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Review

Murine models of asthma in understanding immune dysregulation in human asthma

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1. Introduction

Asthma is a chronic inflammatory disease that has been on the rise in recent years despite increased use of medication. Nevertheless, the fundamental mechanisms that underlie the development and perturbation of the asthmatic state remain elusive. Asthma is characterized by variable airflow obstruction, airway hyperresponsiveness (AHR) and airway inflammation. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall with mast cells, lymphocytes and eosinophils. Although asthma is multifactorial in origin, recent advances suggest that asthma is an immune disease with a prominent role for T lymphocytes in the pathogenesis. In particular, $CD4^+$ T cells producing a Th2 pattern of cytokines (interleukin (IL)-4, IL-5, IL-13, IL-9) have been hypothesized to play a pivotal role in the pathogenesis of this disease (Gerblich et al., 1991; Robinson et al., 1992; Walker et al., 1992). These T cell-derived cytokines work in concert with chemokines and mediators released locally by the airway epithelium to orchestrate the recruitment and activation of the primary effector cells of the allergic response, the mast cell and the eosinophil. Activation of these effector cells results in the release of a plethora of inflammatory mediators that individually or in concert induce changes in airway wall geometry resulting in the symptoms of the disease.

Although considerable descriptive evidence suggests that $CD4^+$ T lymphocytes and Th2 cytokines are important in the pathogenesis of AHR in asthmatic humans, definitive proof is difficult to obtain in humans. Therefore, experimental animal models have been extremely useful in delineation of the role of $CD4^+$ T cells and T-cell-derived cytokines in the pathogenesis of asthma. The murine model has been a particularly valuable model because a wealth of immunologic reagents are available for the study of immune responses in this species. In addition, one of the attractive features of utilizing mice to study the pathogenesis of disease is the availability of over 200 well-characterized inbred strains and the ability

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to delete or overexpress specific genes through knockout and transgenic technologies.

2. Role of T cells in the pathogenesis of asthma

Direct evidence for a causal role for $CD4^+$ T cells in the development of AHR has been provided in mice devoid of CD4 T cells (Gonzalo et al., 1996) and in those treated with anti-CD4 mAb (Gavett et al., 1994). Definitive evidence for a pathogenic role for Th2 cells is provided by the fact that adoptive transfer of Th2 cells into the lungs of naive mice induces AHR and allergic inflammation (Li et al., 1996; Cohn et al., 1998). On the other hand, transfer of Th1 cells results in an inflammatory response, but no AHR (Cohn et al., 1998). Furthermore, studies in which administration of agents such as IL-12 and IFN-γ that inhibit Th2 cytokine production and stimulate Th1 pathways have been shown to prevent the development of antigen-induced AHR and inflammation in murine models (Lack et al., 1996; Gavett et al., 1995).

Despite the recognition that Th2 cytokines play a pivotal role in the development of the allergic diathesis, the exact mechanisms by which they induce asthma and AHR are still unknown. The major focus has been on the paradigmatic type 2 cytokines, IL-4 and IL-5. Both are thought to be central to the development of the allergic phenotype through their ability to drive IgE synthesis by B cells (Finkelman et al., 1988), and their critical involvement in the production, recruitment and activation of eosinophils (Wang et al., 1989). Indeed, early murine studies in which the levels of these cytokines were manipulated either through antibody blockade (Lukacs et al., 1994; Kung et al., 1995; Coyle et al., 1995) or gene targeting (Brusselle et al., 1995; Rankin et al.,1996; Foster et al., 1996) supported a role for these cytokines in allergen-driven pathophysiologic processes. However, more recent studies suggest that perhaps these two cytokines are not necessarily essential for the development of AHR (Coyle et al., 1995; Corry et al., 1998; Hogan et al., 1998). First, several groups showed that while antibody-mediated blockade of IL-4 during allergen sensitization ablates the development of allergic asthma, similar blockade of IL-4 prior to or during antigen challenge inhibits neither allergic inflammation nor AHR (Coyle et al., 1995; Corry et al., 1998). This strongly suggested that, while IL-4 plays its well-recognized immunoregulatory role in generating Th2 deviation in these models, it is not necessary for the expression of allergic asthma. This was further supported by the finding that transfer of Th2 cells derived from IL-4 deficient mice was still able to confer AHR (Cohn et al., 1998). Further evidence that neither IL-4 nor IL-5 were essential for the expression of AHR was provided by the finding that mice rendered deficient in both IL-4 and IL-5 still develop AHR in response to allergen sensitization and challenge (Hogan et al., 1998). Interestingly, Gavett et al. (1997) found that blockade of the IL-4 receptor alpha chain prior to antigen provocation in sensitized mice effectively inhibited AHR, eosinophilic accumulation, and mucus hyperplasia. Furthermore, Kuperman et al. (1998) showed that a deficiency in the signal transducer and activator of transcription-6 (STAT6) molecule, which mediates most of the cellular actions of both IL-4 and IL-13 (Zurawski et al., 1993), abolished antigen-induced eosinophilic inflammation and AHR. These results suggested that the steps distal to IL-4Rα ligation were clearly important in the development of allergic responses, but that IL-4 did not appear to be the ligand. As the T cell-derived cytokine, IL-13, also signals through this pathway, it was proposed that the effectiveness of IL-4 receptor blockade was due to inhibition of IL-13 mediated processes not those mediated by IL-4.

This hypothesis was supported by our recent finding that blockade of IL-13 at the time of antigen challenge in antigen-sensitized mice via administration of a soluble IL-13Rα2-Ig, which only binds IL-13, ablated antigen-induced AHR (Wills-Karp et al., 1998). Interestingly, the reversal of antigen-induced AHR was not associated with suppression in either IgE levels or BAL eosinophil numbers. However, antigen-induced mucus hyperplasia was inhibited by IL-13 blockade. Further proof of its importance in this response was provided by the finding that delivery of the recombinant cytokine to the lungs of naive animals reproduced many features of the allergic phenotype (i.e. AHR, eosinophilia, mucus hyperplasia) (Wills-Karp et al., 1998; Grunig et al., 1998). These findings have been corroborated by Zhu et al. (1999) using IL-13 transgenic mice. In addition, they demonstrated that chronic overexpres-

sion of IL-13 in the lungs of the transgenics also induced subepithelial fibrosis.

3. Role for IL-13 in human asthma

The relevance of these findings to human asthma is supported by the fact that IL-13 has been shown to be elevated in the lungs of asthmatics in several studies (Huang et al., 1995; Ying et al., 1997; Humbert et al., 1997; Till et al., 1997). Interestingly, elevations in IL-13 mRNA and protein levels appeared to be more associated with asthma than with atopy as levels were increased in the lungs of atopic and non-atopic asthmatic patients, but not in atopic-non-asthmatics when they were compared to normal subjects (Humbert et al., 1997). Of note, the *IL-13* and *IL-4* genes are located on human chromosome 5q31 in a region that has been linked with asthma (Marsh et al., 1994). Interestingly, a polymorphism in the IL-4R has recently been shown to be associated with asthma (Hershey et al., 1997).

4. Differential role of IL-4 and IL-13 in the allergic response

The reasons for the apparent differences in the contribution of IL-4 and IL-13 to the effector phase of the allergic response are not well understood. However, reasonable hypotheses include: (1) that there are differences in the kinetics of IL-4 and IL-13 production during the immune response to inhaled antigens; (2) that there are differences in the affinities of these receptors for IL-4 and IL-13; or (3) that these receptors induce different signaling pathways. The simplest of these explanations is that perhaps IL-4 is produced primarily during the differentiation phase of the response, and that IL-13 is either produced later or its production is sustained. Alternatively, the distribution and make-up of the IL-4/IL-13 receptor complex on lung cells may determine the binding affinity of the receptor complex for IL-4 or IL-13. There are currently two known IL-13-binding proteins referred to as IL-13Rα1 and IL-13Rα2 (Caput et al., 1996; Donaldson et al., 1998). The IL-13Rα2 chain specifically recognizes IL-13 with high affinity in the absence of a co-receptor. Its exact role in IL-13 signaling is unknown as it does not appear to serve a signaling function. The functional IL-13R complex is thought to contain the IL-13Rα1 and the IL-4Rα chains. This complex binds both IL-4 and IL-13. On the other hand, the unique IL-4 receptor is composed of the IL-4Rα and the common γ-chain (γc), a shared component of the receptors for IL-2, IL-7, IL-9, and IL-15. It has been shown that depending on the exact composition of these complexes, IL-4 and IL-13 will bind with different affinities and presumably compete for binding of the receptor complexes if both cytokines are present. Specifically, the presence of γc (which is primarily on hematopietic cells) lowers binding affinity of the complex for IL-13 and it is thought that the presence of the IL-13Rα2 may weaken binding of the complex for IL-4. Thus, differences in the make-up of the IL-4/IL-13 receptor complex on resident airway cells may bias the effector responses towards one or the other cytokine.

Signaling through IL-4/IL-13 complexes is thought to occur through the IL-4Rα chain as antibodies directed against the IL-4Rα chain inhibit the binding and the biologic activities of both IL-4 and IL-13. Both cytokines have been shown to activate the JAK-1 and Tyk-2 kinases and induce tyrosine phosphorylation of the IL-4Rα chain and the 170-kd insulin receptor substrate-2, which is the docking site for the Src homology domain containing the PI-kinase in lymphoid cells (Hilton et al., 1996). In contrast to IL-4, IL-13 does not induce the activation of JAK-3 kinase, which associates with the γc of the IL-4R complex after IL-4 binding. Phosphorylation of the IL-4Rα chain after binding of IL-13 or IL-4 results in the recruitment, phosphorylation, and nuclear translocation of STAT6 and the activation of *IL-13* and *IL-4* responsive genes (Zurawski et al., 1993). In the context of the allergic response, we have previously shown that STAT6 molecules are essential for the development of allergen-induced AHR (Kuperman et al., 1998). Thus, although some of IL-13's actions may be mediated via other signaling pathways, IL-13-induced AHR appears to be mediated via the STAT6 signaling pathway.

5. Potential mechanisms of IL-13-induced AHR

Although the exact mechanisms by which IL-13 induces AHR are currently not known, IL-13 has

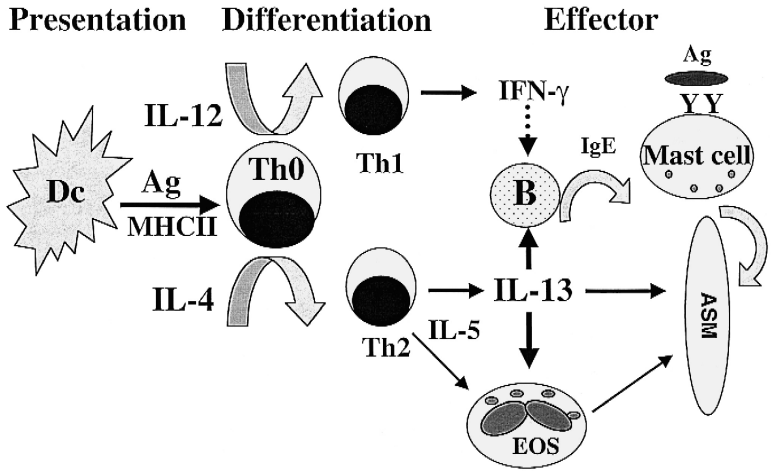

Fig. 1. Schematic representation of the potential mechanisms by which Th2 cells induce the allergic diathesis. In the lungs of asthmatic individuals stimulation of allergen-specific T cells by allergen-derived peptides presented by antigen-presenting cells (dendritic cells) in the context of class II MHC molecules results in differentiation of T cells into Th2 cytokine producing cells. Th2 cells produce IL-4, IL-13, and IL-5, which coordinately regulate the allergic response. IL-4 directs the differentiation of T cells towards a Th2 cytokine profile and acts as a growth factor for the expansion of these cells. IL-5 regulates the differentiation and egress of eosinophils from the bone marrow into the blood. IL-13, likely promotes the recruitment and activation of effector cells in the allergic response via binding to its receptor on numerous cells types such as B cells, eosinophils, and airway smooth muscle. Dc, dendritic cell; B, B cell; Ag, antigen; IgE, immunoglobulin E; MHCII, major histocompatibility complex II; ASM, airway smooth muscle; eos, eosinophil; IL-interleukin; Th, T helper cell.

several known functions, which are potentially important in the development of allergic airway disease: the induction of IgE production (Emson et al., 1998); activation of mast cells (Nilsson and Nilsson, 1995); the induction of VCAM-1 expression on vascular endothelium (Bochner et al., 1995); direct activation of eosinophils (Horie et al., 1997); and induction of chemokine production (Jordan et al., 1997) (Fig. 1). In addition, IL-13 may alter smooth muscle contractility either through one of the pathways highlighted above or independently. In this regard, Chen and Panettieri (1999) have recently reported that IL-13 can augment cholinergically induced contractions of tracheal smooth muscle in vitro suggesting that IL-13 may mediate AHR via direct effects on airway smooth muscle. Future studies will undoubtedly reveal the exact mechanism(s) by which IL-13 mediates the effector phase of the allergic response.

6. Conclusions

For over a relatively short period of time, a wealth of information regarding the immunological, physiological and molecular mechanisms of allergic responses has been derived from the use of murine models. Collectively, the studies to date suggest that Th2 cytokines are clearly important in the pathogenesis of allergic asthma. The individual Th2 cytokines likely have complementary roles in the induction and development of allergen-induced AHR. Specifically, IL-4 is necessary for the differentiation and expansion of Th2 cells, while IL-5 and IL-13 likely mediate the effector phase of the response by activating effector cells such as eosinophils, B cells and perhaps airway smooth muscle cells. The study of murine models should continue to provide insight into the immunological basis of allergic asthma and may ultimately lead to the development of immunotherapeutic strategies to reduce the morbidity and mortality associated with this disease.

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