

Biochemical properties, activities, and presence in biologic fluids of eosinophil granule major basic protein

Pamela Popken-Harris, PhD^a Larry Thomas, PhD^b Claus Oxvig,^c

Lars Sottrup-Jensen,^c Hirokazu Kubo,^a J. S. Klein,^d and

Gerald J. Gleich, MD^{a,c} Rochester, Minn., Chicago, Ill., and Aarhus, Denmark

Eosinophil granule major basic protein (MBP) is a potent toxin for helminths,¹⁻³ protozoa,⁴⁻⁶ bacteria,⁷ and certain types of cells.⁸ Direct application of MBP to guinea pig tracheal rings^{9, 10} causes epithelial desquamation and ciliostasis, an effect that mimics the disease of asthma.¹¹ Further, instillation of MBP directly into the trachea of cynomolgus monkeys provoked transient bronchoconstriction and enhanced sensitivity to inhaled methacholine.¹² Finally, the sputum of patients with asthma contains high levels of MBP,¹³ and MBP is also deposited at sites of tissue damage in the biopsy specimens of patients dying of asthma.¹⁴

In addition to its toxicity and proposed role as a mediator of tissue damage in asthma, MBP also has the ability to activate other cells. For example, MBP causes nonspecific complement activation,¹⁵ basophil histamine release,^{16, 17} neutrophil activation and degranulation,¹⁸ and platelet activation.¹⁹ Because the MBP-mediated histamine release from human basophils is calcium, temperature, and energy dependent, this activity is not due to cell killing.¹⁶ Pretreatment of basophils with pertussis toxin causes a dose-dependent inhibition of the MBP-mediated basophil histamine release, which suggests the involvement of a guanosine triphosphate regulatory protein in the activation mechanism.²⁰

Abbreviations used

cDNA:	Complementary deoxyribonucleic acid
CHOs:	Chinese hamster ovary cells
HES:	Hypereosinophilic syndrome
MBP:	Major basic protein
PAPP-A:	Pregnancy-associated plasma protein A
pI:	Isoelectric point
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

The complementary deoxyribonucleic acid (cDNA) for MBP encodes a prepro-molecule of 222 amino acids including a predicted secretory leader of 15 amino acids, an acidic propiece of 90 amino acids, and a basic MBP portion of 117 amino acids.^{21, 22} The function of the propiece may be to protect developing eosinophils from damage by the toxic MBP during granule processing.²³ Until recently (vide infra), proMBP has not been seen in nature. Interestingly, secretion of proMBP by a T-cell hybridoma has recently been reported.²⁴ In addition, proMBP has been detected in the sera of pregnant women and is tightly complexed to pregnancy-associated plasma protein A (PAPP-A), a placentally derived protein of unknown function.²⁵ However, large quantities of native proMBP from these sources are unavailable. Here we summarize information on the evidence for the presence of proMBP, the biochemical and biologic properties of proMBP, the likely biologic activities of the pro-portion of MBP, the expression, purification, and properties of recombinant proMBP, and the occurrence of proMBP and MBP in human sera.

MBP cDNA CODES FOR AN ACIDIC PRECURSOR

As previously noted, MBP is a 13.8 kd single polypeptide chain, rich in arginine, with a calcu-

From the Departments of Immunology^a and Internal Medicine,^d Mayo Clinic and Mayo Foundation, Rochester, the Department of Immunology/Microbiology,^b Rush-Presbyterian-St. Luke's Medical Center, Chicago and the Department of Molecular Biology,^c University of Aarhus, Aarhus, Denmark.

Supported in part by grants from the National Institutes of Health (AI 15231 and AI 09728). Additional laboratory funding was provided by Incyte Pharmaceuticals, Inc., Palo Alto, Calif., and the Danish Biomembrane Research Center. Reprint requests: Gerald J. Gleich, Department of Immunology, Mayo Clinic, 200 First St. S.W., Rochester, MN 55905.

J ALLERGY CLIN IMMUNOL 1994;94:1282-9.

Copyright © 1994 by Mosby-Year Book, Inc.

0091-6749/94 \$3.00 + 0 1/0/61020

TABLE I. Approximate pIs of MBP from translation of their cDNA sequences

Protein	No. of amino acids	No. of strongly basic amino acids (K.R.)	No. of strongly acidic amino acids (D.E.)	pI
Human MBP	117	17	1	10.9
Pro-portion	90	7	26	3.9
proMBP	207	24	27	6.2

Reproduced from Barker RL, et al. *J Exp Med* 1988; 168:1493-8, by copyright of The Rockefeller University Press.

lated isoelectric point of 10.9.^{21, 22, 26} Because MBP is present in HL-60 cells,²⁷ an HL-60 cDNA library was probed with redundant oligonucleotides derived by reverse translation of MBP amino acid sequences. The resulting cDNA clones revealed an open-reading frame coding for a protein of 117 amino acids with a sequence identical to that of MBP. The MBP sequence was preceded by an additional amino acid sequence of 105 amino acids composed of a 15-amino acid putative signal peptide and a middle sequence of 90 amino acids. Neither of these latter sequences are present in the MBP protein. These data suggested that MBP is initially translated as a preproprotein, which is cotranslationally modified to proMBP and post-translationally modified to MBP.

MBP has 17 strongly basic amino acids, only one strongly acidic amino acid, and an isoelectric point (pI) of 10.9 (Table I). The pro-portion of proMBP has 26 acidic amino acids, seven strongly basic amino acids, and a pI of 3.9. The combination of the acidic and basic peptides in proMBP has a slightly acidic pI of 6.2. Translation of MBP as a proprotein could mask the toxic effects of mature MBP and protect the eosinophil from damage during the time the protein is processed through the endoplasmic reticulum. Prior studies had shown that heparin, an acidic mucopolysaccharide, is reactive with MBP and inhibits its toxicity,¹ suggesting that MBP could be neutralized by an acidic precursor.

BIOLOGIC ACTIVITIES OF MBP ARE NEUTRALIZED BY ACIDIC POLYAMINO ACIDS

A test of the hypothesis that the toxicity of MBP might be neutralized by acidic amino acids was conducted with K562 tumor cells and acidic polyamino acids including homopolymers of aspartic and glutamic acid varying in length from approximately 10 to 80 kd.²³ These results showed that the toxicity of MBP for K562 cells was potentially antagonized by the acidic polyamino acids and that the extent of the inhibition was a

function of the length of the polymer. In addition, the acidic polyamino acids inhibited the toxicity of MBP for respiratory epithelium and prevented both epithelial desquamation and ciliostasis. MBP also caused bronchospasm and a 10-fold increase in bronchial reactivity when instilled into the lungs of cynomolgus monkeys.¹² Nebulization of polyglutamic acid into the lungs of the monkeys prevented the bronchospasm and the increase in airway responsiveness mediated by instillation of MBP into their airways. Thus, these results indicated that polyamino acids might function to neutralize the toxic effects of MBP and support the view that the acidic pro-portion of proMBP serves to neutralize the biologic activities of MBP. Therefore studies of proMBP were conducted to further test this hypothesis.

EXPRESSION, PURIFICATION, AND PROPERTIES OF RECOMBINANT PROMBP

ProMBP was purified from supernatants of Chinese hamster ovary cells (CHO) transfected with the expression vector pSv/DHFR/proMBP. After transfection, dihydrofolate-deficient CHO cells were selected in hypoxanthine-, glycine-, and thymidine-deficient media. Stable transfected lines were established and maintained in selective media, and amplification was accomplished by the stepwise addition of 10 to 1000 nmol/L methotrexate. The proMBP positive CHO cell line, 78.5, was selected from these lines, and 78.5 produced MBP, as judged by Western blotting with monoclonal antibodies. Pro-MBP was purified from the CHO supernatants by affinity chromatography on heparin-Sepharose and by gel filtration with Sephadex G-100. Fractions were analyzed for the presence of proMBP by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie brilliant blue R staining and Western blotting with use of monoclonal antibodies. Fractions positive for proMBP from heparin-Sepharose were concentrated and further purified by two successive passes through columns of Sephadex G-100. Initially, partially puri-

TABLE II. Calculation of the amino acid composition of purified recombinant (33 kd) proMBP

Amino acid	Predicted	Actual
Asx	14	14.5
Thr	13	12.6
Ser	16	16.2
Glx	29	27.8
Pro	9	8.9
Cys	12	12.1
Gly	16	18.1
Ala	10	9.6
Val	12	11.9
Met	2	2.0
Ile	8	7.9
Leu	14	14.1
Tyr	6	6.0
Phe	7	6.9
Trp	8	6.3*
His	5	6.3
Lys	5	5.7
Arg	19	18.4

*Determined by the method of Sottrup-Jensen.²⁸

fied proMBP was obtained and used to immunize mice to obtain the proMBP-specific monoclonal antibody J163-15E. With the use of this antibody, a competitive binding radioimmunoassay for proMBP was established and subsequently used to ascertain the purity of proMBP during purification from the proMBP-rich CHO supernatants. Purification over heparin-Sepharose followed by two passages over a Sephadex G-100 column yielded an 18.3-fold purification of proMBP, but the yield of 1% limited the quantities for experimentation.

The *N*-terminal amino acid sequence of proMBP was LHLRSET, which indicates that cleavage of the signal peptide was not at the predicted Ser 15-Ala 16 site but instead was at Ala 16-Leu 17. The complete amino acid composition of purified recombinant proMBP was determined by a molecular mass estimated from the cDNA coding sequence of 20,942 Da (23,670 Da minus the relative contributions made by tryptophan and cysteine because they are lost during acid hydrolysis). Table II shows that the amino acid composition of the purified 33 kd recombinant form of proMBP is in excellent agreement with the predicted composition.

The purification scheme previously described resulted in the isolation of a predominant 33 kd species of proMBP, which was used for all subsequent analyses. The molecular mass of the 33 kd

species suggested that considerable carbohydrate must be added to the polypeptide chain to account for the approximately 10 kd of mass between the predicted 23 kd of the proMBP polypeptide and the mass of 33 kd found as the predominant species for proMBP. The carbohydrates associated with proMBP accounted for 4856 to 5150 Da, and when added to the predicted cDNA of the peptide of 23,670 Da, yielded an estimated molecular mass between 28,526 and 28,820 Da.

As previously noted, the presence of the propeptide of proMBP may function to protect developing eosinophils from damage by toxic MBP during granule processing. Therefore, we tested whether proMBP had the biologic activities associated with MBP and whether proMBP could inhibit the activities of MBP. Previous results had shown that MBP is a potent stimulator of histamine release from basophils¹⁶ and activates the respiratory burst of neutrophils.¹⁸ Table III shows that as expected, MBP caused histamine release, whereas proMBP failed to cause histamine release. The addition of purified recombinant proMBP, 1 μ mol/L, totally blocked the histamine-releasing activity of 2 μ mol/L MBP. In contrast, proMBP had no effect on immunoglobulin E-induced basophil histamine release. Similarly, Fig. 1 shows that 1.5 μ mol/L MBP causes a time-dependent increase in chemiluminescence,¹⁸ whereas 3.0 μ mol/L proMBP had little effect. The addition of 3.0 μ mol/L proMBP nearly completely blocked the ability of 1.5 μ mol/L MBP to stimulate chemiluminescence. Finally, the toxicity of MBP to K562 cells was measured by its ability to inhibit protein synthesis. Inhibition of protein synthesis was caused by 1 μ mol/L MBP, whereas 1 μ mol/L proMBP had no inhibitory effect. These results indicate that proMBP lacks the phlogistic activities of MBP and indeed is able to inhibit MBP.

OCCURRENCE OF MBP AND proMBP IN BLOOD

As noted earlier, the plasma level of immunoreactive MBP is greatly elevated in pregnancy and, histochemically, MBP has been localized to X cells and giant cells of the placenta.²⁹⁻³² MBP has been purified from placental tissues and is identical to MBP derived from the eosinophil granule.³³ Furthermore, MBP in pregnancy appears to be tightly associated with unknown large proteins.³³ Surprisingly, recent studies of PAPP-A showed that PAPP-A is bound to MBP and that the form of MBP bound to PAPP-A is proMBP.²⁵ PAPP-A is an α -glycoprotein³⁴⁻³⁷ present at a concentration of

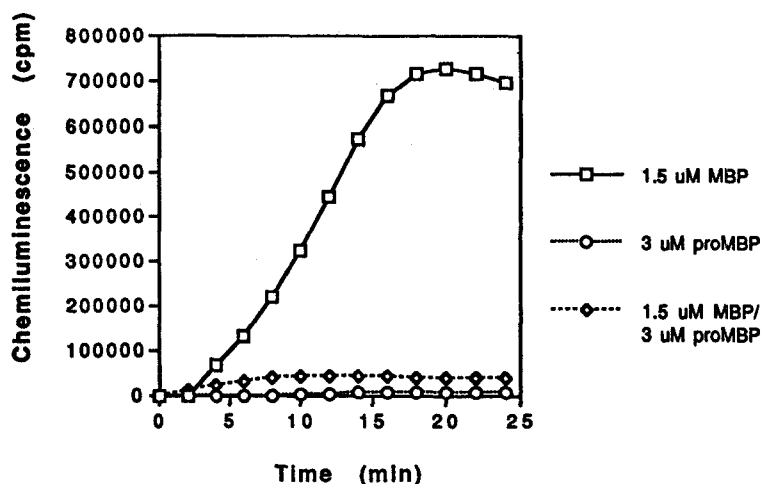


FIG. 1. Neutrophil activation by chemiluminescence. Purified MBP or proMBP was added alone or in combination to purified human neutrophils in the dark. Starting at time 0, neutrophil activation as a function of time was measured by chemiluminescence as described.¹⁸

TABLE III. Basophil histamine release with proMBP, MBP, proMBP + MBP, and anti-IgE

ProMBP ($\mu\text{mol/L}$)	0 $\mu\text{mol/L}$ MBP	1 $\mu\text{mol/L}$ MBP	2 $\mu\text{mol/L}$ MBP	Anti-IgE 0.1 $\mu\text{g/ml}$
0	ND	19*	24,24†	63
1.0	0,0	0	1,5	63
2.0	0	1	0	ND

ND, Not determined.

*Percent histamine release from one experiment.

†Percent histamine release from duplicate experiments.

approximately 50 mg/L in pooled third-trimester sera^{38, 39} whose physiologic role is not known. PAPP-A was believed to consist of two disulfide-bridged 250 kd subunits.³⁴⁻³⁷ PAPP-A has been localized to the trophoblastic tissue of the placenta^{40, 41} and this is believed to be the main source of circulating PAPP-A in pregnancy.⁴⁰ During studies of cDNA cloning of PAPP-A, PAPP-A tryptic peptides isolated from pregnancy sera were characterized and, surprisingly, several peptides revealed amino acid sequences identical to MBP.²⁵ This suggested that proMBP and PAPP-A were tightly associated. Analyses of this possibility by SDS-PAGE and Western blotting shown in Fig. 2 revealed that unreduced PAPP-A/proMBP does not appreciably enter the gel (Fig. 2A, lane 1), whereas reduced PAPP-A/MBP forms a major band at approximately 250 kd. Below the PAPP-A subunit, a smear corresponding to species of approximately 50 to 90 kd was seen with this reduced material but not with the nonreduced material. Fig. 2B shows a Western blot of the same lanes shown in Fig. 2A using a monoclonal antibody reactive with denatured

MBP, J6-8A4, which reveals intense reactivity with the MBP-positive control shown in lane 3 and faint reactivity with material in lanes 2 and 4. Fig. 2C shows a Western blot analysis of the same samples as in Fig. 2A with use of the proMBP specific monoclonal antibody J163-15E10. This antibody reacts strongly with materials in lanes 2 and 4, indicating that antigenic determinants present on the propiece are present in reduced PAPP-A/proMBP (lane 2) and on a subunit of the PAPP-A/proMBP complex derived from chromatography (Fig. 3).

¹⁴C-carboxymethylated PAPP-A/proMBP was subjected to denaturing gel chromatography to separate subunits, and the results are shown in Fig. 3. About 84% of the radioactivity associated with protein eluted in fractions 20 to 29, the remainder in fractions 30 to 37. Analyses by SDS-PAGE revealed that the material in pool 1 has the expected size of the PAPP-A subunit (results not shown), whereas the material in pool 2 (Fig. 2A, lane 4) migrated as a smear corresponding to species of approximately 50 to 90 kd rather than 23.4 kd expected for proMBP.^{21, 22} The pool 2 material evi-

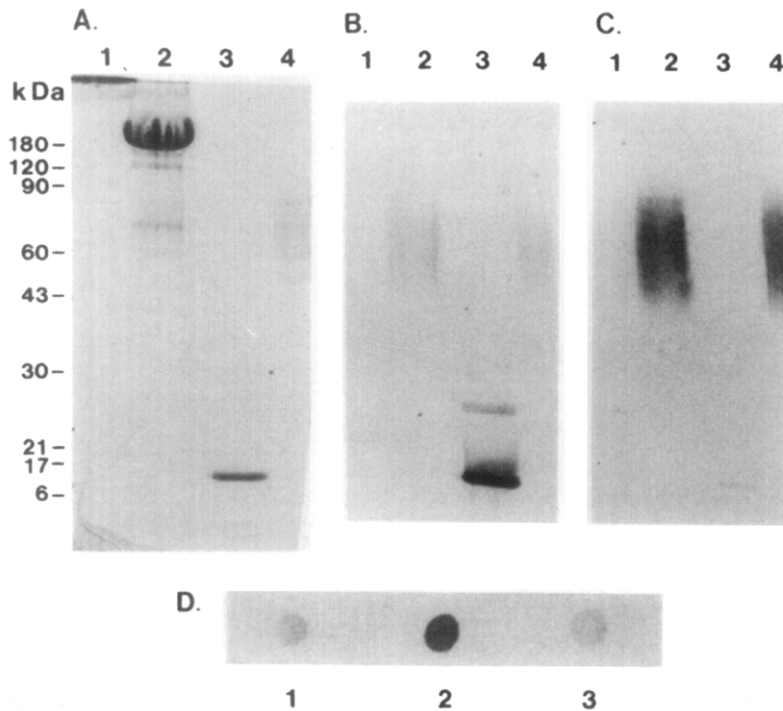


FIG. 2. SDS-PAGE and Western blot analysis of PAPP-A and MBP. *Panel A:* Lane 1, 8 μ g of nonreduced PAPP-A/proMBP; lane 2, 10 μ g of reduced PAPP-A/proMBP; lane 3, 0.5 μ g of reduced MBP; lane 4, 3 μ g of Sepharose CL-6B fraction 2 (Fig. 3). *Panel B:* Western blot analysis of same samples with MBP monoclonal antibody J6-8A4. *Panel C:* Western blot analysis of same samples with MBP propiece monoclonal antibody J163-15E10; weak response in lane 3 is caused by nonspecific binding of secondary antibody caused by high load of MBP. *Panel D:* Dot blot assay of PAPP-A/proMBP and MBP with anti-PAPP-A A230. PAPP-A/proMBP (0.5 μ g; 2) and 0.5 μ g of MBP (1 and 3) were spotted on PVDF. (From Oxvig C, et al. *J Biol Chem* 1993;268:12243-6, by permission of The American Society for Biochemistry and Molecular Biology.)

dently corresponded to the 50 to 90 kd material obtained from PAPP-A/proMBP by reduction (Fig. 2A, lane 2). Sequence analysis further confirmed that the PAPP-A subunit constituted the material in pool 1 and that proMBP, apart from a minor contamination that was a fragment from PAPP-A, constituted material in pool 2.

These results, as well as studies of the peptides derived from tryptic digestion of the PAPP-A/proMBP complex,²⁵ indicate that proMBP and MBP antigenic determinants are present in the PAPP-A complex. Therefore it seems likely that commercial antisera produced to PAPP-A also contain antibodies to MBP; Fig. 2D shows that this is the case. Thus, PAPP-A/proMBP circulates as a covalently bonded complex with links between the sulfhydryls of proMBP and PAPP-A. Furthermore, commercial polyclonal anti-PAPP-A is polyspecific and also reacts with MBP.

Finally, recent analyses of proMBP and MBP have been conducted analyzing sera from patients with diseases associated with eosinophilia and

sera from normal human pregnancies. These analyses consisted of Western blotting with the monoclonal antibody to MBP, J6-8A4, and the monoclonal antibody, J163-15E10, reactive with a determinant in the propiece of proMBP. With these antibodies, the molecular forms of MBP in the serum or plasma of 10 patients with the hypereosinophilic syndrome (HES), two patients with eosinophilic pneumonia, eight pregnant women, and nine normal persons were determined. proMBP was detected in seven of 10 samples from patients with HES, and in five of these, a 14 kd band corresponding to MBP was identified. Sera of one of two patients with eosinophilic pneumonia contained proMBP. Neither MBP nor proMBP was detected in normal serum. In addition, neither MBP nor a 33 kd proMBP could be demonstrated in sera from pregnant women, including sera from both the second and third trimesters. As previously noted during pregnancy, proMBP likely circulates in the blood as a high molecular-weight species complex with

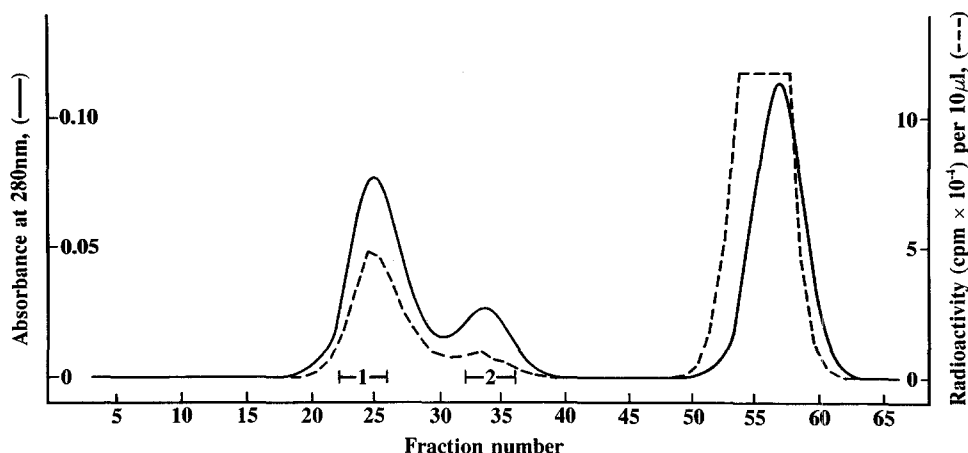


FIG. 3. Sepharose CL-6B gel chromatography of 1 mg of ^{14}C -carboxymethylated PAPP-A/proMBP. A 1×30 cm column was equilibrated and eluted at a flow rate of 1.8 ml/hr with 6 mol/L guanidinium chloride and 20 mmol/L Tris-HCl, pH 8.0. Fractions of 0.45 ml were collected. Absorbance at 280 nm (solid line) and radioactivity (dashed line) are shown. An aliquot of material in peak fractions (bars) was desalted on a PD10 column (eluent 0.1% trifluoroacetic acid) and used for analysis. Carboxymethylated soybean trypsin inhibitor (20 kd) peaked in fractions 39 and 40. (From Oxvig C, et al. J Biol Chem 1993;268:12243-6, by permission of The American Society for Biochemistry and Molecular Biology.)

PAPP-A and has a variety of molecular masses that roughly range from 50 to 90 kd.

SUMMARY

The existence of a proform of MBP is predicted from the sequence of MBP cDNA clones. ProMBP has been purified from the supernatants of CHO cells transfected with cDNA encoding prepro MBP.⁴² Purification involved heparin-Sepharose affinity purification followed by two sequential size fractionation steps over Sephadex G-100 and yielded proMBP with a molecular mass of 33 kd. Recombinant proMBP from the heparin-Sepharose column was subjected to isoelectric focusing followed by SDS-PAGE and Western blot analysis. The results indicated that most of the 33 kd form of proMBP focused predominantly between pI 4.2 and 5.1, with a major peak at a pI of approximately 4.9. Analyses of the carbohydrates associated with the purified 33 kd form of recombinant proMBP indicated the addition of 4856 to 5150 Da by carbohydrates characteristic of the complex type.

Consistent with the hypothesis that the function of the propiece is to neutralize MBP toxicity during granule processing,²¹⁻²³ proMBP lacked MBP cytoestimulatory properties and actually blocked the effect of MBP in two different systems, basophil histamine release and neutrophil activation. In addition, as a measure of toxicity, proMBP did not inhibit protein synthesis, whereas

MBP markedly reduced protein synthesis. The mechanisms by which MBP exerts its actions both as a cytoestimulant and as a toxin are not known; however, it is known that cationic MBP readily reacts with acidic lipids.⁴³ Using artificial liposomes as targets, MBP caused a disordering of the lipid bilayer membrane, resulting in fusion and lysis. Therefore, MBP may act both as a cytoestimulant and as a toxin because of its marked cationicity and its ability to disorder lipid membranes.

Finally, immunoreactive MBP is elevated in the blood of all pregnant women^{29, 31} and levels of MBP peak before labor in about 80% of pregnant women³² and rapidly decline after parturition.³¹ Analyses of PAPP-A indicate that PAPP-A and proMBP are present as a complex in the sera of pregnant women.²⁵ The most likely source of proMBP in the sera of pregnant women is the placental X cell, because proMBP has been localized to X cells by immunofluorescence³⁰ and by in situ hybridization.⁴⁴ Of additional interest is the observation that proMBP has been recently found at microgram per milliliter levels in the sera of patients with HES, and 14 kd MBP was also present in these sera.

REFERENCES

1. Butterworth AE, Wassom DL, Gleich GJ, Loegering DA, David JR. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. J Immunol 1979;122:221-9.
2. Hamann KJ, Barker RL, Loegering DA, Gleich GJ. Com-

- parative toxicity of purified human eosinophil granule proteins for newborn larvae of *Trichinella spiralis*. *J Parasit* 1987;73:523-9.
3. Hamman KI, Gleich GJ, Checkel JL, Loegering DA, McCall JW, Barker RL. In vitro killing of microfilariae of *Brugia pahangi* and *Brugia malayi* by eosinophil granule proteins. *J Immunol* 1990;144:3166-73.
 4. Molina HA, Kierszenbaum F, Hamann KJ, Gleich GJ. Toxic effects produced or mediated by human eosinophil granule components on *Trypanosoma cruzi*. *Am J Trop Med Hyg* 1988;38:327-34.
 5. Villalta F, Kierszenbaum F. Role of inflammatory cells in Chaga's disease. I: uptake and mechanism of destruction of intracellular (amastigote) forms of *Trypanosoma cruzi* by human eosinophils. *J Immunol* 1984;132:2053-8.
 6. Kierszenbaum F, Ackerman SJ, Gleich GJ. Destruction of bloodstream forms of *Trypanosoma cruzi* by eosinophil major basic protein. *Am J Trop Med Hyg* 1981;30:775-9.
 7. Lehrer RI, Szklarek D, Barton A, Ganz T, Hamann KJ, Gleich GJ. 1989. Antibacterial properties of eosinophil major basic protein and eosinophil cationic protein. *J Immunol* 1989;142:4428-34.
 8. Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. *J Immunol* 1979;123:2925-27.
 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. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis* 1989;139:801-5.
 11. Frigas E, Gleich GJ. The eosinophil and the pathophysiology of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:527-37.
 12. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470-3.
 13. 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.
 14. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 1982;2:11-6.
 15. Weiler JM, Edens RE, Gleich GJ. Eosinophil granule cationic proteins regulate complement. I: activity on the alternative pathway. *J Immunol* 1992;149:643-8.
 16. 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* 1991;157:1981-91.
 17. 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.
 18. Moy JN, Gleich GJ, Thomas LL. Nontoxic activation of neutrophils by eosinophil granule major basic protein: effect on superoxide anion generation and lysosomal enzyme release. *Immunology* 1990;145:2626-32.
 19. Rohrbach MS, Wheatley CL, Slifman NR, Gleich GJ. Activation of platelets by eosinophil granule proteins. *J Exp Med* 1990;172:1271-4.
 20. Thomas LL, Zheutlin LM, Gleich GJ. Pharmacological control of human basophil histamine release stimulated by eosinophil granule major basic protein. *Immunology* 1989;66:611-5.
 21. Barker RL, Gleich GJ, Pease LR. Acidic precursor revealed in human eosinophil granule major basic protein cDNA. *J Exp Med* 1988;168:1493-8.
 22. McGrogan M, Simonsen C, Scott R, et al. Isolation of a complementary DNA clone encoding a precursor to human eosinophil major basic protein. *J Exp Med* 1988;168:2295-308.
 23. Barker RL, Gundel RH, Gleich GJ, Checkel JL. Acidic polyamino acids inhibit eosinophil granule major basic protein toxicity: evidence of a functional role for proMBP. *J Clin Invest* 1991;88:798-805.
 24. Yoshimatsu K, Ohya Y, Shikata Y, et al. Purification and cDNA cloning of a novel factor produced by a human T-cell hybridoma: sequence homology with animal lectins. *Mol Immunol* 1992;29:537-46.
 25. Oxvig C, Sand O, Kristensen T, Gleich GJ, Sottrup-Jensen L. Circulating human pregnancy-associated plasma protein-A (PAPP-A) is disulfide bridged to the proform of eosinophil major basic protein (MBP). *J Biol Chem* 1993;268:12243-6.
 26. Wasmoe TL, Bell MP, Loegering DA, Gleich GJ, Prendergast FG, McKean DJ. Biochemical and amino acid sequence analysis of human eosinophil granule major basic protein. *J Biol Chem* 1988;263:12559-63.
 27. Fischkoff SA, Pollak A, Gleich GJ, Testa JR, Misawa S, Reber TJ. Eosinophilic differentiation of the human promyelocytic leukemia cell line, HL-60. *J Exp Med* 1984;160:179-96.
 28. Sottrup-Jensen L. Determination of the half cystine in proteins as cysteine from reducing hydrolyzates. *Biochem Mol Biol Int* 1993;30:789-94.
 29. Maddox DE, Butterfield JH, Ackerman SJ, Coulam CB, Gleich GJ. Elevated serum levels in human pregnancy of a molecule immunochemically similar to eosinophil granule major basic protein. *J Exp Med* 1983;158:1211-26.
 30. Maddox DE, Kephart GM, Coulam CB, Butterfield JH, Benirschke K, Gleich GJ. Localization of a molecule immunochemically similar to eosinophil major basic protein in human placenta. *J Exp Med* 1984;160:29-41.
 31. Wasmoe TL, Coulam CB, Leiferman KM, Gleich GJ. Increases of plasma eosinophil major basic protein levels late in pregnancy predict onset of labor. *Proc Natl Acad Sci USA* 1987;84:3029-32.
 32. Wagner JM, Bartemes K, Vernof K, Dunnette S, Offord KP, Checkel JL, Gleich GJ. Analysis of pregnancy associated major basic protein levels throughout gestation. *Placenta* 1993;14:671-81.
 33. Wasmoe TL, McKean DJ, Benirschke K, Coulam CB, Gleich GJ. Evidence for eosinophil granule major basic protein in human placenta. *J Exp Med* 1989;170:2051-63.
 34. Bischof P. Purification and characterization of pregnancy associated plasma protein A (PAPP-A). *Arch Gynecol* 1979;227:315-26.
 35. Sutcliffe RG, Kukulska-Langlands BM, Coggins JR, Hunter JB, Gore CH. Studies on human pregnancy-associated plasma protein A: purification by affinity chromatography and structural comparisons with alpha 2-macroglobulin. *Biochem J* 1980;191:799-809.
 36. Davey MW, Teisner B, Sinosich M, Grudzinskas JG. Interaction between heparin and human pregnancy-associated plasma protein A (PAPP-A): a simple purification procedure. *Anal Biochem* 1983;131:18-24.
 37. Sinosich MJ, Sim RB, Teisner B. Characterization of pregnancy-associated plasma protein-A: comparison with alpha 2-macroglobulin. *Biochem Int* 1990;20:579-89.

38. Folkersen J, Grudzinskas JG, Hindersson P, Teisner B, Westergaard JG. Pregnancy-associated plasma protein A: circulating levels during normal pregnancy. *Am J Obstet Gynecol* 1981;139:910-4.
39. Westergaard JG, Teisner B, Grudzinskas JG. Serum PAPP-A in normal pregnancy: relationship to fetal and maternal characteristics. *Arch Gynecol* 1983;233:211-5.
40. Lin TM, Halbert SP. Placental localization of human pregnancy-associated plasma proteins. *Science* 1976;193:1249-52.
41. Tornehave D, Chemnitz J, Teisner B, Folkersen J, Westergaard JG. Immunohistochemical demonstration of pregnancy-associated plasma protein A (PAPP-A) in the syncytiotrophoblast of the normal placenta at different gestational ages. *Placenta* 1984;5:427-31.
42. Popken-Harris P. Eosinophil granule major basic protein precursor (proMBP), expression, maturation, purification, and biochemical characterization. PhD Thesis, 1994.
43. Abu-Ghazaleh RI, Gleich GJ, Prendergast FG. Interaction of eosinophil granule major basic protein with synthetic lipid bilayers: a mechanism for toxicity. *J Membrane Biol* 1992;128:153-64.
44. Bonno M, Kephart GM, Carlson C, Loegering D, Vernof K, Gleich GJ. Expression of eosinophil granule major basic protein mRNA in placental X cells. *Lab Invest* 1994 (in press).

Cytokines and allergy

**Mathew Vadas, MBBS, PhD, Angel Lopez, MBBS, PhD,
Jennifer Gamble, MSc, Yeesim Khew-Goodall, PhD,
William Smith, MBBS, Christophe Bernard, MD, Gillian Cockerill, PhD,
Peter Cockerill, PhD, Frances Shannon, PhD, Qiyu Sun, MBBS,
Eija Korpelainen, BSc(Hons), Cameron van den Heuvel, BSc(Hons), and
Timothy Hercus, BSc(Hons) Adelaide, South Australia**

The survival and state of activation of eosinophils and basophils depend on peptide growth factors or cytokines. Many of the cytokines that activate the function of mature cells also stimulate the proliferation and differentiation of their precursors and thus act in a different manner along the whole lineage.

FUNCTION OF CYTOKINES

Cytokines act on eosinophils and basophils by binding to specific receptors. In the case of hemopoietic growth factors (including interleukin [IL]-3, granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-5) the receptors belong to the cytokine receptor superfamily. These are either heterodimers or homodimers, but the latter appear to act as functional heterodimers because the same chain binds different parts of a ligand and interacts asymmetrically. In each instance

Abbreviations used

GM-CSF: Granulocyte-macrophage colony-stimulating factor
IL: Interleukin
MAb: Monoclonal antibody
R α : Alpha-chain receptor
TNF: Tumor necrosis factor

there appears to be a specific binding of the ligand to one of the chains of the homodimer or the α chain of the heterodimer receptor and then to the second or the β chain, conferring a higher affinity of binding and signaling capacity to the complex (Table I).

Hemic cells typically display a small number of high-affinity receptors (usually a few hundred) of which occupancy of 10% to 20% is sufficient for signaling. Because IL-3, GM-CSF, and IL-5 share a common β chain, there is no additive activation by a combination of these cytokines. However, typically, strong synergistic effects are seen with cytokines of different receptor families, which act in concert on a given cell type.

Regulation of cytokine production

An important level of control of cytokine effects is at the level of production. Cells have a specific repertoire of cytokines that can be produced (Table II), and because most act in a

From the Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science.

Supported by the National Health and Medical Research Council and The National Institutes of Health (grant No. CA45822).

Reprint requests: Mathew Vadas, MBBS, PhD, Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, P.O. Box 14, Rundle Mall, Adelaide, South Australia 5000, Australia.

J ALLERGY CLIN IMMUNOL 1994;94:1289-93.

Copyright © 1994 by Mosby-Year Book, Inc.

0091-6749/94/\$3.00 + 0 1/0/59918