

MORAXELLA CATARRHALIS INDUCES MAST CELL ACTIVATION AND NUCLEAR FACTOR KAPPA-B-DEPENDENT CYTOKINE SYNTHESIS

G. Krishnaswamy^{1,4}, R. Martin¹, E. Walker^{1,4}, C. Li², F. Hossler³, K. Hall¹ and D. S. Chi¹

¹ The Departments of Internal Medicine, ² Surgery and ¹Anatomy and Cell Biology, East Tennessee State University, Johnson City, Tennessee and ⁴The James H. Quillen V.A. Medical Center, Mountain Home, Tennessee

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
 - 3.1. Mast cell culture and activation
 - 3.2. Preparation of bacterial suspensions
 - 3.3. ELISA for cytokines
 - 3.4. EMSA for nuclear factor kappaB
 - 3.4.1. Extraction of nuclear proteins
 - 3.4.2. Electrophoretic Mobility Shift Assay (EMSA)
 - 3.5. Scanning electron microscopy
4. Results
 - 4.1. Binding of bacteria to mast cells
 - 4.2. Bacterial stimulation of cytokine production
 - 4.3. Direct bacterial contact is necessary for mast cell activation
 - 4.4. Role of nuclear factor kappaB in mast cell activation
5. Discussion
6. Acknowledgment
7. References

1. ABSTRACT

Human mast cells are often found perivascularly and at mucosal sites and may play crucial roles in the inflammatory response. Recent studies have suggested a prominent role for mast cells in host defense. In this study, we analyzed the effects of a common airway pathogen, *Moraxella catarrhalis* and a commensal bacterium, *Neisseria cinerea*, on activation of human mast cells. Human mast cell leukemia cells (HMC-1) were activated with either phorbol myristate acetate (PMA) and calcium ionophore or with varying concentrations of heat-killed suspensions of bacteria. Supernatants were assayed for the cytokines interleukin-4 (IL-4), granulocyte macrophage colony stimulating factor (GM-CSF), IL-6, IL-8, IL-13 and monocyte chemotactic protein-1 (MCP-1). Nuclear proteins were isolated and assayed by electrophoretic mobility shift assay (EMSA) for nuclear factor kappaB (NF- κ B) nuclear binding activity. In some experiments, NF- κ B inhibitor, Bay-11 was added to determine functional significance. Both *M. catarrhalis* and *N. cinerea* induced mast cell activation and selective secretion of two key inflammatory cytokines, IL-6 and MCP-1. This was accompanied by NF- κ B activation. Neither spun bacterial supernatants nor bacterial lipopolysaccharide induced cytokine secretion, suggesting need for direct bacterial contact with mast cells. Scanning electron microscopy revealed active aggregation of bacteria over mast cell surfaces. The NF- κ B inhibitor, Bay-11, inhibited expression of MCP-1. These findings suggest the possibility of direct interactions between human mast cells and common bacteria and provide evidence for a novel role for human mast cells in innate immunity.

2. INTRODUCTION

Mast cells are multifunctional, tissue-dwelling cells capable of secreting a wide variety of mediators. While typically associated with immediate hypersensitive responses, mast cells (1-3) are also likely to play a critical role in immune surveillance and contribute to host defense (3,4). Data from several laboratories suggest that mast cells are capable of initiating both innate and acquired immune reactions (5,6). Among various immune functions, they can phagocytose foreign particles, present antigen and also express receptors such as intercellular adhesion molecule-1 (ICAM-1) and ICAM-3, CD 43, CD 80, CD 86, and CD 40L allowing interaction with T and B lymphocytes. There is also good evidence to suggest that mast cells are capable of phagocytosis of a large range of bacteria (7). Mast cells enhance the development of Th2 cells and allow B cells to class switch to IgE. By influencing both humoral and cell mediated immune mechanisms, mast cells regulate host defense. Moreover, it should be recognized that complement products as well as neuropeptides can induce mast cell degranulation, thereby allowing interaction with the innate immune system and neuroimmune mechanisms.

Mast cells can secrete cytokines and chemokines, such as IL-1, IL-5, IL-8, and particularly TNF alpha, that activate lymphocytes (7,8). By secreting cytokines and functioning as antigen presenting cells, mast cells modulate cell mediated specific immune responses (9). The initial demonstration that mast cells are capable of cytokine expression was by Plaut *et al.* (10). Since then, mast cells have been demonstrated to express a spectrum of cytokines

and chemokines (11-15). They also produce lipid mediators and histamine that can have profound effects on vascular endothelium allowing other circulating immune cells to migrate into the tissues. Most of these roles are tissue independent but clearly there are some site-specific roles for macrophages. Mast cells play a very important role in host defense at the site of the lung. Here, mast cells reside in an intraepithelial location or near blood vessels, bronchioles, and mucous secreting glands (8). It has been shown that in mast cell-deficient mice, pathogenic bacteria survived ten-fold more than in mice with mast cells (16). In addition, mast cells release prestored TNF alpha which serves as a powerful neutrophil chemoattractant (17,18). Hence, mast cells are likely to play a crucial role in the innate immune response to common bacterial pathogens.

In this study we analyzed the expression of key cytokines and chemokines following activation of a human mast cell line, HMC-1, by *Moraxella* (*Branhamella*) *catarrhalis* (*M. catarrhalis*) and *Neisseria cinerea* (*N. cinerea*). *M. catarrhalis* and *N. cinerea* are Gram-negative, human nasopharyngeal colonists with pathogenic potential. *M. catarrhalis* can cause otitis media, sinusitis and exacerbation of bronchitis (19) and *N. cinerea* (20) is occasionally reported as the causal agent in pediatric ocular infections. We show that both types of bacteria bind mast cells and activate synthesis of IL-6 and MCP-1. This appears to be mediated, at least partially, by the transcription factor, NF- κ B and requires direct bacterial binding. These data suggest that human mast cells may provide pivotal support of host innate immune responses as well as allow interactions with resident bacteria that may mediate physiological processes such as angiogenesis or tissue remodeling at mucosal sites.

3. MATERIALS AND METHODS

3.1. Mast cell culture and activation

HMC-1 cells, established from a patient with mast cell leukemia, were graciously provided by Dr. Butterfield (Mayo Clinic, Rochester, MN). These cells were maintained in RPMI 1640 media (GibcoBRL, Rockville, MD), supplemented with 2×10^{-5} M 2-mercaptoethanol (Sigma Chemical Company, St. Louis, MO), 10 mM HEPES (GibcoBRL), Gentamycin 50 μ g/ml (Sigma), 5 mg/ml insulin transferrin (Sigma), 2 mM L glutamine (GibcoBRL) and 5% heat inactivated fetal bovine serum (Atlanta Biologicals, Atlanta, GA), at 37°C and in 5% CO₂ mixture (1). The cells were seeded at 2×10^4 cells/ml density and recultured every 4 days. Mast cells for this experiment were seeded at 1×10^6 cells/ml in 24 well Costar tissue culture dishes. Cells in media alone serve as negative controls. Cells treated with phorbol myristate acetate (PMA) (50 ng/ml) and 5 μ M ionomycin served as positive controls reflecting maximal activation. In some experiments, mast cells were also activated with varying concentrations of heat-killed suspensions of *M. catarrhalis* (ATCC #25238) or *N. cinerea* (ATCC #14685) (1.0×10^5 , 1.0×10^6 , and 1.0×10^7 bacteria/ml). Bacteria were obtained from the American Type Culture Collection (ATCC), Manassas, VA. In order to determine a role of NF- κ B in mast cell activation by bacteria, Bay-11 (10 μ M

final concentration; Biomol Chemicals, Plymouth Meeting, PA), an inhibitor of NF- κ B activation, was added to mast cell cultures. Bay-11 (BAY-11-7082, Biomol Research Laboratories, Plymouth Meeting, PA) has been shown to inhibit cytokine-induced NF- κ B specific inhibitory protein I κ B α phosphorylation and nuclear factor kappaB-dependent expression of adhesion molecules on endothelial cells (21). The concentrations used in studies with Bay-11 inhibitor have varied between 5-20 μ M (product data sheet, Biomol Research Laboratories) (21).

Supernatants were collected after 24 hours and assayed for cytokine proteins by ELISA. In other experiments, HMC-1 cells were activated with lipopolysaccharide (LPS, 10 ng/ml, Biomol Research Laboratories, Plymouth Meeting, PA) to determine whether LPS was capable of inducing secretion of cytokines in similar magnitudes as bacterial suspensions. In selected experiments, filtered supernatants of bacterial suspensions were added to mast cell cultures in order to detect soluble factors capable of mast cell activation. Following culture for 24 hours, mast cell cultures were centrifuged at 250xg for 10 minutes to remove cell debris, and cell-free supernatants were collected and stored at -70°C until ready for assay.

3.2. Preparation of bacterial suspensions

The type strains for *M. catarrhalis* (ATCC #25238), and *N. cinerea* (ATCC #14685) were cultured in brain heart infusion broth overnight at 37°C. An aliquot of the overnight culture was subjected to serial dilutions to estimate the number of colony forming units/ml (cfu). The remaining bacterial cells were concentrated by centrifugation at 1800xg, and the broth supernatant was stored at -60°C. The bacterial cells were washed three times by suspension in endotoxin-free phosphate-buffered saline followed by centrifugation at 14,000xg. The cell pellet was weighed, resuspended in 2 mls of murine clone media, and heat-killed at 60°C for 2 hours (22). A Gram stain was performed to assess bacterial morphology, and cell death was confirmed by culture of heat-treated bacteria. The suspension was adjusted to 5mg/ml in murine clone media and stored at -60°C. The number of bacterial cells extrapolated from determinations of cfu/ml to cfu/ μ g was 3.5×10^3 cfu/ μ g for *M. catarrhalis* and 4.2×10^3 cfu/ μ g for *N. cinerea*. Equal volumes of bacterial cells (5 mg/ml, 500 μ g/ml, and 50 μ g/ml) were mixed with murine clone media containing 1.5×10^5 HMC-1 cells/ml. Assay conditions corresponded to 120, 12 or 1.2 *M. catarrhalis* cells/HMC-1 cells, and the bacterial concentration falls within the range reported in sputum from patients with bronchiectasis (23).

3.3. ELISA for cytokines

Cytokine ELISA was performed for the following cytokines: IL-4, IL-6, IL-8, IL-13, MCP-1 and GM-CSF. ELISA was carried out on cell-free culture supernatants using commercially available ELISA kits, according to manufacturers instructions as earlier described (R&D Systems, Minneapolis, MN; Immunotech, Westbrook, ME; Genzyme, Cambridge, MA). Results were analyzed on an ELISA plate reader (Dynatech MR 5000 with supporting software) (24,25).

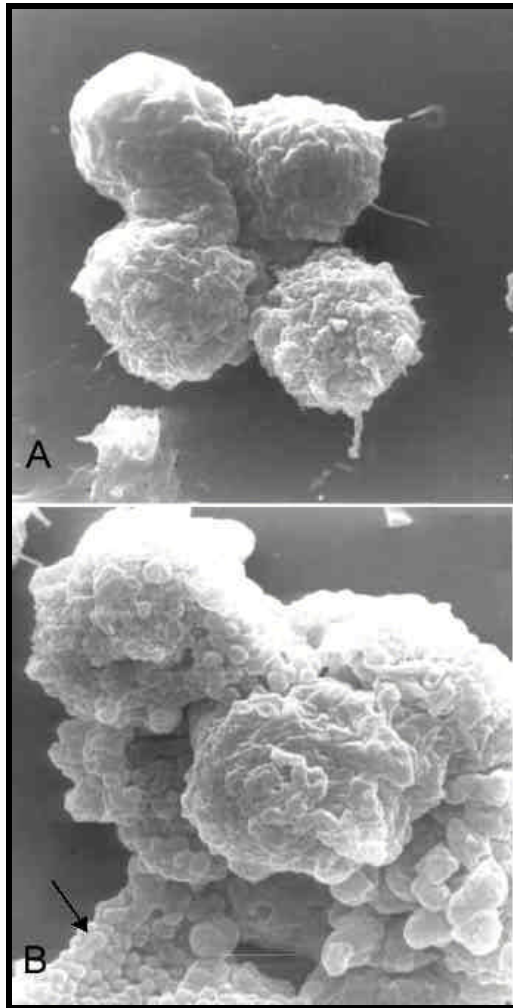


Figure 1. Scanning electron microscopy of the mast cells. Control mast cells displayed microvilli-like processes on their surfaces (1A, 4000 x). Treated mast cells had aggregations of bacteria (arrow) on their surfaces (1B, 5000 x). These experiments were repeated several times and the data shown is representative of typical experiments.

3.4. EMSA for NF- κ B

3.4.1. Extraction of nuclear proteins

Nuclear proteins were isolated using a previously described method (26,27). The Tris buffered saline (TBS) washed cells were mixed with 0.4 ml of ice-cold hypotonic buffer (10 mM HEPES pH7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 1 μ M aprotinin, 1 μ M pepstatin, 14 μ M leupeptin) and allowed to swell in an ice bath for 20 min. After adding 25 μ l of 10% Nonidet P-40, the homogenates were vortexed for 30 seconds, and centrifuged in an Eppendorf centrifuge at 4°C for 2 min. The pellets were washed once with the hypotonic buffer without Nonidet P-40 and re-suspended in an ice-cold hypertonic salt buffer (20 mM HEPES pH 7.9, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 μ M aprotinin, 1 μ M pepstatin, 14 μ M

leupeptin). The samples were then incubated on ice for 30 min with frequent mixes and centrifuged for 15 min at 4°C. The supernatants were collected as nuclear proteins and stored at -80°C. The concentration of total proteins in the samples was determined by using a Pierce protein assay reagent (Pierce Chem. Co., Rockford, IL).

3.4.2. Electrophoretic Mobility Shift Assay (EMSA)

Transcription factor NF- κ B binding activity was performed according to the manufacture's protocol (Promega, Madison, WI) (26,27). The binding reaction was carried out in 15 μ l volume containing 1 x binding buffer [0.2 μ g of double-stranded poly (dI-dC) in 20 mM HEPES (pH 7.9), 5% glycerol, 1 mM EDTA, 0.5 mg of BSA/ml, 1% Nonidet P-40, 5 mM DTT], 5 μ g of nuclear proteins and 35 fmole of NF- κ B oligonucleotide (5'-AGT TGA GGG GAC TTT CCC AGG C-3') labeled with [γ - 32 P]ATP using T4 polynucleotide kinase (Promega). The binding reaction mixture was incubated at room temperature for 20 min and analyzed by 5% non-denaturing acrylamide gel electrophoresis. After electrophoresis, the gels were dried (Gel-Drier; Bio-Rad) and exposed to Kodak X-ray films at -70°C. The optical density of the binding bands on the X-ray films were then analyzed by a Bio-Image Analysis System (MILLIPORE Imaging System, Ann Arbor, MI).

3.5. Scanning electron microscopy

For scanning electron microscopy of mast cells, an equal volume of fixative (2.5 % glutaraldehyde, 1 % p-formaldehyde, 0.1M cacodylate buffer, pH 7.3) was added to cell suspensions growing in tissue culture wells, and fixation was continued overnight under refrigeration (4°C). The supernatant was drawn off the visibly settled cells, and the cells were washed twice by gentle centrifugation and resuspension in an equal volume of 0.2M cacodylate buffer containing 0.1 % CaCl_2 , at pH 7.3. The cells were post-fixed by resuspension in 2 % OsO_4 in 0.1M cacodylate buffer containing 0.1 % CaCl_2 , at pH 7.3, for 3 hr at ambient temperature. The cells were rinsed briefly in distilled water, then placed in 50 % ethanol overnight. Cells were then dehydrated through an ethanol series up to 100%. A drop of cell suspension was allowed to settle onto glass cover slips, and then air dried. Cover slips were attached to aluminum stubs with colloidal carbon, sputter coated with gold, and viewed and photographed in a DSM 940 scanning electron microscope (Carl Zeiss, Inc., Thornwood, NY) at 20 kv.

4. RESULTS

4.1. Binding of Bacteria to Mast Cells

Incubation of heat-killed suspensions of bacteria with mast cells resulted in their aggregation around mast cells. Scanning electron microscopy was performed in resting and heat-killed *M. catarrhalis*-treated HMC-1 cells and the results are shown in Figure 1 A and B, respectively. Control mast cells displayed microvilli-like processes on their surfaces (Figure 1A). Treated mast cells had aggregations of bacteria on their surfaces (Figure 1B). The interactions appeared to be quite tight as the mast cells could not be disengaged from the bacteria even by brief vortexing or vigorous pipetting. We have not analyzed

Table 1. The production of the various cytokines in HMC-1 cells following activation

	Activation [^]			
	Medium Control	PMA + Ionomycin	<i>Moraxella catarrhalis</i>	<i>Neisseria cinerea</i>
GM-CSF	0.3 ± 0.5	748.1 ± 19.2*	6.6 ± 1.1	10.1 ± 1.7
MCP-1	438.5 ± 31.0	3,112.3 ± 225.1*	972.2 ± 40.8*	1,661.2 ± 121.9*
IL-4	5.8 ± 0.4	376.2 ± 59.2*	11.9 ± 2.2	10.5 ± 1.5
IL-6	0	308.2 ± 5.4*	35.9 ± 0.5*	108.8 ± 5.8*
IL-8	3.4 ± 5.8	1,687.1 ± 674.8*	12.9 ± 5.7	27.4 ± 8.2
IL-13	0	334.5 ± 54.3*	0	21.4 ± 18.7

[^] HMC-1 (1 x 10⁶ cells/ml) were activated with PMA (50 ng/ml) + ionomycin (5 uM), *Moraxella catarrhalis* (1 x 10⁸ bacteria/ml), and *Neisseria cinerea* (1 x 10⁸ bacteria/ml). The result was expressed as Mean ± Standard deviation (pg/ml).

* Statistically significant (at least p < 0.05), when compared with the medium control group.

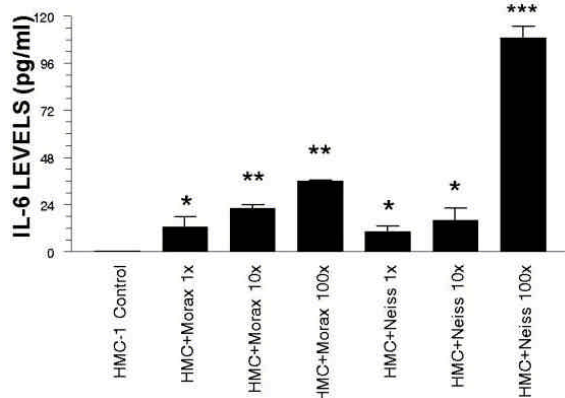


Figure 2. Activation of mast cells by varying concentrations of *M. catarrhalis* and *N. cinerea* produced a dose-dependent secretion of IL-6, with maximal levels seen with 100 x concentrations (1 x 10⁸ bacteria/ml). Cultures with *M. catarrhalis* (Morax) at 1 x (* p < 0.05), 10 x (** p < 0.005) and 100 x concentrations (** p < 0.005), and *N. cinerea* (Neiss) at 1 x (* p < 0.05), 10 x (* p = 0.05) and 100 x (*** p = 0.0009) significantly enhanced IL-6 secretion from HMC-1 cells compared to control cells alone. These experiments were repeated several times and the data shown is representative of a typical experiment.

actual phagocytosis of bacterial particles by HMC-1 cells and these studies are currently in progress.

4.2. Bacterial Stimulation of Cytokine Production

Mast cells were activated with either PMA and ionomycin or with varying concentrations of heat-killed suspensions of *M. catarrhalis* and *N. cinerea*. Supernatants were collected 24 hours after activation and assayed for IL-4, IL-6, IL-8, IL-13, MCP-1 and GM-CSF by ELISA. Mean values for the various cytokines following activation are shown in Table 1. PMA and ionomycin induced significant secretion of all cytokines measured, including IL-4, IL-6, IL-8, IL-13, MCP-1 and GM-CSF. On the other hand, *M. catarrhalis* induced no secretion of IL-13 and minimally increased secretion of IL-4, IL-8 and GM-CSF. *M. catarrhalis*, however, induced a significant secretion of both MCP-1 and IL-6 from mast cells. The results for IL-6 and MCP-1 are shown in Figures 2 and 3 respectively. Activation of mast cells by varying dilutions of *M.*

catarrhalis produced a dose-dependent secretion of IL-6 (Figure 2), with maximal levels seen with 100x concentrations (1 x 10⁸ bacteria/ml). In the case of MCP-1, a dose response curve was again seen with varying concentrations of *M. catarrhalis*, with maximal levels with the 100 x concentration of the bacterium (Figure 3). No additive or synergistic effects were seen when *M. catarrhalis* was added to PMA and ionomycin. Similarly, *N. cinerea* activated IL-6 and MCP-1 secretion from HMC-1 cells (Table 1). The dose responses to *N. Cinerea* for IL-6 and MCP-1 secretion are shown in Figures 2 and 3 respectively, with maximal effects seen at 100 x concentrations.

4.3. Direct bacterial contact is necessary for mast cell activation

In order to determine whether direct bacterial contact or a soluble factor produced by bacteria was responsible for the cytokine secretion, mast cells were activated with either nonfiltered supernatants (containing bacterial fragments) or filtered supernatants from bacterial cultures. As shown in Figure 4A, filtration of the supernatant produced a profound reduction in the amount of MCP-1 secreted suggesting a role for direct bacteria-mast cell interactions in this phenomenon. This suggested that bacterial fragments were also capable of inducing mast cell activation and that no soluble factor was secreted by the bacteria that could result in mast cell activation. Since lipopolysaccharide is capable of binding to cell surface receptors and signaling inflammatory cytokine synthesis in cells of the mononuclear-macrophage lineage, the effects of LPS on mast cell activation needed to be ascertained. As shown in Figure 4B, LPS at 10 ng/ml did not produce any major increase in MCP-1 production. Accordingly, neither bacterial lipopolysaccharide nor a soluble mediator appeared to be instrumental in inducing MCP-1 secretion and physical contact of bacteria with mast cells seems essential.

4.4. Role of Nuclear Factor KappaB in Mast Cell Activation

Nuclear proteins were extracted from mast cells activated for 24 hours by PMA and ionomycin or heat killed-bacterial suspensions and assayed for NF-κB nuclear binding activity by EMSA. The results are shown in Figure 5. In summary, weak activity of NF-κB is seen in

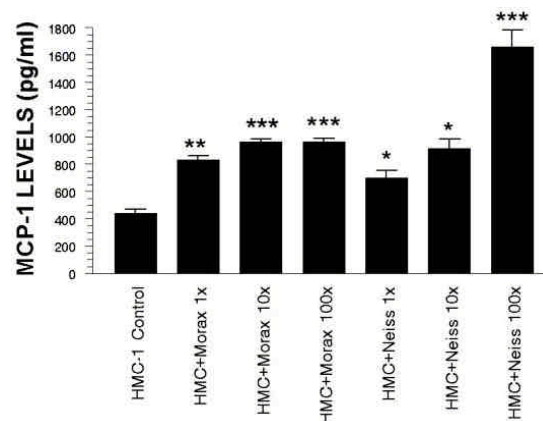


Figure 3. Activation of mast cells by varying concentrations of *M. catarrhalis* and *N. cinerea* produced a dose-dependent secretion of MCP-1. A dose response curve was again seen with varying concentrations of *M. catarrhalis* and *N. cinerea*, with maximal levels with the 100 x concentration (1 x 10⁸ bacteria/ml) of the bacterium. Cultures with *M. catarrhalis* (Morax) at 1 x (** p = 0.007), 10 x (*** p = 0.001) and 100 x concentrations (*** p = 0.001), and *N. cinerea* (Neiss) at 1 x (* p < 0.05), 10 x (* p < 0.05) and 100 x (*** p = 0.001) concentrations significantly enhanced MCP-1 secretion from HMC-1 cells compared to control cells alone. These experiments were repeated several times and the data shown is representative of a typical experiment.

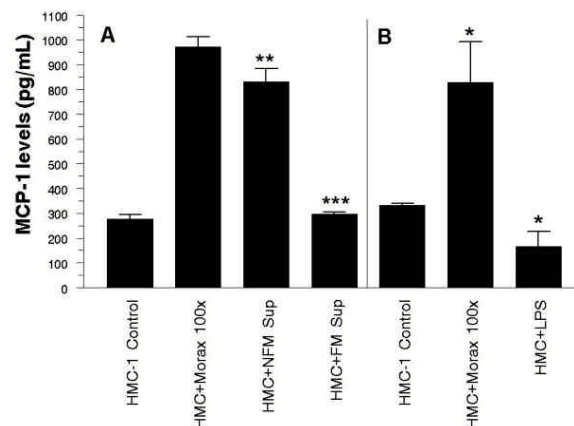


Figure 4. (A) HMC-1 cell activated with *Moraxella catarrhalis* secreted enhanced amounts of MCP-1 compared to controls. Bacterial culture supernatants which were unfiltered (NFM Sup) also induced significant MCP-1 secretion from HMC-1 cells (** p = 0.002 compared to the control). However, filtration of the supernatant (FM Sup) produced a profound reduction in the amount of MCP-1 secreted bringing the levels back to baseline (*** p = 0.17 compared to the control). This suggested that bacterial fragments were also capable of inducing mast cell activation and also that no soluble factor was secreted by the bacteria that could result in mast cell activation. (B) Effects of lipopolysaccharide (bacterial LPS) on MCP-1 production from HMC-1 cells. Again, heat-killed *Moraxella* activated MCP-1 production from HMC-1 cells (* p < 0.05 compared to the control) whereas LPS at 10 ng/ml, on the contrary, inhibited constitutive production of the chemokine (* p < 0.05 compared to the control). These experiments were repeated several times and the data shown is representative of a typical experiment.

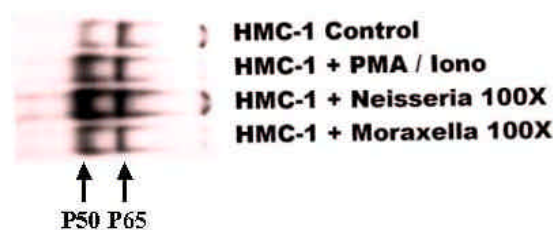


Figure 5. Representative EMSA showing nuclear factor kappaB binding activity in HMC-1 following stimulation. Weak binding activity of NF-κB is seen in unstimulated cells (Control). However, stimulation with either PMA and ionomycin (Iono) or with bacterial suspensions (both *Moraxella catarrhalis* and *Neisseria cinerea*) increased nuclear binding activity of NF-κB.

Moraxella catarrhalis induces mast cell activation

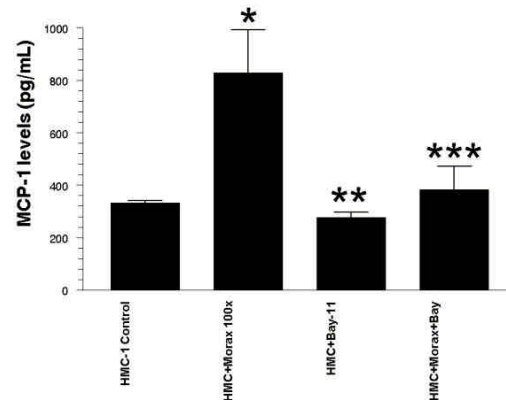


Figure 6. Effects of the NF- κ B inhibitor, Bay-11 on MCP-1 production by HMC-1 cells. To confirm the functional significance of NF- κ B activation, mast cells were incubated with the NF- κ B inhibitor, Bay-11. *Moraxella* (Morax 100 x) activated MCP-1 production from mast cells (* $p = 0.03$ compared to the control). Bay-11 induced a statistically significant inhibition of MCP-1 production both constitutively (** $p = 0.02$, as compared to the control) and following activation induced by *M. catarrhalis* (***) $p = 0.03$, as compared to the Morax 100 x).

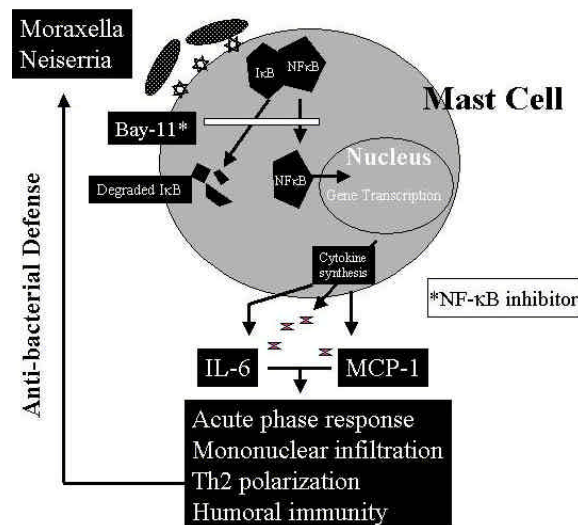


Figure 7. Putative mechanisms governing the regulation of mast cell activation by *M. catarrhalis* and *N. cinerea*. Binding of cell surface receptors on mast cells leads to cellular activation, nuclear translocation of nuclear factor kappaB and stimulation of the expression of proinflammatory cytokine/chemokine genes. The secretion of cytokines such as IL-6 leads to an accentuation of the acute phase response characterized by fever, elevated erythrocyte sedimentation rate and elaboration of opsonic proteins such as C-reactive protein. IL-6 also enhances B cell immunity and production of immunoglobulins by B lymphocytes. The chemokine, MCP-1, on the other hand, enhances mononuclear recruitment and Th2 lymphocyte development.

unstimulated control cells (integrated intensity [II] were 6.9 and 0.2 for p65 and p50, respectively). This may represent the leukemic nature of this cell line. However, marked increase of NF- κ B nuclear binding activity is observed in samples from cells stimulated with either PMA and ionomycin (II were 12.3 and 24.9 for p65 and p50, respectively) or with *M. catarrhalis* (II were 8.1 and 21.5 for p65 and p50, respectively) and *N. cinerea* (II were 26.1 and 50.4 for p65 and p50, respectively). To confirm the functional significance of NF- κ B activation, mast cells were incubated with the NF- κ B inhibitor, Bay-11 (See MATERIALS AND METHODS). The results are shown in Figure 6. Bay-11 caused a statistically significant inhibition of MCP-1 production induced by bacterial cultures.

5. DISCUSSION

In this study, we demonstrate that mast cell activation by *M. catarrhalis*, a respiratory pathogen and *N. cinerea*, an ocular pathogen, leads to selective induction of MCP-1 and IL-6 secretion. This appears to be mediated at least partially by a transcription factor, NF- κ B. The selective induction of IL-6 and MCP-1 may be related to other complex intracellular mechanisms that regulate cytokine/chemokine expression by mast cells. As summarized by Bondeson *et al.* (28), heterogeneity of mechanisms regulating both proinflammatory and antiinflammatory cytokines exist within a given cell type. Thus, the cytokine synthetic response of a given cell to an activating stimulus may depend upon net competing influences of multiple signal transduction pathways.

Bacterial aggregation to mast cell surfaces is seen by scanning electron microscopy, suggesting physical interactions between the bacteria and mast cell membranes. Filtered bacterial culture supernatants were unable to induce cytokine release suggesting the need for cell contact. Moreover, the absence of an LPS effect in terms of cytokine secretion from HMC-1 cells suggests that this was not mediated by interactions of bacterial LPS with its cell surface receptors on mast cells. The functional role of NF- κ B in mast cell-bacterial interactions was confirmed by the demonstration of inhibition of MCP-1 production in mast cells following addition of the NF- κ B inhibitor, Bay-11. The focus of this paper is on the role of mast cell-bacterial interactions on mast cell cytokine synthesis. However, studies on induction of tryptase and histamine from mast cells by bacterial interactions are currently ongoing.

N. cinerea produced as robust a response from mast cells as did *M. catarrhalis*. We used heat killed suspensions of bacteria in order to circumvent problems arising from actual infection of mast cells leading to cell death or necrosis. Our data is compatible with the presence of cell surface receptors on human mast cells for common bacteria. These need further analysis and study. We also did not ascertain actual phagocytosis of the bacteria by mast cells and these studies are ongoing.

The production of immunoregulatory cytokines from mast cells in response to bacterial infection is interesting as it could have many consequences on innate immune responses. IL-6 mediates the acute phase response and enhances immunoglobulin production from B cells. Consequences such as fever, catabolic states, production of fibrinogen, thrombocytosis and synthesis of C reactive protein, an opsonin, are all related to IL-6 production. MCP-1 is a monocyte chemoattractant and induces histamine release from mast cells (an autocrine effect in this case) which all lead to enhanced inflammatory cell influx and tissue infiltration. MCP-1 has also been shown to enhance development of the Th2 subset of T cells that can further enhance humoral immune responses (29). Figure 7 shows the putative role of human mast cells in the innate immune response to bacterial pathogens. In this scenario, direct binding of bacteria to mast cell surface receptors (uncharacterized) leads to activation of NF- κ B in these cells (30,31). This further activates inflammatory cytokine gene expression leading to the elaboration of inflammatory cytokines such as IL-6 and MCP-1 (32,33). These cytokines and chemokines activate acute phase responses characterized by fever and elaboration of opsonic proteins such as CRP. The chemokines may also enhance infiltration of mucosal tissues by mononuclear cells leading to involvement of more specific and targeted immune responses. Moreover, IL-6 also enhances immunoglobulin synthesis by B lymphocytes that accentuates phenomena such as antibody-mediated cytotoxicity and other antibacterial defense mechanisms. Other phenomena such as necrosis, tissue remodeling, angiogenesis and repair could follow the inflammatory event. This suggests that a host-parasite interaction in the body could lead to low level persistent mast cell activation leading to pivotal processes

such as tissue remodeling, angiogenesis and/or immune surveillance. In conclusion, we show that mast cells can be activated by common pathogenic and commensal bacteria leading to NF- κ B activation and inflammatory cytokine synthesis. This suggests a role for bacterial-mast cell interactions in innate immune responses.

6. ACKNOWLEDGMENTS

This study was supported by NIH grants AI-43310 and HL-63070, RDC grant (ETSU), The Cardiovascular Research Institute (ETSU), State of Tennessee Grant 20233, and Ruth R. Harris Endowment (ETSU).

7. REFERENCES

1. Krishnaswamy, G., T. Lakshman, A. R. Miller, S. Srikanth, K. Hall, S. K. Huang, J. Suttles, J. K. Smith, and R. Stout: Multifunctional cytokine expression by human mast cells: regulation by T cell membrane contact and glucocorticoids. *J Interferon Cytokine Res* 17, 167-176 (1997)
2. Kelley, J. L., D. S. Chi, W. Abou-Auda, J. K. Smith, and G. Krishnaswamy: The molecular role of mast cells in atherosclerotic cardiovascular disease. *Mol Med Today* 6, 304-308 (2000)
3. Krishnaswamy, G., J. Kelley, D. Johnson, G. Youngberg, W. Stone, S. K. Huang, J. Bieber, and D. S. Chi: The human mast cell: functions in physiology and disease. *Front Biosci* 6, d1109-1127 (2001)
4. Abraham, S. N., K. Thankavel, and R. Malaviya: Mast cells as modulators of host defense in the lung. *Front Biosci* 2, d78-87 (1997)
5. Henz, B. M., M. Maurer, U. Lippert, M. Worm, and M. Babina: Mast cells as initiators of immunity and host defense. *Exp Dermatol* 10, 1-10 (2001)
6. Galli, S. J., M. Maurer, and C. S. Lantz: Mast cells as sentinels of innate immunity. *Curr Opin Immunol* 11, 53-59 (1999)
7. Arock, M., E. Ross, R. Lai-Kuen, G. Averlant, Z. Gao, and S. N. Abraham: Phagocytic and tumor necrosis factor alpha response of human mast cells following exposure to gram-negative and gram-positive bacteria. *Infect Immun* 66, 6030-6034 (1998)
8. Abraham, S. N. and R. Malaviya: Mast cells in infection and immunity. *Infect Immun* 65, 3501-3508 (1997)
9. Mekori, Y. A. and D. D. Metcalfe: Mast cell-T cell interactions. *J Allergy Clin Immunol* 104, 517-523 (1999)
10. Plaut, M., J. H. Pierce, C. J. Watson, J. Hanley-Hyde, R. P. Nordan, and W. E. Paul: Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature* 339, 64-67 (1989)
11. Church, M. K. and F. Levi-Schaffer: The human mast cell. *J Allergy Clin Immunol* 99, 155-160 (1997)
12. Bradding, P. and S. T. Holgate: The mast cell as a source of cytokines in asthma. *Ann N Y Acad Sci* 796, 272-281 (1996)
13. Bradding, P.: Human mast cell cytokines. *Clin Exp Allergy* 26, 13-19 (1996)
14. Smith, T. J. and J. H. Weis: Mucosal T cells and mast cells share common adhesion receptors. *Immunol Today* 17, 60-63 (1996)

15. Kawaguchi, M., L. F. Onuchic, X. D. Li, D. M. Essayan, J. Schroeder, H. Q. Xiao, M. C. Liu, G. Krishnaswamy, G. Germino, and S. K. Huang: Identification of a Novel Cytokine, ML-1, and Its Expression in Subjects with Asthma. *J Immunol* 167, 4430-4435 (2001)
16. Malaviya, R., T. Ikeda, E. Ross, and S. N. Abraham: Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature* 381, 77-80 (1996)
17. Gordon, J. R., P. R. Burd, and S. J. Galli: Mast cells as a source of multifunctional cytokines. *Immunol Today* 11, 458-464 (1990)
18. Gordon, J. R. and S. J. Galli: Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. *Nature* 346, 274-276 (1990)
19. Catlin, B. W.: Branhamella catarrhalis: an organism gaining respect as a pathogen. *Clin Microbiol Rev* 3, 293-320 (1990)
20. Dolter, J., J. Wong, and J. M. Janda: Association of Neisseria cinerea with ocular infections in paediatric patients. *J Infect* 36, 49-52 (1998)
21. Pierce J. W., R. Schoenleber, G. Jesmok, J. Best, S. A. Moore, T. Collins and M. E. Gerritsen: Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects *in vivo*. *J Biol Chem* 272, 21096-21103 (1997)
22. Keller, R., R. Gehri, R. Keist, E. Huf, and F. H. Kayser: The interaction of macrophages and bacteria: a comparative study of the induction of tumoricidal activity and of reactive nitrogen intermediates. *Cell Immunol* 134, 249-256 (1991)
23. Klingman, K. L., A. Pye, T. F. Murphy, and S. L. Hill: Dynamics of respiratory tract colonization by Branhamella catarrhalis in bronchiectasis. *Am J Respir Crit Care Med* 152, 1072-1078 (1995)
24. Krishnaswamy, G., J. K. Smith, R. Mukkamala, K. Hall, W. Joyner, Y. L. and D. S. Chi: Multifunctional cytokine expression by human coronary endothelium and regulation by monokines and glucocorticoids. *Microvasc Res* 55, 189-200 (1998)
25. Krishnaswamy, G., J. K. Smith, S. Srikanth, D. S. Chi, J. H. Kalbfleisch, and S. K. Huang: Lymphoblastoid interferon-alpha inhibits T cell proliferation and expression of eosinophil-activating cytokines. *J Interferon Cytokine Res* 16, 819-827 (1996)
26. Battle, J., T. Ha, C. Li, V. Della Beffa, P. Rice, J. Kalbfleisch, W. Browder, and D. Williams: Ligand binding to the (1 --> 3)-beta-D-glucan receptor stimulates NFkappaB activation, but not apoptosis in U937 cells. *Biochem Biophys Res Commun* 249, 499-504 (1998)
27. Du, X., K. Stocklauser-Farber, and P. Rosen: Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? *Free Radic Biol Med* 27, 752-763 (1999)
28. Bondeson, J., K. A. Browne, F. M. Brennan, B. M. J. Foxwell and M. Feldmann: Selective regulation of cytokine induction by adenoviral gene transfer of IkB α into human macrophages: Lipopolysaccharide-induced, but not zymosan-induced, proinflammatory cytokines are inhibited, but IL-10 is nuclear factor-kB independent. *J. Immunol.* 162, 2939-2945 (1999)
29. Lukacs, N. W., S. W. Chensue, W. J. Karpus, P. Lincoln, C. Keefer, R. M. Strieter, and S. L. Kunkel: C-C chemokines differentially alter interleukin-4 production from lymphocytes. *Am J Pathol* 150, 1861-1868 (1997)
30. Barnes, P. J.: Nuclear factor-kappa B. *Int J Biochem Cell Biol* 29, 867-870 (1997)
31. Baldwin, A. S., Jr.: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 14, 649-683 (1996)
32. Andoh, A., H. Takaya, T. Saotome, M. Shimada, K. Hata, Y. Araki, F. Nakamura, Y. Shintani, Y. Fujiyama, and T. Bamba: Cytokine regulation of chemokine (IL-8, MCP-1, and RANTES) gene expression in human pancreatic periacinar myofibroblasts. *Gastroenterology* 119, 211-219 (2000)
33. Dokter, W. H. A., S. B. Koopmans, and E. Vellenga: Effects of IL-10 and IL-4 on LPS-induced transcription factors (AP-1, NF-IL6 and NF-kappa B) which are involved in IL-6 regulation. *Leukemia* 10, 1308-1316 (1996)

Key Words: Mast cells, *Moraxella catarrhalis*, *Neisseria cinerea*, Inflammation, Cytokines, Interleukin 6, Monocyte Chemoattractant Protein-1, Nuclear Factor KappaB, Signaling Mechanisms

Send correspondence to: Guha Krishnaswamy, M.D., Professor, Department of Internal Medicine, Box 70622, East Tennessee State University, Johnson City, Tennessee 37614-1709 Tel: 423-439-6288, Fax: 423-439-6387, E-mail: krishnas@etsu.edu