Brief Definitive Report

Interleukin 10: A Novel Stimulatory Factor for Mast Cells and Their Progenitors

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Summary

We have characterized the mast cell stimulating activity of murine cytokine synthesis inhibitory factor, referred to as interleukin 10 (IL-10). It was found that IL-10 alone failed to support the growth of mast cell lines and mast cell progenitors. Nevertheless, it dramatically enhanced their growth when combined with IL-3 or IL-4. Moreover, IL-4 plus IL-10 supported the proliferation of mast cells as well as IL-3, suggesting that these two factors may provide a pathway for their development independent of IL-3. However, optimal mast cell growth was stimulated by the combination of IL-10, IL-4, and IL-3. This particular set of cytokines are coordinately produced by activated T cells and may constitute an effective network regulating early and late stages of mast cell development during certain immune responses.

Early studies with mutant strains of mice suggested that T cell factors are necessary for the growth of mucosal mast cells (1). Several of these factors have been molecularly defined and characterized in various bioassays. Of these, only IL-3 has been shown to support the growth of both long-term mast cell lines and freshly isolated mast cell progenitors (2). In contrast, IL-4 is only weakly stimulatory for most cell lines (3) and is unable to stimulate progenitor cells (4). Nevertheless, IL-4 potentiates their IL-3-dependent growth, suggesting that it functions primarily as a cofactor. Recently, IL-9/P40/MEA was also shown to enhance the IL-3-dependent growth of mast cells, similar to IL-4 (5). These studies indicate that the optimal growth of mast cells is dependent on multiple factor interactions. Identification of such cytokine networks may help explain the profound regulatory effects that T cells exert on this lineage during certain allergic and inflammatory responses.

In the present study, we have assessed the mast cell stimulatory activities of a new T cell–derived factor called IL-10 (8). Although this factor was first described as cytokine synthesis inhibitory factor (CSIF) (6), subsequent studies have revealed its pleiotropic activities (7, 8). Apparently, this factor is also produced by B cells and may account for the novel mast cell stimulatory activity detected in their supernatants (9). Herein, it is shown that IL-10 is a potent coregulator of mast cell growth.

Materials and Methods

Animals. C57BL/6 mice and Wistar rats were purchased from Simonsen Laboratory (Gilroy, CA).

Cytokines and Antibodies. Purified murine rIL-3 (sp act = 1.5×10^8 U/mg) was a gift of Dr. J. Schreurs (DNAX). Purified murine rIL-4 (sp act = 6×10^7 U/mg) was generously provided by Schering-Plough Research (Bloomfield, NJ). One standard unit of activity is defined as the amount of factor necessary to produce a half-maximal response by the MC/9 mast cell line. Purified rIL-10 (sp act = 2×10^7 U/mg) was a gift of B. Castle and W. Dang (DNAX). Anti-CD4 (GK1.5) and anti-Thy-1 (30H12) mAbs were gifts of Dr. M. Kehry (DNAX).

MC/9 Mast Cell Line. The murine mast cell line, MC/9, was maintained in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS, 50 mM 2-ME, and 5% supernatant from Con A–activated T cells.

Infection with Nippostronglylus brasiliensis (Nb). *Nb* larvae were maintained by passage through rats as described (10). Mice were infected subcutaneously with 1,000 stage 3 larvae (L3), and 14 d later were killed by CO_2 asphyxiation.

Clonal Mast Cell Assay. Mesenteric lymph nodes from C57BL/6 mice infected with 1,000 L3 of *Nb* were depleted of T cells after treatment with anti-CD4 (GK1.5) and anti-Thy-1 (30H12) antibodies and goat anti-rat IgG–coated magnetic beads (Advanced Magnetics Inc., Cambridge, MA). The unbound cells usually represented 10–20% of all mesenteric lymph node cells (MLNC) and contained <5% T cells. The T cell–depleted MLNC were cultured at 4×10^5 cells/ml in modified Iscove's medium (Gibco Laboratories) supplemented with 20% FCS, 0.8% (wt/vol) methylcellulose, 50 mM 2-ME, and saturating concentrations of various growth factors. After 14 d in culture, the size and number of colonies were scored.

Staining of Cultured Cells. Individual colonies from methylcellulose cultures were transferred onto glass slides and stained with Wright's-Giemsa for morphological analysis. To identify the presence of connective tissue mast cells (CTMC), colonies were picked

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and stained with alcian blue/safranin (Rowley Biochemical Institute, Rowley, MA) or with berberine sulfate (Sigma Chemical Co., St. Louis, MO) to determine heparin-containing granules. Mature CTMC have been shown to stain alcian blue$^{+/-}$, safranin^{+}, and berberine sulfate^{+}.

Electron Microscopy. Mast cell colonies were prepared and examined by electron microscopy by Dr. R. Sibley, and P. Huie (Stanford University, Stanford, CA).

Proliferation Assay. 5×10^3 MC/9 mast cells were plated in flat-bottomed 24-well plates (Falcon Labware, Oxnard, CA) containing 1 ml of RPMI 1640, 10% FCS, 50 mM 2-ME, and varying concentrations of cytokines. After 3 d of culture, cell counts were performed on triplicate cultures using a cell counter (Coulter Electronics Inc., Hialeah, FL).

Results and Discussion

IL-10 Enhances the IL-3- and IL-4-dependent Growth of a Mast Cell Line, MC/9. The mast cell stimulatory activity of IL-10 was assessed in a 3-d proliferation assay. It was found that IL-10 failed to induce the proliferation of MC/9 cells, although it extended their viability for 1–2 d (data not shown). Nevertheless, IL-10 (at >10 U/ml) caused a modest enhancement in their growth when combined with an optimal concentration of IL-3 (Fig. 1). In separate experiments, we observed that IL-10 enhancement was more pronounced when sub-optimal amounts of IL-3 were used (data not shown). IL-10 was also found to increase the growth of MC/9 cells in response to IL-4 (Fig. 1). In this case, marked synergy was observed at sub-optimal as well as saturating concentrations of IL-4. Because IL-4 is less stimulatory for various mast cell lines than IL-3 (3), it was of great interest that the combination of IL-10 and IL-4 was almost as effective as IL-3 (Fig. 1). These results suggest that IL-10 and IL-4 are able to support the growth of mast cells in the absence of IL-3. This may be important in certain in vivo conditions where IL-3 is either absent or extremely limited. It has already been demonstrated that the combined actions of IL-4 and IL-3 are much more effective in stimulating mast cell growth than each factor alone (3). Here, we show that IL-10 further augments the stimulatory effects of saturating levels of IL-4 + IL-3 (Fig. 1).

IL-10 Is a Potent Cofactor for Colony Formation by Mast Cell Progenitors. To assess whether IL-10 could affect the growth of mast cell progenitors, clonal assays in semi-solid medium were performed with T cell–depleted MLNC isolated from *Nb*-infected mice. MLNC from parasitized mice are an enriched source of mast cell progenitors and do not contain significant numbers of other lymphoid precursors (personal observation). Our results show that progenitor cells were completely unresponsive to IL-10 and responded poorly even to high concentrations of IL-3 or IL-4 (Fig. 2). However, the combination of any two of these factors resulted in a marked increase in mast cell colony formation. For example, when IL-10 was combined with IL-3 or IL-4, colony numbers were increased 13- or 15-fold, respectively (Fig. 2). The most profound growth was elicited by a combination of all three factors,

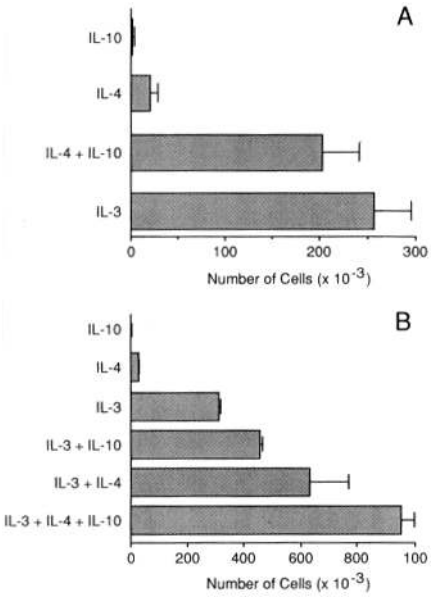

Figure 1. IL-10 enhances the factor-dependent growth of MC/9 mast cells. MC/9 mast cells (at 5×10^3 cells/ml) were cultured for 3 d with IL-3 (200 U/ml), IL-4 (200 U/ml), IL-10 (25 U/ml) or combinations of these factors as indicated. Cell numbers were determined using a cell counter (Coulter Electronics Inc.). Data are reported as mean ± SD of triplicate cultures.

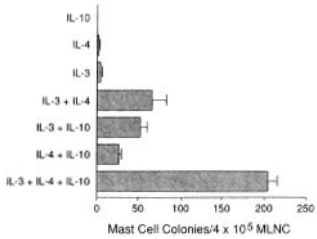

Figure 2. IL-10 is cofactor for mast cell progenitor growth. T cell–depleted MLNC from *Nb*-infected mice were cultured with IL-3 (200 U/ml), IL-4 (300 U/ml), IL-10 (300 U/ml), or combinations thereof. Colonies (containing >50 cells) were enumerated on day 14 of culture. Data are reported as mean ± SD of triplicate cultures.

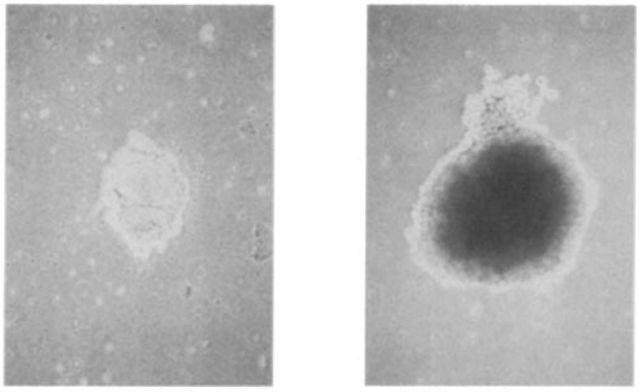

Figure 3. IL-10 augments colony size. Shown are typical colonies found in cultures stimulated with IL-3 + IL-4 in the absence (*A*) and presence (*B*) of IL-10 (×150).

suggesting that some mast cell progenitors require multiple factor interactions in order to initiate clonal growth. Moreover, the combination of IL-3 + IL-4 + IL-10 not only increased the number of colonies, but it also dramatically increased their size. This is illustrated in Fig. 3, where the average diameter and density of colonies supported by IL-3 + IL-4 + IL-10 is shown to be greater than those supported by IL-3 + IL-4. This result indicates that the continuous proliferation of daughter cells within individual colonies was enhanced by the presence of IL-10. This outcome is analogous to results of studies showing that optimal growth of other hematopoietic lineages is also dependent on interactions with more than one factor (11).

Next, individual colonies from each experimental group

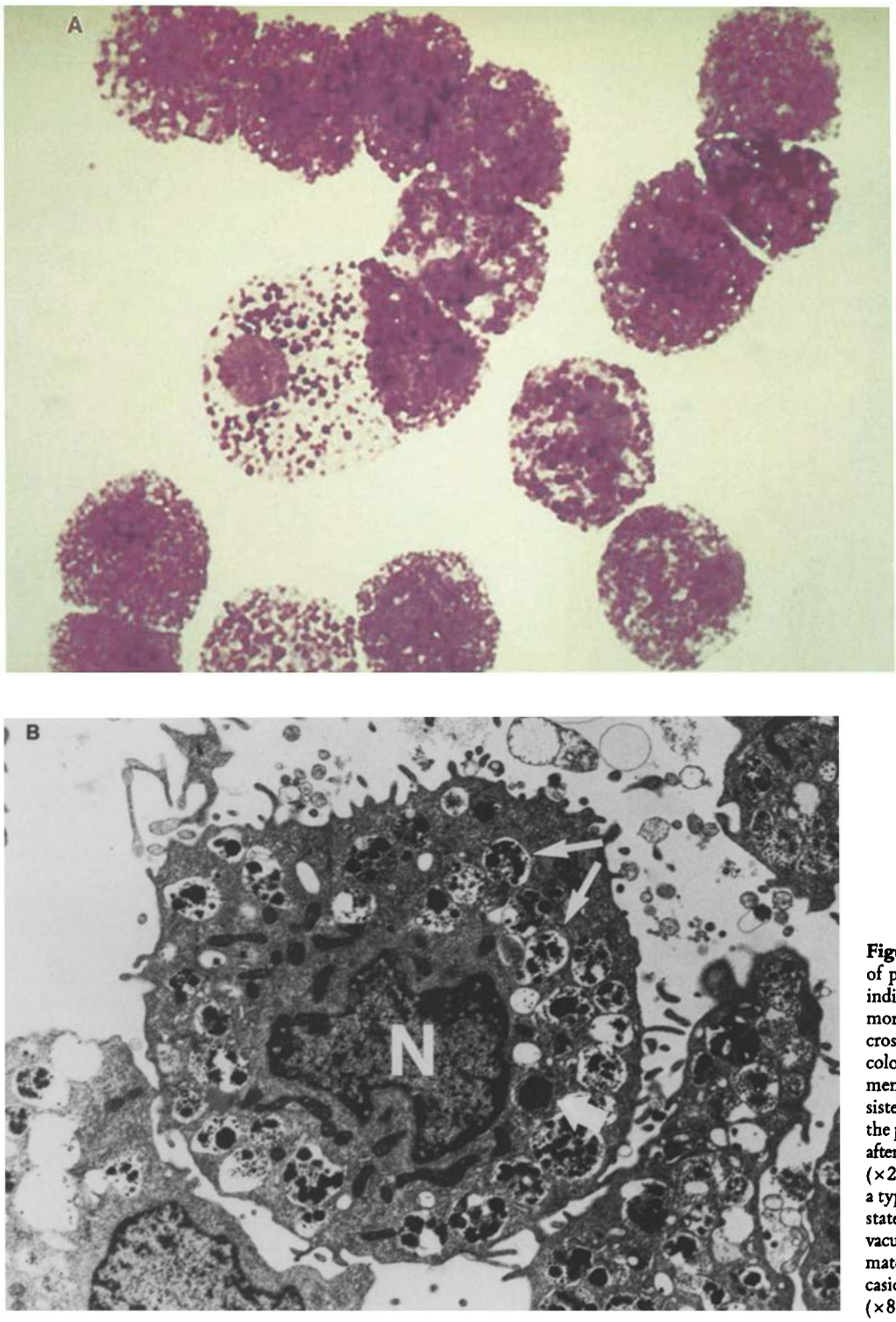

Figure 4. IL-10 augments production of pure mast cell colonies. Cells from individual colonies were analyzed for morphology by light and electron microscopy. Greater than 98% of all the colonies generated in cultures supplemented with IL-3 + IL-4 + IL-10 consisted of pure mast cells identified by the presence of metachromatic granules after staining with Wright's-Giemsa (*A*) (×2,000). An electron micrograph of a typical cell (*B*) confirms its immature state by the presence of cytoplasmic vacuoles incompletely filled with dense material (*small arrows*) and only an occasional dense granule (*large arrow*) (×8,500). N = nucleus.

were analyzed for cellular composition and were found to consist entirely of mast cells (Fig. 4 *A*). To determine whether CTMC were present, cells were stained with berberine sulfate to detect heparin. All colonies tested were negative. Furthermore, none of the cells stained with safranin, while all stained with alcian blue (data not shown). Thus, it appears that the colonies generated by various concentrations of IL-3, IL-4, and IL-10 were composed of mucosal-like mast cells. This was confirmed by the ultrastructural appearance of cytoplasmic vacuoles containing small electron-dense progranules (Fig. 4 *B*) similar to those previously described for several immature mucosal mast cell lines (1).

Because IL-10 has been shown to be a product of activated T cell (6) and B cell subsets (9), it is likely to play a role in certain immune responses, especially those associated with an overt mastocytosis. For example, a T cell–dependent increase in mast cells occurs within the intestinal tract and lungs of mice infected with *Nb*. Among those T cell factors affecting mast cell growth, IL-3, IL-4, and, recently, IL-10 have been shown to be produced during *Nb* infection (12, 13; and R. Coffman, personal communication). Here, we show that the optimal growth of mast cell progenitors isolated from *Nb*-infected mice depends on the presence of all three factors. The sum of these studies provides indirect but compelling evidence that IL-10 may be an important part of the cytokine network that determines the magnitude of a parasite-induced mastocytosis.

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