Mast cells produce ENA-78, which can function as a potent neutrophil chemoattractant during allergic airway inflammation

Nicholas W. Lukacs, Cory M. Hogaboam, Steven L. Kunkel, Stephen W. Chensue, Marie D. Burdick,* Holly L. Evanoff, and Robert M. Strieter*

*University of Michigan Medical School, Department of Pathology and *Department of Internal Medicine, Division of Pulmonary and Critical Care, Ann Arbor*

Abstract: The inflammatory response during allergic airway inflammation involves the recruitment of multiple leukocyte populations, including neutrophils, monocytes, lymphocytes, and eosinophils. All of these populations likely contribute to the pathology observed during repeated episodes of allergic airway inflammation. We have examined the role of a human neutrophil-specific chemokine (C-x-C), ENA-78 , in a model of allergic airway responses and identified murine mast cells as a cellular source of an ENA-78-like molecule. Within this allergic airway model, neutrophil infiltration into the airway occurs within 4–8 h post-allergen challenge, persists within the airway until 24 h, and resolves by 48 h post-challenge. Neutrophil influx precedes the eosinophil infiltration, which peaks in the airway at 48 h post-allergen challenge. In this study the production of ENA-78 from challenged lungs demonstrated a significant increase in the allergen-, but not vehicle-, challenged lungs. In vivo neutralization of ENA-78 by passive immunization demonstrated a significant decrease in peak neutrophil infiltration at 8 h, with no effect on the eosinophil infiltration at 48 h post-challenge. Because ENA-78 has been shown to be chemotactic for neutrophils and given the involvement of mast cell degranulation in allergic responses, we examined mast cells for the presence of ENA-78. Cultured mast cells spontaneously released ENA-78, but on activation with IgE + antigen, N^G-L-arginine methyl ester or compound 48/80 produced significantly increased levels of ENA-78. Supernatants from sonicated MC-9 mast cells induced an overwhelming influx of neutrophils into the BAL by 4 h post-intratracheal injection into mice, suggesting that the mast cell is a significant source of neutrophil chemotactic factors. Mast cell supernatant-mediated neutrophil infiltration was substantially decreased by preincubation of the supernatant with antibodies specific for ENA-78. These data indicate a major neutrophil chemotactic protein produced by mast cells during allergic responses may be mast cell-derived ENA-78. *J. Leukoc. Biol.* 63: 746–751; 1998.

Key Words: *chemokines · allergic inflammation*

INTRODUCTION

Allergic airway inflammation can have long-term detrimental effects on an individual and may be induced by mechanisms contributed by multiple leukocyte populations that infiltrate during allergen-specific responses. The inflammation induced during allergic airway inflammation is mediated by the coordination of several immune specific activational events. The first leukocyte that appears to enter a site of allergic inflammation is the neutrophil. The initial induction of IgE-mediated mast cell degranulation, up-regulation of adhesion molecules, and the production of inflammatory and chemotactic cytokines leading to the infiltration of specific leukocyte subsets is orchestrated in a sequential manner [1–3]. The activation and degranulation of local mast cell populations is an immediate response in the airway mediated both by antigen-specific, surface-bound IgE and by cytokine-induced activational pathways. The relevance of neutrophils to airway hyperreactivity and subsequent late-phase reactions and airway damage in atopic asthma has traditionally been a controversial topic [4]. However, results collected over recent years have provided data that suggest that neutrophils, at the very least, have a role in inducing airway damage leading to lung dysfunction [5–8]. The recruitment of neutrophils to the airway during allergic responses has been well documented. Neutrophils are a source of several inflammatory mediators as well as destructive proteases capable of damaging surrounding tissue in the lungs [9]. Therefore, the investigation into the recruitment of neutrophils into the airway during allergic inflammation may be crucial to fully understand the pathology and related airway responses.

Leukocyte recruitment during inflammation has been shown to be mediated by nonspecific activators, such as leukotriene B_4 (LTB_4), platelet-activating factor (PAF), C5a, etc., however, specific leukocyte populations can be recruited via inflammation-induced chemokines [10, 11]. Chemokines have been

---

Abbreviations: LTB_4, leukotriene B_4; PAF, platelet-activating factor; IL-8, interleukin-8; SEA, soluble egg antigen; PBS, phosphate-buffered saline; BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay.

Correspondence: Nicholas W. Lukacs, Ph.D., University of Michigan Medical School, Department of Pathology, 1301 Catherine, Ann Arbor, MI 48109-0602.

Received September 17, 1997; revised February 1, 1998; accepted February 4, 1998.

divided into two separate families based on their activity and amino acid homology [11]. The C-x-C family of chemokines, which includes interleukin-8 (IL-8), ENA-78, GRO α, β, γ, as well as several others, is primarily chemotactic for neutrophils. The C-C family of chemokines, including monocyte chemoattractant protein (MCP)-1, -2, -3, -4, -5, macrophage inflammatory protein (MIP)-1α/β, eotaxin, RANTES, as well as several other newer members, are chemotactic for lymphocytes, monocytes, eosinophils, and/or basophils. Chemokines are generated by a number of tissue resident cells, including endothelial cells, smooth muscle cells, fibroblasts, epithelial cells, and mast cells. The identification and regulation of these chemokines during diseases may lead to development of novel therapeutic interventions designed to interrupt specific phases of inflammatory responses.

In this study we have utilized an established model of allergic airway inflammation to examine the presence, activation, and specific sources of neutrophil chemotactic proteins during allergic airway responses. The results from this study indicate that an ENA-78-like protein, a C-x-C chemokine, plays a major role in allergic neutrophil infiltration. Furthermore, it appears that a significant early source of ENA-78 may be mast cells activated during the allergen specific response.

MATERIALS AND METHODS

Animals

Female CBA/J mice purchased from Jackson Laboratories (Bar Harbor, ME) were maintained under standard pathogen-free conditions.

Egg isolation and soluble egg antigen (SEA) protein preparation

SEA were prepared from acutely *Schistosoma mansoni*-infected mice as previously described [12]. Briefly, eggs were isolated from livers of infected mice after a 3-day incubation and ground on ice to release the soluble antigens from the egg. The preparation was then spun in an ultracentrifuge at 100,000 *g* for 2 h and the supernatant collected. The antigens in the supernatant are primarily glycoproteins that have been characterized as inducing a TH2 type granulomatous response in schistosomiasis [13, 14].

Sensitization and induction of the airway response

To induce a TH2 type response [13, 14] the following procedure was established in normal CBA/J mice [15]. Briefly, the mice were immunized with 5000 isolated *S. mansoni* eggs intraperitoneally at days 0 and 7 of the protocol. On day 14 the mice were given an intranasal challenge of 10 μg of SEA in 10 μL of phosphate-buffered saline (PBS) to localize the response to the airway. This initial intranasal challenge with antigen induced little cellular infiltrate into the lungs of the mice on histological examination. Mice were then rechallenged 6 days later by intratracheal administration of 10 μg of SEA in 25 μL of sterile PBS or with PBS alone (vehicle). The magnitude of infiltration in both the vehicle control and SEA-challenged mice was examined histologically. Only the SEA-challenged mice displayed a significant inflammatory response that included neutrophil and eosinophil infiltration, as previously described [15].

Quantification and differentiation of leukocyte populations in bronchoalveolar lavage (BAL) and lungs

Mice immunized and challenged with SEA or saline vehicle were subjected to a 1-mL BAL with PBS containing 50 mM EDTA at various time points post-challenge. The cells were then counted, dispersed using a cytospin, and differentially stained with Wright-Giemsa stain. The cell types (mononuclear phagocytes, lymphocytes, neutrophils, and eosinophils) were expressed as a percentage based on 200 total cells counted/sample.

Lung homogenates

Isolated whole-lung tissue was homogenated on ice using a tissue-tearer (Biospec Products, Racine, WI) for 30 s in 1 mL of PBS containing 0.05% Triton X-100. Previous results indicate that there was no interference in enzyme-linked immunosorbent assay (ELISA) measurements with this buffer. The resulting supernatant was isolated after a high-speed spin (10,000 *g*) and subsequent filtration through a 1.2-μm syringe filter (Gelman Sciences, Ann Arbor, MI). The resultant supernatants were immediately frozen at −70°C until tested in the ENA-78 ELISA.

MC-9 mast cell line

The MC-9 mast cell line (ATCC no. CRL 8306) is an established long-term, nontransformed, IL-3-dependent cell line that was derived from fetal liver and represents a mast cell phenotype. MC-9 mast cells are maintained in Dulbecco's modified Eagle's medium supplemented with 1 mM L-Glu, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), antibiotics, and 10% fetal calf serum combined with 10% concanavalin A (Con A)-stimulated rat splenocyte supernatants. MC9 cells were added to six-well tissue culture plates at a density of 1.0×10^5 cells/well before each experiment. To duplicate wells containing MC-9 mast cells, one of the following compounds or combinations of compounds (all obtained from Sigma Chemical Co., St. Louis, MO) were added: 48/80 (1 μg/mL), L-NAME (1 mM), D-NAME (1 mM), L-NAME + 48/80, or D-NAME + 48/80. Control samples were left untreated. Compound 48/80 is a potent nonspecific activator of mast cells. Previous studies have shown that mast cells degranulate after the inhibition of endogenous nitric oxide production by L-NAME treatment [16]. D-NAME is the structural enantiomer of L-NAME and has no inhibitory effect on nitric oxide synthesis [17]. Cell-free supernatants were removed from the culture plates at 2 and 24 h after the start of culture, and assayed for ENA-78 (see below).

ENA-78 ELISA

ENA-78 was quantitated by ELISA using a modification of a double-ligand method as previously described [18]. The antisera used for these assays were specific for human ENA-78 but cross-react with a murine molecule that is not MIP-2, KC, GCP-2, or any other C-x-C chemokine that we have examined. Therefore we have referred to this molecule as ENA-78. Briefly, flat-bottomed 96-well microtiter plates (Nunc Immuno-Plate I 96-F) were coated with 50 μL/well of rabbit anti-human ENA-78 polyclonal antibody for 16 h at 4°C and then washed with PBS, pH 7.5, 0.05% Tween-20 (wash buffer). Nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer and diluted (1:2 and 1:10) cell-free supernatants in duplicate were added, followed by incubation for 1 h at 37°C. Plates were washed four times, followed by the addition of 50 μL/well biotinylated rabbit anti-ENA-78 antibody (3.5 ng/μL in PBS, pH 7.5, 0.05% Tween-20, and 2% fetal calf serum), and plates incubated for 30 min at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) added, and the plates incubated for 30 min at 37°C. Plates were again washed four times and chromogen substrate (Bio-Rad) added. The plates were then incubated at room temperature to the desired extinction, and the reaction terminated with 50 μL/well of 3 M H_2SO_4 solution. Plates were read at 490 nm in an ELISA reader. Standards were 0.5 log dilutions of recombinant ENA-78 from 10 pg/mL to 100 ng/mL. This ELISA method consistently detected ENA-78 concentrations above 50 pg/mL. The ENA-78 antibody ELISA did not cross-react with mIL-3, mIL-1α, mTNF, mMIP-2, mKC, mMIP-1α, IL-6, mJE, mMIP-1β, mIP-10, mGCP-2, hMCP-1, hIL-8, hGCP-2, hRANTES, hMIP-1α, hTNF, and hMIP-1β.

Mast cell lysates and airway inflammation

To ascertain the ability of mast cells to induce an inflammatory response, cell-free freeze/thaw lysates of the MC-9 mast cell line (1×10^7 cells/mL) were injected intratracheally. Four hours after the injected supernatant 1 mL BAL was harvested from the mice and examined by differential staining for leukocyte

populations. In separate studies, anti-ENA-78 (1:20 dilution; 10^6 titer) or control antibody was used in the supernatant and preincubated for 30 min before the intratracheal injection.

Statistics

Statistical significance was determined by analysis of variance and significance was determined with P values < 0.05.

RESULTS

Correlation of ENA-78 production and neutrophil accumulation in allergen-challenged mice

The accumulation of neutrophils and eosinophils in the airspace during the allergic response is expressed in **Figure 1**. Neutrophil infiltration in BAL fluid samples began between 1 and 4 h, peaked at 8 h, plateaued at 24 h, and dropped to background levels by 48 h post-allergen challenge. In contrast, eosinophil accumulation began at 24 h, peaked at 48 h, and subsequently dropped at later time points. The production of neutrophil specific chemokines during allergic airway inflammation may be critical for the recruitment of neutrophils leading to lung damage. To determine the production of ENA-78, whole lung homogenates were made from the lungs of mice at various times after allergen challenge, 1, 4, 8, 24, and 48 h. The debris-free supernatants were assayed using a specific ELISA for ENA-78 (**Fig. 2**). The production of ENA-78 in whole lung homogenates demonstrated a significant increase in ENA-78 as early as 1 h post-allergen challenge, peaking by 4 h, and plateuing at 8 and 24 h post challenge. By

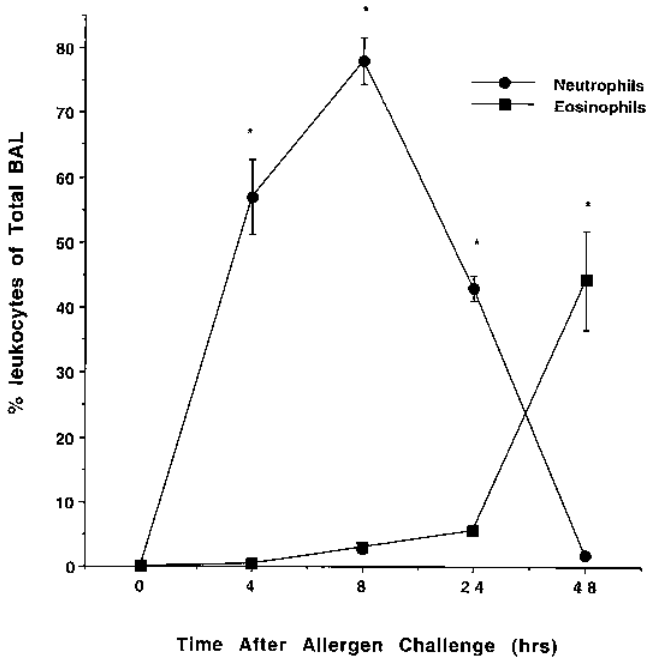

Fig. 1. Accumulation of granulocytes after allergen challenge. Sensitized mice were challenged with SEA and 1 mL BAL was harvested at various time points, cytospun, differentially stained, and counted. Vehicle-challenged animals had 10.5 ± 0.5% neutrophils and no eosinophils at 8 and 48 h post-challenge, respectively. Results represent mean ± SE of at least four mice/time point. *Significant increases over vehicle-challenged animals, $P <$ 0.05.

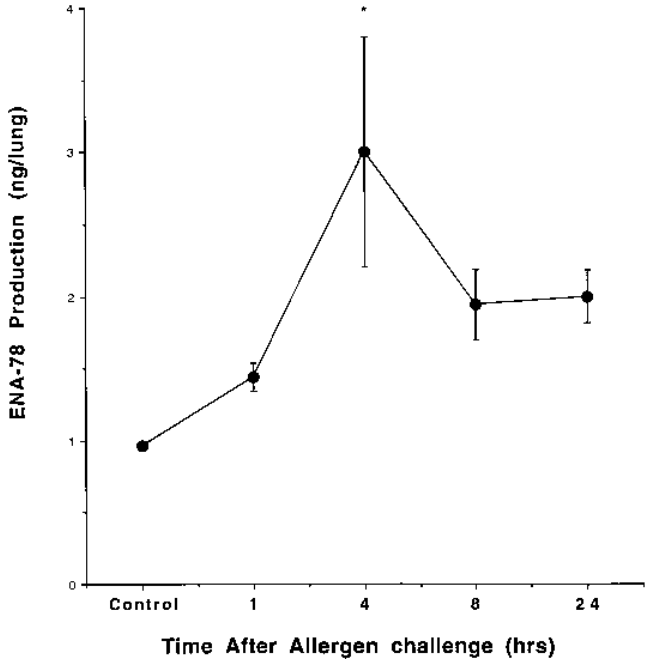

Fig. 2. ENA-78 production in whole lung homogenates during allergic airway inflammation. Whole lungs from specific time points after allergen challenge were homogenated in PBS containing 0.1% Triton X-100 and anti-proteases. The cell-free supernatants were assayed using an ENA-78 specific ELISA. Data represent mean ± SE from six individual mice. *Significant increases over vehicle-challenged animals, $P < 0.05$.

48 h, ENA-78 levels had returned to control background levels. The preceding expression of ENA-78 protein in the lung homogenates correlated with the accumulation of neutrophils ($r^2 = 0.79$), but not eosinophils during the allergic response ($r^2 < 0.1$).

Neutralization of ENA-78 decreases neutrophil infiltration during allergen challenge

The role of ENA-78 was determined by pretreating (1 h) allergic animals with specific neutralizing anti-ENA-78 or control antibody before allergen re-challenge. One-milliliter BAL fluid samples were harvested from the treated animals at 8 h post allergen challenge. The results in **Figure 3** demonstrate a significant reduction in neutrophil accumulation in the BAL at 8 h post challenge, the time of peak neutrophil influx. Compared with control antibody-treated animals the neutrophilic response in the anti-ENA-78-treated animals was reduced by 40–50% at 8 h post treatment, the peak time of response. In contrast, when animals were treated with an anti-MIP-2 antibody (another neutrophil-specific C-X-C chemokine) no decrease in neutrophil accumulation was observed. Furthermore, when eosinophil accumulation was examined within this model, there was no reduction in the anti-ENA-78-treated animals at 48 h, the time of peak eosinophil accumulation within the airway (data not shown).

Role of mast cell-derived ENA-78 in allergic inflammation

Classical studies have previously described the production and release of neutrophil chemotactic factors from activated and

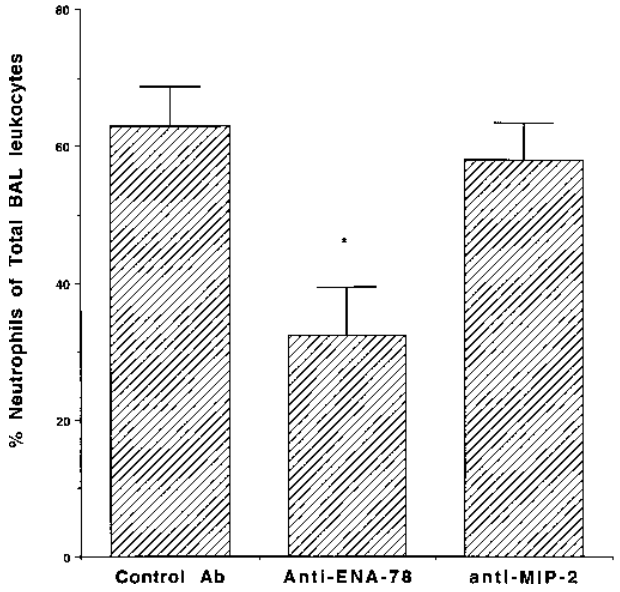

Fig. 3. Neutralization of ENA-78 specifically attenuates neutrophil accumulation in the airways at 8 h post allergen challenge. One-half milliliter of control or anti-ENA-78 specific serum was injected intraperitoneally into mice 1 h before allergen challenge. One milliliter BAL was harvested from each mouse at 8 h post-challenge. Results represent mean ± SE of at least six individual mice in each group. *Significant increases over control ab-treated animals, $P < 0.05$.

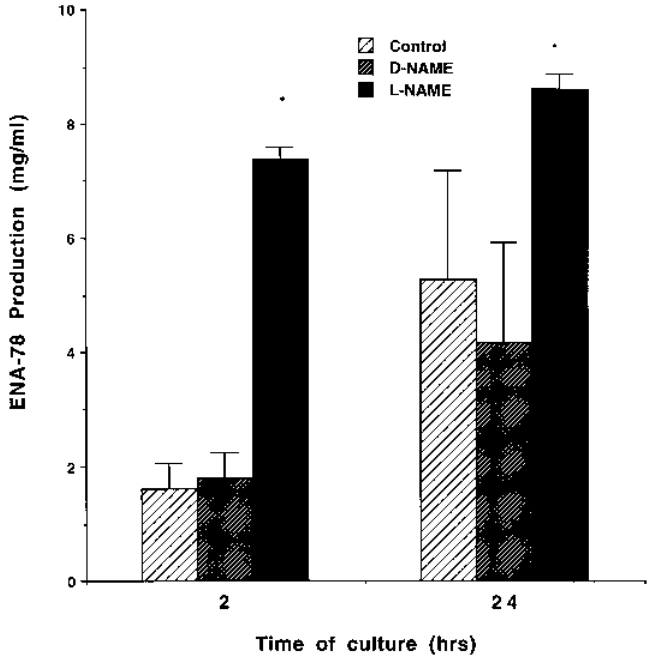

Fig. 4. Release of ENA-78 from MC-9 mast cells in the absence or presence of N^G-nitro-D-arginine methylester (D-NAME) or N^G-nitro-L-arginine methyl ester (L-NAME) for 2 or 24 h. MC-9 cells (1.0×10^5 cells/well) were left untreated or cultured in the presence of 1 mM D-NAME or L-NAME. Cell-free supernatants were removed at 2 or 24 h of culture and ENA-78 levels were measured using ELISA (see Materials and Methods). Results are mean ± SEM of duplicate wells from three separate experiments. *Significant increase compared to untreated or D-NAME-treated values, $P < 0.05$.

degranulated mast cell populations [3]. To examine the potential of mast cells producing ENA-78 during our allergic response, allergic mice were pretreated (1 h before allergen challenge) with 20 mg/kg of cromolyn sodium. Use of cromolyn sodium significantly reduced the ENA-78 production ($P < 0.05$) at 4 h post-challenge by >45% compared with control-treated animals (data not shown). Using a mature MC-9 nontransformed mast cell line, the presence and role of ENA-78 was evaluated in mast cells. Supernatants from unstimulated and stimulated MC-9 mast cells were assayed for the presence of ENA-78 protein using a specific ELISA (**Figs. 4** and **5**). Inhibition of NO has previously been shown to cause degranulation of mast cell populations [16]. The spontaneous release of 1.6 ± 0.44 ng/mL of ENA-78 was observed in cultures of untreated or D-NAME-treated MC-9 mast cells after 2 h, and these levels were increased by threefold after 24 h. In contrast, L-NAME treatment stimulated the release of 7.3 ± 0.21 ng/mL of ENA-78 after 2 h. Only a minor increase above this concentration was observed at the 24-h timepoint in the L-NAME-treated MC-9 cultures (Fig. 4). Approximately 10 ng/mL of ENA-78 was released over a 24-h period after degranulation of MC-9 mast cells with compound 48/80 in the absence (7.4 ± 2.7 ng/mL) or presence (12.6 ± 1.6 ng/mL) of D-NAME (Fig. 5). However, levels of ENA-78 reached 35.3 ± 0.32 ng/mL when MC-9 mast cells were activated with compound 48/80 and L-NAME over a similar culture period (Fig. 5). In separate studies, bone marrow-derived mast cells (1×10^5/mL) were activated by IgE + antigen (DNP) and released significant amounts of ENA-78 compared to controls (1.36 ± 0.22 vs. 0.243 ± 0.081 ng/mL, respectively). Taken together, these results indicate that mast cells have the ability to release

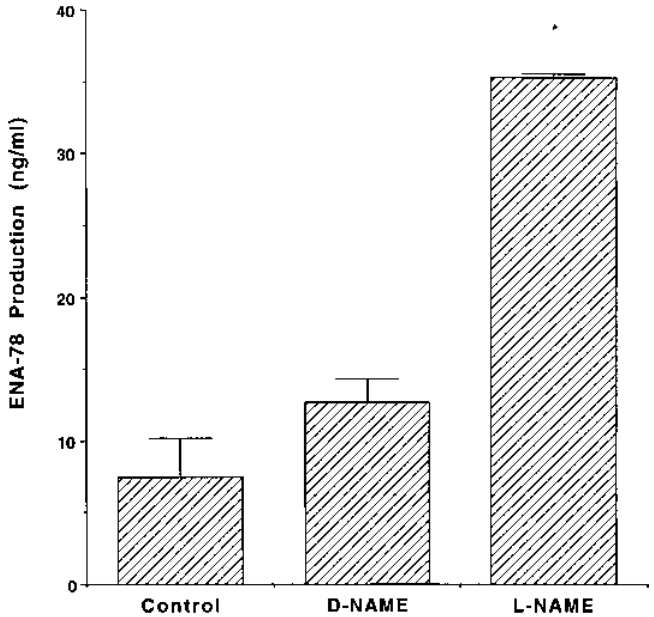

Fig. 5. Compound 48/80-stimulated release of ENA-78 from MC-9 mast cells in the absence or presence of N^G-nitro-D-arginine methyl ester (D-NAME) or N^G-nitro-L-arginine methyl ester (L-NAME) for 24 h. MC-9 cells (1.0×10^5 cells/well) were stimulated with compound 48/80 (1 μg/mL) in the absence or presence of 1 mM D-NAME or L-NAME. Cell-free supernatants were removed after 24 h of culture, and ENA-78 levels were measured using ELISA (see Materials and Methods). Results are mean ± SEM of duplicate wells from three separate experiments. *$P < 0.05$ compared to untreated or D-NAME treated values.

ENA-78 spontaneously, and produce ENA-78 on activation and degranulation.

To examine the neutrophil chemotactic activity of the mast cells, freeze-thaw lysates were prepared from the MC-9 mast cell line (1×10^7/mL in PBS). These mast cell lysates contained ~10 ng/mL of ENA-78. To determine the effect on neutrophil recruitment in vivo, the lysates were intratracheally injected into the airways of normal mice. After 4 h post-challenge, a 1-mL BAL was harvested from the treated mice and assayed for leukocyte recruitment. The results in **Figure 6** indicate that >80% of leukocytes found in the airspace (via BAL fluid analysis) were neutrophils. When the mast cell lysates were preincubated with either a control or anti-ENA-78 purified antibody, only the anti-ENA-78-treated supernatant significantly inhibited the neutrophil infiltration into the airway. Preincubation of the supernatant with anti-MIP-2 had no effect on the neutrophil recruitment to the airway (data not shown). The >40% inhibition of the neutrophil influx in the mast cell lysate experiments demonstrates similar results as observed by inhibition of ENA-78 in the allergic airway model. Because TNF has previously been shown to be the major inducer of ENA-78, in separate experiments preincubation of the mast cell lysates with anti-TNF did not inhibit the neutrophil accumulation (data not shown). These latter results verify that TNF in the mast cell lysates was not inducing the ENA-78 levels once injected in vivo. Overall, the results indicate that mast cell-derived ENA-78 may play a major role in neutrophil accumulation during allergic airway responses.

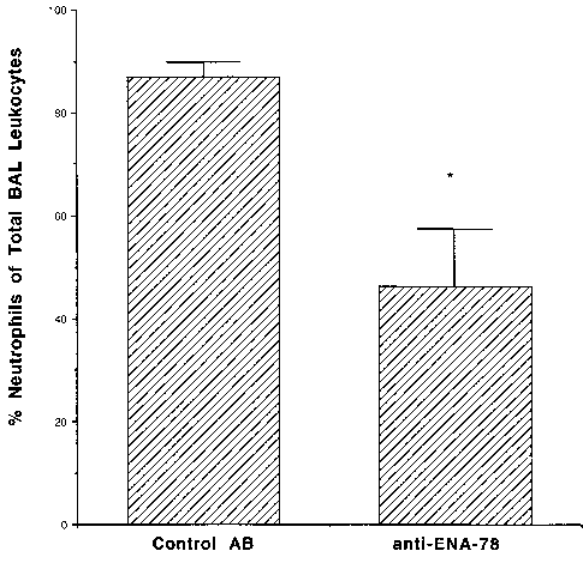

Fig. 6. MC-9 mast cell lysates induce airway neutrophil accumulation that can be partially blocked by neutralization of ENA-78. Freeze-thaw lysates of MC-9 cells (1×10^7/mL) were prepared and 30 μL injected intratracheally into normal mice. Mast cell lysates were preincubated with purified anti-ENA or control IgG for 30 min and then injected into the trachea. One milliliter BAL was harvested from the mice 4 h post-injection. Cytospin samples from the BAL were differentially stained and counted. Control vehicle injections demonstrated only 10–15% neutrophils in the BAL. Results represent mean ± SE of data from at least six mice per group. *$P < 0.05$ decrease compared to control ab-treated mice.

DISCUSSION

The accumulation of multiple cell populations during allergic airway responses leads to the tissue damage that facilitates airway pathology during allergen challenge. In this study, the examination of a neutrophil-specific chemotactic factor, ENA-78, has identified that this mast cell-associated C-x-C chemokine is produced early during the allergic airway response. It is our contention that the early release of ENA-78 by mast cells contributes to the overall accumulation of neutrophils, but not eosinophils, during allergic airway inflammation. Our data indicate that ENA-78 is produced as early as 1–4 h post-allergen challenge, a time when neutrophils are beginning to accumulate into the airspace. The passive immunization of allergic animals with anti-ENA-78 significantly attenuated (>40% decrease) the accumulation of neutrophils, as assayed by BAL analysis. It is interesting to note that the in vivo inhibition of ENA-78 did not affect the eosinophil accumulation. These latter results follow earlier data indicating that ENA-78 is a neutrophil-specific chemotactic factor [19, 20]. Although the specific mouse homolog of ENA-78 has not been isolated (ongoing studies), the human specific anti-sera does not cross-react with any other known human or mouse C-x-C chemokines. Thus, we have designated the molecule in these studies as ENA-78.

One of the earliest cells activated during an allergic response is the mast cell. In fact, the primary difference between allergic responses and other types of immune/inflammatory reactions appears to be mast cell degranulation events. Some of the most intriguing data on allergic responses indicated that degranulated mast cells release chemotactic factors specific for neutrophil and/or eosinophil accumulation [21, 22]. Subsequent studies have identified nonspecific chemotactic factors, such as leukotrienes, etc., which are released by mast cells and contribute to the chemotactic responses. However, mast cells can also produce multiple chemokines that have the ability to recruit specific leukocyte populations [23]. Our data indicate that one of the primary neutrophil chemotactic factors made by mast cells is ENA-78. ENA-78 was spontaneously released by MC-9 mast cell lines, and was further enhanced by activating these cells using IgE + antigen, L-NAME (a nitric oxide synthase inhibitor), and compound 48/80. Inhibition of mast cell activation in allergic mice with cromolyn sodium also significantly reduced the production of ENA-78, suggesting that the mast cell may be an important source of the protein. When mast cell lysates were injected into the airway of normal mice, BAL analysis indicated that >80% of the leukocytes that were found in the airway 4 h post-challenge were neutrophils. Preincubation of the mast cell supernatants with anti-ENA-78 inhibited >40% of the neutrophil influx. These results were similar to the data derived within the allergic airway model. It is interesting to note that when another C-x-C chemokine, MIP-2, was neutralized in vivo as a specificity control no decrease in neutrophil accumulation was observed during the allergic airway response. These results correspond directly to mast cell data demonstrating that ENA-78 protein was detected from MC-9 mast cells, but no significant MIP-2 protein was observed (data not shown). These latter results are quite intriguing because previous data indicate that macrophage-derived MIP-2

is a primary inducer of neutrophil accumulation during endotoxemia [24, 25]. Considering that the previous study utilized the same source of anti-MIP-2 antibody, the results suggest a clear dichotomy of responses between an acute endotoxin-driven response and a mast cell-mediated allergic response. Furthermore, macrophages make much greater levels of MIP-2 than ENA-78, whereas mast cells appear to make much higher levels of ENA-78 than MIP-2. These latter data further strengthen the differences between macrophage (endotoxin) and mast cell (allergic)-mediated inflammatory events.

It is likely that a number of other factors contribute to the neutrophil accumulation within this model. Nonspecific recruitment factors, such as LTB_4, which can be produced early in allergic responses from mast cells and macrophages [9, 10] have also been identified in this model as early as 1 h post challenge [unpublished data]. Other recruitment factors must account for a considerable amount of the neutrophil accumulation because anti-ENA-78 inhibited 40–50% of the neutrophil accumulation within the airway. An interesting aspect of the ENA-78 neutralization studies indicated that only neutrophils were affected and not eosinophils, suggesting a true specificity for leukocyte populations, which follows in vitro chemotaxis data.

The ability of neutrophils to cause damage within tissue upon degranulation and release of proteases, oxygen radicals, and other tissue-damaging products, gives these cells the potential to exert a great deal of damage during an inflammatory response. Neutrophil-induced damage during allergic pulmonary events, such as in asthma, is relatively controversial. However, a number of studies have identified the presence of neutrophils during asthmatic inflammation in patient populations. In particular, in sudden onset fatal asthma neutrophils were the predominant cell population found in the airspace [26]. In an earlier study examining airway reactivity in dogs exposed to ozone, neutrophils were the predominant cell population that infiltrated the airways causing airway reactivity [27]. Supernatants from degranulated neutrophils incubated with human bronchus increase the responsiveness to histamine, suggesting that neutrophil-derived products may be linked to airway hyperreactivity in vivo [5]. Nedocromil sodium, an anti-inflammatory agent used to control asthmatic inflammation, blocks neutrophil-induced bronchial hyperresponsiveness [28]. Altogether, these studies suggest that neutrophil accumulation during allergic airway inflammation may contribute to the overall tissue damage leading to organ dysfunction.

The identification and preferential production of ENA-78 from mast cell populations may indicate that upon allergic inflammatory events neutrophil infiltration may be controlled through the inhibition of mast cell degranulation and release of ENA-78. These data suggest that one possible target for particular forms of allergic asthmatic responses may be ENA-78 and neutrophil accumulation.

REFERENCES

1. Corrigan, C. J., Kay, A. B. (1992) T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* **13**, 501–507.
2. Kay, A. B., and Corrigan, C. J. (1992) Asthma, eosinophils, and neutrophils. *Br. Med. Bull.* **48**, 51–64.
3. Wasserman, S. I. (1989) Mast cell-mediated inflammation in asthma. *Ann. Allergy* **63**, 546–550.
4. Smith, J. A. (1994) Neutrophils, host defense, and inflammation: a double-edged sword. *J. Leukoc. Biol.* **56**, 672–686.
5. Hallahan, A. R., Armour, C. L., Black, J. L. (1990) Products of neutrophils and eosinophils increase the responsiveness of human isolated bronchial tissue. *Eur. Resp. J.* **3**, 554–558.
6. Smith, L., McFadden, E. R. (1995) Bronchial hyperreactivity revisited. *Ann. Allergy Asthma Immunol.* **74**, 454–469.
7. Hughes, J. M., Mackey, K. O., Johnson, P. R., Tragoulias, S., Black, J. L., Armour, C. L. (1993) Neutrophil-induced human bronchial hyperresponsiveness in vitro—pharmacological modulation. *Clin. Exp. Allergy* **23**, 251–256.
8. Montefort, S., Gratziou, C., Goulding, D., Polosa, R., Haskard, D. O., Howarth, P. H., Holgate, S. T., Carroll, M. P. (1994) Bronchial biopsy evidence for induced asthma. *Am. Rev. Respir. Dis.* **128**, 294–296.
9. Ward, P. A., Warren, J. S., Johnson, K. (1988) Oxygen radicals, inflammation, and tissue injury. *Free Rad. Biol. Med.* **5**, 403–408.
10. Lukacs, N. W., Ward, P. A. (1997) Chemotactic molecules and cellular activation. In *Allergy*, 2nd ed. (A. P. Kaplan, ed.), W.B. Saunders, Philadelphia, Pa. 276–286.
11. Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N., Matsushima, K. (1991) Properties of the novel proinflammatory ''intercrine'' cytokine family. *Annu. Rev. Immunol.* **9**, 617.
12. Coker, C. M., Von Lichtenberg, E. (1956) A revised method for isolation of *Schistosoma mansoni* eggs for biological experimentation. *Proc. Soc. Exp. Biol. Med.* **92**, 780.
13. Lukacs, N. W., Boros, D. L. (1991) Utilization of fractionated soluble egg antigens reveals selectively modulated granulomatous and lymphokine responses during murine *Schistomiasis mansoni*. *Infect. Immun.* **60**, 3209–3216.
14. Grzych, J. M., Pearce, E., Cheever, A., Caulada, Z. A., Caspar, P., Henry, S., Lewis, F., Sher, A. (1991) Egg deposition is the major stimulus for the production of Th2 cytokines in murine *Schistosomiasis mansoni*. *J. Immunol.* **146**, 1322–1329.
15. Lukacs, N. W., Strieter, R. M., Chensue, S. W., Kunkel, S. L. (1994) Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. *Am. J. Respir. Cell Mol. Biol.* **10**, 526–532.
16. Hogaboam, C. M., Bissonnette, E. Y., Befus, A. D., Wallace, J. L. (1993) Modulation of mast cell reactivity by interleukin 1-beta: divergent effects on nitric oxide and platelet activating factor. *J. Immunol.* **151**, 3767–3774.
17. Rees, D. D., Palmer, R. M. J., Shulz, R., Hodson, H. F., Moncada, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.* **101**, 746–752.
18. Evanoff, H., Burdick, M. D., Moore, S. A., Kunkel, S. L., Strieter, R. M. (1992) A sensitive ELISA for the detection of human monocyte chemoattractant protein-1 (MCP-1). *Immunol. Invest.* **21**, 39.
19. Colletti, L. M., Kunkel, S. L., Walz, A., Burdick, M. D., Kunkel, R. G. Wilke, C. A., Strieter, R. M. (1995) Chemokine expression during hepatic ischemia/reperfusion-induced lung injury in the rat. The role of epithelial neutrophil activatin protein. *J. Clin. Invest.* **95**, 134–141.
20. Walz, A., Burgener, R., Car, B., Baggiolini, M., Kunkel, S. L., Strieter, R. M. (1991) Structure and neutrophil-activating properties of a novel inflammatory peptide (ENA-78) with homology to interleukin 8. *J. Exp. Med.* **174**, 1355–1362.
21. Holgate, S. T., Benyon, R. C., Howarth, P. H., Agius, R., Hardy, C., Robinson, C., Durham, S. R., Kay, A. B., Church, M. K. (1985) Relationship between mediator release from human lung mast cells in vitro and in vivo. *Int. Arch. Allergy Appl. Immunol.* **77**, 47–56.
22. Malaviya, R., Ikeda, T., Ross, E., Abraham, S. N. (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF alpha. *Nature* **381**, 77–80.
23. Selvan, R. S., Butterfield, J. H., Krangel, M. S. (1994) Expression of multiple chemokine genes by a human mast cell leukemia. *J. Biol. Chem.* **269**, 13893–13898.
24. Standiford, T. J., Strieter, R. M., Lukacs, N. W., Kunkel, S. L. (1995) Neutralization of IL-10 increases lethality in endotoxemia: Cooperative effects of macrophage inflammatory protein-2 and tumor necrosis factor. *J. Immunol.* **155**, 2222–2229.
25. Wolpe, S. D., Cerami, A. (1989) Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB J.* **3**, 2565–2573.
26. Sur, S., Crotty, T. B., Kephant, G. M., Hyma, B. A., Colby, T. V., Reed, C. E., Hunt, L. W., Gleich, G. J. (1993) Sudden onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am. Rev. Respir. Dis.* **148**, 713–719.
27. Holzman, M. J., Fabbri, L. M., O'Bryne, P. M. (1983) Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am. Rev. Respir. Dis.* **127**, 686–690.
28. Hughes, J. M., Mackey, K. O., Johnson, P. R., Tragoulias, S., Black, J. L., Armour, C. L. (1993) Neutrophil-induced human bronchial hyperresponsiveness in vitro—pharmacological modulation. *Clin. Exp. Allergy* **23**, 251–256.