

25. Payan DG, Hess CA, Goetzl EJ. Inhibition by somatostatin of the proliferation of T lymphocytes and MOLT-4 lymphoblasts. *Cell Immunol* 1984;84:433-8.
26. Hachisu M, Hiranuma T, Tani S, Iizuka T. Enzymatic degradation of helodermin and vasoactive intestinal polypeptide. *J Pharmacobio Dyn* 1991;14:126-31.
27. Katz MD, Erstad BL. Octreotide, a new somatostatin analogue. *Clin Pharmacol* 1989;8:255-73.
28. Casale TB, Abbas MK. Comparison of leukotriene B₂-induced neutrophil migration through different cellular barriers. *Am J Physiol* 1990;258(Cell Physiol):C639-C647.
29. Carolan EJ, Casale TB. Degree of platelet activating factor-induced neutrophil migration is dependent upon the molecular species. *J Immunol* 1990;145:2561-5.
30. Ferrante A, Thong YH. A rapid one-step procedure for purification of mononuclear and polymorphonuclear leukocytes from human blood using a modification of the Hypaque-Ficoll technique. *J Immunol Methods* 1978;24:389-93.
31. Gallin JI, Clark RA, Kimball HR. Granulocyte chemotaxis: an improved *in vitro* assay employing ⁵¹Cr-labelled granulocytes. *J Immunol* 1973;110:233-40.
32. Schreiber J, Becher G, Slapke J, Rathsack R, Oehme P. Experimental bronchoalveolar lavage in guinea pigs—a useful tool to investigate the intrabronchial release of substance P. *Biomed Biochim Acta* 1988;47:445-7.
33. Martling CR, Theodorsson-Norheim E, Lundberg JM. Occurrence and effects of multiple tachykinins: substance P, neurokinin A and neuropeptide K in human lower airways. *Life Sci* 1987;40:1633-43.
34. Matis WL, Lavker RM, Murphy GF. Substance P induces the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium. *J Invest Dermatol* 1990;94:492-5.

Eosinophil major basic protein enhances the expression of neutrophil CR3 and p150,95

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Background: We examined the effect of major basic protein (MBP) stimulation on the expression of the neutrophil β_2 -integrins: LFA-1 (CD11a/CD18), CR3 (CD11b/CD18), and p150,95 (CD11c/CD18).

Methods: Incubation of neutrophils with 0.75 to 3.0 $\mu\text{mol/L}$ MBP for 30 minutes at 37°C resulted in concentration-dependent increases in CR3 expression as detected by staining with the CD11b-specific monoclonal antibody, Leu-15.

Results: The expression of CR3 was significantly ($p < 0.001$) higher when neutrophils were stimulated with 1.5 $\mu\text{mol/L}$ (53% $\pm$ 9% increase) or 3.0 $\mu\text{mol/L}$ (100% $\pm$ 17% increase) MBP as compared with unstimulated neutrophils. The kinetics of MBP-stimulated CR3 expression were rapid and were similar to those of 100 nmol/L

N-formyl-methionyl-leucyl-phenylalanine-stimulated enhancement of CR3 expression.

Incubation of neutrophils with reduced and alkylated MBP resulted in significantly lower ($p < 0.05$) increases in CR3 expression as compared with stimulation with native MBP. In addition, neither eosinophil cationic protein nor eosinophil-derived neurotoxin altered neutrophil CR3 expression. MBP stimulated minimal increases in LFA-1 expression. However, staining with the monoclonal antibody Leu-M5 (anti-CD11c) revealed that MBP stimulated significant increases in the expression of p150,95.

Conclusions: These results indicate that MBP stimulates the increased expression of neutrophil adhesion molecules, which in turn may enhance the inflammatory role of neutrophils in the late-phase events of allergic diseases. (*J ALLERGY CLIN IMMUNOL* 1993;92:598-606.)

Key words: Major basic protein, eosinophils, neutrophils, integrins, CR3, p150,95, adhesion

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Abbreviations used

ECP:	Eosinophil cationic protein
EDN:	Eosinophil-derived neurotoxin
FMLP:	N-formyl-methionyl-leucyl-phenylalanine
Hepes:	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
mAb:	Monoclonal antibody
MBP:	Major basic protein
MFC:	Mean fluorescence channel
PBS:	Phosphate-buffered saline
PBS-gel-azide:	Phosphate-buffered saline (pH 7.4) containing 0.1% gelatin and 0.1% NaN ₃

Eosinophilic infiltration is characteristic of allergic reactions in the lungs,¹ nose,² and skin.³ Although a variety of eosinophil products contribute to the pathogenesis of the allergic late-phase response, evidence has accumulated to indicate that major basic protein (MBP) may be a particularly prevalent and important mediator in this context. MBP is a 13.8 kd cationic polypeptide with a predicted isoelectric point of 10.9.⁴ MBP levels have been shown to be elevated in the serum and sputum of patients with bronchial asthma.⁵ Increased levels of MBP have also been found in the nasal lavage fluid of late-phase responses after antigen challenge,⁶ and there has been evidence of MBP deposition in lesions of atopic dermatitis.⁷ Findings that prolonged exposure to high concentrations of MBP immobilized respiratory cilia⁸ and that such exposure was cytotoxic to respiratory epithelium⁹ and human nasal sinus mucosa¹⁰ implied that the pathogenic role of MBP was a consequence of the cytotoxic properties of the molecule. Other studies, however, have shown that micromolar concentrations of MBP stimulated histamine release from human basophils and rat mast cells in a noncytotoxic manner.^{11, 12} More recently, we demonstrated that noncytotoxic concentrations of MBP stimulated neutrophil chemiluminescence, as well as the release of superoxide anion and lysozyme.¹³

Along with eosinophil-derived proteins, neutrophils and their granular proteins have been implicated as important contributors to the inflammation of pulmonary,¹⁴ nasal,¹⁵ and cutaneous^{16, 17} late-phase reactions. Essential early events in the initiation of the neutrophil component of the inflammatory response include the adherence of circulating neutrophils to the microvascular endothelium and subsequent transendothelial migra-

tion to the inflammatory site. The processes of neutrophil adhesion and diapedesis are dependent on the increased expression of CD11/CD18 molecules.¹⁸ This family of molecules is composed of three members: LFA-1 (CD11a/CD18), CR3 (Mac-1; CD11b/CD18), and p150,95 (CD11c/CD18). These molecules are glycoprotein heterodimers composed of a specific α -subunit and a common β -subunit (CD-18). Neutrophil expression of CR3 and p150,95 can be greatly increased by exposure to a number of different stimuli, including N-formyl-methionyl-leucyl-phenylalanine (FMLP), platelet activating factor, and leukotriene B₄.^{19, 20} The latter two stimuli are synthesized and released by various inflammatory cells involved in allergic late-phase reactions.²¹

The expression of CR3 has been shown to be increased on circulating granulocytes in individuals with antigen-induced asthma.²² Given the elevated levels of MBP in the sputum and serum⁴ of patients with asthma and our earlier findings that MBP activates neutrophils,¹³ we postulated that MBP stimulation might also enhance neutrophil expression of both CR3 and other members of the CD11/CD18 family of adhesion molecules.

METHODS

Material

The MBP, reduced and alkylated MBP, eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN) were prepared as described previously^{23, 24} and were generously provided by Dr. Gerald J. Gleich (Mayo Clinic and Foundation, Rochester, Minn.). Ficoll, FMLP, and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) were purchased from Sigma Chemical Co. (St. Louis, Mo.). The monoclonal antibodies (mAbs) Leu-M5 (anti-CD11c), phycoerythrin-conjugated Leu-15 (anti-CD11b), and phycoerythrin-conjugated mouse IgG2a (isotype control for Leu-15) were purchased from Becton Dickinson Immunocytometry Systems (Mountain View, Calif.). CLB-171, which recognizes the α -chain of LFA-1, was purchased from Accurate Chemical and Scientific Corp. (Westbury, N.Y.). TS1/18, which recognizes the common β -chain CD18, was a gift from Dr. Robert Rothlein of Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, Conn.). The F(ab')₂ goat anti-mouse IgG fluorescein isothiocyanate was purchased from Tago, Inc. (Burlingame, Calif.).

Determination of neutrophil CD11/CD18 expression

The expression of CR3 on human neutrophils was determined by direct immunofluorescence with a phycoerythrin-conjugated mAb (Leu-15) against CD11b and by flow cytometry. Neutrophils were isolated from

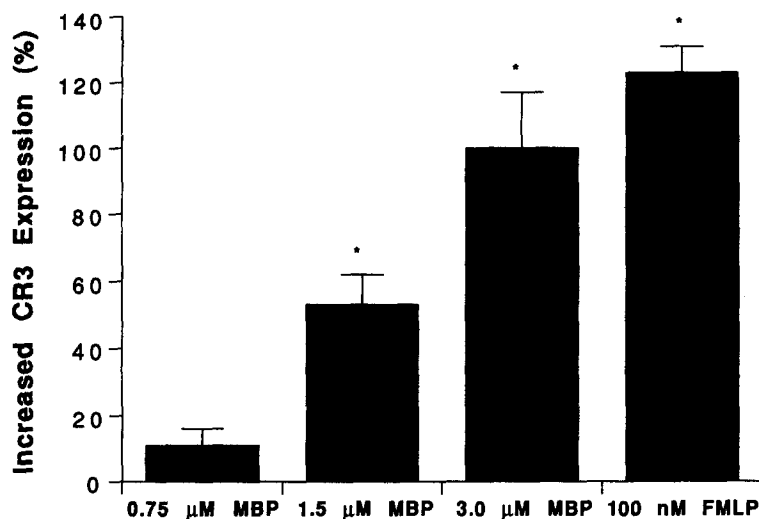


FIG. 1. Enhancement of neutrophil CR3 expression after stimulation with MBP or FMLP. The results are expressed as the mean $\pm$ SEM of multiple experiments, each of which used cells from a different donor. For 0.75 μ mol/L MBP, $n = 4$; 1.5 μ mol/L, and 3.0 μ mol/L MBP, $n = 13$; FMLP, $n = 8$. *Significantly different from unstimulated neutrophils, $p < 0.001$.

the venous blood of adult volunteers, of whom some were known to have positive IgE skin tests to aeroallergen extracts, by Ficoll-Hypaque density gradient centrifugation as described previously.²⁵ The neutrophils (10^6) were incubated in 100 μ l of Hepes-albumin-glucose buffer (pH 7.4) containing 0.6 mmol/L CaCl_2 and 1 mmol/L MgCl_2 with the stated concentrations of MBP, reduced and alkylated MBP, ECP, EDN, or FMLP for 30 minutes (or as indicated) at 37° C in a gently oscillating water bath. After incubation, the neutrophils were washed twice with 1 ml of ice-cold phosphate-buffered saline (PBS) (pH 7.4) containing 0.1% gelatin and 0.1% NaN_3 (PBS-gel-azide). After the second centrifugation, the cell pellet was resuspended in 40 μ l of phycoerythrin-conjugated Leu-15 for 30 minutes at 4° C. A phycoerythrin-conjugated mAb of the same IgG subclass as Leu-15 was used as a control for nonspecific fluorescence. After incubation with the mAb, the cells were washed twice with 1 ml of PBS-gel-azide and were resuspended in 0.2 ml PBS-gel-azide containing 1% paraformaldehyde for flow cytometric analysis.

The expression of CD11a, CD11c, or CD18 was determined by indirect immunofluorescence and flow cytometry after incubation of the neutrophils with MBP, FMLP, or buffer alone as described above. The neutrophils were washed twice with PBS-gel-azide and incubated with 20 μ g/ml of the mAbs (CLB-171 for CD11a, Leu-M5 for CD11c, or TS1/18 for CD18) in a total volume of 40 μ l for 30 minutes at 4° C. After two washes, the cells were incubated with 40 μ l of F(ab')_2 fluorescein isothiocyanate-goat anti-mouse IgG (diluted 1:50) for 30 minutes at 4° C. The cells were then washed and fixed with paraformaldehyde as described above for flow cytometric analysis. A purified mAb belonging to the same subclass as the specific mAb was

used in each case to assess nonspecific fluorescence.

Integral log green (fluorescein isothiocyanate) or integral log red (phycoerythrin) fluorescence was measured with an Epics C flow cytometer (Coulter Electronics, Hialeah, Fla.). For each sample, 5000 cells were analyzed, and fluorescence intensity was displayed on a 256-channel, 3-decade log scale delineated in arbitrary log units. Specific fluorescence associated with binding of antibody to the CD11/CD18 adhesion molecules was calculated by subtracting the mean fluorescence channel (MFC) of the cells stained with the subclass control mAbs from the MFC of cells stained with specific mAb. An increase of 25 units in MFC represents a 100% increase in fluorescence intensity.

Analysis of data

After conversion of the MFC from a logarithmic to a linear value, the percent increase in the level of neutrophil CD11/CD18 expression was calculated according to the formula:

$$\text{Increase in CD11/CD18 expression (\%)} = \frac{\text{MFC of stimulated neutrophils} - \text{MFC of control}}{\text{MFC of control}} \times 100$$

The percent enhancement is expressed as the mean $\pm$ SEM. Statistical analyses were performed with Student's t test.

RESULTS

Effect of MBP on neutrophil CR3 expression

Incubation of neutrophils with 0.75 to 3 μ mol/L MBP for 30 minutes at 37° C produced a concentration-dependent increase in the level of CR3

expression, as detected by phycoerythrin-conjugated Leu-15 staining. Fig. 1 shows that incubation of neutrophils with 0.75 $\mu\text{mol/L}$, 1.5 $\mu\text{mol/L}$, and 3.0 $\mu\text{mol/L}$ MBP resulted in increases of $11\% \pm 5\%$ ($n = 4$, $p > 0.05$), $53\% \pm 9\%$ ($n = 13$, $p < 0.001$), and $100\% \pm 17\%$ ($n = 13$, $p < 0.001$), respectively, in CR3 expression above unstimulated neutrophils. The percent increase in CR3 expression stimulated by 100 nmol/L FMLP was $123\% \pm 8\%$ ($n = 8$, $p < 0.001$). The histograms in Fig. 2 are from a representative experiment and show that the fluorescence intensity on the neutrophil surface increased with increasing concentrations of MBP. In this experiment the MFC for unstimulated neutrophils was 114 and increased to 140 after stimulation with 3.0 $\mu\text{mol/L}$ MBP. In the same experiment, incubation of neutrophils with 100 nmol/L FMLP increased the MFC to 145.

Time course of MBP-stimulated CR3 expression

The time course of CR3 upregulation of MBP-stimulated neutrophils was compared with that of FMLP-stimulated neutrophils in four experiments, each of which used neutrophils from a different donor (Fig. 3). At the earliest time point evaluated (2 minutes), neutrophils incubated with 3.0 $\mu\text{mol/L}$ MBP displayed an increase of approximately 20% in expression of CR3. In two of the four experiments, MBP-stimulated expression of CR3 had plateaued between the 15- and 30-minute time points. An additional experiment revealed that incubation of neutrophils with 3 $\mu\text{mol/L}$ MBP resulted in little increase of CR3 expression beyond that seen at the 30-minute time point (Fig. 3, *inset*). Neutrophils stimulated with 100 nmol/L FMLP showed kinetics of CR3 upregulation that were similar to the kinetics of MBP-stimulated neutrophils. At each time point, the increased expression of CR3 stimulated by FMLP was significantly higher than that stimulated by MBP.

Effect of reduced and alkylated MBP, EDN, and ECP on neutrophil CR3 expression

Previous results demonstrated that reduction and alkylation of the MBP molecule abrogated the ability of MBP to stimulate neutrophil activation.¹³ Therefore the influence of reduced and alkylated MBP on CR3 expression was examined in the present study. Fig. 4 shows that neutrophils incubated with 1.5 $\mu\text{mol/L}$, 2.0 $\mu\text{mol/L}$, or 3.0 $\mu\text{mol/L}$ MBP resulted in concentration-dependent increases of $91\% \pm 6\%$, $112\% \pm 29\%$, and

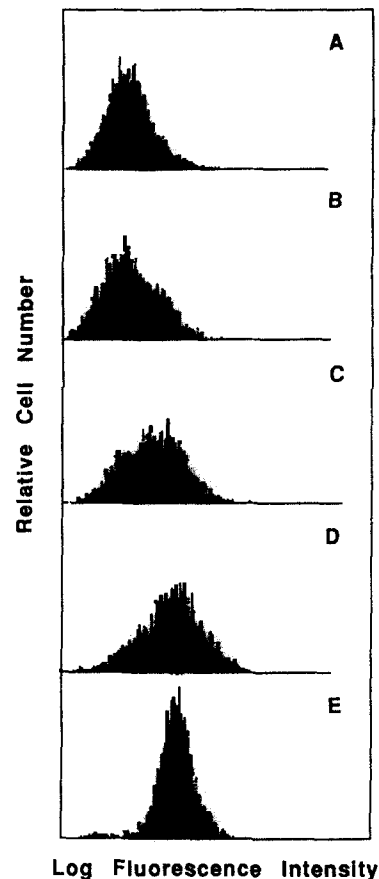


FIG. 2. Histograms of flow cytometric measurement of neutrophil CR3 expression. Neutrophils were incubated with MBP at 37° C for 30 minutes, and CR3 expression was assessed by phycoerythrin-conjugated Leu-15 staining. **A**, Unstimulated neutrophils. Neutrophils stimulated with: **B**, 0.75 $\mu\text{mol/L}$ MBP; **C**, 1.5 $\mu\text{mol/L}$ MBP; **D**, 3.0 $\mu\text{mol/L}$ MBP; and **E**, 100 nmol/L FMLP.

$173\% \pm 30\%$ in CR3 expression, respectively, whereas incubation with reduced and alkylated MBP resulted in small increases ($17\% \pm 4\%$, $16\% \pm 8\%$, and $18\% \pm 6\%$ for 1.5 $\mu\text{mol/L}$, 2.0 $\mu\text{mol/L}$, and 3.0 $\mu\text{mol/L}$, respectively) in CR3 expression, which were not affected by the concentration of reduced and alkylated MBP. These small increases were not significantly different from the expression of CR3 on unstimulated neutrophils. For all three concentrations, the increase in CR3 expression stimulated by native MBP was significantly ($p < 0.05$) higher than the increase stimulated by reduced and alkylated MBP. We have also previously shown that EDN and ECP did not stimulate neutrophil activation.¹³ In the present study neutrophils incubated with 3.0 $\mu\text{mol/L}$ EDN or ECP did not increase the level of CR3 expression above that of unstimulated neutrophils. In contrast, 3 $\mu\text{mol/L}$ MBP increased

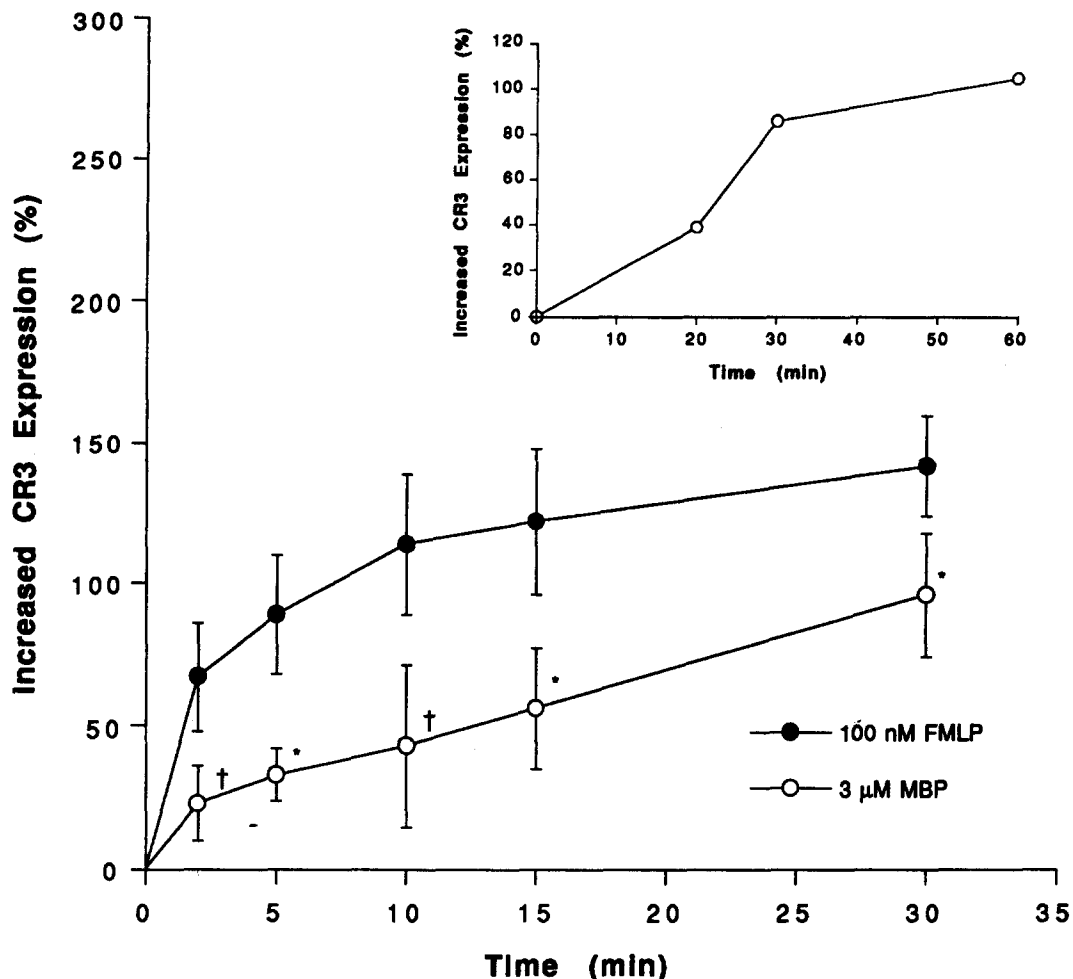


FIG. 3. Time course of enhanced CR3 expression on neutrophils stimulated with MBP or FMLP. Neutrophils were incubated with 3 $\mu\text{mol/L}$ MBP or 100 nmol/L FMLP for the times indicated and stained with phycoerythrin-conjugated Leu-15. The results are the mean $\pm$ SEM of four experiments, each of which used cells from a different donor. *,†Significantly different from FMLP-stimulated neutrophils: * $p < 0.05$; † $p < 0.01$. *Inset:* results of a fifth experiment. Neutrophils were incubated with 3 $\mu\text{mol/L}$ MBP for up to 60 minutes, and CR3 expression was determined by staining with phycoerythrin-conjugated Leu-15.

CR3 expression by 104% and 73% in the same two experiments (data not shown).

Effect of MBP stimulation on the expression of LFA-1 and p150,95

On the basis of the CR3 findings, additional experiments were performed to determine whether neutrophil stimulation with MBP increased the expression of the other two members (LFA-1 and p150,95) of the CD11/CD18 family of adhesion molecules. In five experiments incubation of neutrophils with 1.5 $\mu\text{mol/L}$ MBP increased the expression of CD11a (α -subunit of LFA-1), CD11c, and CD18 (common β -subunit) by 3% $\pm$ 2%, 18% $\pm$ 8%, and 38% $\pm$ 10%, respectively (data not shown). The changes in ex-

pression induced by 1.5 $\mu\text{mol/L}$ MBP of these three molecules were not significantly ($p > 0.05$) different than their baseline expression. In the same experiments 100 nmol/L FMLP increased the expression of CD11a, CD11c, and CD18 by 13% $\pm$ 4%, 64% $\pm$ 18%, and 110% $\pm$ 18%, respectively (data not shown). To assess the effect of MBP concentration on the expression of p150,95, neutrophils from another six donors were studied (Fig. 5). Incubation of neutrophils with 0.5 to 1.5 $\mu\text{mol/L}$ MBP enhanced the expression of p150,95 by 7% $\pm$ 2% to 33% $\pm$ 5%, respectively. In the same experiments stimulation with 100 nmol/L FMLP increased p150,95 expression by 44% $\pm$ 8%. The increases in p150,95 expression stimulated by all of the concentrations of

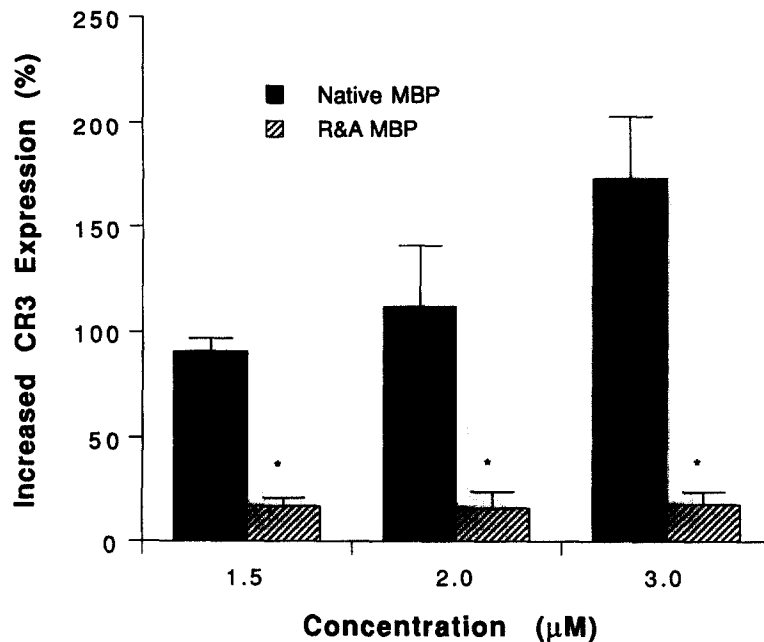


FIG. 4. Comparison of the influence of MBP and reduced and alkylated MBP (*R&A MBP*) on CR3 expression. Results shown are the mean $\pm$ SEM of three experiments. *Significantly different from native MBP, $p < 0.05$.

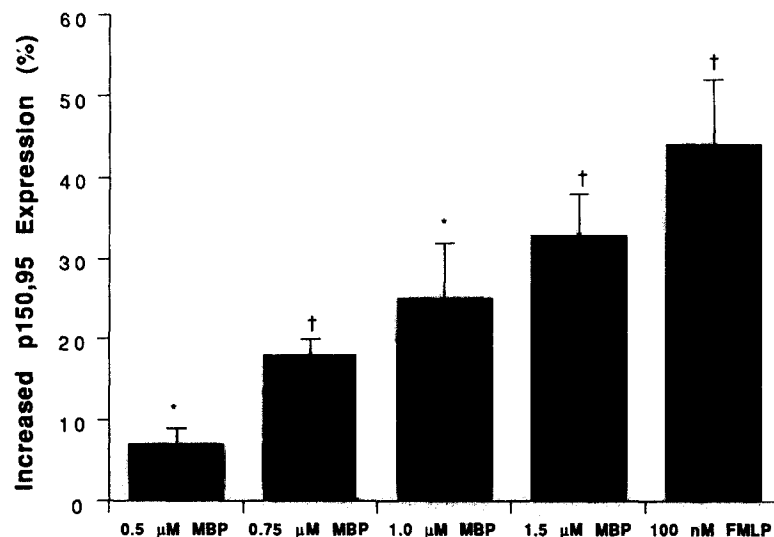


FIG. 5. The effect of MBP on neutrophil expression of p150,95. Neutrophils were incubated with the stated concentrations of MBP at 37° C for 30 minutes, and p150,95 expression was assessed by Leu-M5 staining and flow cytometry. The results shown are the mean $\pm$ SEM of six experiments, each of which used cells from a different donor. *,†Significantly different from unstimulated neutrophils: * $p < 0.05$; † $p < 0.001$.

MBP and FMLP tested were statistically significant ($p < 0.05$) when compared with p150,95 expression on unstimulated neutrophils.

DISCUSSION

Arm et al.²² have shown that the increased expression of neutrophil CR3 may be important in

allergic asthma. Other investigators have demonstrated the importance of endothelial cell intercellular adhesion molecule-1 in the promotion of neutrophil, eosinophil, and basophil migration to sites of late-phase allergic reactions.^{26, 27} Both CR3 and LFA-1 are counter-receptors for intercellular adhesion molecule-1 in neutrophil-endo-

thelial adhesion.^{28, 29} Adherence to the endothelium and transendothelial migration of neutrophils require the increased expression of CR3.³⁰ Inflammatory mediators, such as platelet activating factor and leukotriene B₄, can increase neutrophil CR3 expression.^{31, 32} The present findings indicate that MBP may also contribute to the increased expression of this integrin.

The degree of CR3 enhancement was directly related to the concentrations of MBP tested (0.75 to 3 $\mu\text{mol/L}$). These concentrations were chosen to approximate the concentrations of MBP detected in sputum of patients with asthma. In the study by Frigas et al.⁵ seven of the 25 hospitalized patients with asthma had MBP sputum levels greater than 10 $\mu\text{g/ml}$ (0.71 $\mu\text{mol/L}$) including one patient with a level of 93 $\mu\text{g/ml}$ (6.7 $\mu\text{mol/L}$). In our study incubation of neutrophils with 3.0 $\mu\text{mol/L}$ MBP increased CR3 expression by two-fold to threefold. This was comparable to the increase seen with 100 nmol/L FMLP in the same experiments. A number of neutrophil stimuli, including C5a, leukotriene B₄, and platelet activating factor, are known to increase CR3 expression from twofold to 10-fold.^{31, 32} Although some investigators have reported an enhancement of CR3 expression of as much as 14-fold with FMLP-stimulation,³¹ others have reported a threefold to fourfold enhancement with FMLP.^{32, 33} In our study MBP enhancement of CR3 expression was at the lower end when compared with previous reports on other neutrophil stimuli. This is consistent with our earlier report that MBP was a moderate agonist for neutrophil superoxide anion and lysozyme release.¹³

The time course of the MBP-stimulated increase in CR3 expression was similar to that previously reported^{19, 34} for FMLP stimulation. Although the increase in CR3 expression had not plateaued at the 30-minute time point in two of the first four experiments, a fifth experiment showed that at 60 minutes there was only a minimal increase above the 30-minute time point. The rapid increase in CR3 expression (50% of maximal enhancement after 10 minutes of incubation) suggests that the mechanism of MBP-stimulated enhancement of CR3 is similar to that of other neutrophil agonists, specifically, the recruitment of CR3 from intracellular stores in the tertiary and specific granules.³⁵

Like CR3 expression, p150,95 expression can also be augmented by neutrophil agonists.^{31, 36} In our study MBP also enhanced the expression of p150,95 along with CR3. In the initial set of five

experiments, incubation of neutrophils with 1.5 $\mu\text{mol/L}$ MBP resulted in an increase of $18\% \pm 8\%$ in the expression of CD11c. Subsequently, neutrophils from six donors other than the earlier five donors were used to assess the effect of MBP concentration on CD11c expression. The difference in the percent enhancement ($18\% \pm 8\%$ vs $33\% \pm 5\%$) stimulated by 1.5 $\mu\text{mol/L}$ MBP between the two sets of experiments most likely reflects donor variability. In addition to an increase in the expression of CD11c, an increase in CD18 expression also occurred with MBP stimulation. Although CD11c increased by $18\% \pm 8\%$, CD18 increased by $38\% \pm 10\%$. The discrepancy could be explained by the assumption that an increase of CD18 (common β -subunit) above the 18% for CD11c (α -subunit of p150,95) is needed to accompany the upregulation of the α -subunit of CR3. The function of p150,95 is not well defined, but p150,95 might also act as an adhesion molecule for the binding of neutrophils to endothelial cells.³⁷ Thus the upregulation of both CR3 and p150,95 by MBP could greatly increase the adhesive properties of neutrophils.

Neither MBP nor FMLP increased the expression of LFA-1 in our study. Our findings are in agreement with what others have reported for the effect of neutrophil agonists on the expression of LFA-1. Although calcium ionophore A23187, FMLP, or C5a-desArg increased the expression of CR3³⁸ and p150,95³⁶ by three to 10 times and two to three times, respectively, these stimuli had little to no effect on the expression of LFA-1. Given the above facts, the dose effects of MBP were not studied for LFA-1.

Among the eosinophil granule cationic proteins tested, the unique effect of MBP on neutrophil CR3 expression was underscored by the results obtained with ECP and EDN. Neither ECP (which is similar to MBP in molecular weight, isoelectric point, and arginine content⁴) nor EDN, at concentrations equal to the highest concentration of MBP tested, enhanced CR3 expression. The present results also show that after reduction and alkylation, the ability of MBP to enhance CR3 expression was reduced by 80% to 90%. Whereas the increase in CR3 expression correlated with increasing concentrations of native MBP, the small degree of CR3 upregulation stimulated by the same three concentrations of reduced and alkylated MBP did not occur in a concentration-dependent manner. These findings are in full agreement with our previous report that reduced and alkylated MBP, ECP, and EDN

did not stimulate neutrophil activation.¹³ This is also consistent with the initial findings that reduced and alkylated MBP, ECP, and EDN did not mimic the ability of MBP to stimulate basophil histamine release.¹² The same findings have also been reported for MBP activation of platelets.³⁹ Together with the failure of ECP or EDN to activate neutrophils, these findings suggest that the conformation of the molecule, rather than simply the primary structure or ionic charge, may be important in the activation of neutrophils by MBP.

Subsequent to the initial studies that identified MBP as a noncytotoxic activator of basophils and mast cells,^{11, 12} additional evidence has been provided by others to substantiate that MBP is more than merely a cytotoxic mediator in allergic diseases. MBP has been shown to stimulate human platelets,³⁹ and noncytotoxic concentrations of MBP have been shown to enhance respiratory smooth muscle contractility.⁴⁰ The present study demonstrates that MBP enhances CR3 and p150,95 expression. Although we did not test the neutrophils for viability after stimulation with MBP in these experiments, we previously showed that 1.5 $\mu\text{mol/L}$ MBP did not cause cytotoxicity as assessed by lactate dehydrogenase release.¹³ Additionally, we have recently found that 2 $\mu\text{mol/L}$ and 5 $\mu\text{mol/L}$ concentrations of MBP were not cytotoxic to neutrophils as assessed by chromium 51 release experiments.⁴¹

Together with our previous report that MBP stimulates neutrophil secretory processes,¹³ these findings provide further evidence for the noncytotoxic role of MBP in allergic diseases. In conjunction with the induction of release of toxic oxygen radicals and lysozyme, the upregulation of adherence molecules suggests important roles for the interaction of MBP and neutrophils in the late-phase allergic response.

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REFERENCES

1. Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
2. Pelikan Z. The changes in the nasal secretions of eosinophils during the immediate nasal response to allergen challenge. *J ALLERGY CLIN IMMUNOL* 1983;72:657-62.
3. Atkins P, Green R, Zweiman B. Histologic studies of human skin test exposure to ragweed, compound 48/80, and histamine. *J ALLERGY CLIN IMMUNOL* 1973;51:263-73.
4. Gleich GJ, Loegering DA. Immunobiology of eosinophils. *Ann Rev Immunol* 1984;2:429-60.
5. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin Proc* 1981;56:345-53.
6. Bascom R, Pipkorn U, Proud D, et al. Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. *J ALLERGY CLIN IMMUNOL* 1989;84:338-46.
7. Leiferman KM, Ackerman SJ, Sampson HA, Haugen HS, Venecie PY, Gleich GJ. Dermal deposition of eosinophil-granule major basic protein in atopic dermatitis. *N Engl J Med* 1985;313:282-5.
8. Hastie AJ, Loegering DA, Gleich GJ, Kueppers F. The effect of purified human eosinophil granule major basic protein on mammalian ciliary activity. *Am Rev Respir Dis* 1987;135:848-53.
9. Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab Invest* 1980;42:35-43.
10. Hisamatsu K-i, Ganbo T, Nakazawa T, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J ALLERGY CLIN IMMUNOL* 1990;86:52-63.
11. O'Donnell MC, Ackerman SJ, Gleich GJ, Thomas LL. Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. *J Exp Med* 1983;157:1981-91.
12. Zheutlin LM, Ackerman SJ, Gleich GJ, Thomas LL. Stimulation of basophil and rat mast cell histamine release by eosinophil granule-derived cationic proteins. *J Immunol* 1984;133:2180-5.
13. Moy JN, Gleich GJ, Thomas LL. Noncytotoxic activation of neutrophils by eosinophil granule major basic protein. Effect on superoxide anion generation and lysosomal enzyme release. *J Immunol* 1990;145:2626-32.
14. Carlson M, Hakansson L, Peterson C, Stalenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. *J ALLERGY CLIN IMMUNOL* 1991;87:27-33.
15. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. *Am Rev Respir Dis* 1988;138:406-12.
16. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granular proteins in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 1990;62:579-89.
17. Zweiman B, Kucich U, Shalit M, Von Allmen C, Moskovitz A, Weinbaum G, Atkins PC. Release of lactoferrin and elastase in human allergic skin reactions. *J Immunol* 1990;144:3953-60.
18. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;83:2008-17.
19. Tonnesen MG, Anderson DC, Springer TA, Knedler A, Avdi N, Henson PM. Adherence of neutrophils to cultured human microvascular endothelial cells. Stimulation by chemotactic peptides and lipid mediators and dependence upon the Mac-1, LFA-1, p150,95 glycoprotein family. *J Clin Invest* 1989;83:637-46.
20. Springer TA, Miller LF, Anderson DC. P150,95, the third

- member of the Mac-1, LFA-1 human leukocyte adhesion glycoprotein family. *J Immunol* 1986;136:240-5.
21. Kay AB. Asthma and inflammation. *J ALLERGY CLIN IMMUNOL* 1991;87:893-901.
 22. Arm JP, Walport MJ, Lee TH. Expression of complement receptors type 1 (CR1) and type 3 (CR3) on circulating granulocytes in experimentally provoked asthma. *J ALLERGY CLIN IMMUNOL* 1989;83:649-55.
 23. Slifman NR, Loegering DA, McKean DJ, Gleich GJ. Ribonuclease activity associated with human eosinophil-derived neurotoxin and eosinophil cationic protein. *J Immunol* 1986;137:2913-7.
 24. Wassom DL, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J Clin Invest* 1981;67:651-61.
 25. Schmeichel CJ, Thomas LL. Methylxanthine bronchodilators potentiate multiple human neutrophil functions. *J Immunol* 1987;138:1896-1903.
 26. Bochner BS, Luscinskas FW, Gimbrone MA Jr. Adhesion of human basophils, eosinophils, and neutrophils to interleukin-1 activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991;173:1553-6.
 27. Wegner CD, Gundel RH, Reilly P, Haynes N, Letts LG, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
 28. Diamond MS, Staunton DE, deFougerolles AR, et al. ICAM-1 (CD54) - a counter-receptor for Mac-1 (CD11b/CD18). *J Cell Biol* 1990;111:3129-39.
 29. Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;83:2008-17.
 30. Jutlia MA, Rott L, Berg EL, Butcher EC. Function and regulation of the neutrophil Mel-14 antigen in vivo: comparison with LFA-1 and Mac-1. *J Immunol* 1989;143:3318-24.
 31. Miller LJ, Bainton DF, Borregard N, Springer TA. Stimulated mobilization of monocyte Mac-1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. *J Clin Invest* 1987;80:535-44.
 32. Shalit M, Von Allmen C, Atkins PC, Zweiman B. Platelet activating factor increases expression of complement receptors on human neutrophils. *J Leukoc Biol* 1988;44:212-7.
 33. Vedder NB, Harlan JM. Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. *J Clin Invest* 1988;81:676-82.
 34. Phillips MR, Buyon JP, Winchester R, Weissmann G, Abramson SB. Up-regulation of the iC3b receptor (CR3) is neither necessary nor sufficient to promote neutrophil aggregation. *J Clin Invest* 1988;82:495-501.
 35. Lacal P, Pulido R, Sanchez-Madrid F, Mollinedo F. Intracellular location of T200 and Mo1 glycoproteins in human neutrophils. *J Biol Chem* 1988;263:9946-51.
 36. Lanier LL, Arnauout MA, Schwarting R, Warner NL, Ross GD. P150/95, third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. *Eur J Immunol* 1985;15:713-8.
 37. Stacker SA, Springer TA. Leukocyte integrin p150,95 (CD11c/CD18) functions as an adhesion molecule binding to a counter-receptor on stimulated endothelium. *J Immunol* 1991;146:648-55.
 38. Arnaout MA, Spitz H, Ternhorst C, Pitt J, Todd RF. Deficiency of a leukocyte surface glycoprotein (LFA-1) in two patients with Mo1 deficiency. Effects of cell activation on Mo1/LFA-1 surface expression in normal and deficient leukocytes. *J Clin Invest* 1984;74:1291-1300.
 39. Rohrbach MS, Wheatley CL, Slifman NR, Gleich GJ. Activation of platelets by eosinophil granule proteins. *J Exp Med* 1990;172:1271-4.
 40. Flavahan NA, Slifman NR, Gleich GJ, Vanhoutte PM. Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle: role of the epithelium. *Am Rev Respir Dis* 1988;138:685-8.
 41. Moy JN, Thomas LL, Haskell MD, Ware AE. Eosinophil major basic protein (MBP) stimulates human neutrophil elastase (HNE) release [Abstract]. *J ALLERGY CLIN IMMUNOL* 1993;91:333.