ELEVATED SERUM LEVELS IN HUMAN PREGNANCY OF A MOLECULE IMMUNOCHEMICALLY SIMILAR TO EOSINOPHIL GRANULE MAJOR BASIC PROTEIN*

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In 1956 Gansler (1) observed infiltration of the rat uterus by eosinophils coincident with the estrus cycle. This observation was confirmed and extended by the work of Bassett (2) and Ross and Klebanoff (3). Since then, various investigations have shown (*a*) that injection of estrogen into castrated or immature rodents leads to immediate uterine eosinophilia (4–10), (*b*) that the uterine eosinophil is marrow derived (11), (*c*) that the uterine eosinophil number varies >100-fold during the normal estrus cycle (12), (*d*) that uterine content of eosinophil peroxidase varies directly with the estrus cycle (13, 14), (*e*) that eosinophils possess a unique cell surface estrogen receptor (4–9), and (*f*) that estrogen-induced uterine eosinophilia apparently has no dependence upon uterine mast cell activity (15–18).

In contrast, there are few observations relating eosinophils to human reproductive physiology. Cyclic eosinopenia correlating with ovulation has been reported (19–21) and cyclic variations in endometrial eosinophils and their uptake of tritiated estradiol have also been observed (22). However, no role for the eosinophil in normal human reproduction is presently recognized. During the course of studies of hypersensitivity diseases in pregnancy we found that serum levels of a molecule immunochemically similar to the eosinophil granule major basic protein (MBP)[1] were elevated in all pregnant women, increasing during pregnancy and decreasing to normal levels after parturition. Because eosinophilia is not a feature of normal pregnancy, and serum levels of other eosinophil proteins are not elevated, the results of this study suggest that the immunoreactive MBP in pregnancy serum is derived from a source other than the eosinophil.

Materials and Methods

Dithiothreitol, iodoacetamide, equine liver catalase, sodium borate, boric acid, *Staphylococcus aureus* protein A, chloramine T, protamine sulfate, bovine serum albumin, Trizma

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[1] *Abbreviations used in this manuscript:* CLC, Charcot-Leyden crystal; DARIA, double antibody radioimmunoassay; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; MBP, major basic protein; PPF buffer, protamine phosphate fetal calf serum buffer; TSIRA, two-site immunoradiometric assay.

base, and sucrose were purchased from Sigma Chemical Co., St. Louis, MO. Purified human chorionic gonadotrophin and purified beta subunit of human chorionic gonadotrophin were gifts from Dr. R. Ryan, Mayo Clinic. Purified, radiolabeled human prolactin was a gift from Dr. N. Jiang, Mayo Clinic. Sephadex G-50 and Sepharose 6B-CL were from Pharmacia Fine Chemicals, Piscataway, NJ. Iodine-131 and iodine-125 (carrier free) were protein iodination grade from New England Nuclear, Boston, MA. Microelisa 96-well flexible plastic plates were from Dynatech Laboratories, Inc., Alexandria, VA. Gamma counting was performed on either a Nuclear Chicago gamma spectrometer or a gamma counter (Tracor Analytic Inc., Elk Grove Village, IL). Data were analyzed on a model 9845B computer (Hewlett-Packard Co., Palo Alto, CA).

Double Antibody Inhibition Radioimmunoassay (DARIA) for MBP, Eosinophil Cationic Protein (ECP), Eosinophil-derived Neurotoxin (EDN), and Charcot-Leyden Crystal Protein (CLC). DARIA for MBP and CLC have been described (23–25). ECP and EDN were assayed by the same method. Briefly, human ECP and CLC (lysophospholipase [26]) were isolated and purified as described elsewhere (25),[2] and rabbit antisera to the individual proteins were prepared. Proteins were radiolabeled with ^{131}I by the chloramine T method (27), and in preliminary experiments the dilution of antiserum required to bind 50% of radiolabeled protein was determined. The assay was performed as follows: (*a*) assay buffer (0.1 M Na_2HPO_4-KH_2PO_4, pH 7.4, made 1 mg/ml in protamine sulfate and 0.5% vol/vol in either fetal calf or newborn calf serum [PPF buffer]), 500 μl, was added to 100 μl of test sample and 100 μl of the appropriate dilution (1:1,000–1:10,000) of rabbit antiserum; (*b*) after incubations of 30 min at 37°C and 15 min at 4°C, 1 ng of ^{131}I-labeled MBP, ECP, EDN, or CLC in 100 μl PPF buffer was added, and the tubes were incubated overnight at 4°C; and (*c*) immune complexes were precipitated by the addition of 100 μl of a 1:20 dilution of normal rabbit serum, followed by 200 μl of neat burro anti-rabbit IgG antiserum. After 3 h of incubation at 4°C, precipitation was complete and the tubes were centrifuged for 20 min at 2,500 *g*. The supernatants were decanted and radioactivity in the precipitates was counted. Controls included tubes to determine total counts added and tubes in which specific antibody was omitted to determine nonspecific trapping of counts. Normal serum and serum from a patient with the hypereosinophilic syndrome were included in all assays as internal standards. Radioimmunoassay data were analyzed using a radioimmunoassay program (HP No. 9845-14254) from Hewlett-Packard Co. In those instances where human serum was tested in the guinea pig MBP DARIA (23), performance of the assay was exactly the same as described above.

Two-Site Immunoradiometric Assay for MBP. In the two-site immunoradiometric assay (TSIRA) (28), specific rabbit IgG antibody was bound to a plastic surface and incubated with MBP-containing solutions; bound MBP was detected by radiolabeled affinity-purified antibody to MBP. The IgG fraction of anti-MBP antiserum for coating plates was prepared by equilibrating rabbit anti-human MBP serum with pH 7.8, 0.1 M phosphate buffer on a Sephadex G-25 column. Void volume fractions were pooled and applied to a staphylococcus protein A-Sepharose 4B column. After washing, bound IgG was rapidly eluted with 0.58% acetic acid into tubes containing pH 8.4, 0.4 M Na_2HBO_4-NaH_2BO_4 buffer. Elution was monitored by absorbance at 277 nm, and fractions with peak absorbance were pooled and diluted with 0.002 M, pH 8.2 phosphate buffer.

Microelisa flexible plastic plates (well volume, 0.3 ml) were coated with 100 μl per well of anti-MBP IgG, 3.2 mg/ml, in pH 8.2, 0.001 M phosphate buffer (coating buffer) by overnight incubation at 4°C. Remaining protein-binding sites on the plastic were blocked by incubation with coating buffer made 10 mg/ml in bovine serum albumin, 200 μl per well, for 6 h at room temperature. Wells were aspirated, washed with coating buffer, and stored at 4°C.

The second stage of the assay used anti-human MBP antibody purified by affinity chromatography. Reduced and alkylated MBP (3.8 mg) was coupled to cyanogen bromide-activated Sepharose 6B-CL (8 ml swollen gel) as described (29), and a column was poured.

[2] Ackerman, S. J., D. A. Loegering, P. Venge, I. Olsson, J. B. Harley, A. S. Fauci, G. J. Gleich. Distinctive cationic proteins of human eosinophil granule: major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin. Manuscript submitted for publication.

Immune rabbit serum (25 ml) was applied to the column, the solid phase was washed with citrate-phosphate buffer, pH 7.4, and bound protein was eluted with a linear gradient using a pH 2.2 citrate-phosphate limit buffer. Fractions were collected into 0.2 M, pH 8.4 phosphate buffer for immediate neutralization. Elution was monitored by absorbance at 277 nm, and tubes from the final absorbance peak at pH 2.8 were pooled and shown to possess anti-MBP activity in a modified DARIA. The affinity-purified antibody to MBP was radioiodinated by the iodine monochloride method of Bale et al. (30) as modified for microtechnique (31), and specific activities of 12–15 mCi/mg protein were typically obtained.

The assay was performed by adding 100 μl of purified reduced and alkylated MBP, diluted serially in PPF buffer, to antibody-coated wells for a standard binding curve. Reduced and alkylated samples, 100 μl, were dispensed into wells for analysis. Nonspecific binding was measured in wells receiving only PPF buffer. Plates were incubated overnight at 4°C in a humidified chamber and wells were aspirated and washed thrice with coating buffer. Radiolabeled affinity-purified antibody, diluted in PPF buffer to ~20,000 cpm/100 μl, was added to each well. Plates were incubated for 4–6 h at room temperature; the wells were aspirated, washed 10 times with coating buffer made 1% in Tween 20, dried, and cut out for gamma counting. All samples were assayed in duplicate, and before data analysis, counts in control wells containing only PPF were subtracted.

Reduction and Alkylation of Serum. Measurement of maximal MBP levels in serum requires reduction and alkylation before assay (24). Therefore, before MBP measurement by the DARIA or the TSIRA, sera were reduced and alkylated unless otherwise specified. 50 μl of serum was diluted with 130 μl 0.1 M Tris-hydroxymethylaminomethane, 0.12 M NaCl, 0.01 M EDTA pH 8.2 buffer; dithiothreitol solution (0.075 M) was added to a final concentration of 0.0075 M. After 60 min incubation at room temperature, alkylation was accomplished by the addition of sufficient 0.15 M iodoacetamide to achieve a final concentration of 0.015 M. Samples were incubated for 20 min at room temperature and assayed immediately.

Gel Filtration of Sera. A 1.6 × 50-cm column of Sephadex G-50 fine was poured and equilibrated with acid-saline (0.01 M HCl, 0.15 M NaCl, pH 2). Before chromatography, serum or clotted plasma was reduced, alkylated (see above), and acidified to pH 2 by dropwise addition of 2 M HCl with vigorous mixing. Acidification resulted in the formation of precipitate, and samples were clarified by centrifugation at 40,000 *g* for 40 min before application to the column. Samples were made 5–10% in sucrose and applied under the eluent to the column. The column was eluted with acid saline, at 5 ml · $cm^{-2}hr^{-1}$ at 4°C, and 2.7-ml fractions were collected. Elution was monitored by absorbance at 277 nm. After neutralization in PPF buffer, a sample of each fraction was assayed for MBP in the TSIRA.

Clinical Specimens. 413 serum samples from 397 pregnant women in various stages of gestation were available from a previous unrelated study. Cord serum and amniotic fluid were available from the obstetrical unit at the Rochester Methodist Hospital, Rochester, MN. Paired serum samples were obtained from six women at delivery and at 6 wk postpartum. One woman volunteered serum samples at monthly intervals throughout gestation, and after a second gestation, specimens were obtained every other day for 6 wk postpartum.

Statistical Analyses. Statistical analyses were performed using the regression analysis (HP 09845-15031), analysis of covariance (HP 09845-15171), general statistics (HP 09845-15011), and statistical graphics (HP 09845-15021) programs from Hewlett-Packard Co.

Results

MBP Levels in Pregnancy and Cord Sera. Table I shows MBP levels in sera from pregnant women, paired cord sera, and control sera. MBP levels in the pregnancy sera were >15-fold above normal levels, similar to values found in patients with the hypereosinophilic syndrome. In contrast, cord serum contained normal levels

TABLE I
Measurement of Serum MBP by DARIA

Condition	Patient	Sample source	MBP
			ng/ml
Pregnancy	1	Maternal*	12,671
		Cord	468
	2	Maternal	10,721
		Cord	428
	3	Maternal	12,160
		Cord	488
	4	Maternal	11,521
		Cord	610
	5	Maternal	8,818
		Cord	494
Hypereosinophilic syndrome	1		13,600
	2		14,400
	3		7,200
	4		8,172
Normal‡			415

* The maternal sera were obtained during labor.
‡ Normal serum included as internal standard. MBP levels in 105 normal blood bank donor sera had a mean of 454 ng/ml and a range of 312–800. 95% of values were <600 ng/ml (24).

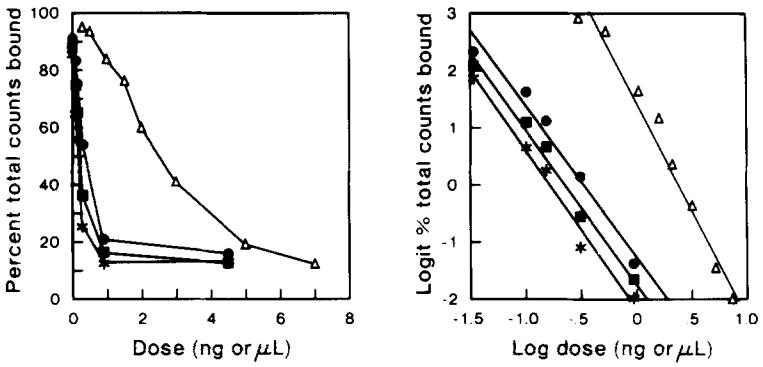

FIGURE 1. Comparison of dose-response curves for pregnancy sera (*, ●, ■) and purified MBP (△) in the DARIA. (*A*) Untransformed data, (*B*) the same data after logit-log transformation. Linear regression analysis showed $r^2 = 0.98$ for purified MBP and a mean r^2 of 0.97 (range 0.96–0.99) for the pregnancy sera. One-way analysis of covariance indicated that the slopes for titrations of pregnancy sera differ from that of pure MBP ($F_{3,15} = 5.86$, $P < 0.01$).

of MBP. Amniotic fluid obtained from one patient during the first stage of labor contained 971 ng/ml of MBP.

Specificity of the Immunochemical Detection of MBP. To determine whether MBP in pregnancy sera was immunochemically similar to purified MBP, we titrated three such sera in the DARIA. Fig. 1 shows that the slopes of the regression lines for the pregnancy sera and MBP standard were quite similar, though not identical, and the pregnancy sera completely inhibited the binding of ^{131}I-MBP

to antibody. Table II shows that pregnancy sera required reduction and alkylation to demonstrate maximal MBP immunoreactivity, as is the case for MBP in sera of patients with eosinophilia. Previous experiments have shown that only 1% of MBP added to serum is recovered in the DARIA if the serum is not reduced and alkylated before assay (24). Table II also shows that the heat lability of MBP in pregnancy serum depended upon antecedent reduction and alkylation, as is the case for MBP in sera of patients with eosinophilia. Heating of pregnancy serum without prior reduction and alkylation did not alter immunoreactivity, in keeping with prior findings (24).

A number of pregnancy-associated serum proteins have been described (32–34), and we investigated whether an acidic pregnancy-associated protein might compete for the basic radiolabeled MBP in the inhibition assay. This would result in inhibition in the DARIA and lead to a spurious MBP elevation. We tested this in two ways: (*a*) by assaying pregnancy sera in the guinea pig MBP DARIA, and (*b*) by developing a TSIRA that detects MBP by direct multivalent ligand binding rather than inhibition. Although human and guinea pig MBP are quite similar in their physicochemical properties, our antisera to these proteins do not appreciably cross-react (24). If a hypothetical acidic pregnancy-associated protein were competing for labeled MBP on the basis of charge, then the species of origin of the radiolabeled MBP should have no influence, and an analysis of pregnancy sera in the guinea pig MBP assay should show elevated levels of guinea pig MBP. Table III shows that pregnancy sera failed to show any inhibition in the guinea pig assay. In the case of the TSIRA, the hypothetical acidic pregnancy-associated

TABLE II

*Effect of Reduction, Alkylation, and Heating on MBP in Pregnancy Sera**

Test specimen	Treatment	MBP
		ng/ml
Pregnancy serum 1	None	51
	Reduced & alkylated	5,914
Pregnancy serum 2	None	36
	Reduced & alkylated	4,805
Pregnancy serum 3	Reduced & alkylated	5,425
	Reduced & alkylated, then heated at 56°C for 3 h	1,945
	Heated at 56°C for 3 h, then reduced & alkylated	5,431
Hypereosinophilic syndrome serum	Reduced & alkylated	5,592
	Reduced & alkylated, then heated at 56°C for 3 h	1,313
	Heated at 56°C for 3 h, then reduced & alkylated	6,982
MBP (500 ng)	None	500
	Heated at 56°C for 3 h	0

* Test substances were reduced and alkylated as described in Methods and analyzed by the DARIA.

TABLE III

Detection of MBP in Pregnancy Sera by DARIA for Human and Guinea Pig MBP

Serum	Patient	Week of gestation	Human MBP	Guinea pig MBP
			ng/ml	
Pregnancy	1	10	2,794	7
	2	13	2,350	11
	3	15	3,454	7
	4	11	2,303	12
Hypereosinophilic syndrome	1		13,600	9
	2		14,400	11
	3		7,200	13
	4		8,172	33

TABLE IV

Measurement of MBP in Pregnancy and Normal Sera by the TSIRA

Serum	Patient	MBP
		ng/ml
Pregnancy*	1	4,268
	2	4,487
	3	4,297
	4	4,242
	5	4,428
	6	4,063
	7	4,643
	8	4,429
	9	4,512
	10	4,122
	11	4,457
	12	4,424
	13	3,937
Hypereosinophilic syndrome		8,040
Normal‡		412

* Mean level, 4,331 ng/ml ± 200 (1 SD).

‡ Assay of 40 normal blood bank donor sera (20 males, 20 females) by the TSIRA gave a mean level of 449 ± 177 (1 SD); there was no statistically significant difference between levels in normal males and normal females.

protein would diminish serum MBP. As shown in Table IV, pregnancy sera showed elevated levels of MBP in the TSIRA. Fig. 2 shows that the slopes of the dose-response curves for normal, hypereosinophilic, and pregnancy sera in the TSIRA were the same and did not differ from purified MBP. The maximal binding obtained with pregnancy serum was also similar to that obtained using serum from a patient with the hypereosinophilic syndrome.

Prior studies found a correlation between the serum levels of MBP and peripheral blood eosinophilia (35, 36). Therefore, we obtained simultaneous

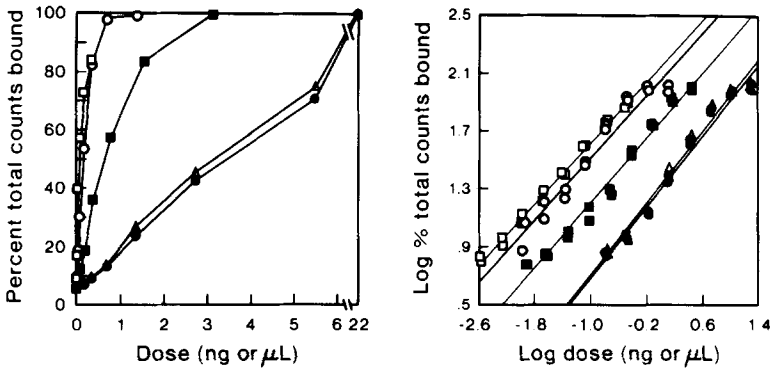

FIGURE 2. Comparison of dose-response curves for pregnancy serum and MBP in the TSIRA. Pregnancy serum (□), hypereosinophilic syndrome serum (○), normal serum (△), purified MBP (■) and blood bank normal donor serum (●). (*A*) Untransformed data, (*B*) the same data after log-log transformation. Linear regression analysis showed $r^2 = > 0.97$ for all curves. One-way analysis of covariance indicated that slopes of all regressions do not differ ($F_{4,31} = 1.31$; not significant).

TABLE V

*Correlation of MBP Levels and Blood Eosinophil Counts**

Gravid patient	MBP‡	Week of gestation	Eosinophils per microliter§
	ng/ml		
1	872	10	116
2	1,028	10	15
3	1,375	12	64
4	1,271	13	41
5	3,401	17	163
6	5,127	23	42
7	9,002	33	31
8	10,655	33	63
9	6,850	35	150

* There was no correlation between MBP levels and eosinophil counts ($r = 0.15$, $F_{1,7} = 0.164$; not significant).

‡ Pregnancy plasma was analyzed in the DARIA.

§ Based on an automated 10,000 cell differential count.

plasma MBP levels and peripheral blood eosinophil counts from nine women, three in each trimester of pregnancy. However, as shown in Table V, there was no correlation between MBP levels and eosinophil counts. Prior studies also found a positive correlation between serum levels of MBP and CLC protein (lysophospholipase) (35, 36). Therefore, we determined whether the levels of CLC and two other eosinophil-associated proteins, ECP and EDN (37–39), were elevated in pregnancy serum. Table VI shows that CLC levels were not elevated, and that the levels of ECP and EDN in the pregnancy sera were depressed compared with normal sera ($P < 0.001$ in both cases). In contrast, all four of the eosinophil proteins were elevated in the serum of a patient with the hypereosinophilic syndrome.

Finally, we found no inhibition by human chorionic gonadotrophin or its beta

TABLE VI

*Measurement of Eosinophil-associated Proteins in Pregnancy Sera by DARIA**

	MBP	CLC	ECP	EDN
		ng/ml		
Pregnancy‡	4,271 ± 2,411	45 ± 19	9 ± 5	20 ± 11
(Range)	(1,344–8,036)	(23–80)	(3–21)	(8–49)
Hypereosinophilic syndrome	7,814	7,420	411	8,916
Normal blood donors§	454 ± 90	41 ± 37	18 ± 9‖	74 ± 53
(Range)	(312–800)	(8–199)	(5–40)	(17–191)

* Values are reported as $\bar{x} \pm 1$ SD.

‡ A group of 20 consecutive pregnancy sera were selected from the larger group of 413 (see Fig. 3) for assays of MBP, ECP, and EDN. The CLC assays were performed on 12 other arbitrarily chosen sera from the group of 413.

§ Normal values for MBP and CLC are those previously published (24, 25); values for ECP and EDN are for 20 normal female blood bank donors.

‖ Levels reported here for ECP in normal sera are quite similar to those reported by Venge et al. (40): mean, 31 ng/ml; range 5–55 ng/ml.

subunit in the DARIA, or binding activity in the TSIRA. Also, purified MBP and serum from a patient with the hypereosinophilic syndrome failed to show activity in a radioimmunoassay for human chorionic gonadotrophin (41). Purified radiolabeled prolactin was not bound by anti-MBP antibody.

Changes in MBP Levels During and After Pregnancy. The above results established that MBP was elevated in sera of pregnant women. We next determined the relationship between the elevation of MBP and the time of gestation. Fig. 3 shows the MBP levels in 413 sera according to the week of gestation; MBP was elevated (>600 ng/ml) in all sera. MBP levels were elevated early in gestation, peaked rapidly to about a 10-fold elevation above normal by the end of the first trimester, and remained relatively close to that level for the remainder of gestation. Analysis of the relationship between MBP level and time showed a significant positive correlation ($r^2 = 0.64$, $F = 86.9$, $P < 0.001$).

In one pregnant woman, MBP was measured in serial samples obtained throughout gestation. Fig. 4 shows that MBP levels were elevated by week 12 (when the diagnosis of pregnancy was first made) and remained between 6,000 and 8,000 ng/ml until after week 34, when they increased to a maximum of almost 14,000 ng/ml. When the MBP levels from the patients in Fig. 3 were examined for a similar near-term rise, a suggestive trend was observed; however, mean MBP levels at 37 and 41 wk were not statistically different.

To determine the change in MBP levels after pregnancy, we analyzed serum samples from six women on the day of delivery and at 6 wk postpartum in the DARIA. Levels fell from a mean of 11,732 ± 2215 ng/ml (1 SD) on the day of delivery to a mean of 761 ± 150 ng/ml (1 SD) by the 6th wk. Fig. 5 shows serum MBP levels after parturition in one patient. The level dropped sharply in the 2 wk after delivery, with a calculated half-time of 13.7 d.

Behavior of MBP in Pregnancy Sera on Gel Filtration. Previous work demonstrated that gel permeation chromatography of serum that has been reduced,

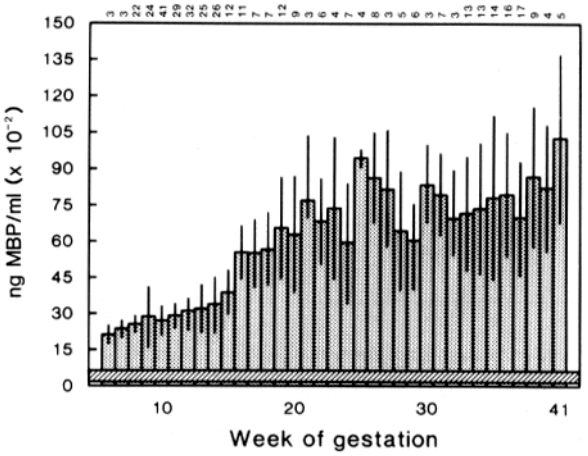

FIGURE 3. Time-related changes of serum MBP during gestation. Arithmetic mean values for MBP levels were analyzed by the DARIA in 413 serum samples from 397 women according to the week of gestation. Data from weeks 32 and 33 are pooled. The horizontal shading across the bottom indicates the 5th to 95th percentiles for MBP levels in normal subjects, and the verticle error bars denote 1 SD. No samples before the 5th wk of gestation were available for analysis. The numbers of samples analyzed in each week are given at the top of the graph. Week of gestation was determined by back-calculation from the date of delivery, taking into account the pediatrician's estimate of the gestational age of the delivered infant. Polynomial regression on all the data together with residual analysis identified cases in which MBP levels were >2 SD from the equation for the line; medical records for all cases were reviewed, and no relationship was found between extreme levels of MBP and complications of gestation.

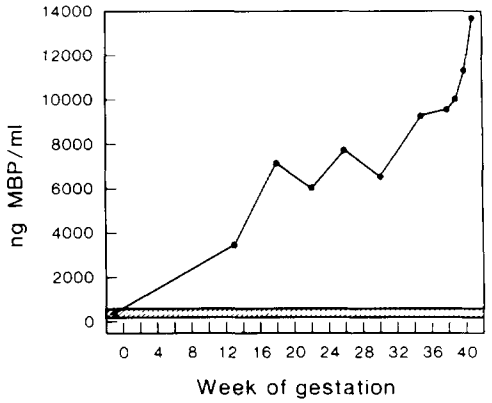

FIGURE 4. Serial determinations of serum MBP in a gravida 1, para 0 pregnant woman by the DARIA. The first data point represents a serum specimen before conception. The shaded area at the bottom of the plot represents the 5th to 95th percentiles for MBP levels in the normal population.

alkylated, and acidified results in the resolution of MBP at an elution volume corresponding to the molecular weight (9,300) of monomer MBP (24). We tested the ability of Sephadex G-50 gel chromatography to resolve such an MBP peak from pregnancy sera. Fig. 6 shows that under conditions in which serum MBP from a patient with hypereosinophilic syndrome eluted at the expected volume for monomeric MBP, the MBP in pregnancy serum eluted at the void volume of

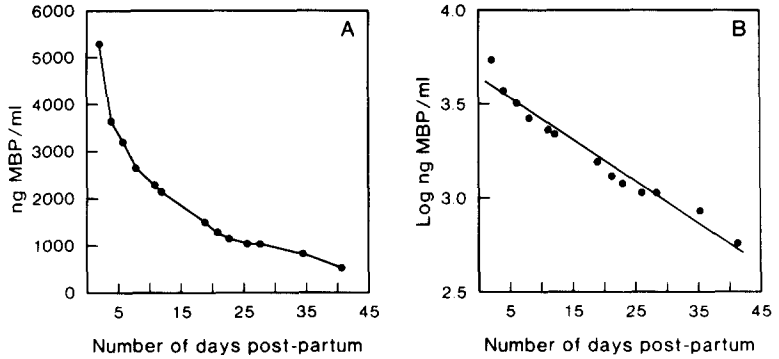

FIGURE 5. Postpartum changes in gestational MBP levels. Data are from sera of a single subject tested in the DARIA. (*A*) Level vs. time, (*B*) linear regression of log-transformed levels vs. time. For this regression, r = −0.98; the line is described by the equation y = 0.22 x + 3.65. T½ for MBP was 13.7 d.

the column. Six separate experiments, using six different pregnancy sera, gave identical results.

Discussion

The ultrastructural localization of eosinophil granule MBP within the eosinophil, its physicochemical properties, and its biological activities have been reviewed elsewhere (42, 43). Briefly, human MBP is a low molecular weight (9,300), extremely basic polypeptide (isoelectric point >10) that, in the guinea pig, forms the electron-dense crystalloid core of the eosinophil-specific granule. It contains at least two sulfhydryl groups and it readily self-associates through disulfide linkages to form polymeric molecules. In biological fluids, such as serum and sputum, MBP is protein bound, and these fluids must be reduced and alkylated to demonstrate maximal immunoreactivity. MBP is elevated in the sera of patients with eosinophilia and in the sputum of patients with asthma. MBP is toxic to several helminths, normal mammalian cells, and tracheal epithelium. Further, immunofluorescence examinations of parasites in tissues reveal large quantities of MBP localized around and on the surface of the parasites (44). Similar examinations on lung tissues from asthmatic patients reveal MBP as a prominent component of the mucus plugs that obstruct airways (45). MBP has therefore been postulated as a mediator of eosinophil killing of parasites and of the respiratory epithelial damage observed in bronchial asthma. Accordingly, the finding of elevated serum levels of an immunoreactive form of this toxic molecule during pregnancy was unanticipated.

We found elevations in serum MBP during human gestation, with levels correlated with the stage of gestation. At the earliest time measured (5 wk), levels were already elevated more than threefold above the upper normal limit (600 ng/ml). As gestation progressed, levels increased until a plateau of 7,500 ng/ml was reached at about the 20th wk of pregnancy. The immunochemical specificity of serum MBP elevation was tested in several ways. In the DARIA, pregnancy serum could fully displace radiolabeled MBP from specific antibody. In the TSIRA, pregnancy serum displayed maximal binding comparable to that

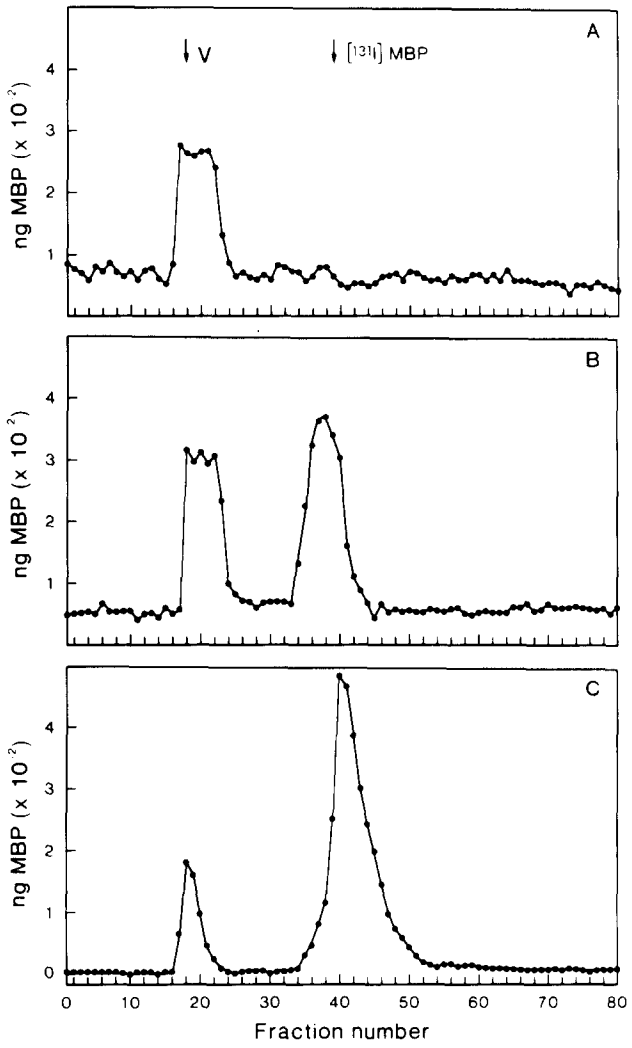

FIGURE 6. Gel-permeation chromatography of pregnancy serum MBP. A single column of Sephadex G-50 fine gel equilibrated with 0.15 M NaCl/0.01 M HCl was used. The arrow marked "v" denotes column void volume and the other arrow indicates the elution volume for pure radiolabeled MBP. (*A*) Elution profile when reduced and alkylated, acidified pregnancy serum was chromatographed, and resultant fractions were neutralized and assayed for MBP by the TSIRA. Under these conditions, recovery of MBP (based on that in the initial serum sample) was quantitative. (*B*) Elution profile for pregnancy serum prepared as in *A* except with 4 μg of purified reduced and alkylated MBP added; fractions were analyzed as in *A*. (*C*) Elution profile for serum (treated as in *A*) from a male with the hypereosinophilic syndrome.

obtained with serum from patients with hypereosinophilic syndrome, and the slopes of pregnancy serum and pure MBP standard dose-response curves were identical. Further, the same pattern of heat lability and an absolute requirement for reduction and alkylation before immunochemical detection was shown for the molecule in pregnancy sera, in keeping with prior results for MBP in serum from patients with eosinophilia (24). We showed that nonspecific factors in

pregnancy serum were not responsible for results obtained in the DARIA, because pregnancy serum did not inhibit detection of guinea pig MBP. We tested for the possibility of cross-reaction among two pregnancy-associated proteins, human chorionic gonadotrophin and prolactin, and MBP, and have found no evidence of such cross-reactivity.

In spite of these lines of evidence supporting the immunochemical identity between the MBP detected in pregnancy sera and the MBP from the eosinophil granule, four anomalous findings have emerged. First, we found no correlation between numbers of eosinophils in peripheral blood and MBP levels in pregnancy serum. Second, measurement of three other eosinophil-associated proteins in pregnancy serum yielded normal or subnormal values, in contrast to the marked elevations of these proteins in serum of patients with eosinophilia. Third, although very similar, the slopes of dose-response curves for pregnancy sera and MBP standard in the DARIA were not identical. Fourth, gel permeation chromatography of pregnancy serum resulted in the elution of MBP immunoreactivity at the void volume of a Sephadex G-50 column, indicating a molecular size greater than ~30,000. These findings suggest that the MBP molecule in pregnancy serum differs from that in the eosinophil granule, and suggests the possibility of a non-eosinophil source for the MBP molecule in pregnancy. For these reasons we have referred to the MBP in pregnancy serum as immunoreactive MBP to indicate that it likely differs from the MBP derived from the eosinophil granule.

The data presented here show that immunoreactive MBP levels in pregnancy increase in a manner similar to the increase in placental size during gestation and decrease following delivery of the placenta. Furthermore, MBP levels in cord sera and amniotic fluid were a great deal lower than in maternal serum. Most of the known pregnancy-associated proteins are produced by the placenta (32–34) so it seemed reasonable to investigate this tissue as a source of the molecule in pregnancy serum. We have recently demonstrated immunoreactive MBP in the human placenta, both by immunofluorescence (46) and chemical extraction.[3] These findings suggest that the MBP detected in human gestation is of placental origin. If so, considerable sequence homology must exist between the eosinophil granule protein and the placental protein because of the remarkable immunochemical similarity between the two. There is ample precedent for multiple tissue sources of various proteins that, through gene conservation, demonstrate major sequence homology with one another (47, 48). However, in most of these instances, a shared function is demonstrable as well. At present, there is no obvious explanation for the placental elaboration of a molecule whose counterpart in the eosinophil appears to function as a helminthotoxin or cytotoxin.

Summary

We have shown that serum levels of a molecule immunochemically similar to eosinophil granule major basic protein (MBP) are elevated in pregnant women throughout gestation. MBP levels increase during gestation and plateau at

[3] Maddox, D. E., G. M. Kephart, J. H. Butterfield, C. B. Coulam, K. Benirschke, and G. J. Gleich. Localization of eosinophil major basic protein in human placenta. Manuscript in preparation.

~7,500 ng/ml by the 20th wk (>10-fold above normal). Levels return to normal after delivery, with a T½ of 13.7 d. The MBP in pregnancy serum is remarkably similar to the eosinophil granule MBP in that: (*a*) pregnancy MBP fully inhibits the binding of radiolabeled MBP standard in a double antibody radioimmunoassay; (*b*) this inhibition reaction is specific for human MBP because pregnancy serum produces no inhibition of the binding of radiolabeled guinea pig MBP in the guinea pig MBP radioimmunoassay; (*c*) in a two-site immunoradiometric assay for MBP, slopes of dose-response curves for pregnancy serum, purified MBP, and serum from a patient with hypereosinophilic syndrome are identical, and maximal binding is comparable; (*d*) reduction and alkylation of pregnancy sera increases measured MBP 100-fold, as previously shown for eosinophil granule MBP in serum; and (*e*) the MBP in pregnancy serum demonstrates the same pattern of heat lability as has been previously reported for MBP.

Four observations have raised the possibility that the eosinophil is not the source of the MBP in pregnancy serum: (*a*) no correlation between serum MBP level and peripheral blood eosinophil count exists in pregnant women, in contrast to previous studies of patients with eosinophilia; (*b*) levels of three other eosinophil-associated proteins are normal or low in pregnancy sera, whereas the serum levels of these proteins are elevated in patients with eosinophilia; (*c*) the slopes of dose-response curves for pregnancy sera and MBP standards differ in the double antibody radioimmunoassay; and (*d*) the molecule in pregnancy serum elutes from Sephadex G-50 columns at the void volume, while eosinophil granule MBP and the MBP in serum of patients with eosinophilia elute at a volume consistent with the previously established molecular weight of 9,300. These findings suggest that the MBP in pregnancy serum is derived from a source other than the eosinophil.

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