INTERLEUKIN 3-DEPENDENT AND -INDEPENDENT MAST CELLS STIMULATED WITH IgE AND ANTIGEN EXPRESS MULTIPLE CYTOKINES

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Mast cells (MC)[1] are widely distributed throughout vascularized tissues and certain epithelia. They represent a source of potent mediators of inflammation (reviewed in references 1–4). These mediators are released after sensitization with IgE immunoglobulins, which are bound to IgE receptors (FcεRI) on the MC, and crosslinking with specific multivalent antigen (4). Such activation causes MC to degranulate releasing histamine, heparin, and other sulphated proteoglycans and certain neutral proteases. Activated MC also elaborate newly synthesized mediators such as products of the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (reviewed in references 2–4). MC are widely regarded as critical effector cells in the inflammatory reactions underlying disorders of IgE-dependent immediate hypersensitivity and in the expression of protective immunity involving IgE (reviewed in references 1–4).

Studies in mice indicate that MC are derived from multipotential bone marrow-derived hematopoietic precursors which complete their program of differentiation and maturation in vascularized tissues, epithelia, and serosal cavities (reviewed in references 1, 5). This process results in the generation of mast cell populations which vary in multiple aspects of their phenotype, including morphology, mediator content, and sensitivity to regulation by cytokines affecting proliferation and maturation (reviewed in reference 1). One such population, referred to as "mucosal" mast cells (MMC) because they occur in the mucosal layer of gastrointestinal tissues, appears to be exquisitely sensitive to regulation by the T cell-associated cytokines IL-3 and IL-4 (1). IL-3 probably represents the major cytokine regulating proliferation of this subset (6, 7), whereas in vitro studies indicate that IL-4 can act as a costimulant of proliferation (8). Thus, the mouse MMC population is regulated by products

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[1] *Abbreviations used in this paper:* AbMuLV, Abelson murine leukemia virus; Ag, antigen; BMCMC, bone marrow-derived cultured mast cell; DNP_{30-40}-HSA 2,4-dinitrophenyl-human serum albumin; FcεRI, cell surface receptor for the Fc portion of IgE; GM-CSF, granulocyte/macrophage colony-stimulating factor; MC, mast cell; MIP, macrophage inflammatory protein; MMC, mucosal mast cell; PKC, protein kinase C.

of the same $CD4^+$ T cells (9, 10), which, through production of IL-4, critically regulate the generation of the IgE response (11).

Techniques for purifying mouse MMC have not been described. However, IL-3-dependent mouse MC which share many phenotypic characteristics with MMC can be generated from normal mouse hematopoietic cells *in vitro* and these primary MC cultures can be used to isolate cloned MC lines of similar phenotype (1, 12, 13). Such clones may spontaneously become IL-3-independent (14, 15). IL-3-independent mouse MC lines can also be generated by infecting mouse hematopoietic cells with Abelson murine leukemia virus (AbMuLV) (16–19). Studies of AbMuLV-transformed MC lines indicate that some of these populations transcribe mRNA and/or release GM-CSF (17, 18, 20), IL-3 (18, 20), IL-4 (18), and IL-6 (20). Moreover, some unstimulated IL-3-dependent MC lines transcribe low levels of IL-4 mRNA, but do not secrete detectable levels of IL-4 (18).

In addition, Young et al. (14) reported that several IL-3-dependent and -independent mouse MC clones, as well as freshly isolated peritoneal MC, contain and secrete upon stimulation a protein with many immunological and functional characteristics of the monocyte/macrophage-associated cytokine TNF-α (cachectin). Richards et al. (21) showed that a rat basophilic leukemia cell line also produced a TNF-α-like cytolytic agent, and that release of this activity could be enhanced by stimulation of the cells with IgE and specific antigen. Plaut et al. (22) reported that stimulation with IgE and antigen induced gene expression and/or secretion of IL-3, IL-4, IL-5, and IL-6 in IL-3-dependent mast cell lines.

Taken together, the observations about MC-derived activities previously regarded as characteristic of T cells or monocytes/macrophages raised the possibility that MC might represent an important alternative source of cytokines during IgE-mediated immunological and inflammatory responses. We therefore assessed RNA levels after activation with IgE plus antigen for a large panel of T cell and/or monocyte/macrophage-associated cytokine genes in both IL-3-dependent and -independent mouse MC populations. We found that growth factor-dependent and -independent MC lines can be induced to transcribe a variety of cytokine genes and secrete their products in response to activation via specific crosslinking of FcϵRI. These data suggest that during IgE-mediated hypersensitivity responses, MC may serve as a potent source of growth, regulatory, and inflammatory agents having a broad range of biologic effects.

Materials and Methods

Cell Lines and Tissue Culture. Cl.MC/2D4 and Cl.MC/C57.1 are cloned growth factor-independent mast cell lines derived from BALB/c mouse spleen cells and C57BL/6J mouse bone marrow cells respectively (14). PT18 is a growth factor-dependent cell line derived from C3H.SW mouse spleen cells (23). Cl.MC/9 is a growth factor-dependent MC line derived from a 13 d (C57BL/6J × A/J) F_1 fetus (12, 13). The Cl.MC/9 cells used for this work were recently (~4 mo before this study) grown from stocks frozen in 1982. Cl.MC/9.2 is a growth factor-dependent subline of Cl.MC/9 obtained from D. Rennick (DNAX Research Institute, Palo Alto, CA) (24) that has been maintained in continuous culture. All cell lines were periodically monitored for growth factor dependence or independence and were assessed for maintenance of the mast cell phenotype by light and electron microscopic examination and analysis of histamine content.

Growth factor-containing cell culture supernatants for maintenance of the factor-dependent MC lines were generated by 24-h Con A (4 μg/ml) stimulation of D10.G4 cells (25). Cl.MC/9,

Cl.MC9.2, and PT-18 cells were maintained routinely in factor-conditioned medium (5% Con A-stimulated D10.G4 cell supernatant, 10% FCS, and 5×10^{-5} M 2-ME in DMEM (Gibco Laboratories, Grand Island, NY)). The factor-independent cell lines Cl.MC/2D4 and Cl.MC/C57.1 were maintained in 10% FCS and 5×10^{-5} M 2-ME in DMEM (Gibco Laboratories). IL-3-dependent bone marrow-derived cultured MC (BMCMC) were derived as indicated previously (12, 13), and were maintained in 20% Con A-stimulated murine spleen cell-conditioned medium, 10% FCS, and 5×10^{-5} M 2-ME in DMEM. Cells harvested for RNA (or DNA) analysis were stimulated for 4 h at a density of 3×10^6 cells/ml in the culture medium routinely used for maintenance of that cell population. Where noted, cells were stimulated in the presence of cyclosporin A (2 μg/ml) or cycloheximide (10 μg/ml) (Sigma Chemical Co., St. Louis, MO). Cell supernatants for bioassay were obtained after 24 h of culture in 5% FCS in RPMI 1640 (Cl.MC/9) or DMEM (Cl.MC/C57.1, BMCMC) with an initial culture density of 10^6 cells/ml.

Where noted, MC were stimulated with 50 ng/ml PMA (stock 10 μg/ml in acetone), 2.5 μg/ml Con A (stock 400 μg/ml in RPMI), or 100 ng/ml A23187 (stock 110 μg/ml in DMSO). Monoclonal IgE anti-DNP-producing hybridoma H1-DNP-E-26 (26) was generously provided by Drs. F.-T. Liu and D. H. Katz (Molecular Biology Institute, La Jolla, CA) and was used to generate ascites. MC were sensitized with the ascites at a final concentration equivalent to 3 μg IgE anti-DNP/ml cells. MC were incubated for 30 min at room temperature with IgE antibody, washed with medium, then resuspended with medium containing DNP_{30-40}-HSA (10 ng/ml; Sigma Chemical Co., St. Louis, MO). These conditions of stimulation were shown previously to be adequate for inducing degranulation and mediator release from these clones (12, 27, 28).

Northern Blot Analysis. Total cellular RNA was isolated by guanidine thiocyanate/cesium chloride gradient centrifugation as described elsewhere (29). 20 μg RNA was denatured in 50% formamide and 2.2 M formaldehyde, fractionated on 1.2% agarose gels, and transferred to nylon-reinforced nitrocellulose (MSI, Westboro, MA) as described (30). RNA sizes were determined by comparison to RNA size markers obtained from Bethesda Research Laboratories, Gaithersburg, MD.

Hybridizations were performed at 37–42°C in 50% formamide, 5× Denhardt's solution, 0.66 M NaCl, 0.09 M $NaPO_4$ (pH 7.0), 0.4 mM Na_3EDTA, 0.09 M sodium pyrophosphate, 0.3% SDS, and 130 μg/ml sonicated, denatured herring sperm DNA. Hybridized blots were washed in 30 mM NaCl and 3 mM sodium citrate (pH 7.0), containing 0.25 mM Na_3EDTA, 0.02 M $NaPO_4$, and 0.1% SDS.

cDNA Probes. Cytokines probes were obtained as follows: murine IL-1α was a gift of Drs. P. Lomedico and U. Grubler (Hoffman LaRoche, Nutley, NJ) (31), murine IL-3 and IFNγ were a gift of Dr. G. Freeman (Dana-Farber Cancer Institute, Boston, MA) (32), JE (33) was a gift of Dr. B. Rollins (Dana-Farber Cancer Institute, Boston, MA), IL-5, IL-6 (34) and granulocyte/macrophage CSF (GM-CSF) (35) were obtained from Dr. S. Clark (Genetics Institute, Cambridge, MA), MIP1α/TY5 (36, 37) and MIP1β/mH400 (37, 38) were gifts of Dr. G. Zurwaski (DNAX Research Institute, Palo Alto, CA) (Note that TY5 and mH400 are referred to as MIP1α and MIP1β in this study), and TCA3 was cloned in this laboratory (39).

cDNA inserts were excised by restriction digestion and twice gel purified by NaI/glass treatment according to the recommendations of the manufacturer (Bio 101, La Jolla, CA). Probes were labeled by random hexamer priming using protocols and reagents suggested by the manufacturer (Bethesda Research Laboratories, Gaithersburg, MD).

Bioassays. All the bioassays were performed in 96-well flat-bottomed microplates (Falcon Labware, Oxnard, CA) in 0.2 ml medium. IL-1 production was measured by the D10.G4.1 proliferation assay in the presence of Con A as described (25, 40). IL-4 bioactivity was measured by assaying the proliferative effects of MC supernatants on the HT-2 cell line. IL-4 specificity was confirmed by antibody inhibition; supernatants were incubated with HT-2 cells in the presence of 1:200 dilution of ascites from anti-IL-4 hybridoma 11B11 (41; a gift of Dr. W. Paul, National Institutes of Health, Bethesda, MD). IL-6 activity was measured using the IL-6-dependent myeloma cell line T1165tc as described elsewhere (42). IL-1, IL-4, and IL-6 activities are reported as cpm of target cell proliferation.

Results

Stimulation of Mast Cells via the FcεRI Leads to Cytokine Gene Expression. We examined in unstimulated MC and in MC activated via FcεRI the transcription levels of a number of cytokine and T cell activation-associated genes. Three growth factor-dependent (Cl.MC/9, Cl.MC/9.2, and PT-18) and one growth factor-independent (Cl.MC/C57.1) MC clones were analyzed. Cells were incubated with IgE for 30 min, washed to remove unbound antibody, exposed to specific antigen for 4 h, and harvested for Northern analysis. Unstimulated (resting) cells not incubated with IgE and antigen were also analyzed. Although absolute levels of RNA were not quantitated, when all four lines were tested on the same blot, relative amounts of RNA could be assessed. Actin was used to verify the integrity of the RNA. Our decision to examine transcription at 4 h after stimulation was based on observations that many T cell-specific genes transcribed in response to cellular activating stimuli are "early genes" and are detectable as soon as 1–2 h after stimulation (29, 43), and on observations that stimulation of MC lines with IgE and antigen for 4 h greatly augments expression of TNFα RNA (Gordon, J. R., and S. J. Galli, unpublished observations).

Cl.MC/9 expressed all but one (IFN-γ) of the genes tested in response to activation by IgE and antigen (Fig. 1), but before activation only low levels of JE were detectable (Fig. 2). Cl.MC/9.2 transcribed lower levels of these genes (except for IL-1, IL-3, and JE, which could not be detected) in response to FcεRI signaling and did not express any of the genes before activation. In contrast, only IL-5, IL-6, and IFN-γ RNA were detected in the PT-18 cell line after activation (Fig. 1). IL-6 RNA was also present in untreated PT-18 cells and expression of IL-6 RNA was not significantly enhanced by activation of PT-18 cells (data not shown). Each of the tested genes was induced in the growth factor-independent cell line Cl.MC/C57.1. However, low levels of IL-6 (Fig. 3) and JE (data not shown) were also expressed in resting Cl.MC/C57.1 cells. In separate experiments using another growth factor-independent MC clone, Cl.MC/2D4, we observed activation specific expression of the three lymphokine genes tested, i.e., IL-1, IL-6, and TCA3 (data not shown).

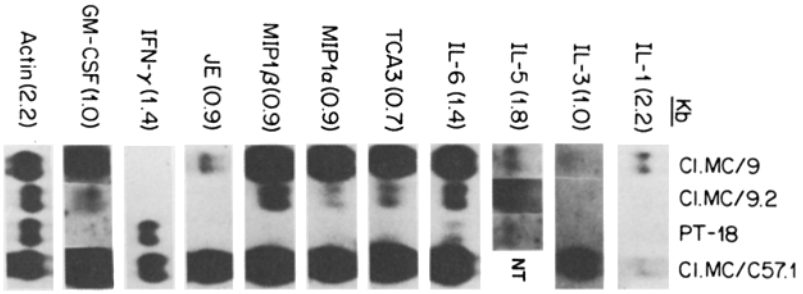

FIGURE 1. FcεRI-mediated lymphokine expression in mast cell lines. 20 μg RNA (whole cell) from mast cell clones Cl.MC/9, Cl.MC/9.2, PT-18, and Cl.MC/C57.1 that had been activated for 4 h with antigen plus IgE were Northern blotted and probed as listed. Sizes of detected RNA species are indicated in parentheses. Data for each cytokine represent single Northern blots containing RNA from each cell type listed (except the data for GM-CSF with clone Cl.MC/C57.1 which was obtained from a separate blot). NT indicates not tested.

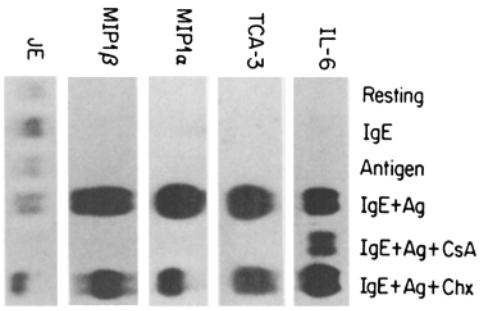

FIGURE 2. Specificity and regulation of FcεRI-mediated gene expression in Cl.MC/9. 20 μg RNA from either resting (unstimulated) cells or cells cultured for 4 h with 3 μg/ml IgE anti-DNP antibody, 10 ng/ml antigen (DNP-HSA), or a mixture of IgE and antigen were Northern blotted. In addition, the effects of addition of 2 μg/ml CsA or 10 μg/ml Chx during the 4-h culture with antigen plus IgE were evaluated.

The combined data indicate that the MC populations examined were capable of expressing numerous cytokine genes and that many of these genes exhibit activation-associated induction via the FcεRI pathway.

Signal Requirements for FcεRI-mediated Gene Induction. We first examined the ability of individual components of the FcεRI-mediated pathway to stimulate gene induction. For this study we chose to examine IL-6, JE, TCA3, MIP1α, and MIP1β which are relatively strongly induced genes in Cl.MC/9. Cells were treated with nothing (resting), antigen alone, IgE alone, or antigen plus IgE and harvested for Northern analysis 4 h later (Fig. 2). Treatment with either antigen or IgE failed to induce transcription of TCA3, MIP1α, or MIP1β, indicating that FcεRI engagement by IgE under noncrosslinking conditions was an insufficient signal for gene induction. Substantially augmented expression of IL-6 RNA also required both IgE plus antigen, but low levels of IL-6 induction in Cl.MC/9 cells were observed with IgE treatment alone. In contrast, JE expression was not significantly enhanced by these treatments. The combined data indicate that, at least for expression of most cytokines, the activating stimulus generated via FcεRI requires both engagement of IgE with its receptor plus binding of antigen to FcεRI-associated IgE. The mechanism responsible for IL-6 induction may be distinct, for as will be indicated below, this gene is constitutively expressed in another MC clone and is relatively resistant to regulation with cyclosporin A (CsA).

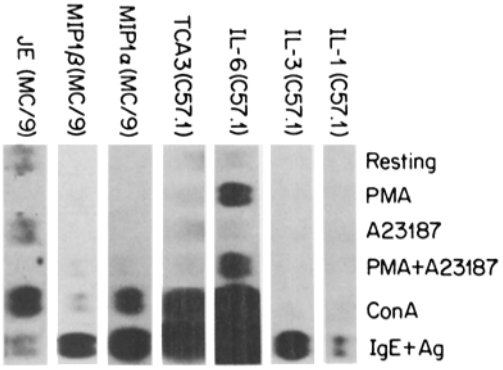

FIGURE 3. Effects of alternative signals on cytokine gene expression. Mast cell clones Cl.MC/C57.1 and Cl.MC/9, indicated in parentheses, were activated for 4 h with either nothing (resting), 50 ng/ml PMA, 100 ng/ml A23187, PMA plus A23187, 2.5 μg/ml Con A, or antigen plus IgE. 20 μg RNA was placed on each lane and Northern blotted with the indicated probes. The smears in the first four TCA3 lanes represent the tails of another probe placed on the same blot but not used in this study.

Regulation of Gene Expression in Mast Cells. The effects of treatment with the immunosuppressive drug CsA and the protein synthesis inhibitor cycloheximide (Chx) upon IgE plus antigen-mediated gene induction on MC gene induction were also evaluated. As shown in Fig. 2 CsA co-treatment abolished IgE-mediated gene induction of TCA3, MIP1α, MIP1β, and JE and partially blocked IL-6 induction. Similar sensitivities to the effects of CsA have also been observed for the three former genes in T cells (39, 43, and data not shown). However, unlike T cells, induction of these genes in MC was only partially sensitive to the effects of Chx cotreatment (Fig. 2).

To examine the nature of the FcϵRI-mediated signal we investigated the ability of different agents to mimic signalling via the FcϵRI in IL-3-dependent Cl.MC/9 or IL-3-independent Cl.MC/C57.1 cells. We evaluated the ability of PMA and calcium ionophore treatment to induce lymphokine gene transcription. We also examined the effect of the lectin Con A, which can stimulate MC via crosslinking of cell-bound IgE and/or cell surface glycoproteins (44, 45). MC cell clones were treated with PMA (50 ng/ml), calcium ionophore A23187 (100 ng/ml), PMA plus A23187, or Con A (2.5 μg/ml) in the absence of IgE and harvested for Northern analysis 4 h later. Untreated resting cells and cells stimulated by IgE and antigen were also examined (Fig. 3). With the exception of JE, all genes tested were best induced by IgE plus antigen stimulation (Fig. 3); IL-1 RNA was not detected after Con A, PMA, or A23187 treatments. Low levels of IL-3 (noted after overexposure of autoradiograph), TCA3, and MIP1α RNA were observed after Con A treatment, but neither gene was induced by PMA and A23187 treatment. MIP1α and MIP1β RNA were strongly induced by IgE plus antigen but were weakly induced by the action of Con A. After prolonged exposure of the autoradiographs MIP1α and MIP1β RNA could be observed in the groups treated with a combination of PMA and A23187. PMA treatment alone induced very low levels of MIP1β expression (noted on prolonged autoradiographic exposure) while it downregulated expression of the JE gene (Fig. 3). IL-6 RNA, while present at low levels in unstimulated Cl.MC/C57.1 cells, was augmented by treatment with Con A or with PMA alone, although not to levels observed for antigen-mediated FcϵRI crosslinking. No detectable increase in IL-6 RNA above the unstimulated levels was observed after A23187 treatment. In contrast, constitutive expression of JE RNA was enhanced after Con A activation (Fig. 3).

Functional Assays of Lymphocyte Production. We next examined the levels of lymphokine bioactivity secreted in response to various activating agents (Table I). Cloned MC lines (Cl.MC/9 and Cl.MC/C57.1) and primary cultures of IL-3-dependent BMCMC (>95% MC by light microscopy) obtained from 6-wk BALB/c bone marrow cultures (see Materials and Methods) were stimulated as indicated (Table I) and culture supernatants were collected for bioassay at 24 h. RNA expression studies of BMCMC have not been performed due to the difficulty in obtaining sufficient quantities of cells. However, supernatants derived from these cells were analyzed to evaluate directly the relevance of the RNA data to mast cell populations generated from short term culture.

A marked increase in IL-1 bioactivity was detected in culture supernatants of Cl.MC/9 and in BMCMC in response to stimulation with IgE plus antigen but was not detectable in supernatants from similarly treated Cl.MC/C57.1. The latter finding is of interest in that IL-1 RNA is induced in Cl.MC/C57.1 by IgE and an-

TABLE I

Activation of MC Populations by IgE and Specific Antigen Results in Lymphokine Release

	Cl.MC/9			Cl.MC/C57.1			Bone marrow MC	
Treatment	IL-1	IL-4	IL-6	IL-1	IL-4	IL-6	IL-1	IL-6
Unstimulated	2.4	1.1	1.1	2.5	1.3	5.9	1.8	0.7
Con A	2.3	1.4	16.7	0.1	1.3	20.3	NT	NT
IgE + Ag	30.2	3.1	51.5	1.0	6.8	45.1	27.8	41.5
IgE alone	NT	NT	NT	NT	NT	NT	4.3	1.4
Media (no cells)	2.7	1.4	1.0	0.7	1.7	0.2	5.5	1.3

The data represent proliferative responses of lymphokine addicted cell lines (D10.G4 for IL-1; HT-2 for IL-4; T1165tc for IL-6) expressed as cpm $\times 10^3$ of thymidine uptake (see Materials and Methods). IL-1, IL-4, and IL-6 bioactivities were tested at 1:100, 1:10, and 1:40 dilutions of culture supernatant, respectively. NT indicates not tested.

tigen stimulation (Figs. 1 and 3). As might be expected from the RNA data (Fig. 3), IL-1 bioactivity was not observed in response to Con A treatment. IL-6 bioactivity was observed in the Cl.MC/9, Cl.MC/C57.1, and BMCMC supernatants after stimulation with IgE and antigen; IL-6 activity was also detected in supernatants from the two Con A-stimulated cell lines tested and in unstimulated Cl.MC/C57.1 cells but not from unstimulated Cl.MC/9 cells as predicted by the RNA data (Figs. 2 and 3). In addition, Cl.MC/9 and Cl.MC/C57.1 cells produce low levels of IL-4 after activation with IgE and antigen, but not with Con A. The IL-4-mediated proliferation could be inhibited with anti-IL-4 antibody (data not shown). The combined results suggest that signals that induce lymphokine gene expression can also result in secretion of lymphokine products.

Discussion

Signaling via FcεRI Induces Gene Expression in MC. In this report we show that specific stimulation of MC via the FcεRI induced gene expression for a wide variety of cytokines. Of the lymphokines tested only IL-2 and IL-4 were not detected by Northern analysis of total cellular RNA, yet IL-4 activity was noted in a highly sensitive bioassay (Table I). Not all clones were capable of producing RNA for each of the cytokines evaluated implying heterogeneity within the population of in vitro propagated MC clones. However, some of the differences in cytokine expression may reflect sensitivity differences. Thus, the MC/9 and MC/9.2 were derived from the same parental clone but Cl.MC/9.2 consistently produced less cytokine RNA and it appeared that the group of cytokines detected in Cl.MC/9.2 represented a subset of those detected in Cl.MC/9.

The production of multiple cytokines by MC suggests that MC may express more complex functions than the simple effector role originally assigned. The following cytokines were expressed by at least two independent MC clones: IL-1, IL-3, IL-4, IL-5, IL-6, IFN-γ, and GM-CSF as well as genes of a new family of cytokines (TCA3, MIP1α, MIP1β, and JE). Members of this family of cytokines have been isolated from T cells (TCA3, MIP1α, and MIP1β), macrophages (MIP1α, MIP1β, and JE) and fibroblasts (JE). Their collective designation as a family has been based upon a striking conservation of structural similarities including positional conservation of cysteine, proline, tyrosine, and phenylalanine residues and a tryptophan-valine-

glutamine motif (37, 38, 46, 47). Members of this family have been shown to possess pyrogenic activity (48) and to act as chemotactic agents for neutrophils (49) and monocytes (50). Their function in MC may relate to these proinflammatory properties. As proposed by Plaut et al. (22), a potential role for the other MC-associated lymphokines may involve regulation of IgE-mediated allergic responses. IL-3 and IL-4 are known to stimulate MC growth and could serve as autocrine growth factors (6–8, 15); IL-1 serves as a cofactor for Th2 cells (51), which release IL-4 and IL-5, which upregulate IgE production (11, 52), while IFN-γ can have an antagonistic effect on IgE production in vitro (53). IL-5 can also induce the eosinophil proliferation often associated with allergic responses (52).

The IgE-mediated signals lead to substantially augmented gene induction of all but one cytokine (JE) and required both FcεRI engagement by IgE as well as cross-linking by antigen (Fig. 2). FcεRI engagement by IgE alone resulted in weak induction of only one cytokine gene (IL-6). Generally, Con A–induced only limited increases in gene expression and phorbol ester plus calcium ionophore had a more limited effect inducing only very low levels of MIP1α and MIP1β under the conditions employed in these experiments (Fig. 3). In contrast, these concentrations of Con A or phorbol ester plus calcium ionophore usually have equally potent effects on induction of T cell lymphokine genes (29, 39, 43).

As has been found in T cells (39, 43), induction of TCA3, MIP1α, and MIP1β was completely blocked and IL-6 partially blocked by CsA co-treatment during IgE plus antigen-mediated stimulation, suggesting that the regulation of these genes in MC and T cells may be similar (43, 54). Furthermore, the data imply that CsA treatment may potentially impair MC-mediated functions related to these products. However, the induction of these genes in MC was only partially sensitive to the effects of Chx, whereas similar treatments of T cells dramatically diminish gene induction (data not shown), suggesting that significant differences exist between the cytokine induction mechanisms regulating expression between T cells and MC.

In addition to the above set of T cell–associated cytokines, MC also produce JE RNA and IL-1 RNA and bioactivity. These genes have not been generally noted in T cells, but are associated with multiple other tissue types, including cells of the monocyte/macrophage lineage. Thus, the MC may have the potential for providing many of the critical cytokines normally associated with distinct immune cell types.

IgE-dependent release of MC mediators may be mimicked by a number of IgE-independent stimuli, including Con A or phorbol ester and calcium ionophore. Although the molecular mechanism leading to IgE-dependent mediator release has been shown to depend upon a calcium flux, IgE-independent mediator release apparently can occur via a variety of pathways depending upon the inducing agent used (reviewed in references 4, 55). Data presented in this study indicate that, when used under conditions that trigger optimal expression of these genes in T cells (29), Con A or PMA and A23187 were not sufficient to induce optimal levels of lymphokine gene expression in either growth factor–dependent or –independent MC lines (Figs. 2 and 3). These results might be interpreted to suggest that the FcεRI-mediated signal is distinct from those generated by calcium flux and PKC translocation. However, without further examination of the effects upon gene induction of different doses of PMA or A23187, we cannot determine the extent to which cytokine gene induction can be triggered independently of the FcεRI.

Origins and Functions of Mast Cells. Based on several lines of indirect evidence, Burnet (56) and others (reviewed in reference 1) suggested that the occurrence of "T cell-dependent" mast cell populations might reflect either the derivation of certain MC from T cells or the influence of T cell-derived factor(s) on mast cell proliferation. The weight of current evidence favors the latter possibility. Nabel et al. (12) and Galli et al. (13) showed that the supernatants of cloned T cells later shown to contain IL-3 and IL-4 (8, 24), promoted the maturation and proliferation of MMC-like cloned MC that lacked surface structures and function characteristic of T cells. In addition, no defects in T cell function have been described in mutant mice genetically deficient in MC (57) and administration of IL-3 repairs the MMC deficiency of athymic nude mice (7).

At the first glance, the expression of multiple T cell-associated cytokines by MC could be regarded as reason to reopen the question of the lineage relationship between MC and T cells. The observation that several of these genes can be regulated by CsA in both cell types is consistent with this interpretation (Fig. 2). On the other hand, IL-1, JE, and TNFα are more characteristic of monocytes/macrophages than T cells, and certain similarities between the natural history of MC and macrophages have been noted (1, 58). MC, T cells, and monocytes/macrophages all are derived from hematopoietic precursors. As a result, some overlaps in the patterns of gene expression in these populations are not necessarily surprising. Much additional analysis will be required to determine if the pattern of cytokine production by MC, T cells, and monocytes will help to clarify lineage relationships among these populations. As expected based upon the large amount of work already performed with Cl.MC/9 (1, 12, 13), Southern blot analysis did not reveal TCR β chain rearrangement, indicating that this clone does not possess a TCR complex (data not shown).

Although the lineage relationships among MC, T cells, and monocytes are of interest, a different but equally important issue is the cell- and tissue-specific regulation of cytokine genes in MC vs. T cells and monocytes. Each of the cytokine genes identified in MC in this report have a wide range of potential biological effects, spanning processes as diverse as hematopoiesis, inflammation, and the regulation of IgE and other elements of immune responses. We have shown that the transcription of these genes can be significantly modulated by signals transduced by the FcϵRI after engagement of receptor-bound IgE with specific antigen, and that this signal also results in release of bioactivities attributable to some of these cytokines. These findings raise the interesting possibility that MC may represent a biologically significant source of these cytokines during IgE-dependent and perhaps other responses. Thus, MC may play an important auxiliary role in regulating inflammatory and immune responses.

Summary

In response to IgE and specific multivalent antigen, mast cell lines (both growth factor-dependent and -independent) induce the transcription and/or secretion of a number of cytokines having a wide spectrum of activities. We have identified IL-1, IL-3, IL-5, IL-6, IFN-γ, GM-CSF, JE, MIP1α, MIP1β, and TCA3 RNA in at least two of four mast cell clones. The production of these products (except JE) is activation-associated and can be induced by IgE plus antigen. In selected instances cytokine

expression can also be induced by activation with Con A or phorbol ester plus ionophore, albeit to levels less than those observed with IgE plus antigen. In addition, long-term mast cell clones and primary cultures of bone marrow-derived mast cells specifically release IL-1, IL-4, and/or IL-6 bioactivity after activation. These findings suggest that in addition to their inflammatory effector function mast cells may serve as a source of growth and regulatory factors. The relationship of mast cells to cells of the T lymphocyte lineage is discussed.

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