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Research Article

Mast cells have been implicated in a number of diseases involving chronic inflammation including asthma, rheumatoid arthritis, and inflammatory bowel diseases. They are a potent source of several cytokines, including IL-6 and TNF-alpha. Freshly isolated rat peritoneal mast cells will produce IL-6 in response to anti-IgE, LPS, PGE1, or PGE2; however, the mechanisms by which such cytokine production is regulated are poorly understood. IL-10 is recognized as an important immunoregulatory cytokine with effects on T cell development and the production of inflammatory cytokines. IL-10 has previously been described to enhance mast cell development in the context of IL-3 and IL-4. In the current study, we have examined the ability of IL-10 to modulate rat peritoneal mast cell IL-6 and TNF-alpha production in response to a variety of stimuli. We have observed that recombinant murine IL-10 can inhibit the production of both IL-6 and TNF-alpha by mast cells without altering the degree of histamine release in response to anti-IgE. Concentrations of IL-10 as low as 0.2 ng/ml were sufficient to inhibit IL-6 production by LPS- or anti-IgE-activated cells significantly. IL-10 also inhibited PGE1- and PGE2-induced IL-6 production. The relative potency of IL-10 as an inhibitor of mast cell IL-6 production was highly dependent upon the stimulus used, with a 10-fold difference in the IC50 for LPS- or anti-IgE-activated cells (0.21 ng/ml) […]



# Interleukin (IL)-10 Inhibits Long-Term IL-6 Production but Not Preformed Mediator Release from Rat Peritoneal Mast Cells

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## Abstract

**Mast cells have been implicated in a number of diseases involving chronic inflammation including asthma, rheumatoid arthritis, and inflammatory bowel diseases. They are a potent source of several cytokines, including IL-6 and TNF-α. Freshly isolated rat peritoneal mast cells will produce IL-6 in response to anti-IgE, LPS, $PGE_1$, or $PGE_2$; however, the mechanisms by which such cytokine production is regulated are poorly understood. IL-10 is recognized as an important immunoregulatory cytokine with effects on T cell development and the production of inflammatory cytokines. IL-10 has previously been described to enhance mast cell development in the context of IL-3 and IL-4. In the current study, we have examined the ability of IL-10 to modulate rat peritoneal mast cell IL-6 and TNF-α production in response to a variety of stimuli. We have observed that recombinant murine IL-10 can inhibit the production of both IL-6 and TNF-α by mast cells without altering the degree of histamine release in response to anti-IgE. Concentrations of IL-10 as low as 0.2 ng/ml were sufficient to inhibit IL-6 production by LPS- or anti-IgE–activated cells significantly. IL-10 also inhibited $PGE_1$- and $PGE_2$-induced IL-6 production. The relative potency of IL-10 as an inhibitor of mast cell IL-6 production was highly dependent upon the stimulus used, with a 10-fold difference in the $IC_{50}$ for LPS- or anti-IgE–activated cells (0.21 ng/ml) and cells activated with a combination of LPS and $PGE_2$ (2.29 ng/ml). This suggests that prostanoids may limit the ability of IL-10 to modulate mast cell IL-6 production in the context of inflammation. These data have important implications for the regulation of mast cell IL-6 in inflammatory diseases involving prostanoid production and the effects of treatment with cyclooxygenase inhibitors. Our results also demonstrate a dual role for IL-10 on mast cells as a growth factor and inhibitor of cytokine production. (*J. Clin. Invest.* 1996. 97:1122–1128.) Key words: mast cells • IL-10 • IL-6 • TNF-α • inflammation**

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## Introduction

Mast cells in the rodent have been demonstrated to produce a wide variety of cytokines upon IgE mediated activation (1–3). Similarly, in human disease, mast cells have been demonstrated to express IL-4, IL-5, IL-6, IL-8, and TNF-α at an immunohistochemical level (4–6). Mast cell cytokine production is not merely an acute response to IgE-mediated cell activation but may persist for many hours after mast cell activation with a variety of stimuli, including bacterial products (7) and prostanoids (8). Mast cell production of some cytokines is not necessarily associated with preformed mediator release (7, 8) and may be an important component in the pathogenesis of inflammatory diseases such as asthma, rheumatoid arthritis, and Crohn's disease. IL-10 is a well-characterized cytokine produced by Th2 cells in the rodent, and it has been shown to have predominately antiinflammatory effects. This cytokine inhibits the production of IFN-γ by lymphocytes (9), TNF-α and IL-6 by macrophages and monocytes (10–13), and TNF-α and GM-CSF production by eosinophils (14). IL-10 also regulates neutrophil chemokine expression (15) and prevents lethal endotoxemia in a mouse model (16, 17). IL-10 is thought to be important in modulating multiple aspects of the Th2 response to certain parasitic infections (18–20). This cytokine can also act as a potent growth factor for mast cells, particularly in the context of IL-3 and IL-4 (21). During mast cell development, it has been shown to induce expression of mouse mast cell protease 2 reversibly both in vivo and in vitro (22). These observations suggest that the mast cell has a functional IL-10 receptor and have been considered as an indication that the overall effect of IL-10 may be to enhance mast cell activities in vivo.

IL-6 and TNF-α are multipotent cytokines produced in the context of inflammation and infection. IL-6 is critical to the development of the acute-phase response during inflammation and has been demonstrated to be necessary for the final stages of plasma cell development as well as participating in a number of other processes (23). The mast cell in both humans and rodents is a potent cellular source of IL-6 (4, 7) and is able to produce this cytokine relatively rapidly compared with the more traditional sources of this cytokine, such as monocytes and macrophages (24). Recent studies of mast cell development from cord blood cells in the human have suggested that IL-6 is important in inducing the expression of high affinity receptors for IgE (FcεR1) (25). Treatment with IL-6 and TNF-α will induce differentiation of the KU-812 immature human basophil-like cell line to express human mast cell tryptase and increased levels of FcεR1 (26).

TNF-α is found preformed within the mast cells of both humans and rodents. Mast cell TNF-α production is thought to

be critical for some of the acute inflammatory events that occur after IgE-mediated mast cell activation, including the local influx of neutrophils observed in the skin (27). Mast cell–derived TNF-α is a major mediator of mast cell–mediated cytotoxicity against tumor cell targets. After IgE-mediated mast cell activation, an initial burst of preformed TNF-α is released within the first 30 min. This is followed by a more sustained production of TNF-α, which persists for several hours (28).

In the current study we have examined the ability of IL-10 to modulate the IL-6 and TNF-α production and histamine release of freshly isolated rat peritoneal mast cells (PMC)[1] activated with anti-IgE, LPS, or the prostanoids $PGE_1$ and $PGE_2$. Our results suggest an important role in the modulation of mature mast cell IL-6 production by IL-10.

## Methods

*Mast cell sources and activation.* Male Lewis rats, 250–300 g, were used for the majority of experiments. In experiments involving anti-IgE activation of mast cells, female Brown Norway rats, 150–200 g, were used in view of their higher serum IgE levels. All rats were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN), housed in the central animal facility of McMaster University, and allowed food and water ad libitum. All experimental procedures were approved by the Animal Research Ethics Board of McMaster University. PMC were purified on a 30/80% discontinuous Percoll gradient (Pharmacia LKB, Uppsala, Sweden). Only those preparations found to contain a minimum of 96% mast cells were used for our studies. The major contaminating cells were found to be neutrophils and eosinophils after May-Grünwald Giemsa staining of cytospin preparations. Standard immunohistochemical analysis was performed using a mouse mAb to the rat macrophage marker ED3 and commercial development kit (Histostain SP; Zymed Laboratories, Inc., San Francisco, CA) with the modification to the manufacturer's instructions that 50% normal rat serum was added to the secondary (anti–mouse IgG) antibodies. One million PMC/ml were resuspended in RPMI 1640 without phenol red (GIBCO BRL, Gaithersburg, MD) supplemented with 5% FCS (vol/vol), 50 U/ml penicillin, 50 μg/ml streptomycin (1% Pen-Strep) (GIBCO BRL), 1.4 mM $CaCl_2$, and 100 μg/ml soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO). These cells were initially activated for 10 min at 37°C using a mouse monoclonal anti–rat IgE (MARE-1; University of Louvain, Louvain, Belgium) at 2.5 μg/ml, LPS (*Escherichia coli* serotype 055-B5; Sigma Chemical Co.) at 5 μg/ml, calcium ionophore A23187, $PGE_1$, or $PGE_2$, $10^{-7}$ M, combinations of these stimuli or media alone as control as previously described (7, 8). Each of these experiments was carried out in the presence or absence of IL-10 at a range of doses between 0.04 and 20 ng/ml. After initial activation and centrifugation, the supernatant was collected for histamine analysis. The cells were washed and resuspended in fresh media containing the activating agent or control at the original concentration and incubated for a further 6 or 18 h at 37°C. Cell-free supernatants were harvested by centrifugation and stored at −20°C before assay.

*Histamine release.* A fluorimetric assay (29) was used to determine the extent of histamine release by PMC as previously described (7, 8). Briefly, after the initial 10-min activation, samples of supernatant were taken for assay. Total histamine content was assessed in a boiled sample of the each original cell suspension assayed in parallel with the supernatants. Samples were diluted 1:50 (vol/vol) in modified Hepes-Tyrodes buffer and placed in a boiling water bath for 5 min to inactivate histaminase. After TCA precipitation of proteins, histamine levels were measured using a CR-15 fluorescence spectrometer (Shimadzu Corp., Tokyo, Japan). Histamine release was expressed as a percentage of the total histamine content calculated by the following formula: (histamine in supernatant/total histamine content) × 100. Spontaneous release of histamine was < 5% in all cases and was subtracted from the induced histamine release.

*B9 bioassay for IL-6.* IL-6 bioactivity was measured by a previously described B-9 hybridoma proliferation assay (30). Briefly, B9 cells were cultured in MEM F-11 (GIBCO BRL) supplemented with 5% FCS, 2-mercaptoethanol, 1% Pen-Strep, and a supernatant source of IL-6. The IL-6 assay was performed in triplicate for each sample or standard in microtiter plates (Nunclon Intermed, Markham, Ontario, Canada). B-9 cells (initially 2,500 per well) were cultured in the described medium in the presence or absence of putative IL-6-containing samples. After a 72-h culture of cells with sample or standard, 10 μl/well of 0.5 mg/ml MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] (Sigma Chemical Co.) was added, and the plates were incubated for a further 18–24 h in the dark. The colored reaction product was assessed at 570 nm by an ELISA reader. Samples were stored at −20°C before analysis. The samples were compared with a standard IL-6 supernatant at several dilutions to assess IL-6 content. Results are expressed as units of IL-6 per milliliter based on the amount of sample required to induce a half-maximal response. One unit is equivalent to ∼ 0.45 pg of IL-6. None of the other known mast cell cytokines have been demonstrated to induce proliferation of this cell line under similar assay conditions. IL-6 standard curves run in the presence of anti-IgE, A23187, $PGE_2$, or IL-10 at each of the concentrations used in this study were not significantly altered from standard curves with IL-6 alone. The specificity of this assay was confirmed as previously described (7, 8) using a specific neutralizing anti–IL-6 antibody on supernatants containing IL-6 induced by each of the activating agents. IL-10 added to samples at a range of concentrations between 1 and 200 ng/ml had no significant effect on detection of the IL-6 standard or of IL-6 within mast cell supernatants.

*L-929 bioassay for TNF-α.* TNF-α bioactivity was assessed using a modification of a method previously described (28), which uses the TNF-α–sensitive L929 (mouse fibroblast) cell line. Briefly, 50 μl/well of $1 \times 10^6$ L929 cells/ml in RPMI 1640 medium supplemented with 5% FCS and 1% penicillin/streptomycin solution were plated onto flat-bottomed 96-well plates and incubated overnight at 37°C. Medium was discarded by suction and replaced with fresh medium further supplemented with 20 μg/ml of cycloheximide and 100 μg/ml of soybean trypsin inhibitor (Sigma Chemical Co.). Mouse recombinant TNF-α (Genentech Inc., San Francisco, CA) was diluted in the same medium and used as a standard. Seven 10-fold serial dilutions starting from 20,000 pg/ml were used to establish the standard curve. 50 μl/well of standard or samples in duplicate were added, and the plates were incubated for 18 h at 37°C. The number of cells in the wells was assessed using MTT. 10 μl of a 5 mg/ml solution of MTT was added and the plates incubated for 4 h. 50 μl of 50% vol/vol *N,N*-dimethylformamide, 20% SDS was added to dissolve the MTT. After an 18-h incubation, the plates were examined using an ELISA plate reader at 570 nm. Medium samples and supernatants from unactivated cells served as negative controls for the assay. The addition of anti-IgE, LPS, prostanoids, or IL-10 at any of the doses used did not significantly alter the standard curve of this assay. TNF-α specificity of the assay system in mast cell supernatants was confirmed by preincubating samples with a specific neutralizing rabbit polyclonal antibody to TNF-α (Genentech Inc.), which completely inhibited the observed cytotoxicity.

*Northern blot analysis.* Highly purified rat PMC (98.7% pure, $1 \times 10^6$/ml) were incubated at 37°C for 4 h in culture medium alone or with the addition of anti-IgE (MARE-1, 5 μg/ml), anti-IgE plus murine recombinant IL-10 (5 ng/ml), or IL-10 with no additional activating agent. RNA was extracted from cell pellets by acid guanidine thiocyanate–phenol chloroform extraction (31) and separated on a 1.2% agarose gel. RNA was transferred onto a UV membrane (Duralon; Stratagene, La Jolla, CA) and hybridized sequentially with full-length cDNA probes for rat TNF-α and rat IL-6 (gifts from Dr. J. Gauldie, Hamilton, Ontario, Canada) or with a cDNA probe for

1. *Abbreviation used in this work*: PMC, rat peritoneal mast cell.

β-actin (Oncor Inc., Gaithersburg, MD). Probes were labeled using a T7 quick prime kit (Pharmacia), and blots were hybridized and washed using standard conditions. Films were exposed for 48 h before development.

*Viability assessment.* Purified PMC were initially activated and incubated for 18 h in the presence or absence of IL-10 as described above. Cell viability was determined by Trypan blue exclusion according to standard protocols. The proportion of cells that took up the dye was determined by counting cells with a hemocytometer at a magnification of 400.

*Statistical analysis.* In view of the variation in baseline cytokine production between individual animals, the IL-6 and TNF-α responses and histamine released under different culture conditions were compared using a paired Student's *t* test.

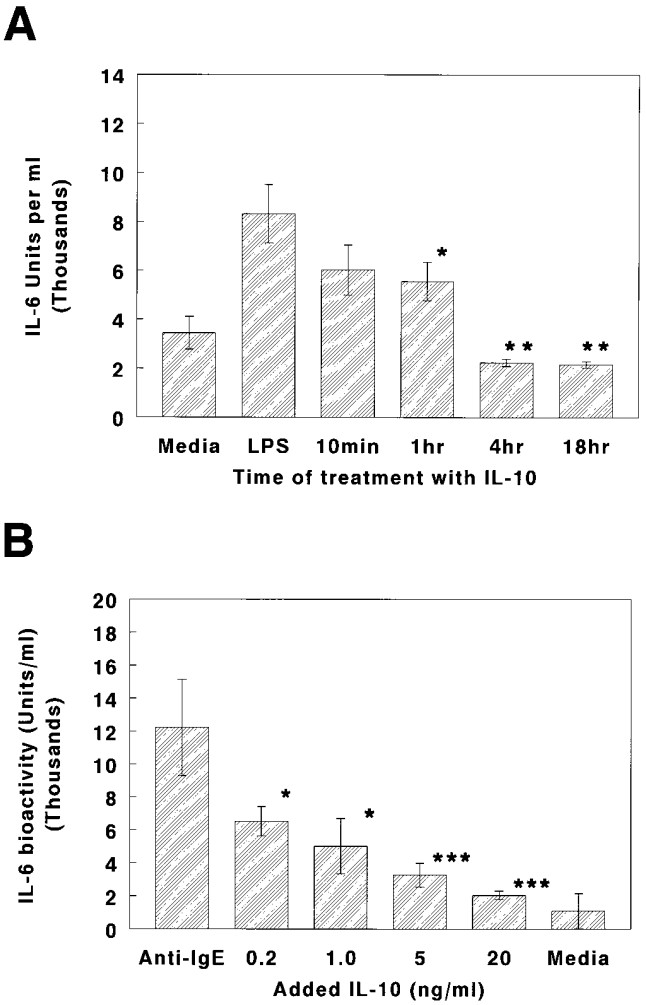


*Figure 1.* The effect of IL-10 on Brown Norway PMC (*A*) histamine release assessed after 10 min, (*B*) IL-6 production assessed after 18 h in response to anti-IgE–mediated activation. Highly purified PMC were activated with 5 μg/ml MARE-1 monoclonal anti–rat IgE either alone (*Anti-IgE*) or in the presence of varying concentrations of recombinant murine IL-10. As a negative control, cells were incubated in parallel with media alone (*Media*). No IL-6 was detectable in association with the cell pellet. Histamine release at 10 min and TNF-α release at 10 min (data not shown) was not altered by the addition of IL-10, whereas IL-6 production at 18 h could be completely inhibited. Each point represents the mean data±SEM. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $n = 4$.

## Results

Anti-IgE activation of Brown Norway rat PMC has been demonstrated to induce short-term histamine and TNF-α release and long-term IL-6 and TNF-α production. We examined the ability of recombinant murine IL-10, at a range of doses, to modulate these responses. In examining short-term mediator release, we observed that the addition of IL-10 concurrent with mast cell activation with an optimal dose of anti-IgE had no significant effect on histamine release observed at 10 min after activation (Fig. 1 *A*). The TNF-α release in response to anti-IgE activation at 10 min was also not inhibited by IL-10 treatment at any dose (data not shown).

The effects of IL-10 on long-term cytokine production, in response to anti-IgE, were more striking. IL-10 effectively inhibited anti-IgE–induced IL-6 (Fig. 1 *B*) production in a dose-dependent manner. At higher doses of IL-10, the production of IL-6 observed at 18 h after addition of anti-IgE was completely inhibited. Significant ($P < 0.05$) inhibition of the IL-6 response was observed at a dose of 0.2 ng/ml IL-10. The production of TNF-α by mast cells at 18 h (examined in separate experiments) was only inhibited at the highest concentration of IL-10 used (anti-IgE alone, 93±33 pg/ml TNF-α; anti-IgE plus 5 ng/ml IL-10, 41±14 pg/ml TNF-α; $n = 4$, $P < 0.02$).

In view of these data, we examined the ability of IL-10 to modulate IL-6 production induced by other stimuli. $PGE_1$ and $PGE_2$ have previously been demonstrated to induce mast cell IL-6 and inhibit mast cell TNF-α production without significant histamine release (8, 32). Maximal induction of IL-6 is observed with a combination of LPS and $PGE_2$, which act synergistically on the mast cell. The production of IL-6 in response to LPS, $PGE_1$, or $PGE_2$ alone was inhibited by the addition of low doses of IL-10. Significant ($P < 0.05$) inhibition was observed at 0.2 ng/ml IL-10 for LPS-driven cultures (Fig. 2 *A*) and at 1.0 ng/ml for $PGE_1$- or $PGE_2$-driven mast cell IL-6 production (Fig. 2 *B*). The mean $IC_{50}$ concentrations for IL-10 inhibition of IL-6 production differed substantially dependent upon the stimulus for mast cell IL-6 production (see Table I). Anti-IgE– and LPS-induced IL-6 were the most sensitive to IL-10 inhibition, whereas $PGE_2$ in combination with LPS induced an IL-6 response that was 10-fold more resistant to IL-10 (Fig. 2 *B*).

To assess the requirement for continued exposure to IL-10 to suppress IL-6 production, the effect of withdrawing IL-10 from LPS-activated mast cells in culture, washing cells extensively, and replacing with LPS-containing media in the absence of IL-10 was examined at various time points. When exposed to LPS alone, PMC will produce a detectable level of IL-6 after 3 h and continue to produce IL-6 for at least 24 h (Fig. 3 *A*). We observed that 1 h of exposure to IL-10 (5 ng/ml) was sufficient to induce a significant inhibition of IL-6 production in response to LPS. Maximal suppression of IL-6 production over 18 h was obtained after a 4-h exposure to IL-10. The ongoing presence of IL-10 was not required for long-term suppression of the mast cell IL-6. LPS activation of PMC also induces a small amount of TNF-α production. A 10-min exposure to IL-10 was sufficient to significantly inhibit this response (Fig. 3 *B*).

The expression of TNF-α and IL-6 at the mRNA level in PMC treated with anti-IgE alone or anti-IgE in combination with IL-10 (5 ng/ml) was examined by Northern blot analysis. Messenger RNA levels for TNF-α showed the expected pattern of enhancement after anti-IgE treatment and inhibition of

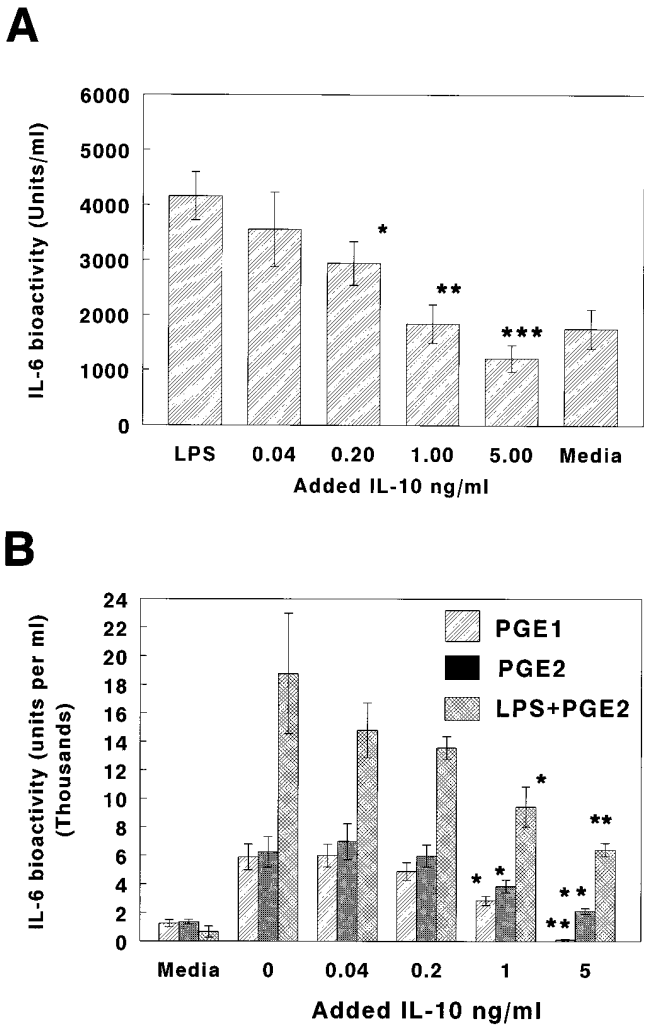


*Figure 2.* The effect of IL-10 on PMC IL-6 production in response to nondegranulating stimuli. (*A*) Highly purified Lewis rat PMC were cultured in the presence of media alone (*Media*), 5 μg/ml LPS alone (*LPS*), or LPS in combination with various concentrations of IL-10 in parallel. A significant ($P < 0.05$) inhibition of IL-6 production was observed in the presence of 0.2 ng/ml IL-10. (*B*) Similar experiments were performed using the prostanoids $PGE_1$ ($10^{-7}$ M), $PGE_2$ ($10^{-7}$ M), or $PGE_2$ ($10^{-7}$ M) in combination with LPS (5 μg/ml) as inducers of mast cell IL-6 production in place of LPS. IL-6 production in response to $PGE_1$ could be completely inhibited by IL-10 at 5 ng/ml. In contrast, significant IL-6 production in response to $PGE_2$ was still observed in the context of 5 ng/ml IL-10. Each point represents the mean data±SEM. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 4$.

this response after the addition of IL-10 (Fig. 4 *A*). The mRNA levels for IL-6 were also inhibited by IL-10 treatment; however, the modulation appeared to be limited to the 2.3-kb band, which represents one of two different detectable mRNA species for rat IL-6 (33). The apparent selective inhibition of this mRNA species was particularly striking in unactivated mast cells treated with IL-10 (Fig. 4 *B*). Northern blots were hybridized with a cDNA probe for β-actin as a control for RNA loading and integrity (Fig. 4 *C*).

To examine the possibility that PMC viability may be reduced in the presence of IL-10, we examined the ability of cells incubated with or without IL-10 at a range of doses for 24 h to exclude Trypan blue dye. Our results demonstrated that IL-10

*Table I. $IC_{50}$s for IL-10 Inhibition of Mast Cell IL-6 Production in Response to Various Stimuli*

| Stimulus | Mean $IC_{50}$ |
|---|---|
| | *ng/ml* |
| Anti-IgE | 0.21 |
| LPS | 0.21 |
| $PGE_1$ | 0.59 |
| $PGE_2$ | 1.38 |
| $PGE_2$ + LPS | 2.29 |

at each of the doses used had no significant effect on mast cell viability. Even at a concentration of 5 ng/ml IL-10, the mean percentage of viable mast cells remaining in the cultures at 18 h was 89.6% compared with 91.3% in control (media alone)-cultured PMC examined in parallel. We also considered the possibility that IL-10 may prevent IL-6 export from the PMC and examined the cell pellet content of LPS- and $PGE_2$-activated PMC in the presence or absence of IL-10 at a range of doses. No cell pellet–associated IL-6 was observed under any or our culture conditions by bioassay of mast cell lysates, although (as previously reported) exogenous IL-6 could be measured when pre-incubated with mast cell lysates (7).

## Discussion

We have demonstrated that IL-10 will selectively inhibit the long-term production of IL-6 and TNF-α by mast cells in response to immunological activation with anti-IgE without altering the degree of short-term preformed mediator release. IL-10 also inhibited the IL-6 production by mast cells in response to other stimuli of relevance to bacterial infection and inflammation, i.e., LPS, $PGE_1$, and $PGE_2$.

In the presence of IL-3 and IL-4, IL-10 enhances the growth of murine mast cells in culture (21) and is important for the full expression of the appropriate protease profile (22). Mast cells are known to be critical for inducing the acute inflammatory events that occur during type 1 hypersensitivity reactions. They are also thought to have an important role in chronic inflammatory processes associated with asthma (34–36), inflammatory bowel disease (37, 38), and rheumatoid arthritis (39, 40). Much of the mast cell's role in chronic inflammatory diseases is thought to be mediated by cytokines such as TNF-α and IL-6. A Th2 cytokine profile has been observed to be associated with allergic disease in which mast cell activation is also an important feature. Thus, it is reasonable to consider that mast cell activation in allergic disease may frequently occur in the context of a local microenvironment rich in IL-10.

IL-10 has been shown to be important in initiating the development of Th2-type responses such as those observed after infection with certain parasites or during allergic disease. This cytokine has also been shown to have potent antiinflammatory properties both in vivo and in vitro (12–17) and is known to decrease IL-1, TNF-α, and IL-6 expression by monocytes, macrophages, and other cell types (12, 13). Mice made deficient in IL-10 by homologous recombination show aberrant inflammatory responses and spontaneously develop an inflammatory intestinal disease, further demonstrating the critical role of this cytokine in modulating inflammatory responses

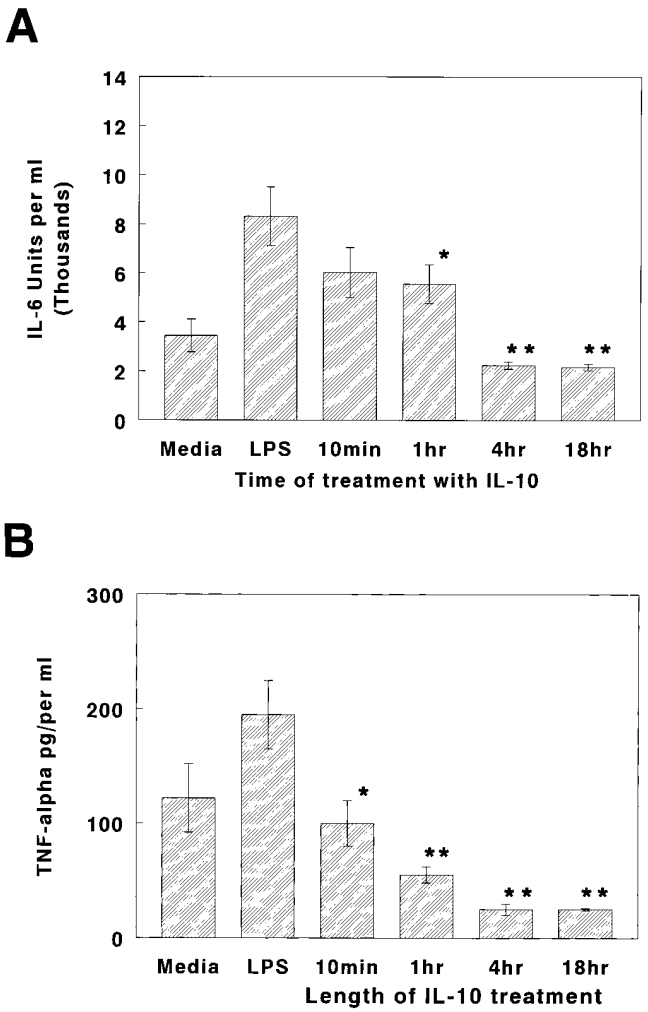


*Figure 3.* The length of mast cell exposure to IL-10 necessary to inhibit (*A*) IL-6 and (*B*) TNF-α production in mast cells activated with LPS. Lewis rat PMC were treated with LPS-containing media (*LPS*) or media alone (*Media*) for 18 h, or they were cultured with media containing IL-10 (5 ng/ml) plus LPS (5 μg/ml) for various periods of time before being washed, and the IL-10–containing media were replaced with LPS-containing media with no added IL-10 for the remains of the 18-h incubation time. 1 h of incubation with IL-10 was sufficient to induce a significant reduction in PMC IL-6 production in response to LPS. A 4-h exposure to IL-10 completely inhibited the mast cell IL-6 response. Each point represents the mean data±SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $n = 4$.

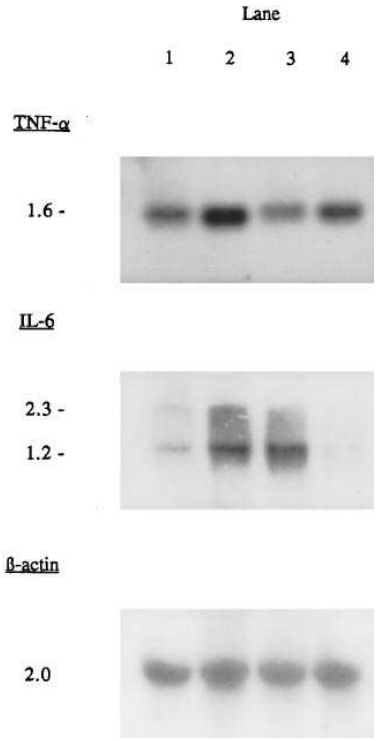


*Figure 4.* Northern blot analysis of PMC RNA after anti-IgE stimulation in the presence or absence of IL-10 (5 ng/ml). PMC (> 98% pure) at a concentration of $1 \times 10^6$ cells/ml were incubated for 4 h with culture media alone (lane *1*) or media supplemented with anti-IgE 2.5 μg/ml (lane *2*), anti-IgE 2.5 μg/ml plus IL-10 5 ng/ml (lane *3*), or IL-10 5 ng/ml alone (lane *4*). RNA was extracted from the cells, and 15 μg of total RNA from each condition was subjected to Northern blot analysis using cDNA probes for rat TNF-α, rat IL-6, and β-actin. Note the enhanced expression of both TNF-α and IL-6 mRNA after anti-IgE–induced activation. TNF-α mRNA levels return to baseline levels when cells are treated with anti-IgE plus IL-10 (lane *3*); however, overall IL-6 mRNA levels or either anti-IgE–treated or untreated cells are less affected by IL-10, with inhibition only apparent in the 2.3-kb band.

(41). We have recently demonstrated that IL-10 can also inhibit eosinophil survival and cytokine production (14). The data in this report suggest that another aspect of the antiinflammatory action of IL-10 is its ability to modulate mast cell production of inflammatory cytokines. Overall, these observations suggest a potent inhibitory role for IL-10 in allergic inflammation acting on the effector cells traditionally associated with Th2 responses. Thus, IL-10 may be viewed as enhancing the initiation of allergic processes but inhibiting the chronic inflammation associated with allergic disease.

It has previously been reported that IL-10 can inhibit the production of a number of proinflammatory cytokines by macrophages including IL-1, TNF-α, and IL-6 (12). The mast cell preparations used in our study were all 97–100% pure, with neutrophils and eosinophils as the major contaminating cell type. Examination of mast cell preparations for macrophage contamination by immunohistochemical staining using an mAb specific for the monocyte/macrophage lineage in rats (ED-3) demonstrated < 0.1% macrophage contamination (data not shown). We have previously demonstrated that mast cells purified to homogeneity also produce large amounts of IL-6 in response to each of the activating agents used (7, 8). We therefore believe that IL-10 has a direct effect on mast cell IL-6 production in these experiments.

Northern blot analysis of TNF-α and IL-6 expression revealed that both TNF-α and IL-6 mRNA levels were reduced after IL-10 exposure. The increase in TNF-α mRNA expression induced by anti-IgE treatment was inhibited by IL-10 treatment, but the constitutive expression of TNF-α was unaffected. However, the degree of IL-10-induced inhibition observed at the mRNA level for IL-6 was small and appeared to be limited to the 2.3-kb mRNA species. This apparent selective regulation may reflect differences in mRNA stability of the two mRNA species. Other mast cell cytokine responses, including the production of IL-3 and GM-CSF, have been shown to be regulated at a posttranscriptional level (42), and our results may reflect a similar mechanism. In previous studies we have observed high levels of IL-6 mRNA expression after calcium ionophore (A23187) treatment under conditions that have not led to IL-6 expression at the protein level (7, 43). Extensive previous studies have failed to demonstrate detectable levels of cell-associated IL-6 in rat PMC under any culture conditions, although recombinant IL-6 added to cell pellet preparations can be readily detected in "spiking" experiments (7).

The degree of sensitivity of IL-6 production to IL-10 inhibition varied in our experiments depending on the stimulus used. $PGE_2$-induced IL-6 production was the most resistant to IL-10 modulation, with a significant amount of IL-6 production remaining in the presence of a 5-ng/ml concentration of IL-10. A number of mast cell mediators including TNF-α and histamine have been demonstrated to induce $PGE_2$ production by other cell types. These observations suggest that prostanoids produced during a chronic inflammatory response may induce IL-6 production that is less susceptible to IL-10 modulation than that induced by acute antigen-mediated mast cell activation. In contrast, the production of TNF-α by mast cells is inhibited by both prostanoids and IL-10.

The in vivo relevance of our findings in the context of human disease remains to be determined. However, recent studies have demonstrated that IL-10 is constitutively expressed in significant amounts by human bronchial epithelial cells (44). IL-10 may form part of an ongoing regulatory process that prevents inappropriate inflammatory responses to ongoing environmental challenge. Further support for this concept is obtained from observations of the development of colitis in IL-10 knockout mice (41). Elevated levels of serum IL-10 for periods of at least 12 h have been observed in mice treated with anti-CD3 in vivo (45) or after parasite infection (18, 20). The local tissue levels of IL-10 are unknown but would be predicted to be higher than those observed in the serum. The sensitivity of mast cell IL-6 and TNF-α to IL-10 regulation is equivalent or greater than that observed in other cell types (14–16).

To date, IL-10 has been considered to have a role in enhancing the development and function of mast cells in the context of a Th2-driven response. The results of this study demonstrate that IL-10 also inhibits aspects of mature mast cell activity such as long-term cytokine production. We believe these observations are important to our understanding of the interaction between IL-10 and mast cells in inflammatory and allergic disease.

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