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EDITORIAL REVIEW

Asthma: new developments in cytokine regulation

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Asthma is a major public health problem caused by the interplay of environmental factors and genetic susceptibility. There has been a dramatic increase in asthma prevalence over the last two decades [1]. It has been widely accepted for many years that asthma involves inflammatory activation in the lower airways of allergen specific Th2 cells [2,3]. The cellular and molecular pathways involved in this are complex and the chronic, inflammatory process encompasses infiltration by eosinophils, neutrophils, mast cells and T cells. The severity of chronic asthma is likely to depend on the extent of eosinophil and mast cell activation and accumulation. The classical Th2 cytokines, IL-4, IL-5 and IL-13 are strongly implicated in the pathogenesis of asthma, both from human studies and studies using cytokine knockout mice [3]. These cytokines have an impact on the many events involved in asthma including production of IgE, eosinophil maturation, mucus over-production and airway hyperresponsiveness (AHR). However, activation of Th2 responses is not in itself sufficient to account for the abnormalities in airway function characteristic of asthma [4]. It was initially envisaged that successful immunotherapeutics for asthma might simply involve re-setting the cytokine balance in the lower airways through the administration of Th1 cytokines or activation of Th1 responses [5]. A number of novel therapeutics currently under investigation derive from a rationale originating in the 'hygiene hypothesis' in terms of trying to shift responses to Th1 through the various forms of microbial products known to stimulate Th1 immunity [6]. However, it has gradually become clear that the complex immunological activation events in asthma cannot be accounted for by a simple Th1-Th2 dichotomy and furthermore, in murine models the pathological consequence of over-compensatory Th1 responses in the lung can be as great as the allergic response itself [5].

Recent publications in the asthma field, including an article in this issue of *Clinical and Experimental Immunology*, have shed light on some of the key processes controlling Th2 cytokine release in the asthmatic lung and may point to possible future directions for novel therapeutic strategies [7–9]. Among the key issues are these: if the simple activation of allergen specific Th2 cells is not sufficient to cause asthma, what else is required and what is special about the lower respiratory tract in supplying this requirement, and if the initial stimulus required for the polarization of antigen specific, IL-4 secreting Th2 cells is IL-4, which cells make the initial IL-4? An important answer to these questions has come from the laboratory of Dale Umetsu at Stanford, and provides one of the first links between NKT cells of the innate immune response and the programming of Th2 cells in asthma [7]. These cells are a population of NK cells that are NK1·1$^+$ but also express conventional T cell receptor, though in a very restricted fashion, such that human NKT cells carry an invariant Vα24-Jα15 TCR while mouse cells have a Vα14-Jα18 receptor [10]. They recognize antigen in the context of the nonclassical MHC molecule, CD1d. To date, the only defined ligand shown to be presented to NKT cells by CD1d molecules is α-galactosyl ceramide, a glycolipid derived from marine sponges. It is presumed that this antigen has structural equivalents in the microbial universe and that this is a basic pathway for the rapid triggering of innate immunity. The ability of these cells, which are activated extremely early in the course of an immune response, to give a burst of cytokines so influencing the Th1/Th2 development of immune responses has previously received much attention with respect to the induction of autoimmune disease [11]. However, the contribution of these cells to asthma is an obvious issue considering that these cells have a critical role in innate immunity to pathogens in the lung [12]. It has now been shown that mice lacking this pathway are protected from the development of AHR. Interestingly though, there is no block on the development of Th2 responses to allergen *per se*. Akbari *et al.* [7] used the ovalbumin-Balb/c mouse lung challenge model of asthma to look at the phenotype of mice that carried a knockout either for the CD1d NKT presenting MHC molecule, or for the invariant NKT TCR. When control mice were sensitized with an ovalbumin aerosol, they showed the expected pattern of AHR on methacholine challenge, while knockout mice showed normal airway responsiveness. Comparison of bronchoalveolar lavage (BAL) cells showed that this lack of AHR was associated with a lack of eosinophils and of antigen specific IgE. When NKT cells selected for the invariant Vα14 receptor were adoptively transferred to knockout mice, susceptibility to AHR was restored. These cells only partially restored the AHR phenotype when taken from either IL-4 or IL-13 knockouts, and cells from the double knockouts lacking both cytokines were unable to restore AHR at all.

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NKT cells from IFNγknockouts, on the other hand, could transfer susceptibility to AHR. The underlying hypothesis is that antigen encountered in the lung somehow changes the mucosal environment of the lung, so exposing self glycolipid antigens that are recognized by NKT cells in the context of Cd1d. The IL-4 and IL-13 rapidly released from the NKT cells activated in this manner then are able to license conventional Th2 cells in the lower respiratory tract for the development of AHR.

With respect to the Th2 cells in the lung which are then activated for induction of the asthma phenotype, much recent work has been devoted to understanding the underlying regulators of cytokine transcription and signalling that might supply new and fundamental therapeutic targets. Kubo's group in Tokyo have recently described an important role for a member of the SOCS (suppressor of cytokine signalling) family in the process [8]. SOCS proteins function to block the Jak/STAT pathways of cytokine signalling [13,14]. Seki *et al.* [8] investigated the function of one of the SOCS family members, SOCS-3, about which relatively little was previously known. They initially showed that SOCS-3 expression is preferentially up-regulated in Th2 cells. Expression promotes Th2 cytokine responses at the expense of Th1, since transgenic mice expressing constitutive SOCS-3 show greatly enhanced Th2 development. The mechanism of action appears to be through the abrogation of IL-12-mediated STAT-4 phosphorylation and IL-2-mediated STAT-5 phosphorylation. Intriguingly, when peripheral blood T cells of asthma patients and controls were compared, it was found that levels of SOCS were significantly higher in asthmatics, with the level of SOCS-3 expression correlating with disease severity. The mouse ovalbumin sensitization model of asthma was then used to investigate the AHR of SOCS-3 transgenics. The AHR of the transgenic mice were considerably enhanced with larger numbers of eosinophils and Th2 cytokines in BAL.

Another upstream regulator of Th1/Th2 cytokine responses in asthma is stem cell factor (SCF), sometimes termed mast cell growth factor or Kit ligand, because it is the ligand of the *c-kit* protooncogene product [15,16]. While the studies from the laboratories of Umetsu and Kubo bear on the driving roles of NKT cells and T cells in asthma, studies on SCF indicate a role for mast cells themselves in driving pathogenesis. In this issue of *Clinical and Experimental Immunology*, Berlin *et al.* [9] report initial studies with therapeutic administration of a monoclonal antibody to SCF in murine asthma. SCF was first described in 1990 and is widely expressed, including synthesis by a variety of cells in the asthmatic lung such as mast cells and eosinophils as well as bronchial smooth muscle cells and lung fibroblasts [15,16]. Thus, in addition to studies such as those described above on activation of NKT cells in the lung, studies on SCF may also help to illuminate what is special about the inflammatory environment of the respiratory tract that can facilitate the action of Th2 cells to trigger asthma. SCF has a well-documented range of effects on several cell types including effects on mast cell development, activation, chemotaxis, degranulation and IL-4 expression. SCF is expressed in membrane-bound and in cleaved, soluble forms and its crystal structure has been solved [17]. A number of previous studies have investigated the role of SCF in murine models of asthma, for example by administering exogenous SCF to enhance AHR. Berlin and colleagues have now added to these findings by producing a monoclonal antibody to SCF which they here administer therapeutically in a murine model of asthma involving intratracheal challenge of primed CBA mice with cockroach antigen. Administration of SCF antibody at the time of allergen challenge significantly reduced AHR, although this was not inextricably linked to a reduction in eosinophils. Cytokine and chemokine analysis showed a significant reduction in IL-5, TNFα, MCP-1 and RANTES. Because of the wide spectrum of cells capable of producing SCF in the lung and of expressing its ligand, *c-kit*, it is difficult to be certain whether these effects on cytokine and chemokine release are due to inhibition of the effects of SCF on mast cells or whether the mode of action is primarily through some other cell type.

Taken together, these studies indicate new levels of complexity in the activation of Th2 cells during an asthmatic response and offer new therapeutic options for modulating the process.

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