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In Activated Mast Cells, IL-1 Up-Regulates the Production of Several Th2-Related Cytokines Including IL-9


Mast cells can play detrimental roles in the pathophysiology and mortality observed in anaphylaxis and other Th2-dominated allergic diseases. In contrast, these cells contribute to protective host defense mechanisms against parasitic worm infections. After IgE/Ag activation, mast cells can produce multiple cytokines that may enhance allergic inflammations, while a similar panel of Th2-related cytokines may support immunological strategies against parasites. Here we report that in primary mouse bone marrow-derived mast cells activated by ionomycin or IgE/Ag, the proinflammatory mediator IL-1 (α or β) up-regulated production of IL-3, IL-5, IL-6, and IL-9 as well as TNF, i.e., cytokines implicated in many inflammatory processes including those associated with allergies and helminthic infections. IL-1 did not induce significant cytokine release in the absence of ionomycin or IgE/Ag, suggesting that Ca-dependent signaling was required. IL-1-mediated enhancement of cytokine expression was confirmed at the mRNA level by Northern blot and/or RT-PCR analysis. Our study reveals a role for IL-1 in the up-regulation of multiple mast cell-derived cytokines. Moreover, we identify mast cells as a novel source of IL-9. These results are of particular importance in the light of recent reports that strongly support a central role of IL-9 in allergic lung inflammation and in host defense against worm infections. The Journal of Immunology, 2000, 164: 5556–5563.

Mast cells participate in a variety of physiological, immunological, and pathological processes including wound healing and angiogenesis, host responses to parasites and tumor cells, fibroses, acute and chronic inflammations, as well as IgE-dependent immediate hypersensitivity reactions (for reviews see Refs. 1–3). During the past decade, it became apparent that these cells are an important source of multifunctional cytokines, a finding expected to contribute to an improved understanding of the physiological and pathological mechanisms associated with the function of mast cells (4, 5).

Upon IgE receptor-mediated or Ca-ionophore-induced activation, primary mouse bone marrow-derived mast cells (BMMC)2 and permanent mast cell lines can produce a panel of different cytokines including IL-3, -4, -5, -6, and -13 as well as GM-CSF and TNF (6–10). Similarly, also human mast cells have been identified as a source of multiple cytokines in vitro as well as in clinically relevant situations in vivo, e.g., in allergic inflammations (reviewed in Refs. 11–13). In these previous studies, the profile of mast cell cytokine secretion displayed a striking overlap with the cytokine pattern produced by a subset of T helper lymphocytes called Th2 (14). Activated Th2 cells are known to mediate humoral immune responses and produce IL-4, IL-5, IL-6, IL-10, and IL-13 (15, 16), while activated Th1 cells and their prototypic cytokine products IL-2 and IFN-γ are involved in cell-mediated immune reactions including delayed-type hypersensitivity responses (17).

Murine IL-9, a multifunctional T cell-derived cytokine (18) previously called P40 (19, 20), T cell growth factor III (21), or mast cell growth-enhancing activity (22–25) has been detected in the supernatants of Th2 but not Th1 clones (26). In the murine system, IL-1 has been identified as an essential costimulatory signal for IL-9 production by activated cultures of established Th2 cells (27). IL-9 production in vitro by murine naive CD4+ T cells was clearly IL-2 dependent, synergistically enhanced by a combination of TGF-β and IL-4, and inhibited by IFN-γ (28). A similar IL-2 dependence of IL-9 expression was found in human naive CD4+ T cells, which produced augmented levels of IL-9 through the autocrine actions of IL-4 and IL-10 (29). Recently, a role of IL-9 as a candidate gene for asthma (30–32) has been further strongly supported by experimental results with IL-9 transgenic mice challenged with allergens in vivo (33, 34).

In this paper, we demonstrate that the proinflammatory mediator IL-1 (35) up-regulates the expression of several cytokine mRNAs and thus promotes enhanced production of the corresponding Th2-related cytokine proteins including IL-9 in primary murine BMMC activated by ionomycin or IgE/Ag. Hence, our study reveals a broad regulatory role for IL-1 in mast cell cytokine expression and we newly identify primary activated mast cells as a source of IL-9. Our results emphasize a potential clinical importance of IL-1 and mast cells in the amplification of Th2-type immune responses during parasitic or retroviral infections (36–38), in Th2-dependent inflammatory skin reactions (39–41), and in allergic diseases (42).

Materials and Methods

Mice

BALB/c mice were bred in our animal facilities under specific pathogen-free conditions until the age of 6 wk and then kept under conventional conditions (GSF, Munich, Germany). Mice of both sexes were used as bone marrow donors at ages of 8–28 wk.
Cytokines and Abs

Recombinant murine (nu) kit-ligand (KL) was expressed in Escherichia coli and purified by affinity chromatography as described by Reisbach et al. (43). The following cytokines were commercially obtained as listed: rmuIL-1β, rmuTNF, and rat anti-mouse IL-4 mAb (code 1688-01; Genzyme, Boston, MA), rmuIL-3 (Bachem Biochemica, Heidelberg, Germany) recombinant human (hu) IL-6 (Life Technologies, Grand Island, NY), rmuIL-1 receptor antagonist (IL-1Ra), and rmuIL-9 (British Biotechnology, Oxon, U.K.). RmuIL-1α was a kind gift from Dr. R. Munker (Med. Klinik III, Universitätsklinikum Grosshadern, München, Germany) and originated from Hoffmann-La Roche (Basel, Switzerland). rmuIL-1β (commercial product from Genzyme) was provided by Dr. R. Munker (Med. Klinik und Poliklinik für Hals-, Nasen- und Ohrenkranke, Universitätsklinikum Grosshadern, München, Germany). The neutralizing rat anti-muIL-3 Ab 19B3.1 (44) was a kind gift of Dr. J. Abrams (DNAX, Palo Alto, CA). The rat anti-muIL-6 Ab 6B4 (45) was provided by Dr. J. Van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium). As a source of rmuIL-3 or rmuIL-5, we used batches of supernatants derived from X63Ag8-653 myeloma cells transfected with a retroviral vector carrying the mouse IL-3 or IL-5 genes (46). These transfected cell lines were kindly provided by Dr. F. Melchers (Basel Institute for Immunology, Basel, Switzerland). Hybridoma cells secreting DNP33-BSA-specific IgE Ab were originally developed at the Weizmann Institute of Science (Rehovot, Israel) (47) and kindly provided by Dr. B. Reck (Max Planck Institut für Immunobiologie, Freiburg, Germany). Anti-DNP-BSA IgE was purified by separation on a protein G column.

Mast cell cultures

BALB/c bone marrow cells were suspended at 5 × 10^6/ml in RPMI 1640 medium containing 20% FCS, 2 mM l-glutamine, 100 U/ml penicillin-streptomycin, 10^{-5} M α-thioglycerol, and 1 μg/ml rmuIL-3 (pretested supernatant from IL-3 gene-transfected X63Ag8-653 cells (46) containing about 1000 U/ml IL-3) and then distributed into 96-well microplates (Nunc, Wiesbaden, Germany) (200 μl/well) and incubated at 37°C in a fully humidified atmosphere (10% CO_{2} in air). After 14 days, cell-free supernatants were usually harvested after 24 or 48 h and stored at −20°C until analysis.

Cytokine induction experiments

If not otherwise indicated, primary BMMC (in vitro age, 4 wk) were grown in the presence of IL-3, BMMC were washed twice, suspended at 1 × 10^6 cells/ml in RPMI 1640 medium including 1 U/ml rmuIL-3 (a dose warranting cellular survival) and the other supplements described above, and then transferred into 24-well plates (Nunc) (0.5 ml/well) containing the Ca-ionophore ionomycin (Sigma, St. Louis, MO) and/or the potentially activating cytokines to be tested in 5- or 10-μl volumes (replicate wells/group). Ionomycin and various cytokines were tested at the following range of concentrations (given in parentheses): ionomycin (0.25–4.0 μM), rmuIL-1α (0.1–10 U/ml), rmuIL-1β, rmuIL-1β, and rmuIL-6 (1.0–100 ng/ml, respectively), rmuTNF-α (0.2–20.0 ng/ml). In experiments with rmuIL-1α (20 nm/liter) in the presence of IL-3, mast cells were activated following a standard protocol (for details see the references cited). The following cell lines have been employed: 3DCl2.23 for IL-3 (22), 7TD1 for IL-6 (45), and TS1.C3 (19) or ST2/K9.4a2 (26) for IL-9 measurements. A standard cytotoxicity assay for TNF bioactivity using a TNF-sensitive L929 fibroblast cell line was used as described (50). The specificities of the biological assays were confirmed employing neutralizing doses of specific anti-mouse cytokine mAb. At the relevant concentrations, the agents and cytokines tested in the cytokine induction assays did not interfere with cytokine activities in the different biological assays.

Murine cytokine-specific ELISA tests

The two-site ELISA tests employed in the present study have been described recently as described in the references cited below. The following anti-mouse cytokine Abs have been used: affinity-purified anti-mouse IL-4 mAb 11B11, rabbit anti-mouse IL-4 antisera, as well as biotinylated swine anti-rabbit antiserum (Dakopatts, Hamburg, Germany) (51), affinity purified anti-muIL-5 mAb TRFK5 and biotinylated anti-muIL-5 mAb TRFK4 (26), hamster anti-muIL-9 mAb C12 (gift of Dr. J. Van Snick, Ludwig Institute, Brussels, Belgium), and biotinylated rat anti-muIL-9 mAb 229.4 (28).

Northern blot analysis

Total cellular RNA was prepared from BMMC by the single step acid guanidinium thiocyanate-phenol-chloroform extraction method (52). RNA was glyoxylated and electrophoresed through a 1.0% agarose gel and blotted by vacuum blotting onto nylon membranes (Hybond-N; Amersham, Braunschweig, Germany). Hybridization and stringency washes of blots were performed as previously described (53). The probe used for hybridization was a cDNA fragment of murine IL-9 (20) (0.36-kb NcoI-BamHI fragment; kindly provided by Dr. J. Van Snick), which had been labeled with [32P]dCTP by the random priming method (Megaprime DNA labeling system; Amersham). Transfer efficiency was controlled by an additional hybridization to a mouse 28S rRNA probe, kindly provided by Dr. I. Grummt (German Cancer Research Center, Heidelberg, Germany). Autoradiographic analysis was performed with the Fuji digital imaging system (exposition on Fuji imaging plates and subsequent evaluation with a Fuji BAS1000 Bio-Imaging Analyzer; Fuji, Düsseldorf, Germany). The amounts of IL-9 mRNA were normalized based on 28S rRNA levels.

RT-PCR analysis

The primer pairs and the method used to detect transcripts for the housekeeping gene GAPDH and for specific mouse cytokine genes (IL-3, IL-6, IL-9) have been described previously (54, 55).

Results

IL-1 acts as a potent costimulator of cytokine production in mast cells activated by ionomycin

Because mast cells are regarded as important cellular regulators and effectors of many immunological and inflammatory reactions, which also involve the action of various proinflammatory mediators (e.g., IL-1, IL-6, TNF), we were interested in potential influences of these mediators on mast cell cytokine production. BMMC generated in the presence of IL-3 were activated following a standard protocol (ionomycin (1 μM), 24 h) in the absence or presence of rmuIL-1α (2 U/ml). Mast cell supernatants were then tested for a panel of different cytokines (i.e., IL-3, IL-4, IL-5, IL-6, IL-9, and TNF) employing specific ELISAs or biological assays. As exemplified for IL-3, IL-6, IL-9, and TNF in Fig. 1, there was no substantial constitutive cytokine production and only moderate cytokine production after activation with ionomycin alone. However, when in addition to the Ca-ionophore also rmuIL-1α (2 U/ml) was provided during the induction period, substantially higher concentrations of IL-3, IL-6, IL-9, and TNF were measured in 24-h supernatants (Fig. 1). In contrast, no significant differences were found between ionomycin-induced IL-4 levels of mast cell supernatants in the presence or absence of rmuIL-1α as a coactivating agent (data not shown). In the absence of the Ca-ionophore, IL-1 induced only small amounts of IL-6 but no other cytokines (Fig. 1), indicating that Ca-dependent signaling was required for cytokine induction by IL-1. This IL-1 effect was dose dependent, as
illustrated for IL-3, IL-5, IL-6, and IL-9 induction with a maximum at 1–10 U/ml IL-1α (Fig. 2), and highly specific, as preincubation (1 h) of BMMC with 20 ng/ml rhuIL-1Ra abolished the action of a saturating dose of rhuIL-1α (2.5 U/ml), an effect that could be efficiently counteracted by increasing the IL-1 dose (demonstrated for IL-9 production in Fig. 3). A similar enhancement of cytokine production in ionomycin-activated BMMC was noted with rhuIL-1β or rmuIL-1β (saturating maximum effects at 1.0–10 ng/ml) but not with rhuIL-6 (0.1–10.0 ng/ml) or rmuTNF (0.2–20.0 ng/ml) (data not shown).

To exclude a contribution of non-mast cells to cytokine production in our system, we developed a panel of primary BMMC clones from limiting dilution cultures of BALB/c bone marrow cells in 96-well microtiter plates under conditions (500 cells seeded per well) warranting a statistical probability of 95% that the mast cell populations were of clonal origin. In pilot limiting dilution experiments, the frequency of IL-3-responsive mast cell progenitors in BALB/c bone marrow cells was found to be about 1/5000, slightly lower than in our earlier reports when PWM-stimulated spleen cell-conditioned medium had been used as a source of mast cell growth factors (48, 54). As shown in Fig. 4, most of the 20 primary mast cell clones analyzed were able to produce variable amounts of IL-3, IL-6, and IL-9 upon activation with ionomycin plus IL-1, while some of them produced exceptionally high cytokine levels. All these ionomycin-activated BMMC clones produced substantially lower levels in the absence of IL-1 (data not shown).

**Kinetics of cytokine production after activation of mast cells with ionomycin/IL-1**

When ionomycin/IL-1-activated BMMC were followed over an induction period of 6–72 h, a remarkable difference in the kinetics of IL-9 production was observed compared with all other cytokines analyzed. As shown for IL-3, IL-5, and IL-6, these bio-activities increased almost constantly over time without a significant time delay after activation, whereas IL-9 production was clearly delayed with a very moderate increase from 6 to 24 h and a dramatic increase from 24 to 48 h (Fig. 5).

**Kinetics of IL-9 mRNA expression in mast cells activated by ionomycin or ionomycin plus IL-1**

Fig. 6 shows a typical result of a Northern blot analysis of IL-9 expression in mast cells activated for 12, 24, or 48 h with either
ionomycin alone or with ionomycin plus IL-1. In mast cells activated by ionomycin alone, IL-9 mRNA expression could not be detected before 48 h. In contrast, after activation with ionomycin plus IL-1, a faint IL-9-specific signal was already observed after 12 h and strong additional increases of IL-9 mRNA expression were seen after 24 and 48 h (Fig. 6). An IL-1-mediated enhanced expression of IL-9 as well as IL-3- and IL-6-specific mRNAs was also observed by RT-PCR analysis of activated BMMC (data not shown).

**Autocrine role of IL-4 for cytokine production in ionomycin/IL-1-activated mast cells**

The delayed kinetics of IL-9 mRNA expression (Fig. 6) and IL-9 protein secretion (Fig. 5) in mast cells activated by ionomycin plus IL-1 and the known enhancing effects of IL-4 on IL-9 production by activated murine and human T cells (28, 29) prompted us to investigate a possible autocrine role of IL-4 in IL-9 production by mast cells. As shown in Fig. 7, significantly reduced IL-9 levels were measured 48 h after activation of BMMC with ionomycin/IL-1 when the cells were treated with an Ab blocking the mouse IL-4 receptor (anti-IL-4R mAb). In contrast, the decline of the IL-4 levels observed in the absence of anti-IL-4R mAb from 24 to 48 h after mast cell activation could be completely prevented in its presence, indicating that substantial amounts of endogenously produced IL-4 were consumed by the mast cells 24 to 48 h after activation (Fig. 7). Moreover, this anti-IL-4R mAb also reduced the levels of IL-3 and IL-6, revealing the autocrine potential of endogeneous IL-4 to further enhance the production of these cytokines in activated mast cells (Fig. 7).

**Following maturation toward a connective tissue-type phenotype in vitro, activated BMMC have the capacity to produce higher amounts of cytokines in response to IL-1**

It is generally accepted that BMMC produced and maintained in vitro in the presence of IL-3 represent a population of relatively immature c-kit-expressing mast cells with the potential to further differentiate either along the mucosal or the connective tissue-type...
mast cell lineage both in vitro and in vivo (1). Therefore, we tested the cytokine-producing capacity of BMMC cultured for 2 wk with IL-3 and then switched for another 2 wk to different cytokine conditions (IL-3 or IL-3/IL-4 or IL-3/IL-4/KL). Compared with BMMC grown in IL-3 alone, BMMC generated in the additional presence of IL-4 or IL-4 plus KL are known to contain significantly higher concentrations of histamine (56, 57), and a substantial proportion of them stained positively with Safranine (Table I), indicating the presence of phenotypically more mature cells with some characteristics of connective tissue-type or serosal mast cells. Compared with activated BMMC grown in IL-3 alone, those BMMC grown with IL-3/IL-4 and more pronounced with IL-3/IL-4/KL displayed a strikingly increased capacity to secrete IL-3, IL-6, and IL-9 in response to ionomycin/IL-1, roughly correlating with the grade of Safranine positivity of the different mast cell populations tested (BMMCIL-3/IL-4/KL > BMMCIL-3/IL-4 > BMMCIL-3) (Fig. 8 and Table I). However, rather low and quite comparable amounts of these cytokines were produced by these different groups of BMMC populations after activation with ionomycin alone (Fig. 8).

**IL-1 enhances production of IL-9 and other Th2-related cytokines in mast cells activated by IgE/Ag**

We next determined whether IL-1 would be able to augment cytokine production of mast cells under more physiological conditions in vitro, i.e., following activation via cross-linking of their

### Table I. Proportion of Alcian blue<sup>+</sup> mast cells staining positively with Safranine in BMMC cultures generated in the presence of different cytokines (week 1 and 2, IL-3 only; week 3 and 4, IL-3 or IL-3/IL-4 or IL-3/IL-4/KL) at the following final cytokine concentrations: rmuIL-3 (100 U/ml), rmuIL-4 (10 ng/ml), rmuKL (200 ng/ml).

<table>
<thead>
<tr>
<th>Cytokines Used (wk 3 and 4)</th>
<th>Safranine&lt;sup&gt;+&lt;/sup&gt; Mast Cells (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tr>
<td></td>
<td>+++++</td>
</tr>
<tr>
<td>IL-3</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 + IL-4</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>IL-3 + IL-4 + KL</td>
<td>1.8 ± 0.9</td>
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<tr>
<td>IL-3 + IL-4</td>
<td>0.2 ± 0.4</td>
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<tr>
<td>IL-3 + IL-4 + KL</td>
<td>1.8 ± 0.9</td>
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<td></td>
<td>+++</td>
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<tr>
<td>IL-3</td>
<td>6.2 ± 3.3</td>
</tr>
<tr>
<td>IL-3 + IL-4</td>
<td>1.8 ± 1.9</td>
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<tr>
<td>IL-3 + IL-4 + KL</td>
<td>8.6 ± 6.0</td>
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</tr>
<tr>
<td>IL-3 + IL-4</td>
<td>17.6 ± 3.4</td>
</tr>
<tr>
<td>IL-3 + IL-4 + KL</td>
<td>43.2 ± 6.1</td>
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<td></td>
<td>+++</td>
</tr>
<tr>
<td>IL-3</td>
<td>93.8 ± 2.6</td>
</tr>
<tr>
<td>IL-3 + IL-4</td>
<td>80.4 ± 3.1</td>
</tr>
<tr>
<td>IL-3 + IL-4 + KL</td>
<td>46.4 ± 9.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean values ± SD determined from three independent experiments at different cytokine conditions after the evaluation of 500 cells per cytoslide (2 slides/group/experiment); at least 10 Safranine<sup>+</sup> granula per cell (+), at least 30 Safranine<sup>+</sup> granula per cell (++++), almost all cellular granula Safranine<sup>+</sup> (+++++).
A dose of 1 ng/ml IL-9 measured by ELISA corresponded to 10 U/ml IL-9 in the absence or presence of rHuIL-1 (10 U/ml). Shown are mean values ± SD (n = 3) of IL-9 concentrations measured by ELISA. A dose of 1 ng/ml IL-9 measured by ELISA corresponded to 10 U/ml IL-9 in the bio-assay.

In the present paper, we present evidence that primary murine mast cells activated by ionomycin or IgE/Ag are able to produce enhanced levels of IL-9 and of several other inflammation-associated cytokines (IL-3, IL-5, IL-6, TNF) when costimulated with IL-1 (Figs. 1–3, 8, and 9). Because not all cytokine induction experiments had been performed with the same batch of FCS, the absolute amounts of cytokines produced by IL-1/ionomycin-activated BMMC in particular sets of experiments cannot be compared directly. During these studies, we realized that the capacities of individual FCS batches to support cytokine production in ionomycin- or ionomycin/IL-1-activated BMMC varied considerably. However, with a panel of different FCS batches tested, we could uniformly confirm the coactivating effect of IL-1 in mouse BMMC cultures (not shown). As illustrated for IL-9 (Fig. 6), we could also observe this IL-1-mediated enhancement of mast cell activation at the level of cytokine mRNA expression using Northern blot analysis. In addition, we confirmed by RT-PCR analysis that not only IL-9 mRNA but also transcripts corresponding to all the other cytokine proteins measured (IL-3, IL-5, IL-6, TNF) were up-regulated by IL-1 in ionomycin-activated mast cells (not shown). This finding strongly indicates that IL-1 may enhance the transcriptional activation of cytokine genes and/or promote the stabilization of various cytokine transcripts. Interestingly, it was shown previously that in mouse BMMC IL-1 can induce the de novo synthesis of protein(s) that increase the stability of IL-6 mRNA induced by KL and IL-10 (58). In several other cell systems (e.g., in fibroblasts and B cells), IL-1 was found to increase the expression of cytokine genes by mechanisms also involving mRNA stabilization (59–61). Because we could detect only very low concentrations of IL-6 and no other cytokines in supernatants derived from IL-1-stimulated BMMC in the absence of ionomycin or IgE/Ag, we conclude that a Ca-ionophore- or IgE/Ag-induced influx of Ca$^{2+}$ ions into mast cells is a prerequisite for the observed IL-1-mediated augmentation of cytokine production. We were able to confirm the well-known finding that activated mast cells are a source of IL-4 by demonstrating the presence of this cytokine in supernatants from mast cells stimulated by ionomycin plus IL-1 (Fig. 7). However, while IL-1 caused a remarkable up-regulation of IL-3, IL-5, IL-6, IL-9, and TNF production in ionomycin-activated BMMC at both the mRNA and protein levels, we could neither demonstrate a comparable enhancing effect of IL-1 on IL-4 mRNA expression nor on the amounts of secreted IL-4 protein (data not shown). However, Fig. 7 illustrates that IL-4 produced by ionomycin/IL-1-activated BMMC can enhance the production of other cytokines (e.g., IL-3, IL-6, IL-9) by an autocrine mechanism. A similar autocrine action of IL-4 was previously described in IL-9-producing cultures of murine and human T cells (28, 29). As demonstrated in Fig. 8, the cytokines IL-4 and KL, known to favor the maturation of BMMC toward a connective-tissue-like phenotype (Table I and Refs. 56, 57, and 62), were able to confer a higher degree of IL-1 responsivity to cultured mast cells. Consequently, ionomycin/IL-1-activated BMMC grown in IL-3/IL-4/KL produced strikingly higher amounts of IL-3, IL-6, and IL-9 than relatively immature BMMC grown in IL-3 alone (Fig. 8). It remains to be established whether this increased reactivity to IL-1 paralleling the grade of in vitro maturation of BMMC (Table I) can be explained by the possibility that IL-4 and/or KL modulate the expression of IL-1 receptors on BMMC, e.g., by down-regulating IL-1RII and/or up-regulating IL-1RII expression.

The phylogenetically highly conserved IL-1 system involves the primarily cell-associated IL-1α, the soluble IL-1β proteolytically cleaved from the IL-1 protein precursor by caspase-1, the naturally occurring IL-1Ra, as well as membrane-associated and soluble forms of two different IL-1 receptors (IL-1RI and IL-1RII), with IL-1RII functioning exclusively as a decoy target (35). The IL-1 family of cytokines has profound effects on the pathogenesis of inflammatory and infectious diseases (35). Most of the cytokines described in our present paper (i.e., IL-3, IL-5, IL-6, IL-9, TNF) were reported to be intimately involved in Th2-polarized immune reactions, e.g., in allergic inflammations (63–65) and in host defense reactions against parasites (36, 37, 66).

In contrast, mast cells have long been known to be functionally involved in many inflammatory reactions including allergic inflammation as well as host immune responses to worm parasites (1–3), while their life-saving role in bacterial infections was discovered only recently (67–69).

While we (22–25) and others (70) have previously described effects of IL-9 on the growth and functional activity of murine mast cells in vitro, recent reports on IL-9 transgenic mice confirmed the mastocytosis-inducing activity of this cytokine in vivo (71) and supported the idea that IL-9-driven mast cells can help to resolve experimental helminthic infections (72, 73). Moreover, recently IL-9 was suggested as a candidate gene for asthma (32), a hypothesis additionally strengthened by experimental results with IL-9 transgenic mice challenged with allergens in vivo (33, 34).

We think that IL-1 could provide a powerful coactivating stimulus to mast cells in the course of a variety of inflammatory reactions and infectious diseases, particularly including helminthic infections and allergic inflammations. Mast cells may then augment inflammatory cascades by the enhanced secretion of cytokines (e.g., IL-3, IL-5, IL-6, IL-9, and TNF) with paracrine and even autocrine actions (e.g., IL-3 and IL-9).

Our finding that activated mast cells costimulated with IL-1 are able to secrete high amounts of IL-9 in vitro may have potential clinical implications in the light of recent reports emphasizing important roles of IL-9 both in host defense against worm parasites (72, 73) and in allergic inflammation (32–34).
IL-1 UP-REGULATES CYTOKINES IN ACTIVATED MAST CELLS

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