In vitro and in vivo modulation of human T lymphocytes from allergic asthmatic subjects
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Products from mast cells influence T lymphocyte proliferation and cytokine production - relevant to allergic asthma?

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Abstract

In IgE allergic diseases both mast cells and T lymphocytes play an important role. Whereas mast cells have been implicated in immediate allergic responses, T lymphocytes mediate subsequent late phase responses and chronic inflammation. Here we review possible links between the early mast cell activation and the later T lymphocyte stimulation. Products from mast cells were found to exert effects on T lymphocytes. Human Mast Cell line-1 (HMC-1) mast cells modulated proliferation and cytokine production of a human CD8+ T-cell clone in vitro. Activated mast cells seemed to drive this CD8+ T-cell clone towards a more pronounced T (helper) 1 type of response, simultaneously decreasing T-cell numbers. It is hypothesized that this might be a negative feed back mechanism operating in allergic subjects, by which the Th2-driven IgE production and eosinophilia are counteracted.

Introduction

In patients with allergic asthma, mast cells play a major role in immediate allergic responses. Mast cells can bind allergen-specific IgE to their FceRI, the high affinity receptor for IgE. Crosslinking of those receptors by allergen binding will lead to subsequent release of stored mediators [1] such as histamine, proteolytic enzymes, proteoglycans, and cytokines, so inducing acute inflammatory reactions. In asthmatic patients these reactions are characterized clinically by a drop in the forced expiratory volume in 1 second (FEV₁) within 30 minutes after allergen exposition. The immediate response is often followed by a second drop in FEV₁, the late phase response, that starts 3-4 hours after allergen exposure and reaches maximal intensity by 4-8 hours [2]. This late phase response is immunologically characterized by the infiltration of activated T lymphocytes, activated eosinophils, basophils and neutrophils, shown by studies on bronchoalveolar lavage fluid (BALF) and bronchial biopsies [3]. The exact mechanism of induction of the late phase response remains to be elucidated.

Role of T lymphocytes in asthma

In allergic asthma, T-cells are believed to be involved in regulation of the local inflammation in the lungs. T-cells in the BALF from patients with asthma show signs of activation; expression of interleukin (IL)-2R, HLA-DR and very late antigen-1 was found to be increased [4]. Activation of T-cells correlated with disease severity [5]. Allergen-specific
CD4+ T-cells, cultured from the peripheral blood of subjects with allergic asthma, were generally found to have a T helper 2 phenotype [6,7]. In concentrated BALF from allergic asthmatic subjects raised amounts of IL-4 and IL-5 were found, compared to healthy controls [4]. In the same study enriched BALF derived lymphocytes produced significantly more IL-4 whereas interferon (IFN)-γ was not raised when compared to healthy controls. The Th2-cytokines IL-4, IL-5 and IL-13 may play a pivotal role in the pathophysiology of allergic asthma. IL-4 and IL-13 induce the switch to IgE production by B cells [8,9]. Furthermore, IL-4 activates endothelial cells and induces the expression of the vascular adhesion molecule-1, thereby facilitating the adhesion and influx of very late antigen-4 positive leukocytes, such as eosinophils and lymphocytes [10]. IL-5 promotes chemoattraction, differentiation and survival of eosinophils [11]. However, in case of mild asthma or at early time points after allergen challenge predominance of Th2-cells could not be demonstrated. For example, no qualitative differences were found in the expression of cytokine mRNA when BALF cells from mild asthmatic subjects were compared with healthy non-atopic controls [12]. Furthermore, T-cell clones derived from BALF 6 h after allergen challenge of mild asthmatic subjects were found to exhibit Th0-, Th1- and Th2-like cytokine profiles, with no dominance of either of the groups [13]. Krug et al. [14] observed in freshly isolated and stimulated BAL cells from asthmatic subjects a greatly increased percentage of IFN-γ producing cells as compared to atopic and non-atopic controls. The proportion of BAL cells producing IL-4 was small (range 0-7.8% in the asthmatic group). So, this study points at a more Th1 type of response in asthma. Summarizing, there is most probably not a fixed Th2 response in the asthmatic airways but local influences can direct Th1/Th2 responses. The shift to Th2 might correlate with disease severity.

The role of CD8+ T-cells is less well understood. CD8+ T-cells may exert suppressive regulatory functions. Some studies in mice and rats indicate a role for CD8+ T cells in the in vivo regulation of IgE production [15]. CD8+ T-cells were able to inhibit IgE production by the production of substantial amounts of IFN-γ. Another mechanism by which CD8+ T-cells could down regulate responses is by deletion of autologous CD4+ cells [16]. In humans, it was reported that asthmatic subjects with markedly increased CD8+ T-cell numbers were less likely to develop a late asthmatic reaction [17,18]. This may also point at a suppressive role for this type of cell.

**Regulation of T lymphocyte functions**

How T-cell reactions are regulated in vivo is still subject of ongoing studies. On the one hand, the type of antigen presenting cell (APC) and the type of stimulus may be important.
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Factors in dictating the phenotype of the T-cells present. In vitro, the ratio between the release of IL-12 and PGE₂ from APCs has been shown to determine the cytokine profile of the T-cells [19]. The IL-12/PGE₂ ratio of APCs is, on its turn, highly influenced by series of autocrine and paracrine factors. The latter include factors produced by local accessory cells. Here we will focus on the possible role of mast cells in T-cell regulation.

**Functions of mast cells**

It has become more and more clear that mast cells are able to produce a wide range of mediators [20]. Those mediators can be divided into two groups: the preformed and the newly generated mediators. The preformed mediators are stored in secretory granules and can be released immediately after mast cell activation. To this group belong histamine, proteolytic enzymes (like tryptase and chymase), proteoglycans (like heparin), and cytokines. The presence of preformed cytokines is still being explored. There is evidence for the production of TNF-α, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13 and granulocyte-macrophage colony stimulating factor (GM-CSF). How fast these products are being released is still under investigation.

Activation of mast cells also induces the formation of newly generated mediators which comprise lipids (prostaglandins and leukotrienes), and cytokines. Synthesis and secretion of all these mediators are upregulated after FcεRI activation.

**Role of mast cells in T-cell regulation**

The ability of sensitized mast cells to release cytokines early upon allergen stimulation makes them important candidates for local immunoregulation. The release of products in the microenvironment affects other cells and this may regulate allergic inflammation. Until now the immunoregulatory capacity of human mast cells has not been extensively studied. Because human mast cells were found to express mRNA for IL-4 [21], and release of IL-4 protein was detected [22], they are commonly thought to favor conversion of T-cells to the Th2 phenotype [23]. However, studies with human lung mast cells suggest that they are unable to induce the class switch to IgE in B-lymphocytes in the absence of exogenous IL-4 [24]. Another study reported mast cells' capacity to degrade IL-4 protein [25]. Thus, the in vivo relevance of IL-4 production by mast cells needs further investigation.

Another mast cell product which was found to be of relevance for the allergic reaction is histamine. Histamine was found to cause the release of preformed IL-16 (formerly Lymphocyte Chemoattractant Factor) by CD8⁺ T lymphocytes. IL-16 is a ligand for CD4; it has chemoattractant activity for CD4⁺ cells and might be an important factor causing
migration of these cells into the tissue. Furthermore IL-16 is a growth factor for unsensitized CD4⁺ T-cells [26].

Directly, the immunoregulatory effects of mast cells on T-cells have only been studied in the mouse system [27]. In the supernatant of mouse bone marrow derived mast cells (BMMC, cultured together with IL-3 and IL-4, and stimulated by either cross-linked IgE or ionomycin) IL-4 protein was detected. Co-culturing of T-cells with anti-DNP-IgE sensitized BMMC led to a decrease of IFN-γ production by the T-cells, while at the same time IL-4 was induced. These effects were (partially) blocked by adding anti-IL-4 to the co-cultures. These findings cannot be easily extrapolated to the human system since significant differences have been observed between mast cells from human or mouse origin. Whereas mouse mast cells depend on IL-3 for growth and differentiation, the human mast cells have only very few IL-3 receptors and depend on Stem Cell Factor (SCF) instead. Another difference is that while IL-4 acts as a co-factor for IL-3 in inducing mast cell growth and differentiation in the murine system, it was shown that IL-4 antagonizes the effects of SCF in human mast cell progenitor cells [28,29].

**Effects of HMC-1 on T lymphocytes**

To test the immunoregulatory capacity of human mast cells we studied the effects of cells from the human mast cell line HMC-1 on human CD8⁺ T-cell proliferation and cytokine production [30]. Since we wanted to mimic the in vivo situation and obtain activated mast cells we stimulated the HMC-1 cells with PMA and A23187. This resulted in increased production of several mediators and cytokines, e.g. IL-8. Resting or activated mast cells had different effects on T lymphocyte proliferation and cytokine production. Whereas resting mast cells increased proliferation of a CD8⁺ T-cell clone to 160% of control (= CD8⁺ T-cells with only medium), activated mast cells decreased proliferation to 47% of control. Also the effects on cytokine production were modulated differently by resting and activated mast cells. Resting mast cells increased IFN-γ and IL-5 production to 273% and 140% of control, respectively, whereas IL-4 production was decreased to 43% of control. Stimulated mast cells increased IFN-γ production even to 820% of control, IL-4 production was only slightly raised (114% of control), whereas IL-5 production was decreased to 39% of control. These results showed that HMC-1 cells were able to modulate proliferative and cytokine production responses of a representative CD8⁺ T-cell clone. Activated mast cells seemed to drive the CD8⁺ T-cell clone towards a more pronounced T (helper) 1 type of response, simultaneously decreasing T-cell numbers. We concluded that this might be a negative feedback mechanism operating in allergic subjects by which the Th2-driven IgE production and
eosinophilia can be counteracted.

We realize that the HMC-1 cells have some disadvantages when compared to native mast cells. Firstly, the HMC-1 cell line is a rather immature mast cell line with tryptase levels being 1/10th of normal human lung mast cells [31]. Secondly, cells lack the γ-chain of the FcεRI receptor [32] and have a mutation in the c-kit receptor [33]. Therefore we had to stimulate the cells with the general stimuli PMA and calcium-ionophore A23187 instead of the more physiological way of using cross-linked anti-IgE. We checked the effectiveness of the PMA/ionophore stimulus by assessing several mediators in the supernatant. They were all found to be raised as a result of the stimuli.

Our findings contradict what Huels et al. [27] found for mouse bone marrow derived mast cells. As stated before, they found a decrease of IFN-γ production by the T-cells, (partially) due to IL-4 production by mast cells. When we added IL-4 to the polyclonal CD8⁺ T-cells we also found a (dose-dependent) decrease of IFN-γ production (unpublished results). This indicates that, in case of our HMC-1 cell experiments, factors other than IL-4 might be involved in the regulation of IFN-γ production. As HMC-1 cells were shown to produce PGE₂, PGD₂, TNF-α, IL-1α, IL-1β and IL-8 we added different doses of these mediators separately to CD8⁺ T-cell cultures. Though some of them had a small stimulatory effect on IFN-γ production, none of the mediators could completely account for the increase of IFN-γ production we found when T-cells were cultured with the stimulated HMC-1 cells themselves. Thus, it might be that other factors or maybe a combination of factors is necessary to mimic the effects.

Summary

In conclusion, products from mast cells exerted marked effects on T lymphocytes. In the human system, we found that HMC-1 mast cells were able to modulate proliferative and cytokine production responses of a CD8⁺ T-cell clone in vitro. Activated mast cells seemed to drive this CD8⁺ T-cell clone towards a more pronounced T (helper) 1 type of response, simultaneously decreasing T-cell numbers. A possible implication of these findings might be that together with the decreased proliferation the increased IFN-γ production accounts for a negative feedback system by which mast cells can downregulate allergen-induced Th2 responses via the CD8⁺ T-cells. This skewing to a more Th1-type of response via the CD8⁺ cells also may explain the finding that people with raised CD8⁺ T-cells in the lungs are less likely to have a late asthmatic reaction. To confirm this hypothesis, studies should be
performed using native lung mast cells.

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References

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