inflammation, adoptively transferred ovalbumin-specific CD4 T cells induced eosinophilic airway inflammation and bronchial hyperresponsiveness in naive rats after ovalbumin challenge, while CD8 T cells did not (40). Furthermore, depletion of CD8 T cells was found to increase the late-phase response after bronchial challenge (41) and have no effect on bronchial hyperresponsiveness in allergic rats (42). In contrast,

CD8 T cells were shown to be critical for eosinophil recruitment and bronchial hyperresponsiveness in a murine model of inflammation (43), although other studies have reported that CD8 T cells downregulated bronchial hyperresponsiveness (44) or had no impact on eosinophil recruitment to the airways (45). Hence, the role of CD8 T cells as effector cells which contribute to the bronchial hyperresponsiveness in CD23 knockout mice remains unclear. Another plausible explanation of the increase in airways responsiveness observed to spasmogens in CD23 knockout mice is that it is secondary to the elevated levels of IgE.

In contrast, anaphylactic bronchoconstriction (46) and bronchial hyperresponsiveness to muscarinic agonists after antigen challenge were not altered in IgE- (34) and B-cell-deficient mice (33). Interestingly, an earlier study had shown bronchial hyperresponsiveness to be significantly diminished in B-cell-deficient mice compared with wild-type mice (32). Differences in the strain of mice and antigen challenge protocols may account for these differences and further support the view that bronchial hyperresponsiveness can also be induced by IgE-dependent and -independent mechanisms.

IgE-independent mechanisms

While a number of studies have demonstrated IgE-dependent mechanisms for the recruitment of eosinophils to the airways and bronchial hyperresponsiveness after antigen challenge, it is clear that other mechanisms additional to IgE play a role in this regard. There is a considerable body of evidence supporting a role for CD4⁺ T cells in murine models of inflammation. Thus, pulmonary recruitment of eosinophils is significantly impaired 24–48 h after antigen challenge of mice previously treated with a monoclonal antibody directed against CD4 (45). Interestingly, the recruitment of eosinophils to the lung 9 h after antigen challenge was not inhibited in this model, suggesting that CD4 T-cell-independent mechanisms could play a role in the early recruitment of eosinophils to the airways. The role of IgE-dependent pathways in the recruitment of eosinophils during this acute phase may account for this finding, particularly in view of the discussion outlined above. CD4⁺ T cells have also been implicated in bronchial hyperresponsiveness (47, 48), and a recent study has demonstrated that the adoptive transfer of Th0 and Th2 clones into SCID mice induces both pulmonary eosinophilia and bronchial hyperresponsiveness after antigen challenge (49). In contrast, the adoptive transfer of Th1 cell clones into SCID mice resulted in a monocytic infiltrate into the lung without any attendant bronchial hyperresponsiveness (49).

Similarly, the adoptive transfer of T cells from genetically hyper-responsive mice increased airways responsiveness in hyporesponsive recipient mice (50). The T-cell factors responsible for these findings remain to be established. However, the role of various cytokines released from Th2 cells, including IL-4, IL-5, and IL-13, upon antigen-induced eosinophilia and bronchial hyperresponsiveness have been investigated in a number of studies using knockout models and monoclonal antibodies directed against these cytokines.

While IL-4 has been shown to be important for Th2-cell commitment and isotype switching of B cells to the IgE phenotype (51), the role of IL-4 in allergen-induced recruitment of eosinophils and bronchial hyperresponsiveness is controversial. Thus, eosinophil recruitment is significantly attenuated in IL-4-gene-disrupted mice (52, 53) or in mice treated with monoclonal antibodies to IL-4 during antigen immunization (53) before antigen challenge (54) and in mice treated with IL-4-receptor antibodies before antigen challenge (55). In contrast, other studies have shown that treatment with antibodies directed against IL-4 before antigen sensitization (56) or before antigen challenge (53, 56) had no effect on eosinophil recruitment to the airways. Interestingly, a recent study has shown that eosinophil recruitment to the lungs still occurs in IL-4-deficient mice (35) or in mice after the adoptive transfer of Th2-cell clones from IL-4-deficient mice (57). However, in both cases, the migration of eosinophils from the tissue to the lumen appears to be impaired, although the mechanism of this phenomenon remains to be established (35, 57). Similarly, both IL-4-dependent (52, 56) and -independent (35, 48, 57) mechanisms contribute to the bronchial hyperresponsiveness observed in murine models of inflammation. It would therefore appear that both IL-4-dependent and -independent mechanisms can be expressed in different murine models of inflammation. These differences cannot be solely explained by the use of different strains of mice, since the conflicting data have arisen with mice of a similar genetic background (35, 37, 52). A more plausible explanation is that under the different experimental conditions employed, CD4 T cells may induce antigen bronchial hyperresponsiveness and eosinophilia via IL-4-dependent and -independent pathways. This conclusion is further substantiated by the finding that a subpopulation of Th2 cells is not dependent upon IL-4 for development (48, 58).

Another cytokine which has received considerable attention is IL-5. Monoclonal antibodies directed against IL-5 attenuate eosinophil recruitment in allergic mice and bronchial hyperresponsiveness (45, 59–61). Conversely, eosinophil recruitment is compromised in IL-5-gene-disrupted mice, and bronchial hyperresponsiveness induced by antigen is inhibited (61); if IL-5 is

overexpressed in the lung, bronchial hyperresponsiveness is observed in nonsensitized mice (62). However, in some circumstances, IL-5 is not obligatory for the development of bronchial hyperresponsiveness (48), nor is IL-4, as monoclonal antibodies directed against IL-4, in IL-5 knockout mice, failed to abrogate the hyperresponsiveness after antigen challenge. In contrast, monoclonal antibodies directed against CD4 T cells were sufficient to abolish this response (48). Therefore, other factors released from the T cell may be important, and it has recently been shown that monoclonal antibodies directed against IL-13 were sufficient to inhibit antigen-induced bronchial hyperresponsiveness (63, 64). However, the role of IL-13 in the recruitment of eosinophils to the airways remains controversial (63, 64).

Conclusion

It is evident that IgE-dependent and CD4 T-cell-dependent pathways operate to induce eosinophil recruitment and bronchial hyperresponsiveness in the airways. These pathways can be stimulated independently, or can occur together, but under different time courses. Furthermore, it is increasingly apparent that considerable redundancy exists in the role of Th2 cytokines in the inflammatory response, and the contribution of each cytokine to this response may overlap or occur at different times during the inflammatory response.

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