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Polymorphonuclear Leukocyte Adherence and Chemotaxis in Stressed and Healthy Neonates

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ABSTRACT. Defects in polymorphonuclear neutrophil (PMN) adherence and chemotaxis in neonates are thought to be an important cause of their increased susceptibility to overwhelming bacterial infection. Few studies of these functions have been carried out in stressed neonates who are at even greater risk of infection. PMN adherence and chemotaxis were examined in 33 stressed neonates with acute lower respiratory illness, 13 healthy neonates, and 43 healthy adults using whole blood PMN adherence and chemotaxis assays. PMN chemotaxis was significantly decreased in stressed neonates (locomotion index of 38.4 ± 9.7 μm) compared with that of healthy neonates (48.9 ± 12.8 μm, $p < 0.01$) or adults (61.6 ± 11.9 μm, $p < 0.001$). PMN chemotaxis was studied during illness and recovery in 13 of the 33 stressed neonates and showed significant improvement during recovery (41.6 ± 9.9 and 53.2 ± 11.9 μm, respectively, $p = 0.012$). PMN adherence was decreased in stressed neonates (1.4 ± 1.6%) compared with that of adults (12.3 ± 11.4%, $p < 0.01$) but was similar to that of healthy neonates (1.1 ± 1.4%). These findings suggest that further impairment of PMN chemotaxis in stressed neonates helps account for their increased susceptibility to overwhelming bacterial infection. (*Pediatr Res* 20: 296–300, 1986)

Abbreviations

PMN, polymorphonuclear neutrophil
LI_{20}, locomotion index

Bacterial infections are a major cause of morbidity and mortality in newborn infants primarily because of immaturity of their host defense mechanisms (1, 2). Neutrophils (PMNs) are the major cellular elements which defend against bacterial invasion. They respond to infection by adhering to vascular endothelium, moving toward the site of infection along a chemotactic gradient (chemotaxis) and killing ingested bacteria. The functional capacity of neonatal PMNs have been carefully studied and although results are not entirely consistent, PMN adherence (3–5) and chemotaxis (6–8) appear to be depressed in healthy neonates compared with that of adults while phagocytosis and microbial killing (9–12) are intact. In studies of neonates with underlying illness or stress, PMN phagocytosis has been found to be normal (11–15) while microbial killing has been found to be decreased (11–13, 15, 16). There have been few studies of PMN adherence and chemotaxis in stressed neonates (17). Since further impairment in these functions could help explain the increased susceptibility of stressed neonates to overwhelming bacterial infection, we prospectively studied PMN adherence and chemotaxis in healthy newborn infants and those with respiratory distress syndrome, pneumonia, and other cardiorespiratory illnesses.

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MATERIALS AND METHODS

Subjects. Blood was obtained from 33 stressed neonates with a median chronological age of 3 days (range 1–88 days) and median gestational age of 36 wk (range 25–43 wk), 13 healthy neonates with a median chronological age of 6 days (range 3–88 days) and median gestational age of 36 wk (range 27–42 wk) and 43 healthy adults. Three groups of stressed neonates were studied. Group I consisted of 16 neonates with respiratory distress syndrome, group II included nine neonates with pneumonia, and group III consisted of eight neonates with other cardiopulmonary illnesses including congenital heart disease with congestive heart failure ($n = 4$), meconium aspiration ($n = 3$) and severe birth anoxia ($n = 1$). All but three of the 33 stressed infants (two in group II and one in group III) required assisted ventilation during their acute illness and three died (all in group III).

One to 2 ml of blood was obtained from indwelling arterial or venous catheters or from capillary (heel stick) sampling in neonates and venipuncture in adults. In every experiment blood was simultaneously obtained and PMN function tested in neonates and healthy adults. The PMN adherence and locomotory assays were begun within 20 min of blood collection. Informed consent to sample blood was obtained from all parents or adult subjects in accordance with Hartford Hospital Institutional Review guidelines.

PMN locomotory assay. PMN locomotory response was determined using a whole blood chemotaxis assay as previously described (18). In brief, blood was diluted with Medium 199 to 5×10^5 PMNs/0.7 ml and placed in the upper compartment of a transparent acrylic modified Boyden chamber (Ahlco Corp., Meriden, CT). The PMNs were allowed to penetrate a 13 mm diameter, 5 μm pore size cellulose nitrate filter (Sartorius Filters, Hayward, CA) during a 90-min incubation in a humid 5% CO_2 atmosphere at 37°C. In all experiments either Medium 199 or 3% zymosan activated normal adult serum in Medium 199 was placed in the bottom compartment of the Boyden chamber to test chemokinetic or chemotactic PMN responses, respectively. The filters were removed and soaked in 3% acetic acid to lyse erythrocytes and remove the hemoglobin stain. The filters were then fixed, stained, clarified, and mounted on slides. An automated microsectioning counting technique was used to determine the number of PMNs at 10 μm increments from 20 to 120

μm into the filter. Since we used a mixed leukocyte suspension, the first 20 μm of the filter was not examined in order to avoid inclusion of monocytes and lymphocytes in the cell counts. The mean distance (μm) migrated by the PMNs in 90 min of incubation time, or LI_{20}, was then determined. A mean LI_{20} value of triplicate determinations was calculated for each blood sample. The coefficient of variation for this assay is 0.13.

Cover glass adherence assay. Blood was tested using a whole blood cover glass adherence assay as previously described (4). In brief, blood was collected in chilled plastic tubes and placed on ice in order to prevent clotting prior to the assay procedure. The PMNs were incubated on cover slips for 45 min in a humid 5% CO_2 atmosphere at 37°C. Only a small fraction of the PMNs adhere to the cover slip with the whole blood assay and the number reaches near plateau at 45 min incubation time. After incubation, the clot was removed with forceps leaving a thin layer of PMNs adherent to the glass. The cover slip was gently rinsed with Medium 199, stained with Wright's stain, and mounted on a glass slide. Neutrophils were counted in every other field (0.25 × 0.25 mm grid × 400 magnification) along a horizontal and vertical diameter of the circle of adherent neutrophils. The percent PMN adherence value was then calculated as follows:

$$\%\ \text{PMN adherence} = \frac{\dfrac{\text{PMNs counted}}{\text{area counted}} \times \text{area of entire circle}}{\text{PMNs in original 20 } \mu\text{l sample}} \times 100$$

The coefficient of variation for this assay is 0.29.

Statistical analysis. Statistical significance of differences between groups was performed using the Student's two-tailed t test. Linear regression was used to analyze the possible association between mean neonatal PMN chemotaxis and the percentage of immature PMNs in peripheral blood. A p value of < 0.05 was considered significant in all cases.

RESULTS

Chemotactic response. The PMN chemotactic response in 33 stressed neonates, 13 healthy neonates, and 43 healthy adults is shown in Figure 1. The mean PMN chemotactic LI_{20} value in stressed neonates (38.4 ± 9.7 μm) was significantly decreased compared with that of healthy neonates (48.9 ± 12.8 μm, $p < 0.01$) or adults (61.6 ± 11.9 μm, $p < 0.001$). The mean chemokinetic LI_{20} response was also significantly decreased in stressed neonates (33.4 ± 9.1 μm) compared with that of adults (46.1 ± 10.7 μm, $p < 0.01$) but not compared with that of healthy neonates (35.2 ± 4.7 μm). Mean chemotactic LI_{20} values were similar among the three stressed neonatal groups: group I (respiratory distress syndrome), 38.2 ± 9.2 μm, group II (pneumonia), 39.2 ± 10.8 μm, and group III (other cardiopulmonary), 38.0 ± 9.1 μm. No difference in PMN chemotaxis was noted in 28 neonates with acute illness who had arterial blood catheter sampling (38.4 ± 9.9 μm) compared with four neonates who had capillary heel stick sampling (39.3 ± 6.0 μm).

The PMN chemotactic response was determined during the acute phase of illness and during recovery (3 to 11 days later) in nine neonates in group I and four neonates in groups II and III. The median chronological age of the total group was 3 days (range 1–81 days) during acute illness and 10 days (range 3–88 days) during convalescence. In all but two cases there was an increase in PMN chemotaxis during recovery with chemotactic LI_{20} values similar to those of healthy neonatal controls (median chronologic age was 5 days; range 3–88 days) as shown in Figure 2. Mean acute and convalescent PMN chemotactic LI_{20} values were 41.6 ± 9.9 and 53.2 ± 11.9 μm ($p = 0.012$), respectively, for the stressed neonates compared with 48.9 ± 12.8 μm in the healthy, age-matched controls.

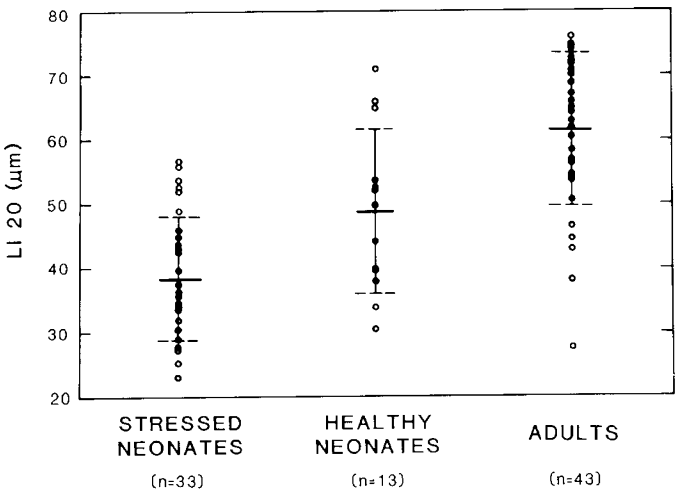

Fig. 1. PMN chemotaxis in stressed neonates, healthy neonates, and adults. The *solid lines* represent mean values. The *hatched lines* represent ±1 SD.

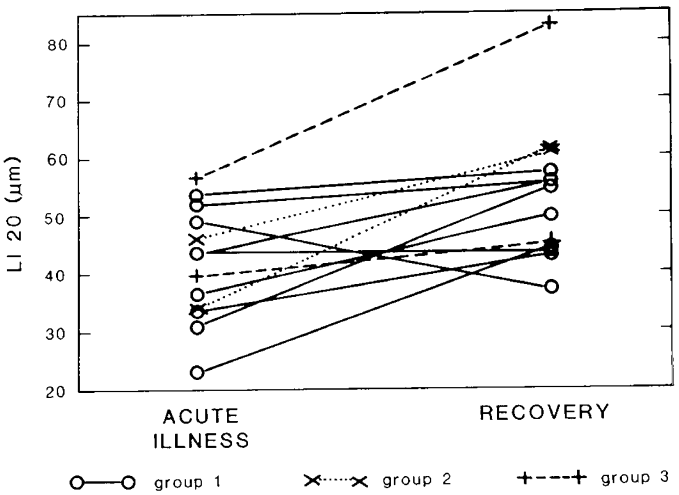

Fig. 2. PMN chemotaxis in neonates during acute illness and recovery.

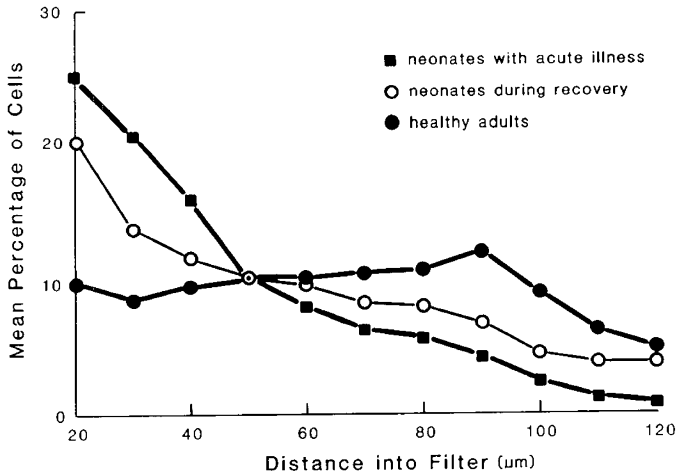

Fig. 3. The distribution of PMNs at different distances into the filter obtained from 13 neonates during acute illness and recovery and from 22 healthy adults. The PMNs were migrating toward a gradient of 3% zymosan activated normal adult serum during a 90-min incubation period.

Previous studies have indicated that depressed PMN locomotory responses may be due to changes in PMN subsets with varied functional characteristics. In order to compare PMN functional subpopulations in neonates and adults, we measured the number of PMNs at various depths in the micropore filter (Fig. 3) in the 13 neonates studied both during illness and recovery and in 22 simultaneously tested adults. We defined slow moving PMNs as those found from 20 to 50 μm and fast moving PMNs as those found 60 to 120 μm into the filter and calculated the percentage of each cell type in the study groups. Neonates with acute respiratory illness had a significantly higher percentage of slow moving PMNs (71.3 ± 6.4%) and lower percentage of fast moving PMNs (28.3 ± 2.7%) than during recovery (55.4 ± 4.5%, $p < 0.01$ and 44.4 ± 2.3%, $p < 0.001$, respectively). Adults had a significantly lower percentage of slow moving PMNs (37.4 ± 0.7%) and a higher percentage of fast moving PMNs (67.5 ± 2.5%) than neonates during illness or recovery with p values of < 0.001 in both cases. Leukocyte differential counts were performed at the time of chemotactic testing in 12 of the 13 neonates studied during acute illness and in six of the 13 during convalescence. Although there was a higher mean percentage immature PMNs during acute illness (11.9 ± 10.7%) than during recovery (5.0 ± 5%), the difference was not statistically significant. Using linear regression analysis, there was no correlation between the percentage of immature PMNs (bands and metamyelocytes) and mean chemotactic LI_{20} in neonates with acute illness ($r = -0.37$, $p = 0.24$) or during recovery ($r = -0.43$, $p = 0.34$). These findings indicate that PMN locomotory responses in the 13 neonates studied during acute illness and recovery cannot be completely explained by the change in percentage of immature PMNs (bands and metamyelocytes) between the two groups.

Adherence response. There was no significant difference between mean PMN adherence values of stressed neonates (1.4 ± 1.6%) and healthy neonatal controls (1.1 ± 1.4%). Both values were significantly decreased compared with that of adults (12.3 ± 11.4%, $p < 0.01$). There was no significant difference in PMN adherence between stressed neonates during acute illness (1.2 ± 1.4%) and recovery (1.0 ± 2.1%).

Clinical correlates of the PMN chemotactic response. In an attempt to identify possible causes of decreased PMN chemotaxis in the stressed neonatal groups, the effects of 11 clinical conditions on chemotaxis were analyzed. These conditions are listed in Table 1. Mean PMN chemotactic LI_{20} values were compared in stressed and healthy neonates with and without each condition. No condition was associated with a significant decrease in PMN chemotaxis. For example, the mean PMN chemotactic LI_{20} values for stressed [36.8 ± 9.0 μm (n = 10)] and healthy neonates [48.8 ± 5.5 μm (n = 5)] who weighed less than 2500 g at birth were not significantly different than values for stressed [39.1 ± 9.6 μm (n = 23, $p > 0.05$)] and healthy neonates [49.0 ± 12.1 μm (n = 8, $p > 0.05$)] with a birth weight of 2500 g or greater. There was also no significant difference in the mean PMN LI_{20} chemotactic values in the three infants who died and the 30 who survived or in the three infants who did not require assisted ventilation and the 30 who did.

Table 1. *Common clinical categories having no significant* effect on PMN chemotaxis*

Sex	Male (19),† female (14)
Gestation	<32 wk (10), ≥32 wk (23); <38 wk (14), ≥38 wk (19).
Birth wt	<2500 g (10), ≥2500 g (23); <1500 g (9), ≥1500 g (24)
Pregnancy	Use of steroids (2), no steroids (31)
Delivery	Cesarean section (3), vaginal delivery (30)
	Local anaesthesia (18), general anaesthesia (6)
Apgar score	<7 (14), ≥7 (19) at 1 min; <7 (10), ≥7 (23) at 5 min
Postnatal age when tested	<1 day (10), ≥1 day (23); <1 wk (14), ≥1 wk (19)
Neonatal therapy	Use of antibiotics (30); no antibiotics (3)
	Use of calcium (8), no calcium (25)
	Use of assisted ventilation for 0 days (3), 1–3 days (2), ≥3 days (28).

* $p > 0.05$ by two tailed t test.

† Number in parentheses refers to the number of subjects.

DISCUSSION

The results of this study clearly show that PMN chemotaxis was decreased in healthy neonates compared with that of adults and was further impaired in neonates with acute respiratory illness. The magnitude of PMN chemotactic impairment in stressed compared with healthy neonates was similar to that of healthy neonates compared with adults. Several *in vitro* studies in healthy human neonates (3, 6–8), have shown decreased PMN motility compared with that of adults. The results of two previous studies of PMN chemotaxis in smaller groups of stressed neonates were also similar to ours. Decreased PMN chemotaxis was noted in premature infants with sepsis (19) and infants of diabetic mothers (20) compared with that of healthy neonates. These data suggest that impairment of PMN motility in neonates is an important cause of their increased susceptibility to overwhelming bacterial infection. They also indicate that a further decrease of PMN motility in stressed neonates helps explain the even greater morbidity and mortality from infection in these infants compared with those who are healthy.

The mechanisms responsible for impaired PMN chemotaxis in the stressed neonates are unclear. The whole blood chemotaxis assay used in this study approximates *in vivo* conditions more closely than separated cell assays, however, it is less useful for determination of the relative effects of serum and cellular factors on PMN chemotaxis. Previous studies in healthy neonates have identified both humoral (6, 8, 21–23) and cellular (3–5, 24–26) abnormalities which could explain decreased PMN chemotaxis in neonates compared with adults. Serum defects have included decreased levels of complement (8, 21–22) and the presence of a chemotactic factor inactivator (8, 23). Cellular defects have included decreased membrane receptors for chemoattractants (24), impaired microtubule assembly (25), decreased adherence (3–5), and decreased membrane deformability (26). Decreased PMN chemotaxis in the stressed neonates might have been due to further impairment in any one or more of these defects or might have resulted from other defects such as the presence of a cell directed inhibitor or activated complement fragments. There have been a number of studies of PMN chemotaxis in stressed populations. In adults with severe bacterial infection, decreased PMN chemotaxis has been described and has been attributed to cellular (27–29) and humoral (30) factors. Interestingly, studies of older children and adults with skin or nonlife-threatening systemic bacterial infections showed an increase in PMN chemotaxis (31–33). Laurenti *et al.* (19) studied neonates with surface infection and sepsis. Those with surface infection had an increase in PMN chemotaxis while those with sepsis had impaired PMN chemotaxis. Serum from neonates with sepsis did not have an inhibitory effect on chemotaxis of PMNs from healthy preterm infants and adults suggesting a cellular cause for decreased PMN chemotaxis in these septic neonates.

Recent attention has focused on the role of PMN adherence in decreased neonatal PMN chemotaxis (3–5). PMN adherence is not only important in PMN margination to vascular endothelium but also provides anchoring of the cell for forward movement. Several children with a PMN membrane glycoprotein

deficiency that prevents normal adherence have been described (34, 35). These children have markedly decreased PMN chemokinesis and chemotaxis and recurrent bacterial and fungal infections. In a more recent study, decreased PMN membrane surface expression of glycoprotein p150, 95 in response to f-Met-Leu-Phe was demonstrated in neonatal compared with adult PMNs. It was suggested that decreased "up regulation" of adhesive glycoproteins in neonates could account for the decrease in "functionally linked" PMN adherence and chemotaxis (36). The results of previous studies of PMN adherence in healthy neonates have shown a decrease (3–5), no difference (8, 37), or an increase (38) compared with that of adults. Anderson *et al.* (3) found no difference in unstimulated PMN adherence between healthy neonates and adults, however, there was augmented adherence in the presence of the chemoattractant f-Met-Leu-Phe with adult but not neonatal PMNs. Harris *et al.* (5) studied PMN adherence in healthy neonates and those with acute respiratory illness using a separated PMN glass cover slip assay. They found no difference in PMN adherence between the two neonatal groups at 5 min incubation but an increase in PMN adherence in stressed compared with healthy neonates when PMNs were incubated for 10 or 20 min. The healthy and stressed neonates had markedly decreased PMN adherence compared with that of adults which was attributed to diminished amounts of PMN fibronectin. The results of our study showed a decrease in PMN adherence in stressed and healthy neonates compared with that of adults using a whole blood cover slip adherence assay. No difference was found in PMN adherence between stressed and healthy neonates, although a more sensitive PMN adherence assay might have detected differences between the two groups. Discordant results in studies of PMN adherence in neonates are thought to be due to differences in experimental conditions including the method of PMN separation, the type of adherent surface used, the presence of chemoattractants, the incubation time during which PMNs adhere, and the method of removing "nonadherent" PMNs. Despite these differences we believe the bulk of evidence indicates that there is a significant decrease in the PMN adherence of healthy neonates compared with that of adults. Differences in PMN adherence between neonates with acute lower respiratory illness and healthy neonates, if present, appear to be more subtle and probably have less of an effect on PMN migration, diapedesis, and chemotaxis.

Alteration in PMN functional subpopulations in the circulation is another possible mechanism which could explain the difference in PMN chemotaxis between stressed and healthy neonates. Gallin (39) has suggested that changes in PMN function in some infectious and noninfectious inflammatory illnesses may result from alterations in PMN subpopulations. In the present study, calculation of the number of PMNs at different depths in the micropore filter suggests that there is PMN functional heterogeneity in neonates and adults. It is unclear whether the observed chemotactic heterogeneity represents true subpopulations of cells derived from distinct stem cells or maturational differences within a common cell line which cannot be distinguished by light microscopy. Regardless of the source, it appears that there may have been a shift to less functional PMN subpopulations in neonates during acute respiratory illness. Recent studies have shown that neonates and adults with various types of noninfectious respiratory illness, including respiratory distress syndrome, have an accumulation of PMNs in the lung (40, 41). It is thus possible that during acute respiratory illness, motile PMNs moved from the circulation to the lungs, leaving behind less motile PMNs. During recovery one would expect a decrease in PMN movement to the lung including the more motile PMN subpopulation. The average distance traveled by the PMNs during recovery (LI_{20}) would thus be expected to be greater than during acute illness.

The observed changes in PMN chemotactic subpopulations might be attributed to changes in the percentage of immature PMNs since previous studies indicate that immature PMNs are slower than mature PMNs (8). Our data suggest that this could not fully account for the PMN functional subpopulation changes observed in our patients. Although there was a higher percentage of immature PMNs in neonates with acute respiratory illness than during recovery, the difference was not statistically significant. Furthermore, variation in the chemotactic response in neonates with acute respiratory illness did not correlate with the percentage of immature PMNs. In recent studies using a monoclonal antibody (31D8) whose heterogeneous binding to PMNs correlates with membrane depolarization, NBT reduction, and chemotaxis (42, 43), we noted significant differences between 31D8 binding to neonatal and adult PMNs (44). Compared with adults, neonates had a lower percentage of PMNs that tightly bound 31D8 (more motile cells) and a higher percentage of PMNs that weakly bound 31D8 (less motile cells). No significant differences in the percentage of immature PMNs were noted between the two 31D8 subpopulations. These data indicate that mature appearing PMNs are functionally heterogeneous and this heterogeneity may help explain changes in PMN chemotaxis in neonates during acute respiratory illness and recovery.

In summary, studies of PMN adherence and chemotaxis in neonates have shown that both functions are significantly decreased compared with that of adults. PMN chemotaxis, but not PMN adherence, was further impaired in neonates with infectious and noninfectious pulmonary disease. The cause of decreased PMN chemotaxis during acute respiratory illness in neonates is unclear but may be related in part to alterations in PMN functional subpopulations. Decreased PMN chemotaxis in stressed neonates may contribute to their increased susceptibility to overwhelming bacterial infection.

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