0031-3998/93/3303-0242$03.00/0
PEDIATRIC RESEARCH

Vol. 33, No. 3, 1993
Printed in U.S.A.

Neutrophil Chemotaxis to Leukotriene B_4 *In Vitro* is Decreased for the Human Neonate

CHRISTIANE DOS SANTOS AND DENNIS DAVIDSON

Division of Neonatal-Perinatal Medicine, Schneider Children's Hospital of Long Island Jewish Medical Center, The Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11042

ABSTRACT. Leukotriene B_4 (LTB_4) is a product of arachidonic acid metabolism and a potent chemoattractant for adult polymorphonuclear leukocytes (PMN). LTB_4 may be an important inflammatory mediator in neonatal lung disorders such as bronchopulmonary dysplasia, but neonatal PMN chemotaxis to LTB_4 has not been studied. We compared total PMN migration and its components, chemotaxis and chemokinesis, to LTB_4 in newborns and adults. PMN from healthy adults and umbilical blood of healthy, full-term newborns ($n = 21$ pairs) were incubated in a 48-well chemotaxis chamber using 10-μm thick polycarbonate membranes. Membranes with pore sizes of either 3 or 5 μm (diameter) were used to assess the influence of PMN deformability on chemotaxis. For both 3- and 5-μm filter pore sizes, total PMN migration increased in a dose-dependent manner from an LTB_4 concentration of 10^{-9} to 10^{-6} M. The increase in total PMN migration was due entirely to chemotaxis (no chemokinesis) for newborns and adults. However, chemotaxis for the newborn was markedly attenuated, specifically, 14 and 24% of adult values at LTB_4 concentrations of 10^{-8} and 10^{-7} M, respectively, with the 3-μm pore size. With the 5-μm filter pore size, newborn chemotaxis significantly increased to 40 and 49% of adult values at LTB_4 concentrations of 10^{-8} and 10^{-7} M, respectively. We conclude that PMN chemotaxis to LTB_4 *in vitro* is lower in newborns than in adults and part of this impairment may be caused by a decreased deformability of the newborn PMN. Decreased PMN chemotaxis to LTB_4 may protect against excessive inflammation, as in bronchopulmonary dysplasia, but may increase susceptibility to infection in the newborn. (*Pediatr Res* 33: 242–246, 1993)

Abbreviations

HBSS, Hanks' balanced salt solution
LTB_4, leukotriene B_4
PMN, polymorphonuclear leukocyte

LTB_4 is a lipid mediator derived from the 5-lipoxygenase pathway of arachidonic acid metabolism in a variety of cells such as macrophages and neutrophils. LTB_4 is one of the most potent endogenous chemoattractants for adult human PMN *in vitro* (1–3) and *in vivo* (2, 4). Increasing evidence indicates that LTB_4 plays a critical role in a variety of inflammatory diseases (5), including neonatal disorders such as bronchopulmonary dysplasia (6). Newborns are known to have lower PMN chemotaxis than adults for several types of stimuli (7) such as the bacterial peptide, N-formyl-methionyl-leucyl-phenylalanine. However, there are no previous reports that have examined PMN chemotaxis to endogenous inflammatory lipid mediators such as LTB_4 in the newborn.

The first aim of the present study was to compare LTB_4-stimulated migration of PMN *in vitro* in newborns and adults. For both groups, PMN migration was measured in terms of baseline unstimulated PMN migration, total stimulated PMN migration, chemokinesis (random stimulated migration), and chemotaxis (directed stimulated migraton). The second aim was to examine whether developmental differences in PMN migration to LTB_4 *in vitro* could be explained by a difference in neutrophil deformability.

MATERIALS AND METHODS

Blood samples. Five to 10 mL of blood were obtained from healthy, adult human volunteers or from the umbilical vein of healthy, full-term infants immediately after delivery by routine cesarean section without general anesthesia. Blood samples were drawn into heparinized preservative-free tubes (Becton Dickinson, Rutherford, NJ). Blood sample collection for this study was approved by the Human Subjects Review Committee of Long Island Jewish Medical Center.

Preparation of LTB_4. LTB_4 50 μg/mL (Biomol, Plymouth Meeting, PA) was diluted with HPLC grade ethanol to 10^{-4}, and 100-μL aliquots were then stored at −75°C. On the day of each experiment, fresh serial dilutions, 10^{-6} to 10^{-9} M, were obtained using HBSS (Gibco, Grand Island, NY).

PMN isolation. Two different methods of PMN isolation were used, corresponding to two sequential sets of experiments using 3- and 5-μm filter pore sizes. Experiments with the 5-μm filter pore size were performed to determine whether neonatal PMN deformability had an effect on chemotaxis. These experiments required a more pure PMN isolation so that lymphocytes would not confound the counting of PMN that migrated through the filter.

The first set of experiments used the method described by Harvath *et al.* (8). Ten mL of blood were mixed with 3.2 mL of 5% dextran T-500 (Pharmacia LKB, Piscataway, NJ) in PBS (Sigma Chemical Co., St. Louis, MO). Blood was left at room temperature to settle for 45 min. The neutrophil-rich plasma was transferred to a 15-mL conical polypropylene tube and centrifuged at 400 × *g* for 5 min. After discarding most of the supernatant, the pellet was resuspended in the residual fluid. Remaining erythrocytes in this mixture were lysed by the addition of 3 mL of 0.034% NaCl for 30 s followed by the addition of 3 mL of 0.28% NaCl and cold Hanks'(−) (no calcium or magnesium) to fill the 15-mL tube. The sample was then centrifuged at 400 × *g* for 5 min. Cells were washed twice with 15 mL of Hanks'(−) and spun down (400 × *g*, 5 min). The pellet was

Received August 26, 1992; accepted November 6, 1992.
Correspondence: Dennis Davidson, M.D., Division of Neonatal-Perinatal Medicine, Schneider Children's Hospital, Long Island Jewish Medical Center, New Hyde Park, NY 11042.
Supported by a Grant-in-Aid from the American Heart Association, New York State Affiliate, and a New York Lung Association Research Grant (D.D.). Dr. Dos Santos was a fellow in the Division of Neonatal-Perinatal Medicine.

resuspended in 1 mL of cold Hanks'(−) and kept on ice for about 30 min. A drop of neutrophil suspension was stained (Diff-Quik, Baxter Scientific Products, McGaw Park, IL) to determine the percentage of the cells that were PMN. For the newborn it was 63 ± 3%, and for the adult it was 75 ± 2%. Final PMN suspensions used for chemotactic assay were adjusted to 10^6 PMN/mL using Hanks' with calcium and magnesium (HBSS). With use of this method of PMN isolation, cell viability assessed by the trypan blue dye exclusion method (Sigma) was 98.4 ± 0.3% for adult PMN and 97.2 ± 0.7% for the newborn.

For the second set of experiments, using the 5-μm pore size filter, we used a modification of the method described by Boyum (9). Six mL of blood were layered over 3 mL of Histopaque 1077 (Sigma) and spun in a centrifuge at 400 × *g* for 25 min. The supernatant and interface, containing primarily lymphocytes and monocytes, were removed, and the remaining mixture of blood cells was reconstituted with Hanks'(−) to 10 mL. Subsequent steps using dextran sedimentation and red blood cell lysis were identical to the first method described above. For the second method, the percentages of neutrophils that were identified as PMN were 92 ± 1% for the newborn and 94 ± 1% for the adult. The viabilities of the PMN using the second method were 94 ± 1% for the newborn and 95 ± 1% for the adult.

Chemotactic assay. A 48-well micro-chemotaxis chamber (Neuro Probe, Cabin John, MD) was used to study PMN motility (10). The chamber contains 25-μL lower wells for the chemotactic stimulus, separated by a filter from the 50-μL upper wells for placement of the PMN suspension. The lower wells were filled with either control solution (HBSS+) or solutions containing LTB_4 10^{-9} to 10^{-6} M. The filter was a polyvinylpyrrolidine-free polycarbonate membrane (Nucleopore, Pleasanton, CA) (8). It was 10 μm thick and was available with pore sizes of 3 or 5 μm (diameter). The upper chamber well was filled with 50 μL of a PMN solution of 10^6 PMN/mL. Chemotactic assays were paired for one adult and one newborn subject within the same chamber. Assays were run in triplicate for each concentration. After the PMN solutions were added to the upper wells, the entire chamber was placed in an incubator for 40 min at 37°C with humidified room air. When the incubation period was completed, the filter was carefully removed. PMN that did not migrate were wiped off the upper surface of the filter against a rubber blade (Neuro Probe). The remaining PMN on the filter were then stained with Diff-Quick. Using an optical grid and magnifications ranging from 100 to 1000×, 50 areas (0.01 mm^2) were counted and averaged. The results were expressed as PMN per mm^2.

The fractions of chemokinesis and chemotaxis that made up the total number of migrated PMN were determined by filling each additional upper well with a PMN suspension containing an LTB_4 concentration equal to that of its respective lower well. This method eliminates the chemotactic gradient for chemotaxis (directed migration). Under these conditions, any PMN migration that occurred could be accounted for by chemokinesis (random stimulated migration) or control, unstimulated migration. Therefore, chemotaxis values were derived from the total migration minus the chemokinetic component. The LTB_4 concentrations that gave a submaximal response, 10^{-8} and 10^{-7} M, were selected for these additional studies. For both one newborn and one adult, the total migration response curve to all LTB_4 concentrations and the additional chemokinesis studies could be performed in triplicate in one 48-well chamber.

Effect of filter pore size on chemotaxis. Chemotaxis data was also analyzed in terms of differences between filters with 3-μm pores and those with 5-μm pores to address the question of whether neonatal PMN may be less deformable than adult PMN. Because of the differences in the number of pores/mm^2 for the two types of filters, chemotaxis in terms of absolute numbers of PMN migrated/mm^2 could not be compared. Therefore, a chemotaxis index was used. This index was defined as the number of PMN that migrated by chemotaxis × 100%, divided by the total PMN migration for a given pore size, LTB_4 concentration, and subject group.

Drop-off studies. The number of PMN that migrated through the filter and then dropped into the lower chamber were determined in a series of chemotactic assays using individual blind well chambers and the same filters as the assays above. These experiments were performed to rule out the possibility that the decrease in neonatal chemotaxis was not artifactual due to neonatal PMN that migrated through but then dropped off the filter. The blind well chamber has a larger lower well (100 μL) so that any PMN that dropped off the filter could be more easily recovered for counting than with the microwell chamber. Migrated cells per mm^2 were counted, and this result was corrected to the total surface area of the filter over the well. The PMN concentration in the lower well was counted with a hemocytometer. The drop-off results express the PMN concentration of the lower well divided by the total PMN on a filter area corresponding to a well multiplied by 100%.

Statistics. A one-way analysis of variance for repeated measures was used to determine whether LTB_4 increased PMN migration for the newborn and adult. Differences in adult *versus* newborn total PMN migration, chemotaxis, chemokinesis, and control unstimulated migration were determined by unpaired *t* testing with a Bonferroni correction for multiple *t* tests (at different LTB_4 concentrations). The overall *p* value of 0.05 was used, but if comparisons were made at five different concentrations then a *p* value of 0.01 was considered significant (11, 12). Differences between chemotaxis indices (for comparison of chemotaxis using filters with differing pore size) were determined by an unpaired *t* test.

RESULTS

Assays using 3-μm filter pore size. The total PMN migration in response to LTB_4, for 12 adults *versus* 12 newborns, is shown in Figure 1. Baseline migration (unstimulated random migration, no LTB_4 stimulation) was slightly but significantly higher in the newborn than the adult. LTB_4 increased total PMN migration in a dose-dependent manner for the newborn and the adult. However, total migration was significantly lower in the newborn

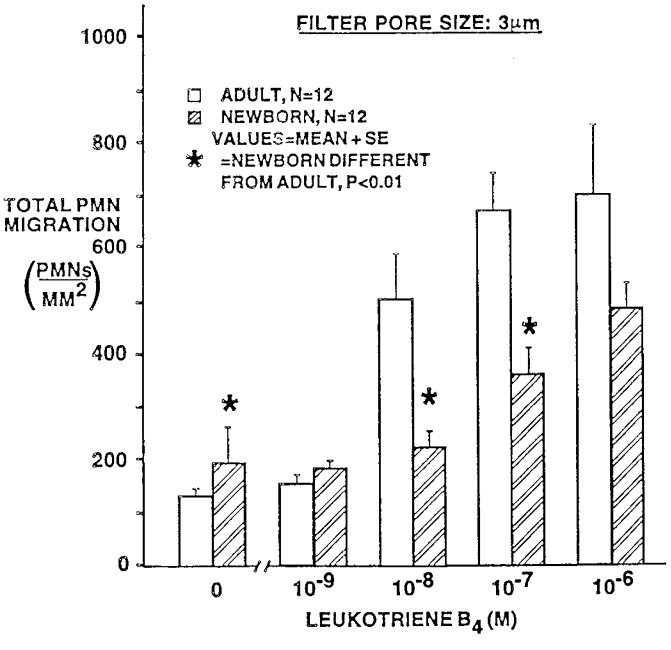

Fig. 1. Comparison between newborn and adult total PMN migration *in vitro* using a 3-μm filter pore size and LTB_4 as the chemoattractant. PMN migration for the newborn and adult increased significantly from baseline as the concentration of LTB_4 increased; however, this response was markedly attenuated for the newborn.

than the adult for the submaximal stimulatory concentrations of 10^{-8} and 10^{-7} M. Figure 2 depicts the components of LTB4-induced PMN migration, chemotaxis and chemokinesis, for six newborns and six adults at 10^{-8} and 10^{-7} M. At both concentrations, the chemotaxis components of total migration were significantly less for the newborn (14 and 38% of adult values, respectively). It was surprising that the chemokinesis component at both concentrations of LTB_4 was no greater than baseline migration for the adult and the newborn. The decreases in total migration and chemotaxis that we observed for the newborn and adult could not be explained by a drop-off of PMN from the bottom of the 3-μm filter into the lower well. Drop-off values for the newborn and the adult, 6.9 ± 0.7% and 9.6 ± 1.7%, respectively, were not significantly different.

Assays using 5-μm filter pore size. The total PMN migration in response to LTB_4 is shown in Figure 3. LTB_4 increased total PMN migration above baseline for both the newborn and the adult in a concentration-dependent manner. The absolute number of cells that migrated was less for the 5-μm filter pore size than for the 3-μm filter pore size at each of the LTB_4 concentrations. This could be accounted for by the smaller number of pores that were counted per microscopic field in the filter with the 5-μm pore size (25% of the number counted for the filter with the 3-μm pore size). As with the 3-μm filter, total migration was markedly reduced for the newborn compared with the adult. At an LTB_4 concentration of 10^{-8} M, this was not significant statistically ($p = 0.056$), but at 10^{-7} and 10^{-6} M total migration was significantly reduced for newborns ($p = 0.01$).

Chemotaxis and chemokinesis using the 5-μm filter pore size are shown in Figure 4. We found that neonatal PMN chemotaxis was also decreased (49% of adult values, $p = 0.001$) using this larger filter pore size at an LTB_4 concentration of 10^{-7} M. For LTB_4 10^{-8} M, the newborn chemotaxis was 40% of the adult chemotaxis; however, these results indicated a trend, inasmuch as statistical significance was not reached ($p = 0.08$). Similar to the 3-μm filter pore size assays, there was no appreciable chemokinetic component of PMN migration above baseline values (no LTB_4).

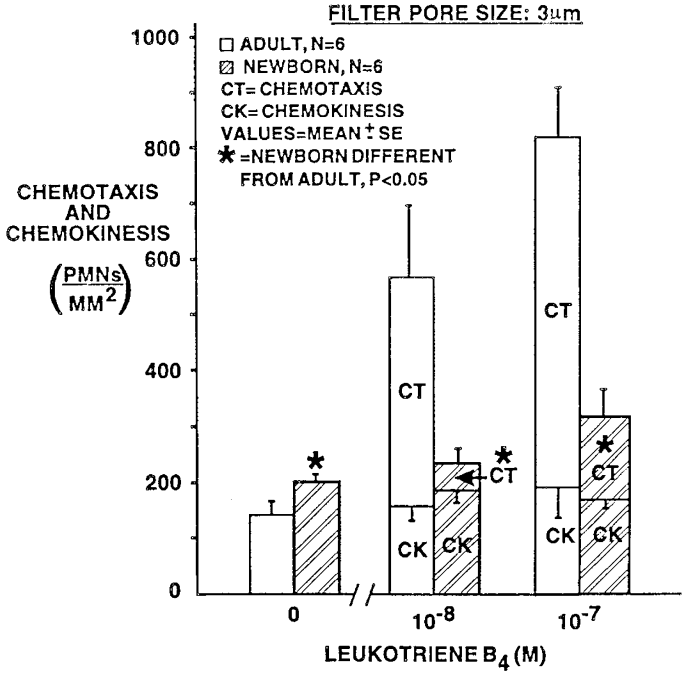

Fig. 2. Components of total migration for newborn and adult PMN *in vitro*, using a 3-μm filter pore size and LTB_4 as the chemoattractant. The whole bar (total migration) is divided into chemotaxis and chemokinesis. Chemotaxis accounted entirely for PMN migration above baseline in the newborn and adult; however, this response was markedly diminished for the newborn.

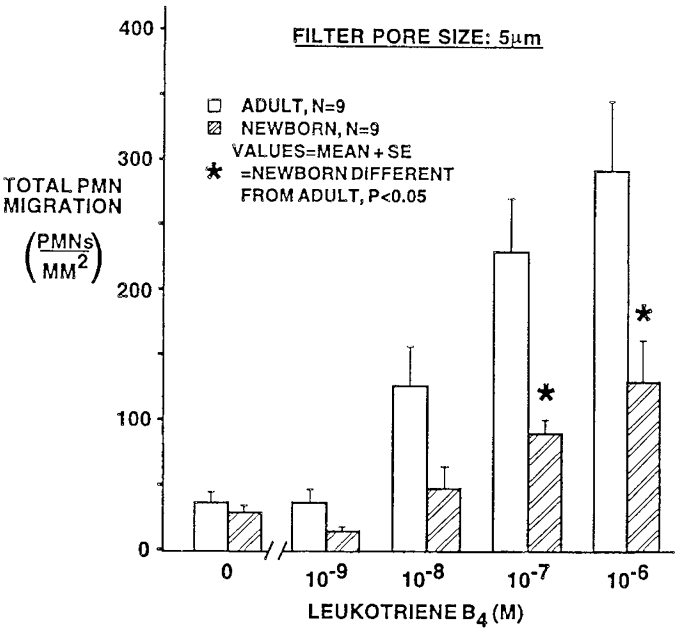

Fig. 3. Comparison between adult and newborn total PMN migration *in vitro* using a 5-μm filter pore size and LTB_4 as the chemoattractant. Total PMN migration increased significantly from baseline as the LTB_4 concentration increased; however, this response was markedly attenuated for the newborn.

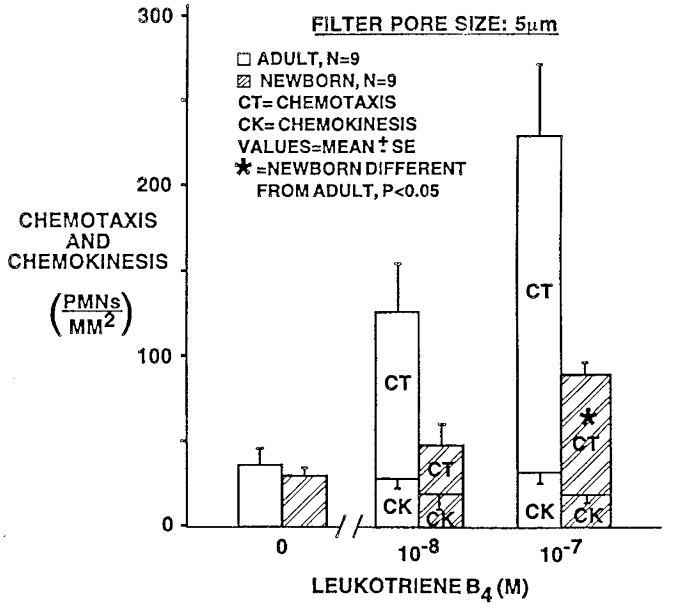

Fig. 4. Components of total migration (chemotaxis and chemokinesis) for newborn and adult PMN *in vitro* using a 5-μm filter pore size and LTB_4 as the chemoattractant. The whole bar (total migration) is divided into chemotaxis and chemokinesis. PMN chemotaxis was significantly lower in newborns than adults. However, newborn PMN chemotaxis represented a greater proportion of total PMN migration than previous experiments using a 3-μm filter pore size.

The chemotaxis index, that is, the chemotactic component of total migration for different filter pore sizes, is shown in Table 1. For newborns, the chemotactic component of total migration increased significantly with the 5-μm compared with the 3-μm filter pore size at both concentrations of LTB_4, 10^{-8} and 10^{-7} M. For adults, there were no changes in the chemotaxis index using the two different pore sizes and the two LTB_4 concentrations.

Table 1. *Chemotaxis component of total PMN migration* to LTB_4 in vitro: effect of filter pore size*

LTB_4 concentration	Patient group (n)	3-μm pore (%)	5-μm pore (%)
10^{-8} M	Newborn (15)	22 ± 8	66 ± 20†
10^{-8} M	Adult (14)	71 ± 10	76 ± 4
10^{-7} M	Newborn (15)	43 ± 9	80 ± 6†
10^{-7} M	Adult (15)	77 ± 5	82 ± 6

*Values = (chemotaxis/total migration) × 100%, mean ± SEM.

† Values different between 3- and 5-μm filter pore size, $p < 0.01$, for same group and LTB_4 concentration.

DISCUSSION

The present study is the first to compare PMN chemotaxis in the newborn *versus* adult for endogenous lipid mediators such as LTB_4. Using a 48-well chemotactic chamber, we found that PMN migration to LTB_4 was decreased for the newborn in the range of 14 to 49% of adult values depending on the LTB_4 concentration and filter pore size that was used. In this chamber, both newborn and adult PMN appeared to migrate toward LTB_4 via chemotaxis (directed stimulated migration) with no appreciable chemokinetic component (random stimulated migration). Accordingly, chemotaxis specifically was found to be decreased in the newborn *versus* adult, and this finding may be partially explained by decreased neonatal PMN deformability based on experiments using differing filter pore sizes.

For over a decade, LTB_4 has been identified as a potent lipid mediator for adult PMN chemotaxis (1–3). On a molar basis, LTB_4 has comparable, if not greater, chemotactic activity to the complement peptide, C5a, and the synthetic peptide F-met-leu-phe (2, 13). Peak chemotactic activity of adult PMN to LTB_4 in a number of previous studies is in the range of 10^{-6} to 10^{-8} M (1, 2, 14). These results are similar to those found in the present study for the adult and newborn PMN. In most of the original studies identifying LTB_4 as a PMN chemotactic stimulus for adult PMN, there were no determinations of the chemokinesis and chemotaxis components of total migration. Our results differ from one previous study in which 70% of total migration of adult PMN to LTB_4 *in vitro* was accounted for by chemokinesis (15). We found no appreciable chemokinetic component of total migration above random unstimulated migration for either the adult or the newborn PMN. Differences in assay methodology might explain these varied results. For example, in our study there was no protein in the upper-well PMN suspension, whereas in the former study a medium with 1% heat-inactivated FCS was used. In the present study, we found that baseline unstimulated migration was mildly but signficantly increased for the newborn using the first PMN isolation method (8) only. Although we did not directly test whether one or the other method preferentially activated neutrophils, it is unlikely that this affected our results regarding stimulated migration because *1*) PMN viability was equally high using both methods, *2*) chemokinesis for the newborn and the adult was the same as baseline regardless of PMN isolation method or LTB_4 concentration used, and *3*) the component of neonatal total migration due to chemotaxis was less for experiments in which PMN isolation was performed by the first as opposed to the second method.

The present finding of decreased neonatal chemotaxis to LTB_4 is consistent with previous work demonstrating a decrease in neonatal PMN chemotaxis compared with adult PNM chemotaxis for nonlipid stimuli such as N-formyl-methionyl-leucyl-phenylalanine, C5a, endotoxin, and serum activated by bacteria or zymosan (7, 13, 16–18). There is a wide range in the magnitude of decrease in neonatal PMN chemotaxis reported previously that may be related to the different assay techniques, filter pore sizes, age of the newborn or infant, and the type of chemoattractant used (7). However, with so many different types of stimuli associated with decreased neonatal chemotaxis, the possibility of a common impaired mechanism involving intracellular signal transduction leading to actin polymerization (19) in the neonatal PMN is strengthened. There may also be impaired redistribution of adhesion sites (13), impaired aggregation (20), or decreased deformability (7, 21).

To address whether decreased deformability could explain the decrease in neonatal PMN chemotaxis to LTB_4 observed in the present study, we compared our assay results for 3- and 5-μm filter pore sizes. The decrease in neonatal PMN chemotaxis with the 3-μm filter pore size persisted for the 5-μm filter pore size at 10^{-7} M LTB_4. For the 10^{-8} M concentration, there was a statistically significant decrease in neonatal PMN chemotaxis compared with the adult using the 3-μm filter but only a strong trend toward a difference using the 5-μm filter. The chemotaxis index was used to account for differences in the number of pores per mm^2 between the two filters. Although direct measurements of PMN deformability were not performed, the results from the present study indicated that chemotaxis increased partially but significantly toward adult values using the 5-μm filter pore size. We conclude that a decrease in deformability may partially explain the decrease in neonatal PMN chemotaxis to LTB_4.

The role of LTB_4-induced chemotaxis of PMN in neonatal inflammatory disorders has not been explored. Bronchoalveolar lavage of newborns with bronchopulmonary dysplasia has high levels of LTB_4 and PMN (6). The striking recruitment of PMN into the air space of preterm infants who will develop bronchopulmonary dysplasia begins within 3 d after birth (22). A positive feedback cycle for PMN recruitment could then start because neonatal PMN appear to have the same capacity to produce LTB_4 as adult PMN (23). *In vivo* LTB_4 has been shown to be a potent mediator of neutrophil recruitment into the air space of the adult human lung (4). In light of the present study, which demonstrates a decrease in neonatal PMN chemotaxis to LTB_4, it could be suggested that newborns may be partially protected from neutrophil-induced tissue injury that may occur with noninfectious inflammatory disorders such as the oxygen toxicity component of bronchopulmonary dysplasia. On the other hand, the impairment of neonatal PMN chemotaxis to LTB_4 may be an important cause of the increased susceptibility to infection in the newborn, especially for the very low birth weight infant who is also prone to bronchopulmonary dysplasia. The decrease in PMN chemotaxis to LTB_4 and other chemotactic stimuli in the early neonatal period may be part of a delicate balance between host defense and prevention of excessive inflammatory responses.

Acknowledgments. The authors thank Dr. P. Lipsitz and Dr. R. Bienkowski for their support and critical review of the manuscript. The preparation of the manuscript by Irene Barling is also gratefully acknowledged.

REFERENCES

1. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith JH 1980 Leukotriene B_4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286:264–265
2. Smith MJH, Ford-Hutchinson AW, Bray MA 1980 Leukotriene B_4: a potent mediator of inflammation. J Pharm Pharmacol 37:517–518
3. Malmsten CL, Palmblad J, Uden AM, Radmark O, Engstedt L, Samuelson B 1980 Leukotriene B_4: a highly potent stereospecific factor stimulating migration of polymorphonuclear leukocytes. Acta Physiol Scand 110:449–451
4. Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA 1989 Effects of leukotriene B4 in the human lung. J Clin Invest 84:1609–1619
5. Henderson Jr WR 1991 Eicosanoids and platelet activating factor in allergic respiratory diseases. Am Rev Respir Dis 143:S86-S90
6. Stenmark KR, Eyzaguirre M, Westcott JY, Henson PM, Murphy RC 1987 Potential role of eicosanoids and PAF in the pathophysiology of bronchopulmonary dysplasia. Am Rev Respir Dis 136:770–772
7. Wilson CB 1990 Developmental immunology and role of host defenses in neonatal susceptibility. In: Remington JS, Klein JO (eds) Infectious Diseases of the Fetus and Newborn Infant. WB Saunders, Philadelphia, pp 40–44
8. Harvath L, Falk W, Leonard EJ 1980 Rapid quantitation of neutrophil chemotaxis: use of a polyvinylpyrrolidone-free polycarbonate membrane in a multiwell assembly. J Immunol Methods 37:39–45
9. Boyum A 1976 Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5(suppl 5):9–15

10. Falk W, Goodwin RH, Leonard EJ 1980 A 48-well micro chemostasis assembly for rapid and accurate measurement of leukocyte migration. J Immunol Methods 33:239–247
11. Neter J, Wasserman W 1974 Applied Liner Statistical Models. Irwin, Homewood, IL, pp 796–808
12. Winer RJ 1962 Statistical Principles in Experimental Design. McGraw-Hill, New York, pp 591–595
13. Anderson DL, Hughes BJ, Smith CW 1981 Abnormal mobility of neonatal polymorphonuclear leukocytes. J Clin Invest 68:863–874
14. Palmblad J, Malmsten CL, Uden AM, Radmark O, Engstedt L, Samuelsson B 1981 Leukotriene B_4 is a potent and stereospecific stimulator of neutrophil chemotaxis and adherence. Blood 58:658–661
15. Ternowitz T, Herlin T, Fogh K 1987 Human monocyte and polymorphonuclear leukocyte chemotactic and chemokinetic responses to leukotriene B_4 and FMLP. Acta Pathol Microbiol Immunol Scand [C] 95:47–54
16. Pahwa SG, Pahwa R, Grimes E, Smithwick E 1977 Cellular and humoral components of monocyte and neutrophil chemotaxis in cord blood. Pediatr Res 11:677–680
17. Krause PJ, Kreutzer DL, Eisenfeld L, Herson VL, Weisman S, Bannon P, Greca N 1989 Characterization of nonmobile neutrophil subpopulations in neonates and adults. Pediatr Res 25:519–524
18. Eisenfeld L, Krause PJ, Herson V, Savidakis J, Bannon P, Maderazo E, Woronik C, Giuliano C, Banco L 1990 Longitudinal study of neutrophil adherence and motility. J Pediatr 117:926–929
19. Zigmond SH 1989 Chemotactic response of neutrophils. Am J Resp Cell Mol Biol 1:451–453
20. Hill HR 1987 Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. Pediatr Res 22:375–382
21. Kawaoka EJ, Miller ME, Cheung ATW 1981 Chemotactic factor-induced effects upon deformability of human polymorphonuclear leukocytes. J Clin Immunol 1:41–44
22. Merrit TA, Cochrane CG, Holcomb K, Bohl B, Hallman M, Strayer D, Edwards III DK, Gluck L 1983 Elastase and α_1-proteinase inhibitor activity in tracheal aspirates during respiratory distress syndrome. J Clin Invest 72:656–666
23. Kikawa Y, Shigematsu Y, Sudo M 1986 Leukotriene B_4 biosynthesis in polymorphonuclear leukocytes from blood of umbilical cord, infants, children and adults. Pediatr Res 20:402–406

Announcement

Meeting

The Society for Behavioral Pediatrics will conduct its 11th Annual Scientific Meeting and Workshops on September 9–13, 1993 at the Providence Marriott in Providence, RI. *For further information and registration forms, please contact* Ms. Noreen Spota at (215) 248-9168.