

Modulation by IL-10 of Antigen-induced Allergic Responses in Mice

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Over the last few years, we examined the anti-allergic properties of interleukin (IL)-10 in different models of inflammation in the mouse, as well as against IgE-dependent activation of mouse bone marrow-derived mast cells (BMMC). We showed that IL-10, concurrently administered with ovalbumin, inhibited inflammatory cell accumulation in the airways and in the peritoneal cavity of sensitized mice, as well as the accompanying cytokine release. IL-10 also blocked antigen-induced cytokine generation by IgE-stimulated BMMC. Together, these results identify a novel biological property of IL-10, as a cytokine with potent anti-allergic activities.

Key words: interleukin-10 - eosinophils - mast cells - TNF- α - interleukin-5

Allergic inflammation involves the attraction and activation of a variety of cell types at the site of antigen stimulation, including mast cells, eosinophils, neutrophils and lymphocytes (Arm & Lee 1992). Attention is particularly focused on eosinophils, since their recruitment in elevated numbers into the airways of asthmatics after allergen exposure (Bousquet et al. 1990, Azzawi et al. 1992) may lead to a long-term damage of the bronchial mucosa (Gleich & Adolphson 1986), as a result of the release of cytotoxic proteins (Gleich 1990). Several mechanisms responsible for the attraction and localization of inflammatory cells at site of allergic reactions have been proposed. Among them, attention is presently focused on Th2-derived cytokines, particularly interleukin (IL)-5, which promotes the growth, differentiation, survival and activation of eosinophils (Sanderson et al. 1985, Clutterbuck et al. 1989, Sanderson 1992) and IL-4, which induces IgE production by B cells (Pene et al. 1988). In murine models of allergic inflammation, infiltration of the airways by eosinophils is regulated by IL-5 and IL-4, since sensitized mice treated with anti-IL-5 (Nakajima et al. 1992) or anti-IL-4 (Lukacs et al. 1994) antibodies display reduced eosinophilia in their bronchoalveolar lavage (BAL) fluid and bronchial tissue following antigen challenge.

IL-10 was initially characterized as a product of CD4⁺ T-lymphocytes of the Th2 subtype and shown to inhibit interferon (IFN)- γ production by

Th1 clones (Fiorentino et al. 1989). Several properties of IL-10 on various cell types have been described since then, including growth stimulation of thymocytes, mast cells and B cells, and inhibition of cytokine production by activated monocytes, macrophages (Moore et al. 1993) and, more recently, by neutrophils (Cassatella et al. 1993, Kasama et al. 1994) and eosinophils (Takanashi et al. 1994). Only a few studies, however, have focused on the *in vivo* allergic modulatory properties of IL-10.

In this report, we evaluated the effects of IL-10 on: (i) Antigen-induced airway inflammation in immunized BALB/c mice; (ii) IgE-dependent activation of mouse bone marrow-derived mast cells (BMMC); (iii) Antigen-induced IL-5 generation, CD4⁺ T-lymphocyte infiltration and activation into the mice peritoneal cavity.

Antigen-induced cellular recruitment into the airways of sensitized mice - The intra-nasal instillation of 10 μ g ovalbumin to sensitized BALB/c mice induced a rise in the number of eosinophils in the bronchial tissue at 24 hr (Fig. 1A). Treatment with 0.1 μ g recombinant murine (rm) IL-10, markedly decreased ovalbumin-induced eosinophil infiltration in the peribronchial wall (Fig. 1B), as well as in the BAL fluid (Zuany-Amorim et al. 1995).

Several lines of evidence designate tumor-necrosis factor (TNF)- α as a cytokine generated by numerous cell types during inflammatory reactions and shock states (Tracey & Cerami 1993). The participation of TNF- α in allergic reactions is linked to different observations, including its release by mast cells, basophils and alveolar macrophages upon IgE-dependent mechanisms (Burd et al. 1989, Gordon & Galli 1990, Ohno et al. 1990,

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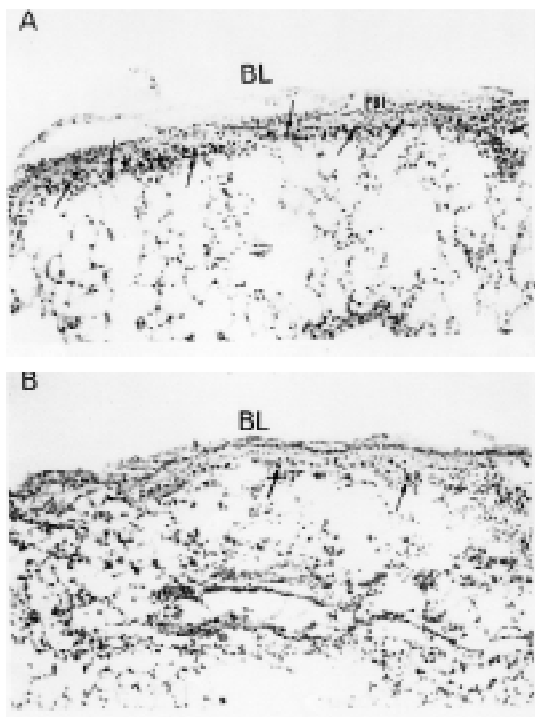


Fig. 1: cyanide-resistant eosinophil peroxidase activity in the bronchial wall of sensitized saline- or antigen-challenged mice. (A) Lung section from an immunized ovalbumin-challenged mouse used 24 hr after the challenge, showing massive eosinophil infiltration concentrated in the bronchial submucosa (arrows); (B) ovalbumin-challenged-rmIL-10 (0.1 $\mu\text{g}/\text{mouse}$)-treated mouse with scant eosinophils in the bronchial submucosa (arrows). BL : bronchial lumen, PBI : peribronchial infiltrate. Original magnifications $\times 100$.

Gosset et al. 1991). High levels of TNF- α have been shown in monocytes and epithelial cells from asthmatics (Mattoli et al. 1991) and BAL fluid from antigen-challenged guinea-pigs (Watson et al. 1993). Finally, treatment of sensitized guinea-pigs with an IL-1 receptor antagonist prevents antigen-induced eosinophil accumulation and TNF- α generation in the BAL fluid (Watson et al. 1993), indicating that TNF- α may modulate allergic airway inflammation. We thus investigated whether antigen challenge induced the release of this cytokine in the BAL fluid of sensitized mice and verified the potential modulatory activity of IL-10 in this process. The intra-nasal administration of ovalbumin was followed by a marked rise in the levels of TNF- α in the BAL fluid, which reached a peak at 1 hr to resolve between 3 and 6 hr after the challenge (Fig. 2A). The simultaneous administration of ovalbumin and rmIL-10 indicated a substantial reduction in the levels of TNF- α , particularly at 1 hr (Fig. 2A), a result extending previous observations showing reduction by IL-10 of LPS-induced *in vivo* TNF- α generation (Howard et al. 1993,

Takanashi et al. 1994). Thus, the observation that TNF- α released upon antigenic stimulation is also susceptible of inhibition by IL-10 may have important consequences for the subsequent invasion of the bronchial wall by inflammatory cells.

Accordingly, failure by IL-10 to modify antigen-induced cellular infiltration when administered 1 hr after the challenge, i.e., the time of the peak release of TNF- α in the BAL fluid, support the hypothesis that blockade of local TNF- α generation and inhibition of leukocyte recruitment are related phenomena. In confirmation, we demonstrate that the local administration of an antiserum to TNF- α markedly reduced antigen-induced eosinophil accumulation in the BAL fluid (Fig. 2B). These results extend those from previous studies showing the participation of TNF- α in leukocyte infiltration associated with IgE-dependent cutaneous inflammation in sensitized mice (Wershil et al. 1991). Together, these results suggest that the inhibitory effect of IL-10 probably involved down-regulation of TNF- α generation in the BAL fluid, since treatment of sensitized mice with a specific anti-TNF- α antiserum drastically reduced airway eosinophilia.

IgE-dependent cytokine generation by mast cells - Contrary to the inhibition of allergic airway inflammation observed when rmIL-10 was given by intra-nasal route, its subcutaneous injection did not modify antigen-induced cellular accumulation in the BAL fluid (data not shown). These results suggest that the target(s) for IL-10 are located in the airways and that the amounts of rmIL-10 reaching the bronchial compartment after its systemic administration are probably not sufficient to display an effect. Since mast cells are one of the major sources of TNF- α in the bronchial wall, we hypothesized that reduced TNF- α release observed in the BAL fluid from antigen challenged IL-10-treated mice resulted from mast cell deactivation by IL-10. To address this question, we tested the extent to which IL-10 could interfere with IgE-mediated *in vitro* mast cell activation, with particular emphasis to cytokine production.

Dinitrophenyl-bovine serum albumin (DNP-BSA)-mediated stimulation of BMDC was followed by TNF- α release, which occurred as early as 1 hr after challenge, peaked at 3 hr and resolved between 6 and 24 hr (Fig. 3A). Incubation of the cells with 50 ng/ml rmIL-10 resulted in a significant inhibition of TNF- α production, particularly at 3 and 6 hr (Fig. 3A). These findings suggest that TNF- α originating from pulmonary mast cells upon antigenic stimulation is one of the main targets for the anti-allergic activity of IL-10. Northern blot analysis revealed a reduction by rmIL-10 of

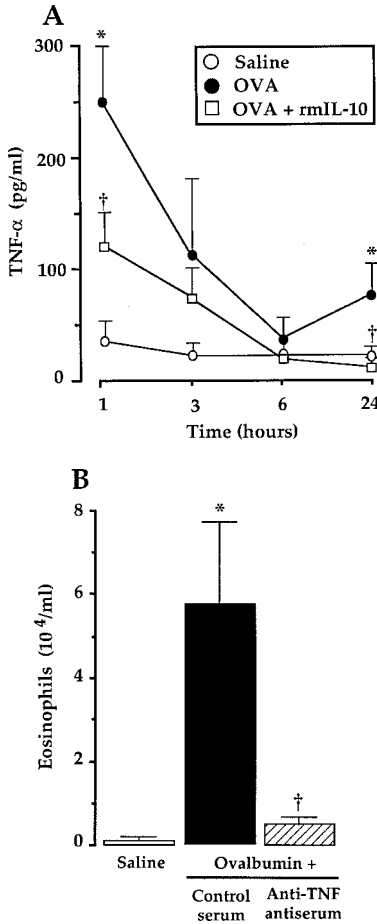


Fig. 2 - A: inhibition by rmIL-10 of antigen-induced TNF- α generation in the BAL fluid from sensitized mice. Sensitized BALB/c mice were challenged with intra-nasal saline (○), or with 10 μ g ovalbumin, administered either alone (●) or with 0.1 (□) μ g rmIL-10 and they were killed at various time-intervals after the challenge. The levels of TNF- α in the BAL fluid were evaluated by a double-sandwich specific ELISA. Results are expressed as means \pm SEM of 5-9 experiments for each group. B: eosinophil distribution in the BAL fluid from sensitized mice challenged with intra-nasal saline (□) or ovalbumin and treated concomitantly, *via* the intra-nasal route, with 50 μ l/mouse of a sheep anti-murine TNF- α antiserum (▨) or with its control preimmune serum (■). Results represent the number of eosinophils $\times 10^4$ /ml BAL fluid. * $p < 0.05$ and † $p < 0.05$, as compared to saline- or ovalbumin-challenged untreated mice, respectively.

TNF- α mRNA expression, a finding consistent with the inhibition of TNF- α protein release (Arock et al. 1995). In contrast, while GM-CSF protein release in the medium was decreased by more than 50 % by 50 ng/ml of rmIL-10 (Fig. 3B), the same concentration of the cytokine failed to reduce GM-CSF mRNA expression (Arock et al. 1995). This suggests that IL-10 interferes rather with post-transcriptional events involved in GM-CSF production than with its gene expression.

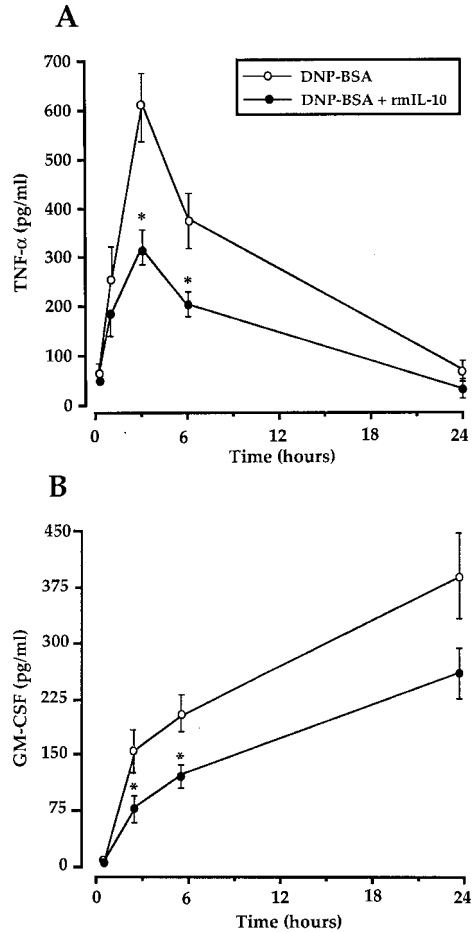


Fig. 3: effect of rmIL-10 on DNP-BSA-induced TNF- α (A) and GM-CSF (B) release from mouse BMMC. IgE-sensitized BMMC were incubated with 50 ng/ml rmIL-10 (●) for 10 min and then challenged with 10 ng/10⁶ cells of DNP-BSA (○). The levels of TNF- α and GM-CSF were determined by a specific ELISA. Values are means \pm SEM of 4-8 experiments. * $p < 0.05$, as compared with sensitized DNP-BSA-stimulated cells.

Antigen-induced IL-5 generation, CD4⁺ T-lymphocyte and eosinophil infiltration into the mice peritoneal cavity - The i.p. injection of 1 μ g ovalbumin to sensitized BALB/c mice induced a marked increase in the number of eosinophils, starting at 6 hr and reaching a plateau between 24 and 48 hr (Fig. 4). No changes in the number of eosinophils were observed in saline-challenged mice at any time point (Fig. 4A).

The administration of 0.1 μ g rmIL-10, concomitantly injected with 1 μ g ovalbumin, reduced eosinophil counts at 6 h and 24 hr after the antigen challenge. At 48 hr, even though the numbers of eosinophils were reduced by 47% by the treatment with rmIL-10, the high variability in cell counts precluded the results from achieving statistical significance (Fig. 4A).

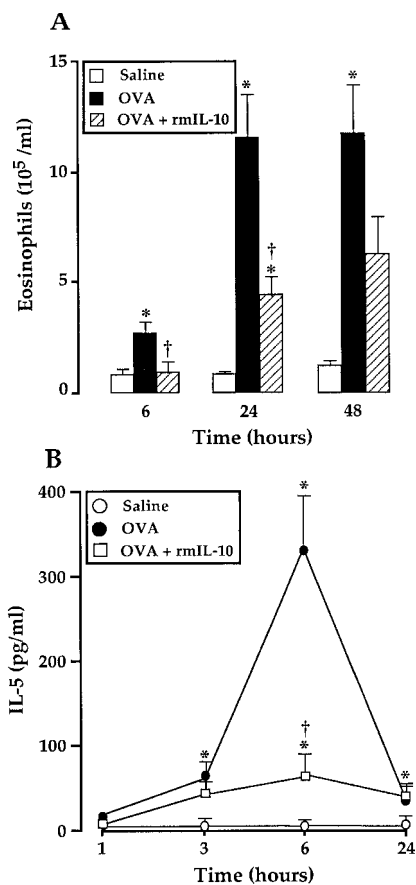


Fig. 4 - A: effect of rmIL-10 on antigen-induced eosinophil accumulation in the PL fluid from sensitized mice killed 6, 24 or 48 hr after the challenge. Animals were injected i.p. either with saline (□), or with 1 μ g ovalbumin alone (■), or mixed with 0.1 μ g/cavity rmIL-10 (▨). B: inhibition by rmIL-10 of antigen-induced IL-5 release in the PL fluid from sensitized mice. Sensitized mice were challenged with saline (○), or with 1 μ g ovalbumin alone (●), or concurrently administered with 0.1 μ g rmIL-10 (◻) and they were sacrificed at various time intervals after the challenge. The levels of IL-5 in the PL fluid were determined by enzyme immunometric assay. Results are expressed as means \pm SEM (vertical bars) of 3-14 experiments. * $p < 0.05$ and † $p < 0.05$, as compared to saline- or ovalbumin-challenged untreated mice, respectively.

Contrary to what we have observed when the animals were challenged *via* the intra-nasal route, no release of TNF- α was detected in the supernatant of peritoneal lavage (PL) fluid after the intra-peritoneal administration of ovalbumin, at any time-point (data not shown). These results indicate that IL-10 reduced antigen-induced eosinophilia in the peritoneal cavity by mechanisms unrelated to the blockade of the local generation of TNF- α and suggest that, depending upon the site of inflammation, IL-10 may exhibit anti-allergic properties by acting on different cell types. Interestingly

enough, the administration of rmIL-10, 3 hr after antigen challenge still inhibits eosinophilia in the PL fluid at 24 hr (Zuany-Amorim et al. 1996). This result differs from our previous findings showing that the intra-nasal instillation of rmIL-10, 1 hr after ovalbumin stimulation, failed to protect sensitized mice against eosinophil accumulation in the BAL fluid (Zuany-Amorim et al. 1995). This discrepancy suggests that mediator(s) and/or cytokine(s) released in the peritoneal cavity later than 3 hr after ovalbumin stimulation are involved in the eosinophil recruitment and are target for the anti-allergic activity of IL-10. The data presented herein show marked down-regulation by rmIL-10 of antigen-induced IL-5 release in the PL fluid at 6 hr (Fig. 4B), a phenomenon temporally correlated with the subsequent inhibition of eosinophil accumulation in this compartment. The IL-5-dependency of allergic eosinophilia in sensitized mice has been widely demonstrated (Kaneko et al. 1991, Okudaira et al. 1991). In particular, the observation that the *in vivo* administration of an antibody to murine IL-5 receptor prevented eosinophilia in IL-5 transgenic mice (Hitoshi et al. 1990), further supports the concept that IL-5 generation and eosinophil recruitment are related events. In confirmation of these findings, we showed a reduced antigen-induced eosinophil accumulation in the peritoneal cavity of sensitized mice after treatment with a neutralizing anti-IL-5 monoclonal antibody (Zuany-Amorim et al. 1996).

Ovalbumin stimulation was followed by a time-dependent rise in CD4⁺ T cells at 24 hr (Fig. 5A). In parallel, a significant increment in the number of CD4⁺ cells bearing IL-2 receptor on their surface (CD25⁺/CD4⁺ T-cells) was observed at 24 hr (Fig. 5B). When 0.1 μ g rmIL-10 were co-injected with ovalbumin, a significant reduction in the number of CD4⁺ T-lymphocytes and in that of CD25⁺/CD4⁺ cells at 24 hr was observed (Fig. 5A, B). These results parallel those of Jinquan et al. (1993), who demonstrated that IL-10 inhibits *in vitro* CD4⁺ T-lymphocyte chemotaxis induced by IL-8.

Accumulating evidence indicates that the recruitment and function of eosinophils may be orchestrated by the products of activated T-lymphocytes. Thus, our observations that IL-5 release in the PL fluid risen at 6 hr, while T-cell numbers increased at 24 hr, suggest that T-lymphocytes residing in the peritoneal cavity, rather than those having infiltrated this compartment in response to antigen challenge, are responsible for the early cytokine release and the accompanying eosinophilia. Alternatively, cell types other than T-lymphocytes, such as mast cells (Plaut et al. 1989), may be involved in ovalbumin-induced IL-5 production at 6 hr. Nevertheless, our observation that

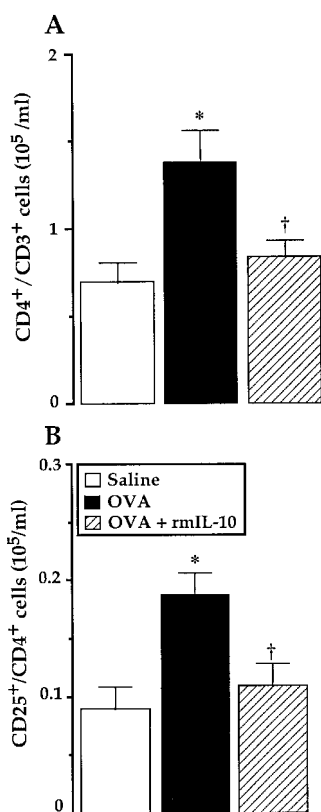


Fig. 5: effect of rmIL-10 on antigen-induced CD4⁺/CD3⁺ and CD25⁺/CD4⁺ T cell accumulation in the PL fluid from sensitized mice killed 24 hR after the challenge. Animals were injected i.p. either with saline (□), or with 1 µg ovalbumin alone (■), or mixed with 0.1 µg/cavity rmIL-10 (▨). Results are expressed as means ± SEM (vertical bars) of 5-11 experiments. **p* < 0.05 and †*p* < 0.05, as compared to saline- or ovalbumin-challenged untreated mice, respectively.

specific depletion of CD4⁺ T-lymphocytes abolished both eosinophil accumulation and IL-5 release in the peritoneal cavity (Zuany-Amorim et al. 1996) suggests that IL-5 originating from T-cells plays an important role in antigen-induced eosinophilia in this model.

To verify whether IL-10 would directly interfere with T-cell activation, we triggered *in vitro* and *in vivo* the CD3/Tcell receptor complex with an anti-CD3 mAb (Hirsch et al. 1989). *In vivo* stimulation of non immunized mice with anti-CD3 mAb led to a time-dependent production of IL-5 in the PL fluid, which was not modified by treatment of the animals with a dose of rmIL-10 effective against ovalbumin-induced eosinophilia and IL-5 release. In parallel, IL-10 failed to inhibit CD3-dependent IL-5 generation from purified spleen CD4⁺ T-lymphocytes (Zuany-Amorim et al. 1996). These findings suggest that mechanisms

unrelated to T-cell activation (possibly antigen-presenting cell deactivation) are targets for the anti-allergic activity of IL-10 in this model.

In recent years, evidence has suggested that allergic inflammation is a Th2-cytokine-mediated disease. As IL-10 is produced by bronchial epithelial cells (Bonfield et al. 1995) and can suppress acute inflammation induced by immune-complexes in mice lungs (Shanley et al. 1995), there has been considerable interest in the role of IL-10 in regulating Th2-like responses leading to allergic lung inflammation. Our present results showing that IL-10 can negatively regulate allergic inflammatory reaction by inhibiting IL-5 and TNF-α release, suggest that this cytokine may have therapeutic value for treating eosinophilic disorders, such as bronchial asthma.

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