In Vitro Defects of Phagocyte Chemotaxis During Pregnancy

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Pregnant women have an increased risk for some infections, particularly during the last trimester. Phagocytic emigration from the circulation into tissues is an important aspect of the initial immune response. Therefore, circulating phagocytes of 42 pregnant and 15 postpartum patients were studied in vitro for random and chemotactic (or directional) migration through membrane filters (Millipore Corp., Bedford, Mass.). Random migration of phagocytes from all 42 pregnant patients studied in each trimester was within normal limits. Chemotactic migration of 25 patients who were between 6 and 33 weeks of pregnancy was also similar to values obtained with control leukocytes (20 nonpregnant, normal females). However, phagocytes of 17 other women studied between week 34 of pregnancy and term showed marked depressions in chemotaxis ($P < 0.001$ from control values). During labor and within 3 days of delivery, chemotactic migration increased to supranormal levels in 14 of 15 women studied. Sera from six pregnant patients with proven chemotactic defects did not reduce migration when incubated with normal phagocytes. These chemotactic defects appear to be intrinsic and may be important in predisposing to infections during late pregnancy.

Most infections during pregnancy affect neither the fetus nor the course of pregnancy, but some (like rubella) can cause profound teratogenic effects (5, 27), or others (such as smallpox and chickenpox) can result in severe natal and maternal infections (8, 20). Pregnant women also appear to be hypersusceptible to influenzal infections. During the Asian influenza epidemic of 1957, 50% of women of childbearing age who died from influenza, and particularly bacterial superinfections, were pregnant (1, 7, 10). Even in this antibiotic era, postpartum bacterial infections, such as in the urinary tract, are an important cause of maternal morbidity (29).

Limited data exist as to the immune status of pregnant females or their incidences of various infections. One model of uterine infections in pregnant animals indicated an enhanced susceptibility to bacterial infections, especially during late gestation (4). The intensity of leukocytic infiltrations into experimentally infected uteri are also less in pregnant than nonpregnant females (12). Deficiencies in phagocytic chemotaxis could be important in determining the early course of infectious challenges. For these reasons, we decided to study phagocytic chemotaxis of healthy, pregnant females.

MATERIALS AND METHODS

Characteristics of patients and controls. Chemotaxis of circulating venous leukocytes from 57 pregnant and postpartum patients was compared with that

of 20 nonpregnant, age-matched female controls. These patients were selected for absences of a personal history of diabetes mellitus, intercurrent infections, and family histories of recurrent infections. Written and informed consent was obtained from all patients and control donors. Patients taking medications other than vitamins or iron supplements were excluded from this study. Gestation in 17 patients ranged between 9 and 20 weeks, and in 8 other patients it ranged between 21 and 33 weeks. Seventeen additional patients ranged between 34 weeks and term, and five patients were in active labor (one patient, labeled B, had been studied at 38 weeks). Ten patients were examined within 3 days of parturition, one of whom had been studied before labor (labeled patient A). Parity of postpartum patients included the present pregnancy. Controls also were not taking any medications.

Migrational assay. In vitro leukocyte chemotaxis was tested by modification of the Boyden method as previously described by Martin et al. (14). In brief, leukocyte-rich plasma was obtained from 25 ml of heparinized venous blood by allowing erythrocytic sedimentation for 1 h in 6% dextran. After the plasma-rich fraction was washed with 0.87% (wt/vol) ammonium chloride (pH 7.4) and centrifuged ($500 \times g$), the cells were suspended in phosphate buffer solution (pH 7.4). Concentrations of segmented and nonsegmented polymorphonuclear leukocytes and monocytes were then adjusted to 5×10^6 phagocytic cells per ml. One-tenth milliliter of this phagocytic suspension was added to 0.25 ml of Hanks balanced salt solution (pH 7.45) after a preincubation for 15 min with autologous or homologous sera. These cells were then deposited by gravity sedimentation onto a 5- μ m filter (SWMP 02500, Millipore Corp., Bedford, Mass.).

A chemotactic field was generated by incubating 100 μ g of lipopolysaccharide B (*Escherichia coli*, O26:B6; Difco Laboratories, Detroit, Mich.) in 1.0 ml of

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normal pooled sera (obtained from four AB blood group, Rh-positive donors) for 30 min at 37°C. One-tenth milliliter of the endotoxin-treated plasma was diluted in 0.9 ml of Hanks balanced salt solution and placed in the bottom of the modified Boyden chamber (10 μ g of endotoxin per ml). Chemotaxis was permitted for 2 h at 37°C in a 5% CO₂ incubator. Chemotaxis in these fields was compared to fields in other chambers which contained no endotoxin but only plasma and Hanks balanced salt solution (random migration). Filters were then removed, fixed, and stained with a hematoxylin dye.

A migrational index (MI) was calculated by multiplying the number of leukocytes found in the leading edge of five ocular fields (high dry objective, X45) by the distance (μ m) this cellular edge had traveled; this product was then divided by 1,000. All assays were always done in duplicate within 3 h of venipuncture; specimens were discarded if this time period was exceeded. Results were averaged for each patient, and data were analyzed by the Student's *t* test, linear regression, and analysis of variance.

RESULTS

Characteristics of the assay system. MIs were computed from leading edges so that both the number of cells and their respective distances traveled in a serum system were considered. These distances were notably consistent in duplicate leukocyte samples run for each patient and control donors and did not vary by more than 15 μ m. The mean migratory distance for control cells was 88 ± 8 μ m (range = 75 to 100 μ m). Phagocytes obtained from all pregnant patients showed a mean migratory distance of 84 μ m plus or minus one standard deviation of 13

μ m (range = 50 to 100 μ m). In particular, the mean of the ranges of migration was 88 μ m for patients who were 0 to 33 weeks pregnant and 80 μ m for women in the last trimester.

Leukocytes from all patients and controls were also studied in chambers which contained no serum of lipopolysaccharide B in the lower chamber. This unstimulated migration probably reflected random migration. Mean distance of random migration for all pregnant patients was 45 ± 15 μ m (range = 20 to 80 μ m), and for control donors it was 49 ± 15 μ m (range equals 30 to 82 μ m). Phagocytes of women in the first two trimesters showed a mean distance of 48 μ m and of 42 μ m in the last trimester.

Differential counts of phagocytes (polymorphonuclear leukocytes and monocytes) contained in the phagocytic suspensions were similar for all patient groups and control subjects.

Effect of duration of pregnancy. Chemotactic activities of phagocytes from pregnant and postpartum patients and nonpregnant controls are shown (Fig. 1). Values of MIs are on the vertical axis, whereas the abscissa is divided into groups for female controls and patients who were studied at 0 to 33 and 34 to 40 weeks of gestation, during labor, and within 3 days of delivery. Twenty control patients had phagocytes with a mean MI of 20.2 ± 4.7 (range = 13.7 to 33.9). Phagocytes from one normal control were studied over 4 consecutive days and showed a mean MI of 20.1 ± 5.2 (range = 15.0 to 25.7). Phagocytes from 25 patients whose gestation was less than 34 weeks showed a mean MI of 18.7 ± 5.8 (range = 9.9 to 35.4). These latter

FIG. 1. MIs during pregnancy, labor, and postpartum phases. A and B indicate patients studied more than once. Δ , A control female who was studied at four different times. Brackets and center dot indicate one standard deviation and the mean for the group, respectively.

values were very similar to those obtained with leukocytes from healthy controls.

Seventeen other patients who were pregnant between 34 weeks and term had lower phagocytic MIs with a mean of 11.3 ± 7.1 (range = 3.5 to 25.3). MIs of this group were significantly lower than MIs of control subjects ($P < 0.001$). Only six of these patients near term had leukocytes with normal values of MIs. Five patients in active labor (without oxytocin administration) had phagocytes which demonstrated a wide range of low, normal, and high MIs (mean = 28.4 ± 17.3 , range = 8.1 to 50.6). Ten postpartum patients were studied within 3 days of delivery. Their leukocytes had a mean MI of 40.3 ± 16.6 (range = 21.7 to 73.0), which was significantly greater than control values ($P < 0.001$).

No associations were found by analysis of variance ($P > 0.4$) between the degree of chemotactic defect and (i) the number of our patients' parity or (ii) if the patient had a positive family history for diabetes mellitus. Ages of our patients and control donors also showed no correlation with the degree of chemotactic defect as determined by linear regression analysis ($n = 78$, $P > 0.1$). Also, no association was seen between the degree of impairment in MI and the number of mature or band-type neutrophils nor the venous leukocyte count before cellular recovery and adjustment.

Effects of sera from pregnant patients. Sera from pregnant patients were added to normal phagocytes to observe if values were caused by serum inhibitors which were not removed by cellular isolation techniques. Samples (0.1 ml) of sera from six pregnant patients with proven, low values of MIs (range = 8.1 to 10.0) were added to 0.9 ml of the phagocyte suspensions obtained from eight normal donors, and 0.25 ml of this mixture was placed in the upper chemotactic chamber. Every patient's serum was tested with phagocytes obtained from three normal healthy donors. Pregnant sera caused little alteration in MIs of these normal phagocytes when compared to MI values found with control sera (by analysis of variance, $F + 0.5$, $P > 0.4$).

DISCUSSION

Several alterations in both cellular and humoral immunity during pregnancy have already been described (2, 18, 21, 24, 28). Circulating phagocytes of some pregnant women show decreased phagocytosis and killing of *E. coli* and *Pseudomonas* (2, 16), and sera from pregnant women can reduce bacterial ingestion of phagocytes from nonpregnant donors (2, 15). Other studies of fixed phagocytes in the reticuloendothelial system have generally demonstrated hy-

perphagocytosis during pregnancy (6, 17, 26). One aspect of acute cellular immunity during pregnancy which has not been fully studied is phagocytic chemotaxis. Defects are suggested by the studies of Persellin et al. which showed that acute inflammation was reduced in the hind paw of pregnant rats after carrageenan injections (19).

Pregnancy or creation of a pseudopregnancy by administering progestational hormones alters acute phases of cellular immunity. Polymorphonuclear leukocyte exudates in uteri of animals given estrogens were less intense than in control animals after induction of bacterial infections (12) or the implantation of nylon sutures (23). Similarly, bacterial elimination after inoculation into uteri of pregnant animals was reduced as compared to clearances seen in control animals.

Our data indicate that significant decreases occur in phagocytic chemotaxis of pregnant women, particularly between 34 weeks of pregnancy and term. These data confirm the recent studies of Björkstén et al., which showed not only an intrinsic depression in chemotaxis during the last half of pregnancy but also decreases in phagocytosis of *E. coli* and responses in mixed lymphocyte cultures (2). These investigators did not separate the periods of gestation nor study patients during or after parturition. We also found that these chemotactic defects appear to reverse during parturition as only one of five patients in labor had depressed chemotaxis. Postpartum patients demonstrated very significant increases in phagocytic chemotaxis when studied within 3 days of delivery, although there was a wide range of values. No depressions were induced in chemotaxis of control phagocytes after incubation in serum obtained from pregnant patients having known chemotactic defects. These results suggest that an intrinsic defect occurs in circulating phagocytes between 34 weeks of pregnancy and term.

This observed defect in chemotaxis may be related to circulating steroidal compounds which increase during gestation, such as estrogens, which appear to be anti-inflammatory in *in vitro* studies (3, 22). Estrogen treatment of animals also produces diminutions in size of granulomas after tuberculous infections (22), smaller sizes of skin-test reactions after tuberculo-protein sensitization (13), and lesser inflammatory responses after croton oil administration (9).

These alterations in levels of hormones which occur during gestation may alter phagocytic migration toward chemotactic stimuli. Several hormones are now known to interact with phagocytic membranes and trigger changes in cellular metabolism (11, 25). Other studies will be necessary to explain the biochemistry of these

chemotactic defects we have described and whether they are clinically relevant and predispose toward infection.

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