

In vitro and in vivo immunostimulatory potential of bone marrow–derived mast cells on B- and T-lymphocyte activation

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Background: Mast cells, which play a unique role in inflammatory and allergic responses, have also been shown to actively participate to the build-up of protective host defense mechanisms. Recently, they have been shown to stimulate resting B cells and to form heterotypic aggregates with activated T cells, resulting in mast cell degranulation.

Objectives: Our aim is to investigate the cytokine requirements and the mechanisms by which murine mast cells activate resting B and T lymphocytes.

Methods: Mouse bone marrow–derived mast cells (BMMCs) or peritoneal mast cells were cocultured with resting splenocytes. Activation of B and T lymphocytes was assessed by measuring cell proliferation, blast formation, and cytokine release. **Results:** We report that addition of IL-4–treated BMMCs to normal spleen cells resulted within 48 hours in a B- and T-cell activation with substantial amounts of the T_{H1} cytokines IFN- γ and IL-12 and no detectable IL-4. We also demonstrate that mature mast cells in the peritoneal cavity are able to induce spleen cell activation and cytokine release. Addition of antileukocyte function–associated antigen 1 and anti-intercellular adhesion molecule 1 to the cocultures completely abrogates mast cell–induced blast formation and cytokine release. Experiments performed in vivo indicate that spleen cells from mice injected with BMMCs sustain their capacity of proliferation and cytokine production in vitro without any further stimulation.

Conclusion: These observations suggest that mast cells may exert a helper effect on B and T lymphocytes, initiate T_{H1}-type immune responses, and may participate, through this mechanism, in the downregulation of allergic responses. (*J Allergy Clin Immunol* 1999;134:42.)

Key words: Mast cells, adhesion molecules, lymphocyte activation, T_{H1} cytokines

Mast cells are mainly known to play a pivotal role in allergic immune responses because of the synthesis of

Abbreviations used

BMMC: Bone marrow–derived mast cell
ICAM-1: Intercellular adhesion molecule 1
LFA-1: Leukocyte function–associated antigen 1
NK: Natural killer

proinflammatory mediators. Degranulation of mast cells has been found in sites at which T cell–mediated inflammatory reactions have occurred.¹ Recent observations led several investigators to examine the possible relationship between mast cells and T and B lymphocytes.^{2,3} Among the diverse functional properties ascribed to mast cells, recent work has focused on their potential to promote both innate (nonspecific) and acquired (specific) immune responses.^{4,5} Interactions between mast cells and B and T lymphocytes have recently been investigated and only partly elucidated. Mast cells and T cells have been shown to share common homing-adhesion receptors and to colocalize with B cells in the lamina propria of the intestinal mucosa.² It has been postulated that the localization of mast cells, B cells, and T cells at the same site of the mucosa implies that they have the potential to regulate the activities of each other and to accomplish concerted immunologic functions. The discovery that mast cells can produce a large panel of cytokines^{6–9} suggested that mast cells could influence T- and B-cell activation and development. Recently, bone marrow–derived mast cells (BMMCs) and rat peritoneal mast cells have been shown to present antigen to T cells^{10,11} in a cytokine-dependent fashion.¹² Moreover, it has become clear that BMMCs selectively activate B cells, upregulate their MHC class II expression, and induce these cells to proliferate.¹³ Regarding noncognate interaction between mast cells and lymphocytes, it has been found that Fc ϵ RI-activated mast cells can form heterotypic and homotypic aggregates with T lymphocytes.¹⁴ More recently, it has been demonstrated that naive BMMCs can be activated for histamine release when cocultured with activated T cells.¹⁵ In this study the inductive signals for mast cell activation were provided by leukocyte function–associated antigen 1 (LFA-1)/intercellular adhesion molecule 1 (ICAM-1)–mediated heterotypic aggregation with T lymphocytes. In the present study we

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demonstrate that a noncognate B- and T-lymphocyte activation can be induced by IL-4-stimulated BMMCs or by mast cells from the peritoneal cavity. This mast cell-dependent lymphocyte activation resulted in IL-12 and IFN- γ production and was dependent on LFA-1 and ICAM-1 interactions. This collaboration between mast cells and T and B lymphocytes may take place at the mucosal barriers for the build-up of T_{H1}-mediated defense mechanisms, particularly against invading infectious agents.

METHODS

Mice

DBA/2, BALB/c, BALB/nu/nu, and C57BL/6 mice were obtained from Janvier (Laval, France). C57BL/6 oLa Hsd-bg mice were obtained from Harlan (Blackthorn, Oxon, England).

Reagents and antibodies

BSA was obtained from Sigma (St Louis, Mo). LPS from *Neisseria meningitidis* was a gift from Dr M. Caroff (Institut de Biochimie, Orsay, France). Mouse recombinant IL-3, IL-4, and IFN- γ were purchased from Immugenex (Los Angeles, Calif).

Anti-mouse IAb^d mAb and rat anti-mouse CD4 (ATCC, Rockville, Md) were prepared from 25-9-17S and GK1.5 clones, respectively. Anti-Thy.1 mAb (clone 9.37) was kindly provided by U. Hämmerling (Sloan-Kettering Institute, New York). Unlabeled anti-IFN- γ (clone AN18), biotinylated anti-IFN- γ (clone R46A2), anti-IL-4 mAbs (clones 11B11 and BVD6), biotinylated anti-CD3 ϵ mAb 145-2C11, and biotinylated anti-mouse c-kit antibody (clone 2B8) were purchased from Pharmingen (San Francisco, Calif). Anti-LFA-1¹⁶ and anti-ICAM-1 clones were kindly provided by Dr G. Milon (Institut Pasteur, Paris, France). Biotinylated goat anti-mouse IgM was purchased from ICN (Lisle, Ill). Biotinylated-anti-B220 mAb was obtained from Dr C. Desaymard (Institut Curie, Paris, France).

Preparation of mast cells and B cells

BMMCs were prepared as described earlier¹² except that IL-3-dependent BMMCs were cultured for the last 48 hours in the presence of 100 U/mL recombinant (r)IL-4. B cells were purified as previously described.¹³

Isolation of peritoneal mast cells

Cells from the peritoneal cavity of DBA/2 mice were incubated at 4°C for 30 minutes with biotinylated anti-mouse c-kit antibody. Cells (1.5×10^7) were then washed twice and incubated in 1 mL of PBS containing 1.5×10^7 streptavidin-coated magnetic beads (Dynabeads M-280; Dynal, Oslo, Finland) under gentle rotation for 1 hour at 4°C. Beads were then removed after 2 passages on the magnet (Dynal MPC-6; Dynal, Oslo, Finland) according to the manufacturer's recommendations. Mast cell purity (90%) was assessed by toluidine blue staining. The cells were then cultured for 48 hours in 3 U/mL rIL-3 with or without 100 U/mL rIL-4.

FACS analysis

The proportion of T- and B-cell blasts in activated spleen cells was determined by FACS analysis, as previously described.¹³

Detection of IL-4, IFN- γ , and IL-12

Flat-bottom 96-well plates (Nunc, Denmark) were coated for 2 hours at 37°C with anti-IL4 mAb 11B11 or with anti-IFN- γ mAb AN18 at 4 μ g/mL or 3 μ g/mL, respectively. After 3 washes in PBS-

Tween (0.5%), plates were saturated for 1 hour at 37°C with PBS-BSA (0.1%). After 3 washes, standards and pure samples were incubated overnight at 4°C. Plates were then incubated for 2 hours at 37°C with 1 μ g/mL biotinylated anti-IL-4 BVD6 or with biotinylated anti-IFN- γ R46A2 mAbs at 1 μ g/mL. After washing, streptavidin-peroxidase conjugate was added for 2 hours at 37°C followed by *o*-phenylenediamine (Sigma). Absorbance was determined by OD at 490 nm. Determination of IL-12 was carried out by using an ELISA kit purchased from R&D Systems (Minneapolis, Minn).

IL-2 assay

The IL-2 in culture supernatants was titrated by using the IL-2-dependent cell line CTLL-2. Supernatants were incubated for 24 hours with 10^4 CTLL-2 cells, and 0.25 μ Ci of tritiated thymidine per well was added 8 hours before cell harvest.

Statistical analysis

Results are expressed as the means $\pm$ SEM from 3 experiments. The Mann-Whitney *U* test was used to compare the mean values between different groups, and the significance was set at a *P* value of less than .05.

RESULTS

Mast cells induce B and T cells to proliferate and to produce IL-2 and IFN- γ in vitro

Resting spleen cells from DBA/2 mice were cocultured for 48 hours with BMMCs at various concentrations. Investigating the cytokine requirements for IL-3-dependent BMMCs to induce lymphocyte activation, IL-4 was found, among several cytokines tested, to be the only one that confers this activity. We have previously shown that mast cells are unable to activate purified splenic T cells or thymocytes and that B cells are the primary targets for cell activation.¹³ Nevertheless, when mast cells were cultured with spleen cells at a ratio of 1%, clusters of activated spleen cells contained an augmented proportion of B-cell and T-cell blasts, as shown by FACS analysis (Fig 1, A). In addition to these phenotypic changes, BMMCs induce spleen cells to proliferate in a dose-dependent fashion up to a BMBC/spleen cell ratio of 1:100 (Fig 1, B). As to the fate of mast cells, cultures in which mast cells represented 1% of the cell population at the onset of the assay contained 4% to 5% after 48 hours of culture, reflecting a mast cell proliferation as a consequence of lymphocyte activation (data not shown). Supernatants from activated spleen cells were analyzed for their IFN- γ content. There was an effective IFN- γ production when spleen cells were activated by BMMCs (Fig 1, B). LPS induced spleen cells to proliferate, but unlike BMMCs, there was no IFN- γ production. No detectable IL-4 was found whether spleen cells were activated with BMMCs or with LPS (data not shown). These data demonstrate that although B cells, but not T cells, are the primary targets for BMMCs, there is a subsequent T-cell activation and INF- γ production.

In vivo lymphocyte activation and cytokine production induced by mast cells

On the basis of the in vitro studies, we attempted to determine whether lymphocyte activation can be induced

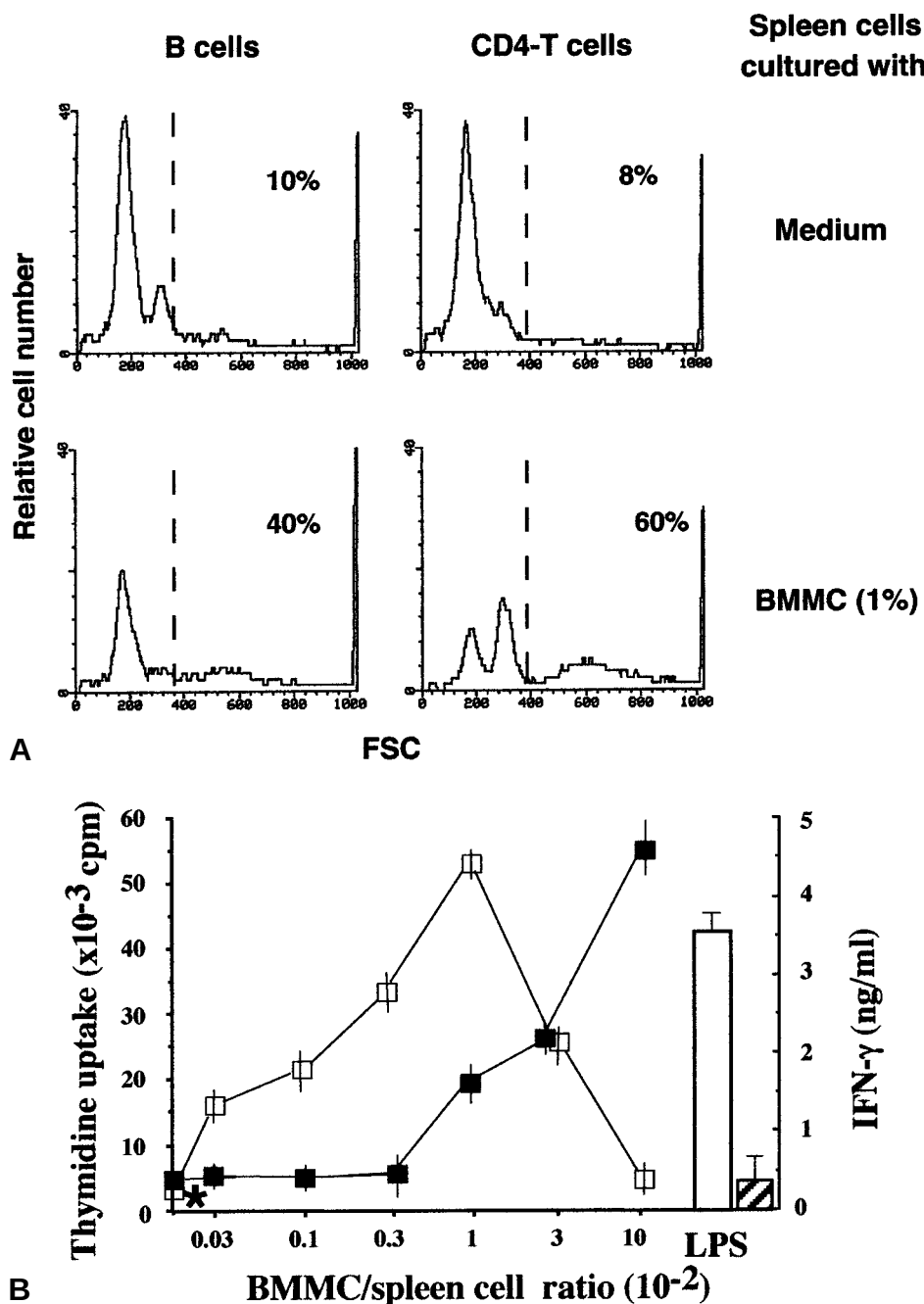


FIG 1. A, BMMCs induce B- and T-cell blast formation. After 48 hours of culture with mast cells at a BMMC/spleen cell ratio of 1:100, spleen cells from DBA/2 mice (3×10^6 /well) were harvested, and the proportion of T- and B-cell blasts was determined by FACS analysis, as described earlier.¹³ The results presented are from one of 4 experiments with similar findings. **B**, BMMCs induce spleen cells to proliferate and to produce IFN- γ . Spleen cells (3×10^6 /well) were cultured alone (*) or in the presence of BMMCs at different BMMC/spleen cell ratios. After 48 hours of culture, spleen cells were harvested and tested for proliferation (open squares) by a tritiated thymidine incorporation assay and for IFN- γ production (filled squares). LPS (5 μ g/mL) was used as a control to induce proliferation (open column) and IFN- γ production (striped column). Data are expressed as mean values $\pm$ SEM from 3 different experiments.

in mice injected with mast cells. Cultures of spleen cells from mice injected with BMMCs contain enhanced proportions of B-cell blasts ($51\% \pm 7\%$, $P < .001$), as well as T-cell blasts ($54\% \pm 8\%$, $P < .006$), in contrast to

spleen cells from mice injected with B cells ($8\% \pm 2\%$ B-cell blasts and $10\% \pm 2\%$ T-cell blasts) or with HBSS ($19\% \pm 4\%$ B-cell blasts and $20\% \pm 5\%$ T-cell blasts). In parallel, active DNA synthesis (Fig 2, A), along with IL-

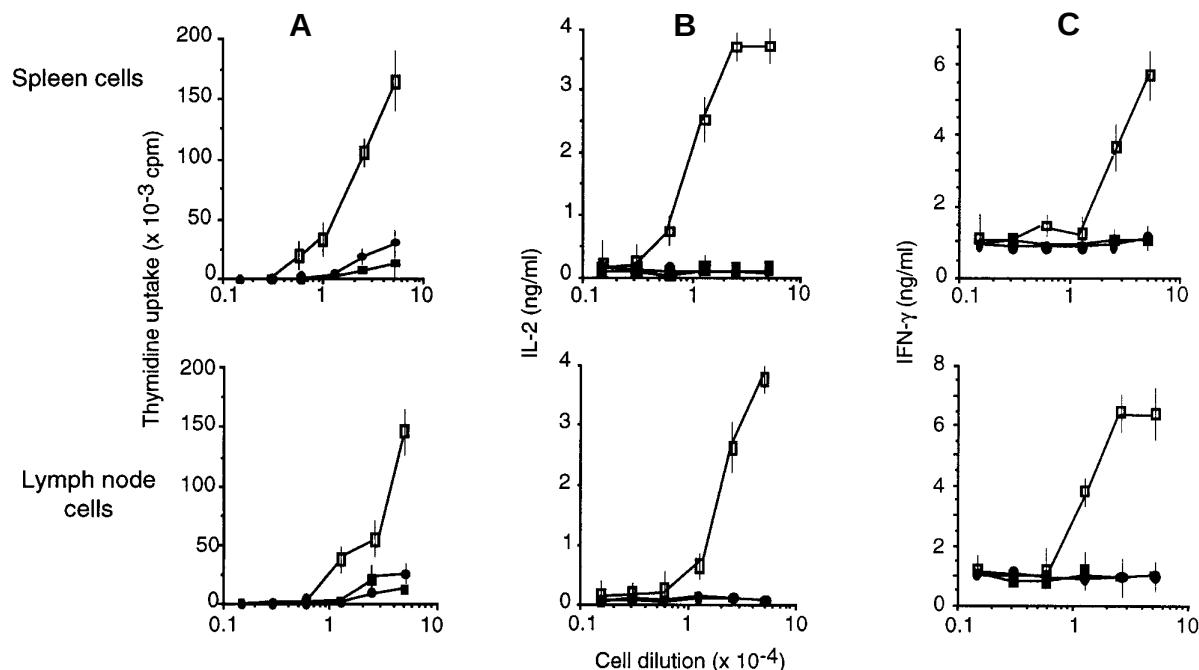


FIG 2. BMMCs induce in vivo lymphocyte activation. DBA/2 mice were injected intraperitoneally with BMMCs (open squares), DBA/2-purified B cells (filled circles), or HBSS (filled squares). A second injection was performed 1 week later. Cell proliferation (**A**), IL-2 (**B**), and IFN- γ (**C**) from cultured spleen cells and mesenteric lymph nodes were estimated by tritiated thymidine uptake and ELISA, respectively. Values are expressed as means of $\pm$ SEM of 3 experiments.

2 (Fig 2, *B*) and IFN- γ (Fig 2, *C*) production, occurred in spleen and lymph node cells from BMMC-injected mice at a significantly higher level than in control mice injected with B cells or with HBSS ($P = .006$). It should be pointed out that this cell activation and cytokine production occurred without any further in vitro stimulation.

Presence of T cells is mandatory for IL-2 and IFN- γ production in mice injected with BMMCs

To demonstrate the active role of T cells in the IL-2 and IFN- γ production on stimulation with mast cells, normal BALB/c and BALB/c nude mice were injected with BALB/c mast cells, and their spleen cells were analyzed for their capacity to undergo blast formation, to proliferate, and to produce IL-2 and IFN- γ . Data summarized in Fig 3 indicate that the absence of T cells in nude mice correlates with the lack of IL-2 and IFN- γ production (Fig 3, *B* and *C*, respectively). Injection of mast cells, however, was effective on blast formation, as well as proliferation (Fig 3, *A*), in nude mice, and that exclusively affected the B-cell population.

Mast cell-dependent IFN- γ and IL-12 production by spleen cells from NK1.1⁺CD3⁻ beige mice

To discriminate between T cells and natural killer (NK) cells, which known as major sources of IFN- γ , we compared the induction by mast cells of IFN- γ production by

spleen cells from NK-deficient C57 BL/6bg/bg mice and their normal litter mates C57BL/6. C57BL/6bg/bg mice express the beige mutation, which causes a lack of NK1.1⁺CD3⁻, a cell subset that produces large amounts of IFN- γ .¹⁷ Cocultures of mast cells from either mice with their respective spleen cells induced substantial amounts of IFN- γ (Fig 4). These results suggest that T cells are the major source of IFN- γ and that NK cells are marginally, if not at all, involved in this process. Interestingly, supernatants from mast cell-activated spleen cells also contained IL-12. The mast cell-dependent IL-12 production raises the possibility of a new player in the mast cell-splenocyte activation cascade. Because IL-12 is produced mainly by macrophages, it can be inferred from these data that these cells are connected to the mast cell-lymphocyte activation network.

Role of adhesion molecules in mast cell-induced lymphocyte aggregation

An early event of mast cell-mediated spleen cell activation consists of homotypic and heterotypic aggregation, as demonstrated by double-staining experiments with anti-B220 and anti-CD4 mAbs (data not shown). In addition, clusters of activated B and T cells contain few mast cells. Adhesion molecules, LFA-1 and its counter-receptor ICAM-1, are largely expressed by B cells, T cells, and BMMCs¹⁸ and have been considered in our system as possible candidates mediating cell aggregation. To test this hypothesis, spleen cells and mast cells

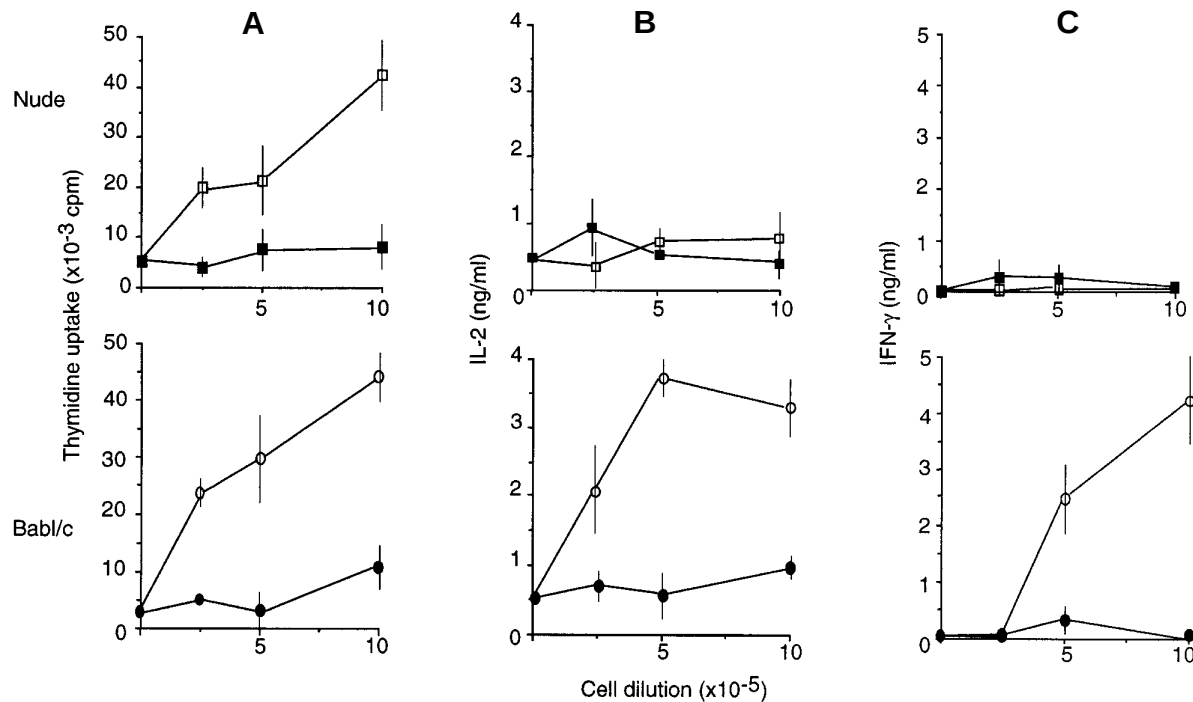


FIG 3. T cells are the major source of IL-2 and IFN- γ production induced by mast cells. Experiments were performed as in Fig 2, except that normal BALB/c or BALB/c nu/nu mice were injected with either BALB/c BMMCs ($\square$) or HBSS ($\blacksquare$). Data for cell proliferation (A), IL-2 (B), and IFN- γ (C) production are shown. Values are presented as means $\pm$ SEM of 3 experiments.

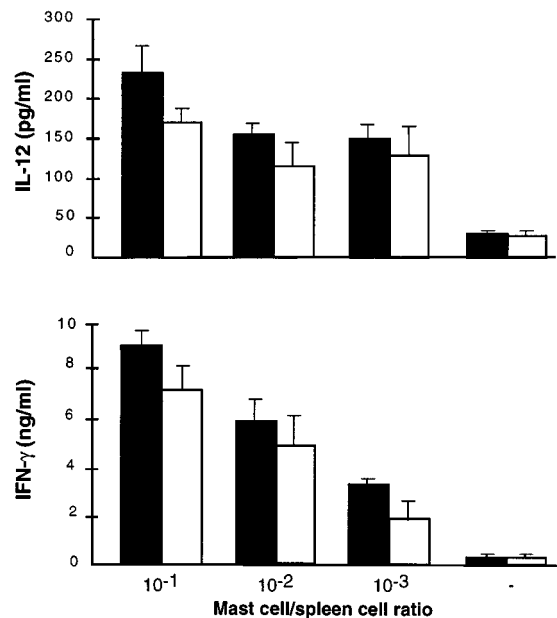


FIG 4. Induction by mast cells of IFN- γ and IL-12 from NK-deficient beige mice. Mast cells from C57Bl/6 (B6; open columns) or C57Bl/6 beige (B6bg/bg; filled columns) mice were cocultured at indicated ratios with 3×10^6 of their respective spleen cells in 1 mL of culture medium for 48 hours at 37°C. Supernatants were then tested for their content in IFN- γ and IL-12 by ELISA. Values are expressed as means $\pm$ SEM of 3 experiments.

were cultured at a ratio of 1:100 in the absence or in the presence of 10 μ g/mL anti-LFA-1, anti-ICAM-1, or both or with an irrelevant rat IgG1 antibody as control. After 48 hours of incubation, cultures were examined under the microscope to assess aggregate formation. As shown on the photomicrographs in Fig 5, the large clusters observed in the absence of antibodies (Fig 5, B) and in the presence of control mAb (Fig 5, F) were almost completely disrupted in the presence of anti-LFA-1 (Fig 5, C) or anti-ICAM-1 (Fig 5, D). However, no alteration in the blast formation could be observed. More interestingly, when both antibodies were combined, not only was blast formation dramatically reduced, but cell aggregates were almost completely disrupted (Fig 5, E).

Mast cell-induced cytokine secretion by activated spleen cells is inhibited by blocking LFA-1 and ICAM-1 molecules

Because aggregate formation is an early event in our model, we decided to investigate whether blocking this event also affects cytokine secretion. To elucidate this point, mast cells and spleen cells were cocultured at a ratio of 1:100 with or without anti-LFA-1 and anti-ICAM-1 antibodies. After 48 hours of culture, cytokine production was assessed by measuring IL-2 and IFN- γ levels in the supernatants. As shown in Fig 6, both antibodies, when examined separately, were effective in

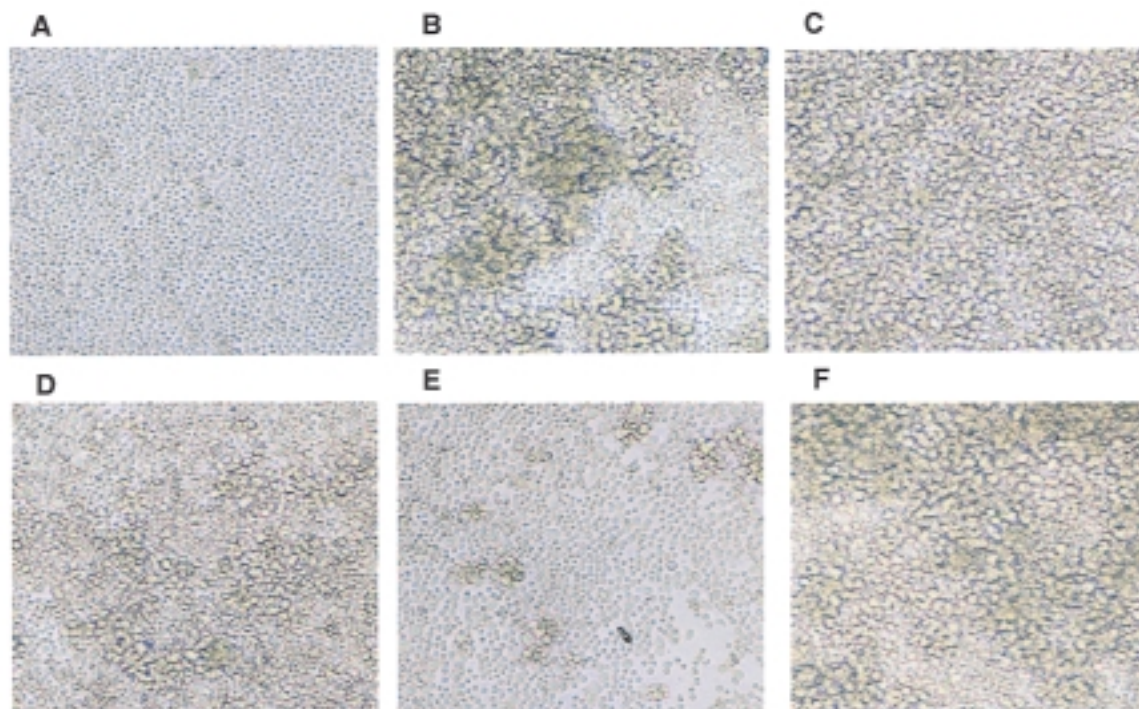


FIG 5. Anti-LFA-1 and anti-ICAM-1 antibodies block mast cell-induced spleen cell aggregation. Spleen cells (3×10^6 /well) were cultured at 37°C alone (**A**) or with 1% mast cells (**B**). In parallel, antibodies anti-LFA-1 (**C**), anti-ICAM-1 (**D**), or both (**E**) were added to these cocultures at 10 μ g/mL. As a negative control, irrelevant rat IgG1 antibody was used at 10 μ g/mL (**F**). After 48 hours of incubation, photomicrographs of cell cultures were taken (magnification, $\times 100$).

reducing the levels of IL-2 ($P = .004$ for anti-LFA-1 and $P = .0036$ for anti-ICAM-1) and IFN- γ ($P = .004$ for both antibodies). When anti-LFA-1 and anti-ICAM-1 were combined, the inhibitory effect was even more pronounced ($P = .006$ for IL-2 and $P = .003$ for IFN- γ).

Mast cells from the peritoneal cavity induce lymphocyte activation

The aim of this study is to assess the physiologic relevance of mast cell-induced lymphocyte activation by considering mature mast cells present in the peritoneum instead of BMMCs. Mast cells, which represent 1% to 2% from the peritoneal lavage fluid of mice, are the only cells among mature hematopoietic cells that are c-kit⁺, whereas most of the remaining cells are Mac-1⁺ macrophages. Peritoneal mast cells were sorted out by using anti-c-kit-coated magnetic beads and cultured for 48 hours in the presence of IL-3 or IL-3 and IL-4 before the culture assay. As controls, mast cell-depleted peritoneal cells were cultured in similar conditions. After 48 hours of culture in the presence of syngeneic spleen cells, peritoneal mast cells were highly effective compared with the mast cell-depleted peritoneal cell population in stimulating IL-2 and IFN- γ production (Fig 7). Similar to BMMCs, pretreatment with IL-4 compared with IL-3 ($P = .001$) was necessary for peritoneal mast cells to acquire this activity.

DISCUSSION

Several reports have mentioned the existence of T cell-mediated mast cell proliferation and activation.^{1,19} More recently, studies in mice¹⁵ and human subjects²⁰ have provided evidence that activated T cells form heterotypic aggregates with mast cells, which are subsequently induced to release histamine and cytokines. Existence of putative bidirectional mast cell-lymphocyte interactions prompted us to explore the mechanisms of mast cell-mediated B- and T-cell maturation and activation.

Mast cell-induced heterotypic adhesion of splenic lymphocytes was first evidenced by immunofluorescence analysis with a double-staining procedure in which aggregates consisted of B220⁺ and CD4⁺ cells (data not shown). In agreement with this observation, FACS analysis of EDTA-disrupted cell clusters shows that both B and T lymphocytes display increased cell size (Fig 1). In our search for mechanisms by which BMMCs activate B and T lymphocytes, IL-6²¹ and IL-4,²² which can be secreted by BMMCs, were thought to be possible candidates. In a previous report we have shown that these 2 cytokines were not implicated in BMDC-induced lymphocyte activation.¹³ Investigation into the cytokine requirements that modulate this mast cell activity revealed that treatment of BMMCs with IL-4, but not

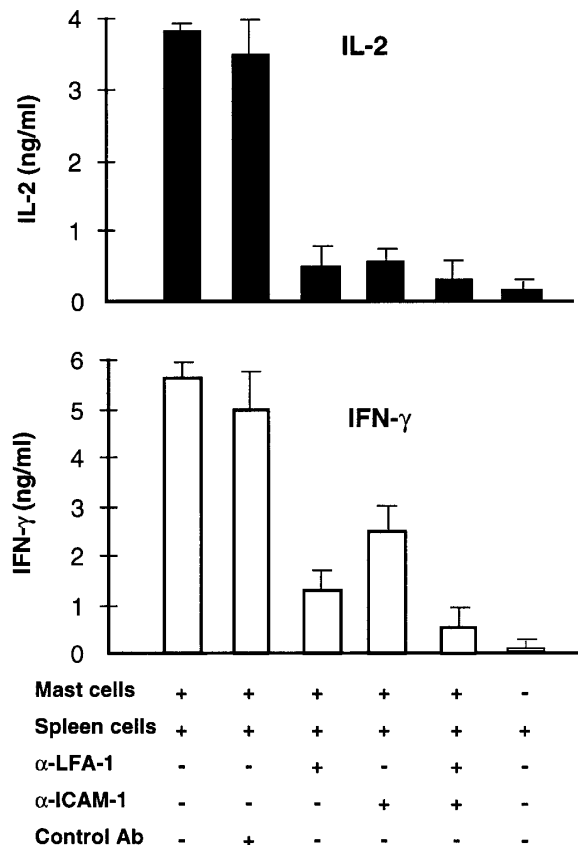


FIG 6. Anti-LFA-1 and anti-ICAM-1 antibodies inhibit cytokine secretion by mast cell-activated spleen cells. Supernatants from the same cultures described in Fig 5 were tested for their IL-2 and IFN-γ contents. Values are expressed as means $\pm$ SEM of 3 experiments. Ab, antibody.

other cytokines, 48 hours before the coculture assay was found to be critical. Among the pleiotropic effects of IL-4, previous studies indicated that mouse BMMCs, as well as airway-derived mast cells, can be induced to proliferate in the presence of this cytokine.²³ More recently, IL-4 has been shown to act as an inhibitory signal for mouse mast cell FcεRI expression through a STAT6-dependent mechanism.²⁴ In an effort to determine the mechanism by which IL-4-treated BMMCs mediate homotypic and heterotypic aggregation and activation of B and T lymphocytes, among several cell surface receptors we have examined, including CD80, CD86, CD40, MHC class II, CD4, CD2, LFA-1, and ICAM-1, only antibodies against LFA-1 and ICAM-1 were able to completely block the formation of cell aggregates and almost completely inhibited IL-2 and IFN-γ production. Interaction between LFA-1 and ICAM-1 was found to be involved in cytokine synthesis and release.²⁵ In this regard it has recently been shown that a heterotypic adhesion between human T cells and mast cells leads to degranulation and cytokine production.²⁰ Furthermore, mast cell degranulation after coculture with murine-activated T cells was found to be dependent on LFA-1 and ICAM-1.¹⁵ Interestingly, LFA-

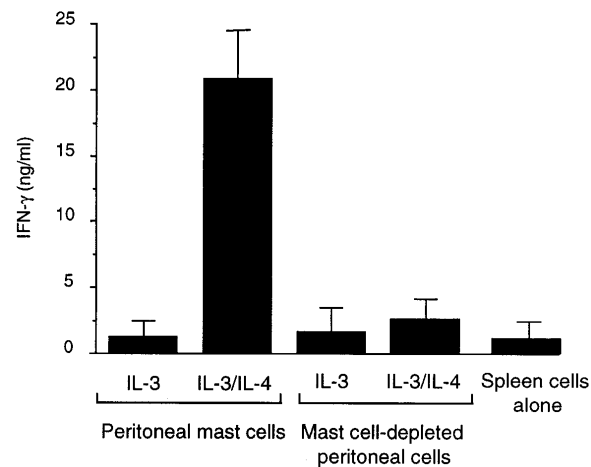


FIG 7. Peritoneal mature mast cells activate resting spleen cells in vitro. Isolated peritoneal mast cells or mast cell-depleted peritoneal cells pretreated with IL-3 or IL-3 and IL-4 were cocultured with spleen cells at a ratio of 1%. As control, spleen cells were cultured alone. After 48 hours of incubation, supernatants were harvested and tested for their IL-2 and IFN-γ contents. Values are expressed as means $\pm$ SEM of 3 experiments.

1 is expressed in inactive form on resting leukocytes and becomes activated after signaling through cell surface receptors.²⁶ According to this process, IL-4 treatment may provide the necessary signals for BMMCs to express activated LFA-1 molecules. This hypothesis is consistent with the observation that BMMCs cultured in the presence of IL-4 display homotypic adhesion. Peritoneal mature mast cells, which represent a more physiologic cell system when treated with IL-4, have also been shown to induce B- and T-lymphocyte stimulation with a similar pattern of cytokine release as that induced by BMMCs. As in in vitro studies, spleen cells from in vivo injected mice were shown to proliferate and produce IL-2 and IFN-γ ex vivo in the absence of any further stimulation. It is questionable why the addition of cultured BMMCs to cultures of mouse spleen cells, which certainly contain mast cells, or their injection into a mouse that already has a full complement of mast cells results in the responses noted. One possibility is that cultured BMMCs or peritoneal mast cells, in contrast to resident mast cells, are in an activated state resulting from the culture system. Indeed, IL-4 is necessary for mast cells to acquire the stimulatory activity on B and T lymphocytes. These data suggest that this novel mast cell property may take place in vivo provided the local IL-4 concentration reaches substantial levels in the vicinity of tissue mast cells. The dialogue between mast cells and B and T lymphocytes may account for the recruitment and activation of immune cells in inflamed tissues, which takes place during the late-phase reaction that follows the mast cell-mediated allergic response.

A characteristic feature of mast cell-induced lymphocyte activation is the production of T_H1 cytokines represented by IFN-γ and IL-12. Our data are consistent with a recent report showing that T_H1 development is promot-

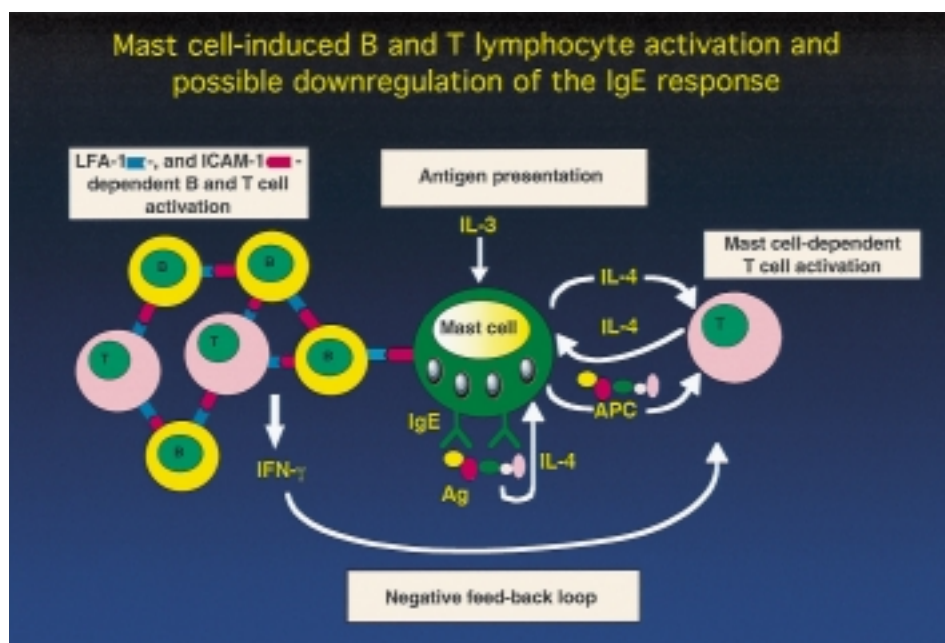


FIG 8. Schematic diagram representing mast cell-mediated B- and T-cell activation. Mast cells can act as antigen-presenting cells and specifically activate T lymphocytes, which in turn induce mast cells to produce IL-4 after T cell receptor-mediated MHC class II aggregation. Alternatively, IL-4 can also be produced by mast cells after antigen-mediated IgE cross-linking. Then IL-4-primed mast cells upregulate their adhesion molecules and directly interact with B lymphocytes, which are activated. Subsequently, neighboring T lymphocytes, which will form heterotypic aggregates with B lymphocytes and mast cells, are also activated and produce significant amounts of IFN- γ . By this mechanism, mast cells may contribute to decelerate IgE-induced allergic responses. Ag, Antigen; APC, antigen-presenting cell.

ed, whereas T_H2 differentiation is suppressed, through the interaction of ICAM-1 and ICAM-2 with LFA-1.²⁷ Mast cell-induced IFN- γ production seems to be restricted to T cells and rules out an involvement of NK1.1⁺CD3⁻ cells in this process, as shown by the unaffected IFN- γ production in NK-deficient beige mice (Fig 4). Production of IL-12 by splenocytes on activation by mast cells points out the existence of a cellular network and a cascade of events leading to IFN- γ production. IL-12 was shown to be the major stimulus of IFN- γ production by T cells.²⁸ Because IL-12 is mainly produced by activated macrophages,²⁸ it will be of interest to investigate the existence of possible relationships between mast cells and macrophages.

What could be the sequence of the cellular and cytokine events that result in mast cell-induced lymphocyte activation and the T_H1 type of response? Clearly, IL-4-induced mast cell activation is a crucial step toward lymphocyte heterotypic adhesion. Upstream to this event, IL-4 could be available after cognate interaction with specific T cells in the presence of immunogenic peptide because mast cells have been shown to produce IL-4 during antigen presentation to T cells²⁹ or through cross-linking of IgE with antigen. Alternatively, IL-4 could be provided through a paracrine pathway as a consequence of an ongoing T_H2 response. Then IL-4-primed mast cells upregulate their adhesion molecules, which may contribute to the attraction and activation of neigh-

boring T and B lymphocytes. On the basis of our previous data showing that BMDCs activate purified B cells, but not isolated T lymphocytes,¹³ the current results suggest that B lymphocytes are the primary targets for cell activation, which in turn directly or indirectly contributes to T-cell activation. As depicted in Fig 8, the mechanism we describe herein by which IL-4-activated mast cells induce T cells to produce IFN- γ may serve as a regulatory feed-back loop to decelerate a T_H2 response that has already been initiated.

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