Regulation of 10P2 Murine Mast Cell Proliferation and Secretory Function by Stem Cell Factor or IL-9 (44254)

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Abstract. **Mast cells are effectors of inflammatory responses. When triggered by immunological or nonimmunological mechanisms, mast cells release potent biological mediators from preformed stores and synthesize others *de novo.* In previous investigations from this laboratory, the signal transduction pathways of cloned 10P2 cytokine-independent mast cells were explored. Results suggested that 10P2 cells undergo activation-secretion coupling assessed as release of stored [^{14}C]serotonin (5-HT) when challenged with IgE-specific antigen, influx of extracellular calcium, release of intracellular calcium stores, or by direct activation of protein kinase C isozymes. In the present investigations, cytokine proliferative effects and modulatory roles on release of stored [^{14}C]5-HT have been explored. Following passive sensitization with anti-dinitrophenol (anti-DNP) IgE and challenge with DNP, mast cells released up to 32% of the stored [^{14}C]5-HT. Pretreatment of cells with 10, 30, or 50 ng/ml stem cell factor (SCF) did not alter the response. SCF did not directly induce [^{14}C]5-HT release. Pretreatment with 25 ng/ml interleukin-9 (IL-9) significantly potentiated the IgE-antigen release by 51.1%, 35.7%, or 31.6% when challenged with 3, 10 or 30 ng/ml DNP-HSA. Treatment of cells with 1–100 ng/ml SCF for 72 hr resulted in significantly enhanced proliferation whereas this did not occur when cells were treated with 1–100 ng/ml IL-9. Collectively, these results suggest that SCF alone has a proliferative effect, does not alter the IgE-specific antigen signal transduction pathway, and does not directly stimulate mast cell degranulation. In contrast, IL-9 potentiates the IgE-antigen signal transduction response but exerts no proliferative response. Reports of effects of orally administered cytokines are now beginning to emerge. This raises the possibility that cytokines may be a future therapeutic approach to treatment of allergic and nonallergic inflammatory diseases. The 10P2 cytokine-independent mast cell line may be a valuable adjunct to existing mast cell models as this avenue of drug discovery is explored.** [P.S.E.B.M. 1998, Vol 217]

Mast cell precursors, derived from multipotential hematopoietic stem cells, migrate in the bloodstream and enter the tissues where they differentiate into morphologically identifiable mast cells (1). Differentiated mast cells have been classified into at least two phenotypically distinct subpopulations, connective tissue–type mast cells (CTMC) and mucosal mast cells (MMC) (2). CTMC preparations are best exemplified by metrizamide-purified rat or mouse peritoneal mast cells and may easily be studied as *ex vivo* preparations (3, 4). Murine bone marrow–derived mast cell (BMMC) model systems result from culturing bone marrow hematopoietic cells with various cytokines (IL-3, IL-5, and stem cell factor) (5, 6). These cytokine-dependent BMMCs were once thought to be mucosal-type mast cell models; however, results of cDNA and protease-6 gene cloning experiments suggest that these cytokine-dependent cells may be immature connective tissue–type mast cells (7). Recently, another murine mast cell model has been derived from midgestation embryonic placenta and transformed *in vitro* with Abelson murine leukemia virus. Cells were subsequently cloned from semisolid agarose and established as the growth-factor-independent line designated 10P2 (8). Results of characterization experiments indicate that 10P2 cells contain histamine, express

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Fc receptors for IgE, and also test positive for the antigen recognized by hybridoma B23.1, which is expressed on mast cells from bone marrow, spleen, and blood. 10P2 cells have also been shown to synthesize chondroitin sulfate but not heparin proteoglycan. Based on these characteristics, it has been suggested that 10P2 cells may be of the MMC type (8, 9).

Mast cells participate in inflammatory responses by synthesizing and releasing a variety of proinflammatory mediators following activation by any of several signal transduction pathways that include crosslinking of IgE bound to surface FcεRI receptors, calcium ionophore, phorbol esters and certain bioactive peptides (3, 4, 10, 11). In previous investigations from this laboratory, the signal transduction pathways of the 10P2 cytokine-independent mast cells were explored. Results suggested that 10P2 cells undergo activation-secretion coupling, assessed as release of [^{14}C]serotonin (5-HT), when challenged with IgE-specific antigen, influx of extracellular calcium, release of intracellular calcium stores, or by direct activation of protein kinase C isozymes (12).

Recently published results have suggested that murine CTMC development and secretory functions are influenced by several cytokines including stem cell factor, IL-3 and IL-4 (13, 14). Stem cell factor (SCF), a recombinant form of the ligand for the c-*kit* receptor, has been shown to induce proliferation of murine peritoneal mast cells *in vitro* (15–17). BMMC did not undergo differentiation when treated with SCF alone. Differentiation was best supported by cotreatment with IL-3, IL-4 and SCF (15, 18). *In vitro* proliferative responses were also noted when murine CTMC cells were treated with combinations of IL-3 and IL-4 (13, 19, 20). Previous results have suggested that IL-4 pretreatment of 10P2 cells significantly potentiated the IgE-specific antigen degranulation response, assessed as release of [^{14}C]5-HT, but did not induce proliferation (12). IL-9, formerly known as P40, is a cytokine originally identified as a murine T-cell growth factor. The spectrum of potential human and murine cell targets was recently shown to include mast cells, megakaryoblastic leukemia cells, and T-cell lines (21–23). Murine IL-9 has recently been found to enhance proliferative responses of certain mast cell lines cultured in the presence of IL-3 (24–27).

In continuing investigations of the cytokine-independent 10P2 murine mast cell model system, proliferative responses to SCF or IL-9 and effects on the IgE-specific antigen signal transduction pathway have now been explored. As a valuable adjunct to existing cytokine-dependent mast cell models, the 10P2 mast cell model system may be useful for increasing our understanding of the effects of a specific cytokine on mast cell proliferative or activation-secretion pathways.

Material and Methods

Materials. Cell culture medium, Hank's balanced salts solution (HBSS), 5-hydroxytryptamine-β-^{14}C ([^{14}C]5-HT) (58.7 mCi/mmol), dinitrophenol human serum albumin (DNP-HSA), stem cell factor (SCF), and interleukin 9 (IL-9) were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was purchased from Atlanta Biologicals (Atlanta, GA), and mouse monoclonal anti-dinitrophenol-IgE (anti-DNP-IgE) was purchased from ICN Pharmaceuticals Inc. (Costa Mesa, CA). All other chemicals were of reagent grade and purchased from Fisher Scientific (Norcross, GA).

Cell Culture. The murine 10P2 mast cell line was purchased from the American Type Culture Collection (Rockville, MD) (ATCC CRL-2034) and propagated in suspension culture in RPMI 1640 supplemented with 0.05 m*M* 2-mercaptoethanol and 10% (v/v) fetal bovine serum at 35°C, 5% CO_2 and 95% Rh. Cells were subcultured by centrifugation with subsequent resuspension in the above medium at 1×10^5 viable cells/ml. Cell viability was assessed microscopically by Trypan blue exclusion.

Cytokine Treatment and Passive Sensitization. Murine 10P2 cells were centrifuged (100g, 5 min), resuspended in the supplemented RPMI 1640 medium, and treated with indicated concentrations of murine recombinant SCF or IL-9. Concomitantly, DNP-IgE, final dilution 1:1000 (v/v), was then added and cells incubated overnight at 35°C, 5% CO_2, 95% Rh (3, 8).

[^{14}C]5-HT Uptake, Release, and Measurement. To evaluate the release of a classical preformed mediator, release of the cytoplasmic granule-associated substance serotonin was monitored. Following cytokine treatment and passive sensitization, 10P2 cells were centrifuged (100g, 5 min) and washed in 10 ml HBSS, and the cell pellet was resuspended in 10 ml supplemented RPMI containing 1 μCi/ml [^{14}C]5-HT. Cells were incubated at 35°C, 5% CO_2, 95% Rh for 3 hr, then washed and resuspended in HBSS at 0.25×10^6 cells/0.225 ml buffer. Aliquots of 0.025 ml of various concentrations of secreting agents were dispensed to polypropylene microcentrifuge tubes, and release was begun by addition of 0.225 ml warmed cell suspension. Release was allowed to proceed for 45 min at 35°C, then halted by addition of 0.5 ml chilled calcium-free HBSS. Tubes were centrifuged in a microcentrifuge for 2 min (2000 rpm) and aliquots (0.2 ml) counted by scintillation spectroscopy to determine the amounts of [^{14}C]5-HT released. Total [^{14}C]5-HT mast cell uptake was similarly determined in separate tubes of cells lysed with 1% triton X-100 in buffer (4).

Cell Proliferation Investigations. To determine proliferative effects of cytokines, murine 10P2 cells were centrifuged, washed in HBSS, resuspended in the culture medium described above, and dispensed (0.1×10^6 cells/ml) into 24-well flat bottom tissue culture plates. Cultures were incubated for 72 hr at 35°C, 5% CO_2 and 95% Rh. At 24, 48, and 72 hr, aliquots were removed and total cells/well quantitated using enhanced Neubauer chambers.

Data Handling. Experiments were conducted in triplicate or quadruplicate on the number of separate occasions

indicated in the respective legends. Data shown are expressed as the mean ± SEM. One-way analysis of variance (ANOVA) was applied to results. When indicated, ANOVA was followed by either the Dunnett multiple comparisons post-test or the Tukey-Kramer multiple comparisons post-test to determine statistical significance ($P < 0.05$) between indicated groups.

Results

Specific Antigen Induces Release of [^{14}C]5-HT from Passively Sensitized 10P2 Mast Cells. 10P2 mast cells are known to express receptors for IgE (9). To characterize the IgE-specific antigen activation-secretion pathway responses of these cells, 10P2 cells were passively sensitized with anti-DNP IgE, incubated with [^{14}C]5-HT, washed, and stimulated with indicated concentrations of the specific antigen DNP-HSA. Results, shown in Figure 1, indicate that 10P2 cells can be sensitized with IgE, are able to take up and store [^{14}C]5-HT and concentration-dependently release the incorporated mediator when stimulated with specific antigen. Over the antigen concentration range of 3–300 ng/ml 10P2 cells consistently released 20%–32% of the total incorporated [^{14}C]5-HT. These results suggest that 10P2 mast cells have a functional immunoglobulin-specific antigen signal transduction pathway. This system was then used to explore the effects of SCF or IL-9 on mast cell activation-secretion.

Stem Cell Factor Does Not Potentiate or Induce Release of [^{14}C]5-HT from 10P2 Mast Cells. In previously published results, we have reported that overnight pretreatment of 10P2 cells with 10 ng/ml of IL-4 significantly potentiated release of [^{14}C]5-HT by 28% from passively sensitized cells stimulated with specific antigen (12). Increasing the cytokine concentrations to 20, 30, or 50 ng/ml increased the degranulation response to 35%–38%. In continuing investigations of the effects of cytokines on 10P2 cell responses, similar concentrations of SCF were used to pretreat 10P2 cells. As shown in Figure 2, SCF did not affect the immunoglobulin-specific antigen induced release of [^{14}C]5-HT. In data not shown, it was found that short pretreatments of 20 or 30 min did not affect IgE-antigen stimulated release of [^{14}C]5-HT. To determine the ability of SCF to induce release of [^{14}C]5-HT directly, cells were stimulated with 1–500 ng/ml SCF. No induced release

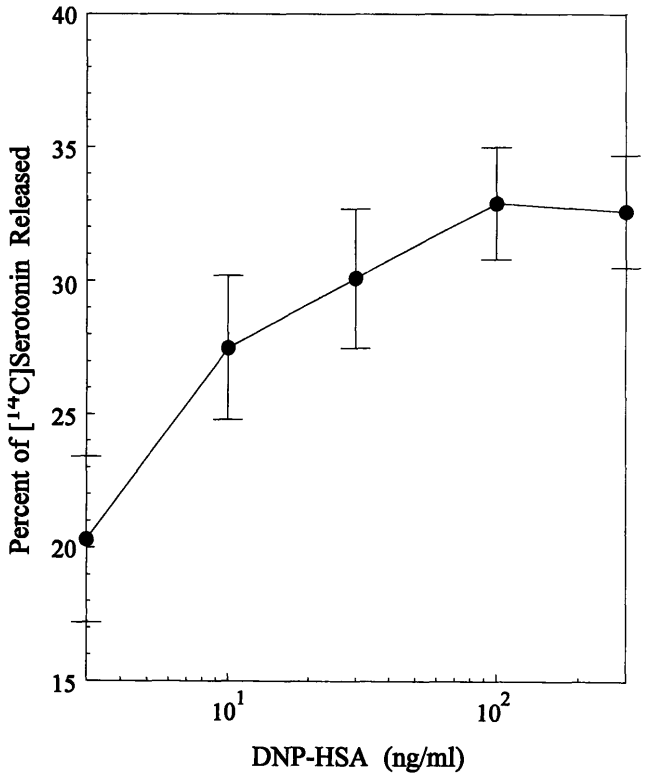

Figure 1. Passively sensitized 10P2 cells release preloaded [^{14}C]5-HT when stimulated with specific antigen. 10P2 cells were passively sensitized with murine anti-DNP IgE (1:1000 dilution) overnight in RPMI with 10% fetal calf serum. [^{14}C]5-HT was added for the final 3 hr of incubation. Cells were washed, resuspended, and stimulated with the indicated concentrations of DNP-HSA. Total incorporation was 1493 ± 51 cpms, and unstimulated release was 185 ± 8 cpm (12.4% of total). Points represent the mean ± SEM of three experiments each performed in triplicate.

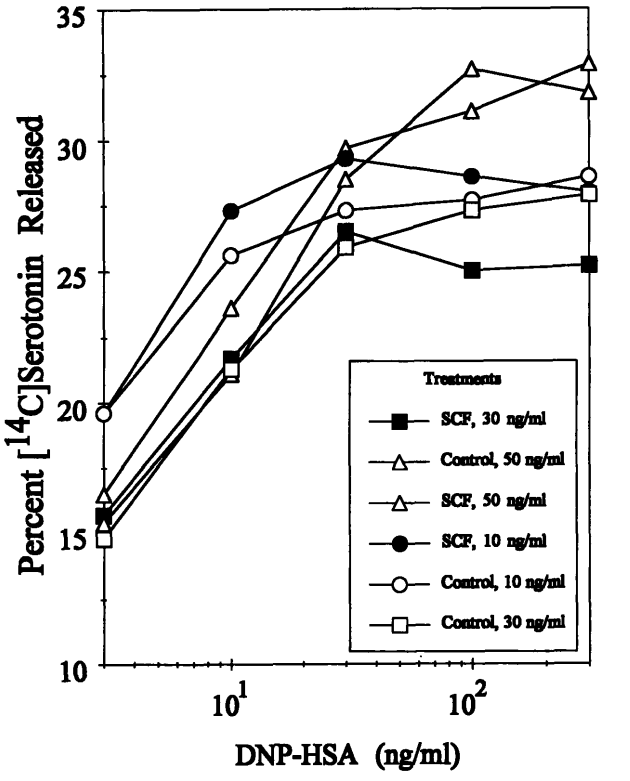

Figure 2. Effect of stem cell factor pretreatment on passively sensitized 10P2 cells stimulated with IgE-DNP. 10P2 cells were treated with (filled symbols) or without (open symbols) stem cell factor (10, 30, or 50 ng/ml) and passively sensitized with murine anti-DNP IgE (1:1000 dilution) overnight in RPMI with 10% fetal calf serum. [^{14}C]5-HT was added for the final 3 hr of incubation. Cells were washed, resuspended, and stimulated with the indicated concentrations of DNP-HSA. Total incorporation and background releases were 1982 ± 267 cpms/0.5 × 10^6 cells and 239 ± 22 cpms/0.5 × 10^6 cells, respectively. Points represent the mean ± SEM of two experiments each performed in triplicate. The range of the SEM for all data was 3.3%–6.4%.

was noted. Collectively, these results suggest that SCF does not potentiate or reduce IgE-specific antigen activation-secretion responses of 10P2 cells, and SCF is not a 10P2 cell degranulating agent.

Stem Cell Factor has a Proliferative Effect on 10P2 Murine Mast Cells. The proliferative effect of SCF was investigated by culturing 10P2 cells in the presence of 1–100 ng/ml of the cytokine for 72 hr. Results of total cell counts following 48- and 72-hr treatments are shown in Figure 3. SCF significantly increased cell numbers at 30 and 100 ng/ml following 48-hr treatments and 3–100 ng/ml following 72-hr treatments. At the highest concentration tested, cell numbers were significantly increased by 22.8% and 25.1% at 48 and 72 hr, respectively. Taken together, results suggest that SCF significantly augmented on-going proliferative effects but does not induce activation-secretion or exert activation-secretion potentiating effects on 10P2 cells.

IL-9 Potentiates Release of [^{14}C]5-HT from Sensitized 10P2 Mast Cells. To explore the effects of IL-9 on the IgE-specific antigen 10P2 cell signal transduction pathway, cells were pretreated with IL-9 at 10 or 25 ng/ml, concentrations shown to be effective for IL-4-induced potentiation. Pretreatment with 10 ng/ml IL-9 slightly, but not significantly, enhanced IgE-antigen induced release of [^{14}C]5-HT. Figure 4 depicts results of passively sensitized cells treated with 25 ng/ml IL-9 and stimulated with the indicated concentrations of DNP-HSA. Untreated cells concentration-dependently released 14.3%–31.4% of the incorporated [^{14}C]5-HT. IL-9 pretreated cells released 18.2%–43.1% of the incorporated [^{14}C]5-HT. Significant potentiation of the immunoglobulin-specific antigen signal transduction response was noted at 3, 10, and 30 ng/ml DNP-HSA. In results not shown, it was determined that culturing 10P2 cells in the presence of 1–100 ng/ml IL-9 for 72 hr did not induce a proliferative response, as assessed by total cell numbers. Collectively, these results suggest that, in contrast to SCF, IL-9 acts to potentiate 10P2 mast cell activation-secretion but does not exert proliferative effects.

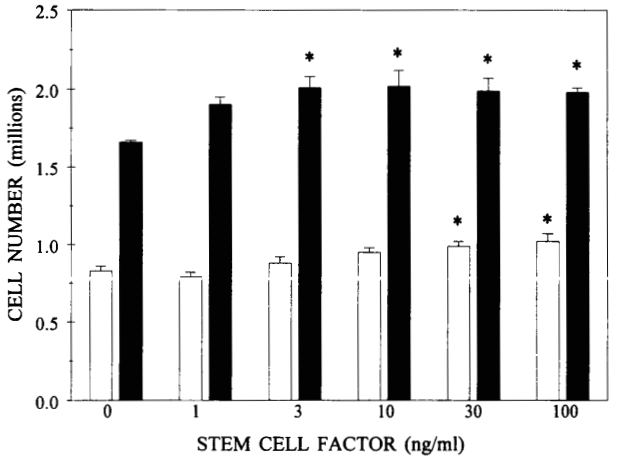

Figure 3. Proliferative effect of stem cell factor on 10P2 cells. Cells were washed and resuspended in RPMI with 10% fetal calf serum at 0.1 x 10^6 cells/ml and seeded into 24-well plates with indicated concentrations of stem cell factor for 48 hr (open bars) or 72 hr (shaded bars). Values represent the mean ± SEM of the total number of cells/ml (millions) from two experiments each performed in quadruplicate. Asterisks indicate those values that differed significantly from untreated controls at $P < 0.05$ as assessed by ANOVA followed by a Dunnett's multiple comparisons post-test.

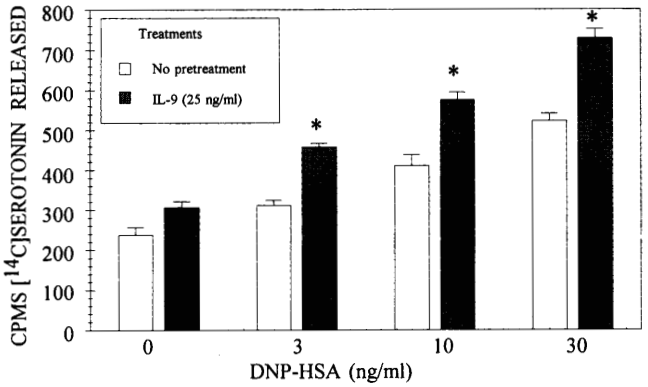

Figure 4. IL-9 pretreatment increases degranulation in passively sensitized 10P2 cells stimulated with IgE-DNP. 10P2 cells were treated with or without IL-9 (25 ng/ml) and passively sensitized with murine anti-DNP IgE (1:1000 dilution) overnight in RPMI with 10% fetal calf serum. [^{14}C]5-HT was added for the final 3 hr of incubation. Cells were washed, resuspended, and stimulated with the indicated concentrations of DNP-HSA. Total incorporation was 1589 ± 48 cpms/0.36 x 10^6 cells. Points represent the mean ± SEM of triplicates of an experiment performed on three separate occasions with the same outcome. Asterisks indicate those values that differed significantly from untreated controls at $P < 0.05$ as assessed by ANOVA followed by a Tukey-Kramer multiple comparisons post-test.

Discussion

The present investigations were conducted to determine responses of the cytokine-independent 10P2 murine mast cell line to SCF or IL-9 treatments. Proliferative effects and potentiation of the IgE-specific antigen signal transduction pathway were assessed. Results suggest that SCF alone has a proliferative effect, does not alter the IgE-specific antigen signal transduction pathway, and does not directly stimulate cellular degranulation. In contrast, IL-9 potentiates the IgE-specific antigen signal transduction response but exerts no proliferative response. While originally developed to investigate signals involved in maturation of immunocompetent cells, the present results suggest that the cytokine-independent 10P2 mast cell line may be a valuable model system for exploring the specific effects of singly applied cytokines.

It is now well accepted that certain cytokines are potent regulators of mast cell development and/or secretory function. When immature murine mast cells, derived by culture of bone marrow cells in WEHI-3 cell-conditioned medium, were cultured with recombinant SCF, sensitized with IgE, and stimulated with antigen, prostaglandin D_2 generation increased 3-fold (28). Similarly, treatment of human lung mast cells with human SCF significantly enhanced release of preformed and *de novo* synthesized inflammatory mediators when cells were activated by IgE receptor stimulation.

SCF did not directly induce release of preformed or *de novo* synthesized inflammatory mediators (29). Recombinant rat SCF induces direct degranulation of rat CTMC both *in vivo* and *in vitro* (30). Similarly, human recombinant SCF induces mediator release from human cutaneous mast cells and enhances IgE-dependent mediator release from both cutaneous mast cells and peripheral blood basophils (31). Results of the present investigations suggest that treatment of 10P2 cells did not directly induce degranulation or affect release of [^{14}C]5-HT from passively sensitized cells challenged with specific antigen. Responses are not those observed in either CTMC models or immature murine mast cells cultured in the presence of IL-3 and other cytokines that may be present in WEHL-3 cell-conditioned medium.

When employed as the sole cytokine, SCF has been shown to induce proliferation of murine CTMC *in vitro* (32). Nakahata *et al.* examined the effects of SCF on mast cell colony formation from murine bone marrow cultured with various combinations of IL-3, IL-4, and SCF for 14 days. When used as sole cytokines, only IL-3 supported BMMC colony formation. However, a combination of IL-4 and SCF synergistically induced colony formation of BMMC (16). To investigate SCF regulaton of 10P2 mast cell proliferation, cells were cultured with SCF for 72 hr. Within this short time frame, on-going cell proliferation was significantly augmented. However, this result may not demonstrate initiation of proliferation by this cytokine. In previous results, it was noted that IL-4 alone did not exert proliferative effects on 10P2 mast cells.

IL-9, a T-lymphocyte derived cytokine, may also be involved in mast cell differentiation, proliferation, and activation (33, 34). Murine IL-9 has recently been found to enhance proliferative responses of certain mast cell lines cultured in the presence of IL-3 (24–27). In murine BMMC, IL-9 is known to act in concert with SCF and IL-3 (provided as WEHI-3 cell-conditioned medium) to enhance IgE-dependent PGD_2 generation. IL-9 did not, however, increase the generation of IgE-dependent LTC_4 generation (34). Results of the present investigations demonstrate that IL-9 treatment potentiates the IgE-specific antigen induced release of [^{14}C]5-HT. Taken together, these results suggest that IL-9 may increase release of preformed inflammatory mediators and selectively enhance the *de novo* generation of proinflammatory mediators when mast cells are stimulated *via* the IgE-antigen signal transduction pathway.

Effects of cytokines, including IL-9, have recently been investigated in suspension cultures of umbilical cord blood mononuclear cells induced to differentiate into mast cells by treatment with SCF and IL-6. Addition of IL-9 (100 ng/ml) to these SCF and IL-6 cytokine-dependent cultures had no survival-promoting or proliferative effect (35). Results of the present investigations demonstrated that treatment with IL-9 (1–100 ng/ml) alone had no proliferative effect on the 10P2 cytokine-independent mast cells. Taken together, these results suggest that IL-9 may exert its specific effects on mast cell activation-secretion events rather than survival or proliferation.

In summary, results of the present investigations with the 10P2 cytokine-independent cloned murine mast cell line suggest it may be a useful model for investigating the effects of cytokines, applied singly or in combinations, on both activation-secretion signal transduction pathways and cell proliferation. Knowledge of the cytokines that modulate mast cell/basophil differentiation, growth, and activation may be very relevant to a wide range of allergic and nonallergic inflammatory or bone marrow diseases including allergic airway disease, certain myeloproliferative disorders, and allergic skin disorders (36). Reports of effects of orally administered cytokines are now beginning to emerge. This raises the possibility that cytokines may be a future therapeutic approach to treatment of allergic and nonallergic inflammatory diseases. The 10P2 cytokine-independent mast cell line may be a valuable adjunct to existing mast cell models as this avenue of drug discovery is explored.

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