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Chemotactic Responses of Various Differentiational Stages of Neutrophils from Human Cord and Adult Blood

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Chemotactic response and responsiveness, that is, the ability to respond to various concentrations of the chemoattractant zymosan-activated serum was measured for cord and adult blood neutrophils. Chemotactic response of neutrophils from adult blood was two to seven times greater than that of neutrophils from cord blood. In addition, neutrophils from cord blood showed poor response to concentrations of 25% chemoattractant or less, compared to those from adults, which responded to concentrations of 10%. Further, it was found that approximately 30% of neutrophils in cord blood were the band form compared to only 9% in adult. Based upon this, a simple method was devised to assay mean migrating distance of various differentiational stages of neutrophils incorporating both distance and magnitude of their responses. The results of these assays showed that all differentiational stages of cord blood neutrophils have a mean migrating distance less than those of adults. In addition, the band form from both cord and adult blood had a mean migrating distance less than the polymorphonuclear form. Adherence studies revealed that all differentiational stages of neutrophils adhered in a similar manner, and no difference was detected between cells from cord and adult blood. Assays to test the ability of cord blood to produce chemotactic activity when activated revealed 50% less activity than that obtained from adult serum which further decreased with dilution of attractant. These data suggest that both the cellular and humoral systems involved in chemotactic responses are less in cord blood compared to adult blood.

Infectious disease remains a major health problem in the pediatric age group, especially in early infancy. The age susceptibility of young infants to infection with a variety of bacteria is now well established. Although the basis of this susceptibility is not entirely clear, deficiencies in many parameters of both specific and nonspecific immunity have been reported. Primary nonspecific immunity involves two major phagocytic cell types: the polymorphonuclear leukocyte (PMN) and the mononuclear phagocyte. The destruction of bacteria during infection represents the final event of a series of responses of these phagocytic cells which begin with their initial influx into the site of inflammation, that is, chemotaxis. A number of investigators have reported diminished chemotactic responses in the normal infant as well as infants with bacterial sepsis (7, 9, 10, 13, 17).

The present studies were performed to evaluate differences in chemotactic function of neutrophils from cord blood compared to that of neutrophils from blood of healthy adults in response to different concentrations of chemoattractant, as well as the chemotactic responsiveness of these neutrophils at their various stages of morphological differentiation. In addition, the ability of cord blood compared to adult sera to generate chemotactic activity was assessed.

MATERIALS AND METHODS

Collection of blood and isolation of cells. Cord blood obtained from 12 healthy full-term infants was collected in polypropylene tubes containing sufficient heparin to attain a final concentration of 20 U/ml. Tests were performed immediately after collection, and in every experiment blood from a healthy adult was tested simultaneously.

Heparinized blood was mixed with an equal volume of 2% dextran in 0.9% NaCl and allowed to settle at room temperature for 15 min. The leukocytes in the supernatant were sedimented by centrifugation at 120 $\times$ *g* for 5 min at 20°C. The erythrocytes were lysed by the addition of three parts of sterile water followed immediately by one part of 3.6% sodium chloride. The cells were then sedimented by centrifugation and washed two times in 0.9% NaCl. The leukocytes were adjusted to a final concentration of 2.5×10^6 leukocytes per ml in medium 199 containing 2% albumin for assay of chemotaxis or in Gey's buffer for adherence studies. The viability of the isolated leukocytes, determined by trypan dye exclusion, was always greater

than 97%. Cytocentrifuge smears were prepared on all final cell suspensions, and were fixed with methanol and stained with Giemsa. The percentages of cell types present on each slide preparation were determined microscopically by examining at least 100 leukocytes.

Preparation of serum. The chemotactic factor employed in all assays was zymosan-activated pooled adult serum, or cord blood serum. The blood was obtained by venipuncture from five healthy adults, or collected from the umbilical vein for cord blood. Blood was allowed to clot at room temperature for 30 min and then centrifuged at 750 × *g* for 10 min at 4°C. The adult sera were pooled; the cord sera were processed separately. Serum samples were either aliquoted and frozen immediately for complement factor determination or activated for use as chemoattractants as described.

Preparation of chemotactic factor. A 3-ml volume of pooled human serum was incubated with 150 mg of zymosan at 37°C for 60 min and then centrifuged at 750 × *g* for 10 min. The supernatants were removed and inactivated at 56°C for 30 min and stored in aliquots at −20°C. The activated serum was thawed and diluted with medium 199 immediately before testing and employed at concentrations of 100 (undiluted), 50, 25, 10, and 1%.

Complement determination. Total complement activity (CH_{100}) was assayed using Quantiplate Single Radial Diffusion Total Complement plates (Kallestad lot no. R310M100, Chaska, Minn.). Each determination was performed in duplicate. C4 and C3 components were determined by Endoplate Single Immunodiffusion plates (Kallestad lot no. 9466, 1406, respectively). Each sample was assayed in duplicate employing both timed diffusion and endpoint methods.

Measurement of leukocyte adherence. Adherence of leukocyte preparations was examined in four cord bloods and in four normal adult donors. Control cytocentrifuge slides were made from each leukocyte suspension as a zero-time reference for the total cell population. Fifty-microliter volumes of Gey's buffer containing 1.25×10^5 leukocytes were placed on the center of a glass cover slip (18 by 18 mm) and incubated at 37°C on a leveled platform for 3, 6, or 10 min. The cover slips were gently washed five times with 1.5-ml volumes of 0.5% NaCl to remove nonadherent cells. The cover slips were air dried and fixed with 100% methanol for 2 min, washed with distilled water, and stained with 8% Giemsa stain for 15 min. Cell counts and differentials were performed under high power (40×) utilizing a 10 mm by 10 mm ocular grid, and the number of cells per 10 random high-power fields was determined. All experiments were performed in triplicate, and the results are reported as the mean values of triplicate determinations.

Measurement of chemotaxis. Two assay procedures were utilized to measure chemotaxis. They consisted of a modification of the agarose plate method (7, 15) and a slide method adapted for this study to measure mean migrating distances of various differential stages of neutrophils. This was accomplished by determining the number of each cell type at measured intervals along their migration path. These methods are described briefly below. The agarose plates were prepared by placing 1.7 ml of 0.75% agarose (ICN Pharmaceuticals, Inc., electrophoresis grade) solution in medium 199 containing 10% heat-inactivated fetal calf serum into a tissue culture multiwell plate (3.5 by 1.0 cm; Limbro). The same solution was added in volumes of 1.5 ml to two-well tissue culture slides (Lab-Tek) for the morphological analysis of migrating neutrophils. After the agarose solution solidified, the plates were incubated at 37°C in 5% CO_2 for 30 to 40 min without moisture. Three aligned wells, 4 mm in diameter, 2 mm apart, were then punched in the agar using a template and a stainless-steel tube after which the agar plugs were removed by suction. The center wells were then immediately filled with the cell suspensions—the right wells with chemoattractant and the left wells with control solution (medium 199). The wells in the Limbro plates and Lab-Tek slides were filled with 14 μl and 25 μl of the appropriate samples, respectively. All assays were performed in duplicate or triplicate. The agarose plates were incubated at 37°C in 5% CO_2 for 3 h and then fixed with 100% methanol and stored at 4°C. The migrating cells were counted microscopically on Limbro plates employing a 10- by 10-mm ocular grid using 150× magnification. The chemotactic responses were determined by subtracting the total number of cells which had migrated towards the buffer from the total number which had migrated towards the chemoattractant for each plate. The mean value of the identical samples was determined, and the results were expressed as mean ± standard error of the response of seven donors of each age group. After overnight fixing at 4°C, the agarose was removed from the Lab-Tek slides by gentle suction and the slides were stained with 8% Giemsa for 25 min. The total number of neutrophils chemotaxing, as well as the number of each differentiational stage of neutrophils, that is, band, bilobed, multilobed, at each 0.1 mm along the total path of migration was determined microscopically with the 10- by 10-mm ocular grid using 400× magnification. The mean migrating distance of various differentiational stages of neutrophils in cord and adult blood to 25, 50, and 100% activated serum was determined on duplicate samples from four donors of each age group. This was calculated utilizing the number of each cell type at each distance and the total number of that type which responded as described in Fig. 1. The results are expressed as mean ± standard error. Statistical analysis was performed by employing the Student's *t* test (3).

RESULTS

Leukocyte isolation. The differential counts of the various leukocyte populations obtained by dextran sedimentation of cord and adult blood are shown in Table 1. The differential proportions of leukocytes, that is, neutrophils, monocytes, and eosinophils, were similar in cord and adult samples (Table 1). In contrast, the differentiational types of neutrophils, that is, bands, bilobed, and multilobed, differed greatly in cord blood, which contained 23% of both bands and multilobed compared to 7% bands and 54% multi-lobed in adult blood. Differentials on blood samples before leukocyte separation showed similar proportions of the various stages of neutrophils, which is similar to previous reports (11). This indicates that the method utilized for sepa-

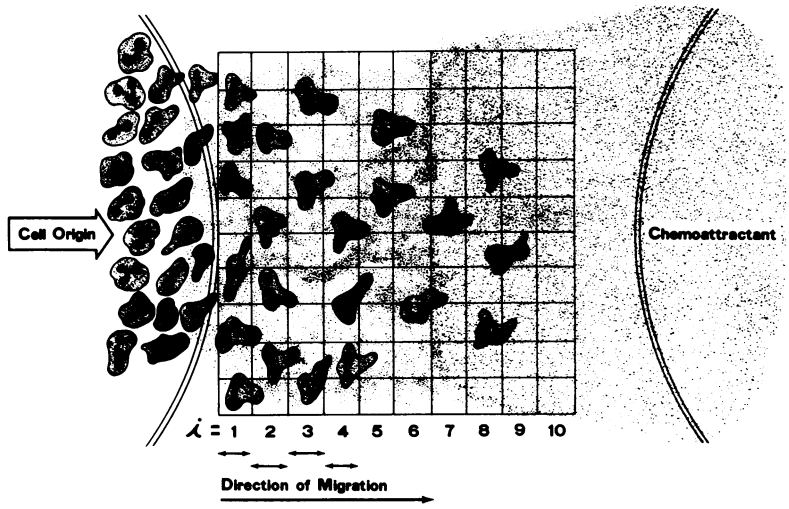

FIG. 1. Schematic representation of the microscopic view of the ocular grid overlaying leukocytes migrating towards activated serum on a Lab-Tek chamber slide. The mean migrating distance ($\bar{X}_{MD}$) for each neutrophil type, that is, band, bilobed, and multilobed, was calculated as follows:

$$\bar{X}_{MD} = \frac{0.1 \sum_{i=1}^{n} (N_i \times i)}{\sum_{i=1}^{n} N_i}$$

where N is the number of a given neutrophil type in each (i^{th}) subdivision (each subdivision of this grid = 0.1 mm), and i is the subdivision number. $\bar{X}_{MD}$ for bands in diagram: $\bar{X}_{MDband} = 0.1\,\Sigma\,(3 \times 1) + (2 \times 2) + (1 \times 3) + (1 \times 4)/7 = 0.2$.

rating neutrophils did not appear to change the proportions found in whole blood.

Adherence. Results of experiments to determine the difference in the numbers of the various differentiational stages of neutrophils from cord or adult blood able to adhere during various time intervals compared to those in the original starting suspensions (zero time) are shown in Table 2. It can be seen that the number of both cord and adult blood leukocytes which adhere after 3, 6, or 10 min are similar. In addition, the various proportions of cell types do not change with incubation compared to their proportion in the original starting suspensions before adherence (zero time). These results indicate that there is no difference in the number of cord blood leuko-

TABLE 1. Percentage of various leukocytes obtained by dextran sedimentation from cord blood and adult peripheral blood

WBC[a] source blood	% Cell type[b]		
	Neutrophils	Monocytes	Eosinophils
Cord	75 ± 2[c] (23 ± 2; 29 ± 2; 23 ± 2)[d]	21 ± 1[c]	4 ± 1[c]
Adult	80 ± 2 (7 ± 0.8; 20 ± 2; 54 ± 3)	17 ± 2	3 ± 0.4

[a] Blood was obtained from either cord blood or adult peripheral blood and anticoagulated with heparin, and leukocytes (WBC) were separated within 15 min of blood drawing.

[b] Leukocytes were purified by dextran sedimentation of either heparinized cord blood or adult peripheral blood obtained from 9 to 14 normal people in each blood group. Cytocentrifuge slides were prepared and stained with 8% Giemsa. The percentage of each cell type was determined microscopically by examining 100 cells from each specimen and expressed as mean % ± standard error.

[c] Mean percentage of types of leukocytes identified ± standard error.

[d] Mean percentage of each type of neutrophil identified by nuclear morphology (band nucleus; bilobed; multilobed, respectively) ± standard error.

TABLE 2. Number and percentage of leukocyte types from cord and adult blood which adhere after various incubation periods

Blood source	Incubation time (min)[a]	Cell no.[b]	% Cell types[c]				
			Band	Bilobed	Multilobed	Monocytes	Eosinophils
Cord	0		18 ± 2	26 ± 4	28 ± 3	22 ± 2	6 ± 2
	3	141 ± 23	17 ± 3	25 ± 1	34 ± 2	20 ± 4	4 ± 1
	6	145 ± 25	16 ± 4	24 ± 1	32 ± 2	23 ± 4	5 ± 2
	10	152 ± 27	18 ± 5	20 ± 2	29 ± 4	28 ± 5	5 ± 3
Adult	0		7 ± 1	24 ± 5	52 ± 5	15 ± 4	2 ± 1
	3	136 ± 19	9 ± 2	25 ± 2	55 ± 5	10 ± 1	1 ± 0.5
	6	139 ± 22	8 ± 2	21 ± 3	57 ± 7	13 ± 4	1 ± 0.5
	10	135 ± 17	6 ± 1	22 ± 5	56 ± 5	14 ± 2	2 ± 1

[a] Cytocentrifuge preparations were made of cell suspensions obtained by dextran separation of four cord and adult blood samples for zero time. Triplicate aliquots of these suspensions were then incubated on cover slips at various times after which they were washed, fixed, and stained.

[b] Cell numbers were determined in 10 high-power fields using a 10 by 10 mm ocular grid. Results are expressed as mean ± standard error.

[c] Percentage of each cell type present was determined by examining 100 cells on each cover slip. Results expressed as mean ± standard error.

cytes which adhere compared to adults and that all cell types regardless of differentiational stages adhere in a similar manner.

Chemotactic response. The chemotactic response of cord blood compared to adult leukocytes to various concentrations of activated pooled adult serum is shown in Fig. 2. It can be seen that the responses of adult leukocytes to activated serum concentrations from 10 to 100% were two to seven times greater than those of cord blood, and these differences were statistically different ($P < 0.05$) Even when a concentration of 25% chemoattractant was employed, the number of adult cells responding was as great as the greatest number of cord cells responding to 100% serum. The number of cord versus adult cells migrating towards the control buffer was not significantly different and was not influenced by the concentration of the activated serum used. The mean number (± standard error of the mean) of cord and adult cells responding to the buffer was 229 ± 10 and 240 ± 37, respectively. These results indicate that the chemotactic response of cord blood neutrophils is less than those from adult blood.

Since it was determined that a greater proportion of cord blood neutrophils are the less differentiated band form, the following experiments were performed to determine if their chemotactic response was less than that of the multilobed form and therefore responsible for the lower total chemotaxis of cord blood neutrophils. A simple method was devised to measure the mean migrating distance of the various differentiational stages of neutrophils since the available assay systems were not entirely suitable. The method incorporates the distance and the magnitude of response while maintaining good cellular morphology. This method also allowed assessment of all cell types on each sample without further cell separation, utilizing Lab-Tek chamber slides and the method depicted in Fig. 1. Firstly, the mean migrating distances of the various differential stages of neutrophils from adult and cord blood were determined (Fig. 3). The mean migrating distances of all the differential stages of neutrophils from adult blood were significantly greater than those from cord blood at concentrations of 50 and 100% chemoattractant ($P < 0.05$). The mean migrating distance of only the adult multilobed neutrophils were significantly different than the multilobed cells from cord blood in response to 25% activated serum. These results show that the chemotactic response of all differentiational stages of neutrophils from adult blood tested was greater than those of cord blood in response to high concentrations of activated serum.

Secondly, in order to further determine if the mean migrating distance changed with neutrophil differentiation, the ratio of the mean migrating distance of the two major circulating differentiational types of neutrophil, that is, PMN (bilobed and multilobed) to band, was determined for each adult and each cord blood specimen, and the mean values of these ratios were analyzed to see if they were statistically different, that is, a ratio of PMN/band of 1 would indicate no difference in mean migrating distances. The results showed that the mean ratio of mean migrating distances of PMN compared to bands from adult blood is significantly greater when responding to 50 and 100% activated serum ($P < 0.05$) but not to 25% serum, whereas this ratio of PMN compared to bands from cord blood is significantly greater only when respond-

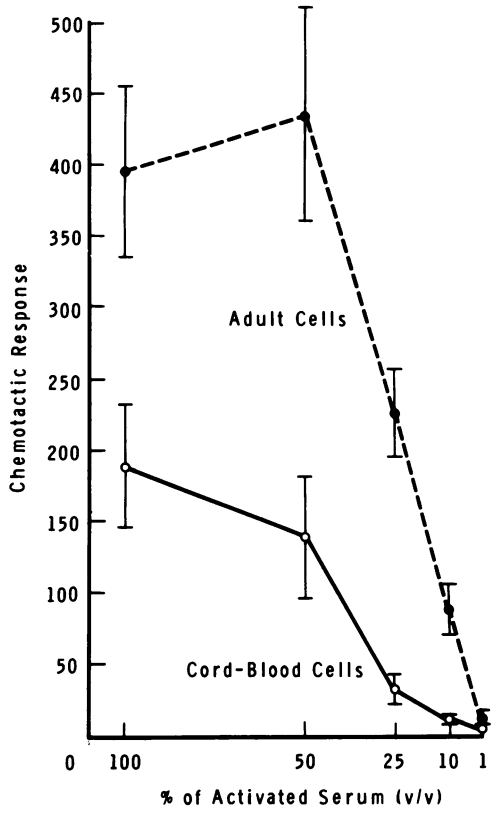

FIG. 2. Chemotactic response of neutrophils from cord and adult blood to various concentrations of chemoattractant (zymosan-activated serum). The chemotactic response is the total number of neutrophils migrating towards chemoattractant minus the total number migrating towards the control medium. The experiments were performed in duplicate. The results are expressed as mean ± standard error of the chemotactic response of seven donors of each age group.

ing to 50% activated serum ($P < 0.05$). These results suggest that the chemotactic activity of neutrophils increases with cell differentiation, and this is only evidenced when optimum serum concentrations are employed.

Because these results indicate that the chemotactic response was not only less in neutrophils from cord blood but also that it decreased significantly when the concentration of chemoattractant was decreased, the capacity of cord blood serum to generate chemoattractants was assayed. The chemotactic activity and complement levels of each serum were tested, and the results of these studies appear in Fig. 4 and Table 3, respectively. The chemotactic responses of the indicator cells to 100% activated adult and cord serum (not shown) were not significantly different than those to 50% activated serum concentrations (Fig. 4). The chemoattractants, which can be generated by zymosan activation, have a significantly lower capacity to attract indicator adult neutrophils ($P < 0.05$) compared to that generated in adult serum. When 10% activated serum was employed, no cells responded to cord serum, but 95 ± 35 (mean ± standard deviation) cells responded to adult serum.

The results of assay of total complement levels and C3 and C4 determinations in these sera revealed that both C3 and C4 were significantly less in cord blood sera compared to adult levels, but the total complement levels were similar and within the normal ranges. Collectively, these results indicate that the capacity of cord blood serum to produce chemoattractants when zymosan activated is less than that of adult blood serum. This correlates with their lower levels of C3 and C4 which are involved in generating some of the complement-related chemoattractants.

DISCUSSION

The results of the present studies indicate that the chemotactic responses of cord blood neutrophils appear to be approximately 20% of the adult levels. Similar results have been previously reported by several other investigators utiliz-

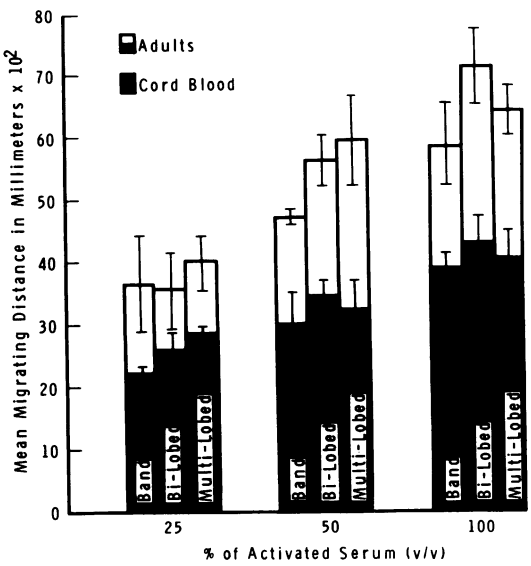

FIG. 3. Mean migrating distance ($\bar{X}_{MD}$) of various differentiational stages of neutrophils from adult and cord blood was derived by the method and formula described in Fig. 1. The results for neutrophils from cord blood are depicted by the shaded area of the bars, and those from adult blood are depicted by the total area of the bars. All experiments were done in duplicate. The results are expressed as the mean $\bar{X}_{MD}$ ± standard error from samples of four donors of each age group.

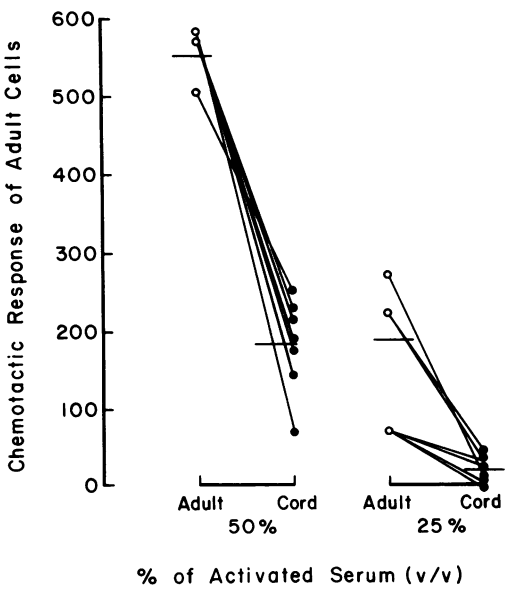

FIG. 4. Capacity of cord blood compared to adult blood serum to generate chemoattractants when activated with zymosan. Neutrophils from three normal adults were used as indicator cells. Their chemotactic response to 50 and 25% activated cord blood serum were compared to their response to similar concentrations of a pool of activated adult serum. All experiments were performed in duplicate, and the mean chemotactic response of the neutrophils to the adult pool and cord blood sera which had been prepared and assayed at the same time are connected with a line. Seven different cord blood sera were analyzed, and the mean chemotactic response of the adults to these are indicated by a horizontal bar.

ing various methods to measure chemotaxis of neutrophils from healthy young infants (7, 9, 10, 13, 17, 24). The present studies, however, address the quantitative responsiveness of these neutrophils under conditions of varying concentrations of chemoattractant and suggest additional differences employing this approach. Further, by devising a simple method of assay which incorporates both distance of migration and number of responding cells, it was also determined that the mean migration distances of all of the differentiational stages of cord blood neutrophils is less than those of adult. In addition, employing this assay, it was found that the mean migration distance of both adult and cord blood bands is less than that of their more mature differentiated PMN form. This increase in the chemotactic response during neutrophil maturation may partially contribute to the smaller total chemotactic response of cord blood neutrophils compared to adults since 31% of cord blood neutrophils are the less differentiated band form versus only 8% of the adult neutrophils. These data suggest that the chemotactic responses of cells in the neutrophil series increase with cell differentiation. This is suggested also by the work from several laboratories in studies with tissue culture systems, such as that of Rabinovitch and DeStefano (20), in which only the mature neutrophils from mouse bone marrow explants responded to a chemoattractant. In addition, the work by Niedel et al. (16) describes the appearance of chemotactic receptors in a cultured myeloid cell line with differentiation. Similarly, Symonds and Sachs (23) have reported activation of chemotaxis in relation to normal differentiation in clones of myeloid leukemia cells. Pike et al. (18) have recently reported the maturational development of specific receptors for chemoattractants and chemotactic responses in a monocyte cell line. Collectively, these data suggest that the state of cell maturation may determine its chemotactic responsiveness. Therefore, it is tempting to speculate that since the neutrophil population is less differentiated in cord blood compared to adult, that this in part may account for their lower chemotactic response.

Although the cellular mechanism(s) responsible for this immaturity in chemotactic function is as yet unclear, there are several possibilities to be considered: these include differences such as cell surface receptors (8), metabolic activity (1, 26), and cell adherence (6), all of which appear necessary to mediate and sustain directed migration. Firstly, the cell surface receptors which interact with the chemoattractant may be decreased in number. In support of this, Klemper and Gallin (8) have reported subpopulations of PMNs in peripheral blood which can be distinguished from one another by the presence of Fc

TABLE 3. Complement levels in cord and adult blood

Serum[a] source	Total C[b] (CH_{100} U/ml)	C4[c] (mg/dl)	C4[c] (mg/dl)
Cord	52 ± 3	14 ± 2	66 ± 4
Adult	55 ± 1	21 ± 2	100 ± 6

[a] Blood was collected from two groups, five healthy adult volunteers and the cord blood of six full-term newborns. After clotting, 4°C serum was removed, divided into volumes, and stored at −70°C until assay.

[b] Total complement was assayed by Quantiplate Single Radial Diffusion Total Complement plates. Each determination was done in duplicate. Results are expressed as mean ± standard error as CH_{100} in units per milliliter.

[c] C3 and C4 were assayed using Endoplate Single Radial Immunodiffusion plates. Each sample was assayed in duplicate both by timed diffusion and endpoint methods. The results (milligrams per deciliter) are expressed as mean ± standard error.

receptors as well as by specific functional differences. These workers have reported a correlation between the presence of Fc receptors and certain functional capabilities, e.g., adherence, chemotaxis, and microbicidal activity, and have demonstrated a decreased chemotactic function by cells showing fewer Fc receptors. In addition, Rabellino et al. (19) and Ross et al. (21) have reported the appearance of Fc and C3 receptors at different times during neutrophil differentiation. This appearance of chemotactic receptors with differentiation is also indicated by the work of Niedel et al. (16). The results of the present studies also support these findings as indicated by the increase in chemotactic response from the band to the PMN form. It therefore appears possible that the receptors for chemoattractants on normal peripheral blood myeloid cells as in cultured human promyelocytic leukemia cells (16) may also follow this maturational development.

A second explanation for the maturational changes in function may derive from cell metabolism. The metabolic event, that is, the "respiratory burst" which is associated with chemotaxis was recently reported to be immature in neutrophils from either cord blood (G. R. Strauss and M. S. Seifert, Pediatr. Res. **11**:495, 1977) or young infants (14) compared to those from adult. This may result in the inability of cord blood neutrophils to migrate as far as adult cells since Strauss and Seifert (Pediatr. Res. **11**:495, 1977) have reported that the decrease in the respiratory burst in cord blood cells appears to derive not from their initial hexose monophosphate shunt activity, but in their inability to sustain this activity.

A final possible mechanism which may explain the maturation in function may derive from cell adherence and aggregation associated with chemotaxis. This has recently been reported to be different in neonatal neutrophils compared to that of adult cells. Mease et al. (A. D. Mease, D. P. Burgess, and P. J. Thomas, Pediatr. Res. **14**:549, 1980) reported that neonatal neutrophils failed to exhibit the biphasic aggregation seen with adult cells, but remained aggregated. They suggest that this may be the cause of the lower chemotactic response. Recently they have reported decreased phytohemagglutinin-induced aggregation and C5a-induced chemotaxis of cord blood neutrophils (12). Although we have not directly tested this cell aggregation, we have repeatedly observed that the nonresponding neutrophils, that is, those which did not migrate out of the origin well towards the chemoattractant, from cord blood showed clumping whereas this was not seen with adult cells. This correlates with the observation of irreversible aggregation exhibited by neonatal neutrophils reported by Mease et al. (Pediatr. Res. **14**:549, 1980). In addition, Miller (13) previously reported a decreased movement and deformability in neonatal PMNs. He recently reported that these cells appear to be unable to reorient cytoplasmic streaming, associated with inability to adhere, and show direct migration (M. E. Miller and A. T. W. Cheung, Pediatr. Res. **14**:549, 1980) which may result in decreased chemotaxis. Although we could detect no differences in cell adherence either with neutrophil differentiation or between neutrophils from neonates or adults, this was not performed while cells were responding to a chemoattractant. It may be that, during such stimulation, neonatal neutrophils may not have the capacity to sustain additional normal functions such as adherence and chemotaxis.

In addition to the decrease in chemotactic function of the cord blood neutrophils, we also found that the capacity of cord blood serum to generate chemoattractants was 50% less than that of adult serum. These results are similar to those reported previously (13, 17). This may be related to the lower levels of some of the complement factors which we detected in these sera and which have been reported in the neonate (4) since the chemoattractants produced by serum activation are predominantly complement related, that is, C5a, C567 (22, 25). This decreased serum activity together with the inability of cells from cord blood to respond to lower concentrations of attractant may be contributing factors in the reported paucity of neutrophils at inflammatory sites in young animals (2, 5).

Collectively, these data suggest that the decrease in chemotactic response of neutrophils may derive in part from both intracellular as well as extracellular deficiencies and that these probably mature with cell differentiation as well as animal maturation.

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