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Comparative Differences and Combined Effects of Interleukin-8, Leukotriene B_4, and Platelet-Activating Factor on Neutrophil Chemotaxis of the Newborn

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ABSTRACT

IL-8, leukotriene B_4 (LTB_4), and platelet-activating factor (PAF) are potent neutrophil chemoattractants that have been identified in inflammatory conditions of the newborn such as chronic lung disease of extreme prematurity. The aims of this study were to determine the relative potency and combined effects of these mediators on chemotaxis of polymorphonuclear leukocytes (PMN) from the newborn and to compare the effect of combining all three mediators on chemotaxis of PMN from newborns and adults. Neutrophils were isolated from cord blood ($n = 17$) or healthy adults ($n = 4$) and incubated in a 3-tier, 48-well chemotaxis chamber. For PMN from newborns, using chemoattractant concentrations ranging from 0.01 to 100 nM, we found that there were significant differences in potency: IL-8 > LTB_4 > PAF. Migration to each of these mediators was almost completely due to chemotaxis as opposed to chemokinesis. At submaximal chemotaxis, using equally effective doses of IL-8 (0.2 nM), LTB_4 (1.0 nM), and PAF (10 nM), the combination of all three mediators increased chemotaxis 2.4-fold above the average individual responses. Further studies indicated this increase in chemotaxis was due to the combination of IL-8 and PAF or IL-8 and LTB_4; but there was no increase in chemotaxis when PAF and LTB_4 were combined. The combination of all three submaximal doses of chemoattractants resulted in PMN chemotaxis that was still 36% of the adult response. We conclude that for neutrophils from the newborn: 1) IL-8 is a potent stimulus that has additive effects on chemotaxis in combination with either LTB_4 or PAF, 2) the combination of LTB_4 and PAF did not have an additive effect on chemotaxis, and 3) in spite of enhanced chemotaxis by more than one stimuli, the response is still significantly lower than for neutrophils from adults. (***Pediatr Res* 38: 11–16, 1995**)

Abbreviations

LTB_4, leukotriene B_4
PAF, platelet-activating factor
PMN, polymorphonuclear leukocyte

Inflammatory disorders in the newborn, such as chronic lung disease of extreme prematurity, necrotizing enterocolitis, and sepsis, are important causes of mortality and morbidity in neonatal intensive care units today (1). Neutrophil chemotaxis plays a central role in these disorders, leading to host defense or neutrophil-mediated tissue injury (2, 3). Potent neutrophil chemoattractants that have been implicated in the pathophysiology of these inflammatory disorders include IL-8, LTB_4, and PAF. Increased concentrations of all three mediators have been measured in extremely low-birth-weight infants with chronic lung disease and these chemoattractants can be produced locally by lung cells or neutrophils recruited into the lung (4–7). In addition, there are known biochemical interactions between these mediators in terms of their production by neutrophils and other cells (8–10). Therefore, in chronic lung disease the neutrophil is exposed to several structurally dissimilar signals that are integrated into a chemotactic response. The relative chemotactic potency of each mediator, individually or in combination, for neutrophils from the newborn, has not been previously studied.

The focus of the present study was on neutrophil chemotaxis in the newborn, which is known to be decreased compared with the adult when a single chemoattractant is tested (11–13). The overall hypothesis was that neutrophil chemoattractants may have a wide variation in potency, and yet together they could produce an additive effect which could partially correct the impaired chemotaxis previously observed for the neutrophil of the newborn when a single chemoattractant was used. The aims were to determine: 1) the relative chemotactic potency of IL-8, LTB_4, and PAF, 2) whether the chemotactic response was additive or synergistic in the presence of combinations of these

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mediators, and 3) whether combined mediator stimulation resulted in chemotaxis that approached the adult response.

METHODS

Materials

Recombinant human IL-8 (R&D Systems, Minneapolis, MN), LTB_4 (Biomol, Plymouth Meeting, PA), and PAF (L-α-phosphatidylcholine β-acetyl-γ-*O*-hexadecyl, Sigma Chemical Co., St. Louis, MO) were dissolved in Hanks' balanced salt solution containing 0.1% bovine albumin (endotoxin-free, Sigma Chemical Co.). A 3-tiered 48-well microchemotaxis chamber was used (Neuro Probe, Cabin John, MD).

Blood Samples

Eight milliliters of blood were obtained from healthy, adult human volunteers or from the umbilical vein of the placentas from healthy full-term infants immediately after delivery by elective cesarean section without general anesthesia. Blood samples were drawn into heparinized, preservative-free tubes. The study was approved by the Human Subjects Review Committee of Long Island Jewish Medical Center.

Neutrophil (Polymorphonuclear Leukocyte) Isolation

We used a modification of the method described by Boyum (14). Eight milliliters of blood from an individual subject was gently layered over 5 mL of Ficoll 1077 (Sigma Chemical Co.) in a 15-mL conical tube and centrifuged at 400 $\times$ *g* for 25 min. The plasma was discarded and the lower red cell layer was diluted with Hanks' minus solution (no calcium or magnesium) to a volume of 10 mL at 4°C. This cell suspension was then mixed with 3 mL of 5% Dextran T500 (Pharmacia Biotech Inc., Piscataway, NJ) in a 50-mL tube and left to settle by gravity for 40 min. The supernatant was transferred (without disturbing the red cell layer) to a 15-mL tube and centrifuged at 400 $\times$ *g* for 5 min. The supernatant was poured off, and a white pellet with a small amount of residual fluid remained in the bottom of the tube. The pellet was resuspended in the residual fluid followed by the addition of 4.2 mL of sterile water. After 20 s of mixing, 1.8 mL of 3% NaCl were immediately added to restore isotonicity. After thoroughly mixing, Hanks' minus solution (4°C) was added to make a volume of 13 mL. This cell suspension was centrifuged at 400 $\times$ *g* for 5 min and the pink supernatant (lysed red cells) was discarded. Washing and centrifugation were repeated twice. After the last centrifugation, the pellet and residual fluid was then resuspended in 2 mL of 0.1% BSA (endotoxin-free) in Hanks' solution at 4°C. Cell concentration was determined by counting cells in a hemocytometer, and a final cell concentration of 1×10^6 cells/mL in 0.1% BSA (Hanks' solution, 4°C) was made. Cell viability was determined by the trypan blue (Sigma Chemical Co.) exclusion method. A neutrophil differential count was determined by staining cells with Diff Quik (Baxter Scientific Products, McGaw Park, IL). The viability of neutrophils before chemotaxis was 98% for newborns and adults. The percentages of neutrophils identified as polymorphonuclear leukocytes were 93% for the newborn and 97% for the adult.

In pilot studies we found that PAF in Hanks' solution did not result in chemotaxis unless a carrier protein such as 0.1% albumin was added to the solution. Therefore, all chemoattractant solutions contained 0.1% albumin.

Chemotaxis Assay

The 3-tiered microchemotaxis chamber was composed of three plates with 48 through holes. This chamber was used to correct for possible differences in neutrophil drop-off from the counting filter for different chemoattractants. The top plate contained the upper wells where the neutrophil suspensions of 50 μL (5×10^4 neutrophils) were placed. Neutrophils migrated through a 3-μm pore polycarbonate filter that was layered over the chemoattractant reservoirs held in the middle plate (15). A 0.2-μm pore polycarbonate filter was placed between the bottom and the middle plate to trap any neutrophils that dropped off the underside of the 3-μm pore polycarbonate filter. After assembly, the chamber was placed in a humidified incubator at 37°C for 60 min.

After incubation, the top plate was removed along with the top filter. The underside of the filter contained cells that have migrated through the filter. Cells on the top side of the filter (that did not migrate) were wiped off along a firm rubber straight-edge (Neuroprobe, Cabin John, MD), and cells on the underside of the filter were stained with Diff Quik. This procedure left only a negligible number of cells on the top side of the filter. The filter was mounted on a glass slide for counting under the microscope. Cell counts were expressed as the number of cells per square millimeter.

Drop-off of cells from the underside of the top filter was determined for all experiments. These cells were recovered by centrifuging the chamber at 400 $\times$ *g* for 15 min, thereby forcing liquid but not cells through the bottom filter (0.2-μm filter pore size). The percent drop-off in this study was never greater than 3.9% with a mean of 2.1 $\pm$ 0.31% (SE). The number of cells that dropped off were added to the number of cells counted on the underside of the upper filter.

Protocols

All assays to a specific chemoattractant concentration were performed in triplicate wells, and the counts were combined as a mean value. An assay on cells from a single newborn subject, exposed to a variety of chemoattractants, was performed within one 48-well chamber. When chemoattractant was placed only in the middle chamber below the filter with the 3-μm pores, cell movement was described as total migration. Total migration was defined as the sum of random (undirected) migration and directed migration that is chemokinesis and chemotaxis, respectively. Chemokinesis was determined after adding the same concentration of chemoattractant into both the neutrophil reservoir and the chemoattractant reservoir, above and below the filter, respectively. Chemotaxis was determined by subtracting the chemokinesis value (mean of triplicate) from total migration.

The specific protocols were undertaken in the following order.

Protocol 1: Relative potencies of individual chemoattractants. To determine the relative potencies of IL-8, LTB_4, and PAF on total migration, dose responses were established for albumin blank (baseline), 0.01, 0.1, 1, 10, and 100 nM. The three smallest concentrations are the most likely to be observed in airway fluid of infants with chronic lung disease (4–6).

Protocol 2: Effect of combining three chemoattractants at submaximal and maximal effective doses. Based on data from protocol 1, we determined the dose of IL-8 (0.225 nM), LTB_4 (1 nM), and PAF (10 nM) that yielded a submaximal (approximately 10 × baseline) but equal level of total migration. Total migration, chemokinesis, and chemotaxis were determined for the individual chemoattractants and the combination of all three. To determine whether there was a maximal neutrophil chemotactic response for the newborn, we combined the maximal IL-8 dose (10 nM) with submaximal doses of LTB_4 (1 nM) and PAF (10 nM). The same assays described above for submaximal stimulation were performed.

Protocol 3: Contribution of individual chemoattractants to total effect of three combined. Total migration for mixtures of IL-8 and LTB_4, IL-8 and PAF, and LTB_4 and PAF were examined at the submaximal effective concentrations of the individual mediators described in Protocol 2.

Protocol 4: Comparison of total migration to combined chemoattractants with neutrophils from newborns *versus* adult. Neutrophil isolation and chemotactic assays were run simultaneously for adult and newborn subjects. We compared total migration to cells from newborns *versus* adult when the combination of the three submaximal concentrations of chemoattractants were used as described in Protocol 2.

Statistical Analysis

Changes in total migration from baseline for an individual mediator at progressively increasing concentrations were determined by analysis of variance. Comparisons of total migration or chemotaxis under different conditions of chemoattraction were made by analysis of variance and the unpaired *t* test or Mann Whitney test (based on distribution of data) followed by a Bonferroni correction for multiple comparisons. The overall significance value before correction was 0.05.

RESULTS

The dose-response curves for the three chemoattractants as measured by total migration of PMN from newborns is demonstrated in Figure 1 (n = number of subjects). The order of chemoattractant potencies was IL-8 > LTB_4 > PAF. At the peak of the IL-8 dose-response curve (10 nM), total migration was significantly lower for LTB_4 and PAF, *i.e.* 59% and 32% of the IL-8, respectively. At the highest concentration of chemoattractant (100 nM), total migration decreased for IL-8 but not LTB_4 and PAF, compared with the respective total migration levels at 10 nM. The dose-response curves indicated that the following doses of each chemoattractant produced an equal, submaximal total migration response that could be used in the next experiments: IL-8 (0.2 nM), LTB_4 (1 nM), and PAF

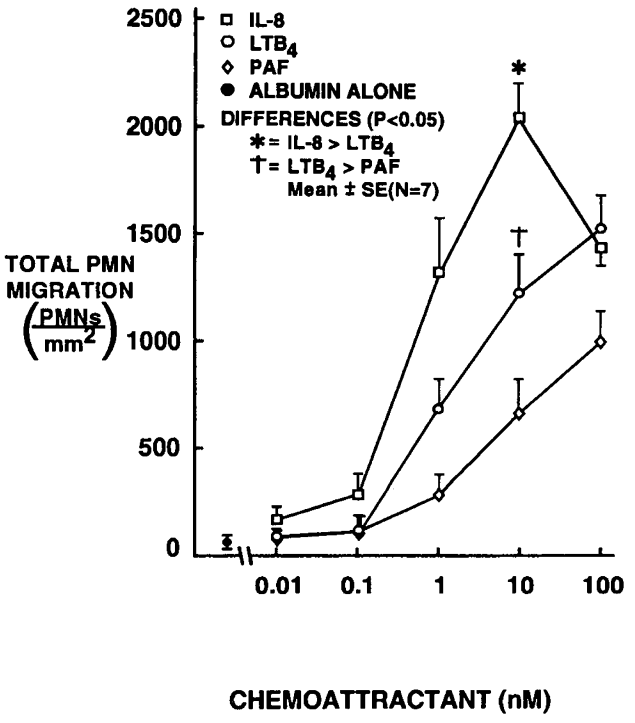

Figure 1. Dose-response comparison of three chemoattractants on total migration (chemotaxis plus chemokinesis) of polymorphonuclear leukocytes from newborns.

(10 nM). Figure 1 shows that equal total migration was the same for LTB_4 (1 nM) and PAF (10 nM); the physiologically equivalent dose for IL-8 (0.2 nM) was interpolated from the IL-8 dose-response curve. In addition, the peak total miagration at 10 nM for IL-8 suggested a maximum migratory

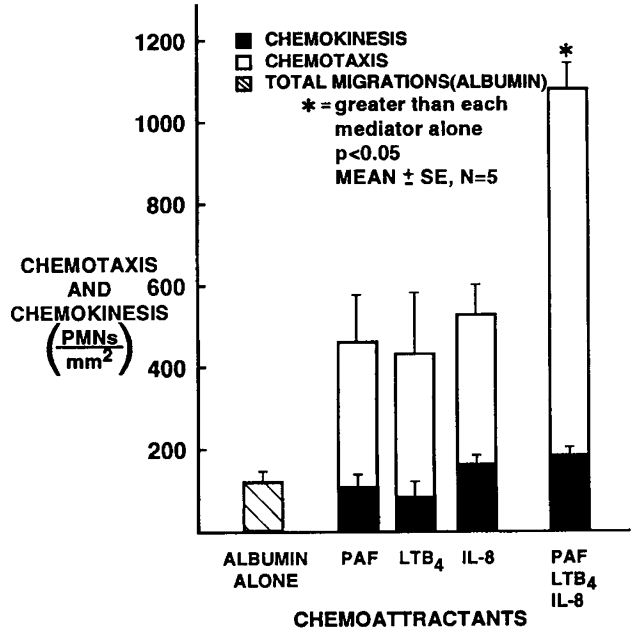

Figure 2. Effect of combining three chemoattractants on chemotaxis and chemokinesis, at concentrations that produce individually equal submaximal migration of polymorphonuclear leukocytes from newborns. Chemotaxis and chemokinesis combined equals total migration for studies involving IL-8, LTB_4, and PAF.

response for the neutrophil of the newborn which could be tested using a combination of chemoattractants.

The combined effect of the three chemoattractants, at doses which separately produce an equivalent submaximal total migratory response, is shown in Figure 2. There were no differences in total migration for IL-8 (0.2 nM), LTB_4 (1 nM), and PAF (10 nM). In addition, total migration for each of these chemoattractants could be explained almost totally by chemotaxis as opposed to chemokinesis. The combination of the three mediators would have been expected to give a mean total migration of approximately 1445 ± 236 PMN/mm^2 if the response was completely additive. A mean total response of 1069 ± 44 was observed and the difference compared with the expected value was marginally significant (0.08); the trend suggesting that the combination of the 3 mediators did not have an additive effect.

When the combined effect of pairs of chemoattractants was studied, it was demonstrated that total migration of neutrophils from newborns did not increase when submaximal, equally effective doses of PAF (10 nM) and LTB_4 (1 nM) were combined (Fig. 3). There were additive effects observed for PAF + IL-8 and LTB_4 + IL-8 compared with their individual effects on total migration; however there was no difference in total migration between these two combinations and total migration in response to all three mediators.

The increased effect of the three mediators (*versus* a single mediator) on total migration of neutrophils of the newborn was observed at submaximal but not maximal doses of IL-8 as shown in Figure 4. When a submaximal dose of IL-8 (0.2 nM) was used, the addition of submaximal equally effective doses of LTB_4 (1 nM) and PAF (10 nM) produced an increase in chemotaxis. However, when the dose of IL-8 that gave the greatest total migration (10 nM) was used, the addition of LTB_4 (1 nM) and PAF (10 nM) had no effect on total migration.

The effects of submaximal doses of IL-8 (0.2 nM), LTB_4 (1 nM), and PAF (10 nM) on total migration of neutrophils from

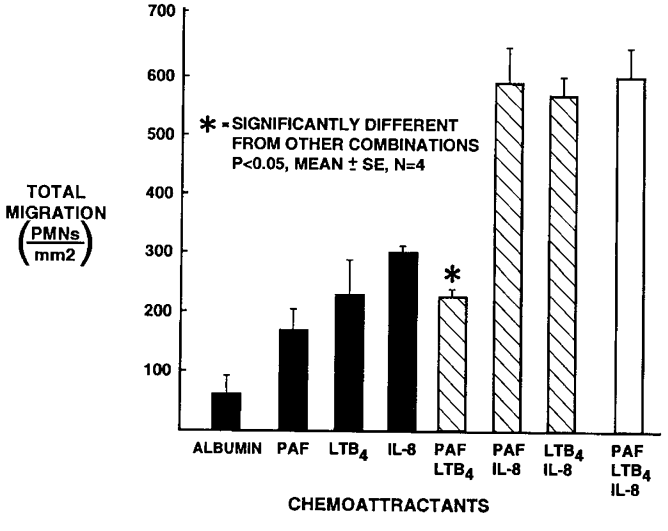

Figure 3. Interaction of different pairs of chemoattractants on total migration (chemotaxis plus chemokinesis) of polymorphonuclear leukocytes from newborns when equally effective, submaximal concentrations of individual chemoattractants are used.

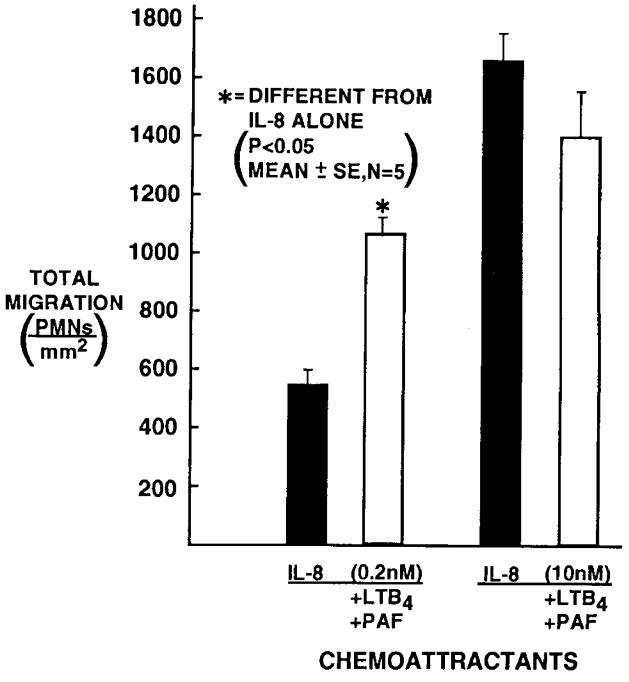

Figure 4. Total migration (chemotaxis plus chemokinesis) of polymorphonuclear leukocytes from newborns after addition of equally effective submaximal concentrations of LTB_4 and PAF to submaximal and maximally effective concentrations of IL-8.

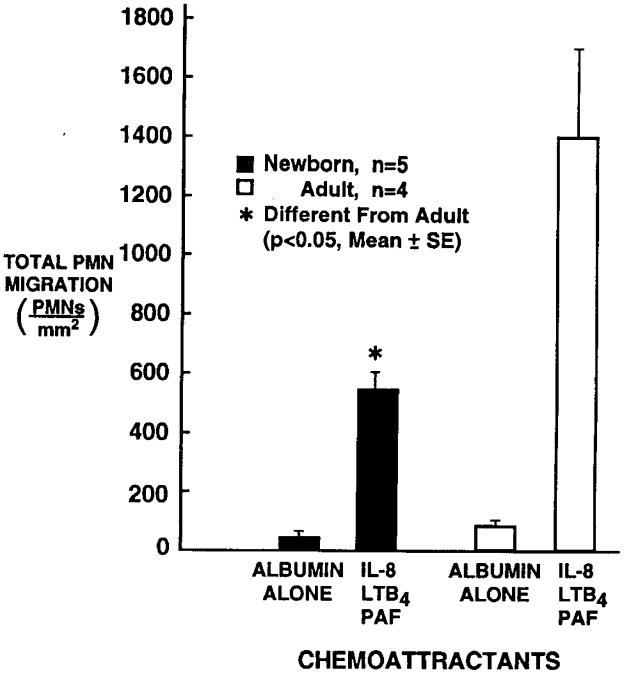

Figure 5. Total migration of PMN (chemotaxis and chemokinesis) to a combination of three chemoattractants, at submaximal concentrations: newborn *vs* adult.

newborns *versus* adults are shown in Figure 5. The response of PMN from newborns was 36% of the adult response (p = 0.005).

DISCUSSION

Although it has been well established that neutrophil chemotaxis is lower in the newborn compared with the adult

(10–13), there have been no previous studies which have examined the relative potency and combined effects of IL-8, LTB_4, and PAF on chemotaxis of neutrophils of the newborn. The present studies provide new information related to neutrophil migration into an inflammatory site of the newborn where multiple chemotactic substances have been described (4–6), e.g. the airway fluid from newborns with acute lung injury or chronic lung disease. We found that the order of chemotactic potency was IL-8 > LTB_4 > PAF. Work with neutrophils from adults suggests the same order of chemotactic potency. In adults, significant neutrophil chemotaxis has been observed for IL-8 at levels of below 1 nM in blood vessel wall constructs (16). Using a skin window preparation in adults, neutrophil migration to LTB_4 was greater than to PAF, with responses observed using solutions containing concentrations of these mediators at greater than 1 nM (17).

Conditions *in vivo* are likely to modulate the potency of the mediators examined in the present study (18). We used cord blood neutrophils from term infants after elective cesarean section. However, recent work indicates mode of delivery does not affect chemotaxis of cord blood neutrophils (19). It should also be noted that, in our pilot studies, PAF had very little chemoattractant activity unless it was prepared in a solution with albumin; this has also been shown by other workers (17). However, PAF and albumin are found together at inflammatory sites because of the important edemogenic properties of PAF. LTB_4, another lipid chemoattractant, does not require albumin to have an appreciable effect on neutrophil chemotaxis (11, 20).

The rationale for examining the effect of combining IL-8, LTB_4, and PAF on neutrophil chemotaxis for newborns was not only because they are identifiable together in an inflammatory condition, but additionally because there are known biochemical interactions between these mediators (8–10). Each of the mediators produced neutrophil migration that was almost completely accounted for by chemotaxis as opposed to chemokinesis; this did not change when neutrophil migration increased in the presence of all three mediators. However, the level of chemotaxis, which was expected when submaximal, equally effective doses of the three chemoattractants where combined, did not appear additive or synergistic. This was unexpected based on previous biochemical work. For example, PAF stimulates neutrophil LTB_4 production (8), IL-8 stimulates LTB_4 production (9), and LTB_4 stimulates neutrophil IL-8 production, although the last reaction usually requires hours (10). The failure to see a definitive, additive, or synergistic effect in the present study was not limited by the total capacity of the newborn neutrophils to migrate based on the protocol design. It is possible that *in vivo* conditions may be more favorable for modulatory interactions between these chemoattractants. For example, PAF-induced LTB_4 production by neutrophils is greatly enhanced by the granulocyte-monocyte colony-stimulating factor (8).

When the interaction of chemotactic mediators were studied by pairs of chemoattractants, additive effects were observed for submaximal doses of IL-8 + LTB_4 and IL-8 + PAF. We were surprised to find no increase in chemotaxis induced by the combination of LTB_4 and PAF above chemotaxis observed for each mediator alone. Taken together, these results suggest that PAF and LTB_4 may be specifically linked by a negative feedback or saturation mechanism which limits chemotaxis. Studies confirming these results *in vivo* may provide important information regarding mechanisms that control early, neutrophil-induced inflammation by these lipid mediators.

In spite of the increase in chemotaxis observed for newborn neutrophils after combining the three submaximal, equally effective doses of IL-8, LTB_4, and PAF, a maximal level of chemotaxis was observed if a dose of IL-8 (10 nM) was substituted into the combination of mediators. Furthermore, the increase in chemotaxis observed with the combined submaximal dose was still 36% of the level observed for adult neutrophils under the same conditions. Explanations for limited chemotaxis in the newborn have included a large subpopulation of immobile cells (21) with decreased actin polymerization (22, 23), decreased membrane fluidity or deformability (11), and decreased interferon-γ-induced calcium entry (24). Although we found an appreciable difference in chemotaxis between the two age groups, our study suggests that motile neutrophil subpopulations may be greater than previously considered for the newborn if more than one chemoattractant is used at submaximal doses or a single but maximal chemoattractant dose is used. It would be of interest to determine, in future studies, whether there are different subpopulations of neutrophils from newborns that respond differently to IL-8, LTB_4, and PAF.

In summary, we found that for neutrophils from the newborn: 1) IL-8 was a potent stimulus that, when combined with LTB_4 or PAF, produced additive effects on chemotaxis; 2) LTB_4 and PAF, together, did not have an additive effect on chemotaxis; and 3) in spite of enhanced chemotaxis by more than one stimulus, the response is still significantly lower than for neutrophils from adults. Our study supports increasing evidence that IL-8 may have a particularly important chemotactic role in neonatal inflammatory disorders such as bronchopulmonary dysplasia. Rapidly produced lipid mediators such as LTB_4 and PAF may play an important initial role in recruiting neutrophils into the airway; then neutrophil production of IL-8 (25) in the lungs of newborns with chronic lung disease may enhance a positive feedback cycle for the development of chronic inflammation. The present study also suggests that the neutrophil of the newborn may also have a down-regulatory mechanism for chemotaxis that may be addressed with future studies of the interaction between LTB_4 and PAF.

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REFERENCES

1. Hack M, Horbar JD, Malloy MH, Tyson JE, Wright E, Wright L 1991 Very low birth weight outcomes of the National Institute of Child Health and Human Development neonatal network. Pediatrics 87:587–595
2. Merritt 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
3. Musemeche C, Caplan M, Hsueh W, Sun X, Kelly A 1991 Experimental necrotizing enterocolitis: The role of polymorphonuclear neutrophils. J Pediatr Surg 26:1047–1049

4. Stenmark KR, Eyzaquirre 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
5. Groneck P, Reuss D, Götze-Speer B, Speer C 1993 Effects of dexamethasone on chemotactic activity and inflammatory mediators in tracheobronchial aspirates of preterm infants at risk for chronic lung disease. J Pediatr 122:938–944
6. Groneck P, Götze-Speer B, Oppermann M, Eiffert H, Speer C 1994 Association of pulmonary inflammation and increased microvascular permeability during development of bronchopulmonary dysplasia: A sequential analysis of inflammatory mediators in respiratory fluids of high-risk preterm neonates. Pediatrics 93:712–718
7. Strieter RM, Kasahara K, Allen RM, Standiford TJ, Rolfe MW, Becker FS, Chensue SW, Kunkel SL 1992 Cytokine-induced neutrophil-derived interleukin-8. Am J Pathol 141:397–407
8. McColl SR, Krump E, Naccache PH, Poubelle PE, Braquet P, Braquet M, Borgeat P 1991 Granulocyte-macrophage colony stimulating factor increases the synthesis of leukotriene B_4 by human neutrophils in response to platelet activating factor. J Immunol 146:1204–1211
9. Thomsen KM, Larsen CG, Thomsen HK, Hirstein D, Shak-Nielsen T, Ahnfelt-Ronne I, Threstrop-Pedersen K 1991 Recombinant human interleukin-8 is a potent activator of canine neutrophil aggregation, migration and LTB_4 biosynthesis. J Invest Dermatol 96:260–266
10. McCain RW, Holden EP, Blackwell T, Christman JW 1993 Leukotriene B_4 stimulates human polymorphonuclear leukocytes to produce interleukin-8. Am Rev Respir Dis 147:A754
11. Dos Santos C, Davidson D 1993 Neutrophil chemotaxis to leukotriene B_4 *in vitro* is decreased for the human neonate. Pediatr Res 33:242–246
12. Anderson DL, Hughes BJ, Smith CW 1981 Abnormal mobility of neonatal polymorphonuclear leukocytes. J Clin Invest 68:863–874
13. Hill HR 1987 Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. Pediatr Res 22:375–382
14. Boyum A 1976 Isolation of lymphocytes, granulocytes, and macrophages. Scand J Immunol 5(suppl 5):9–15
15. Harvath L, Falk W, Leonard EJ 1980 Rapid quantitation of neutrophil chemotaxis: Use of a polyvinyl pyrrolidone-free polycarbonate membrane in a multiwell assembly. J Immunol Methods 37:39–45
16. Huber BB, Kunkel SL, Todd RF, Weiss SJ 1991 Regulation of transendothelial neutrophil migration by endogenous interleukin-8. Science 254:99–102
17. Michel L, Mencia-Huerta J-M, Benveniste J, Duberteit L 1987 Biologic properties of LTB_4 and PAF-acether *in vivo* in human skin. J Invest Dermatol 88:675–681
18. Casale TB, Abbas MK 1991 Effects of various barriers on platelet-activating factor-induced neutrophil chemotaxis. J Allergy Clin Immunol 87:565–574
19. Herson VC, Block C, Eisenfeld LI, Maderazo E, Krause PJ 1992 Effect of labor and delivery on *neonatal* polymorphonuclear leukocyte number and function. Am J Perinatol 9:285–288
20. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith JH 1980 Leukotriene B_4, a potent chemotactic and aggregating substance released from polymorphonuclear leukocytes. Nature 286:264–265
21. Krause PJ, Kreutzer DL, Eisenfeld L, Herson VC, Weisman S, Bannon P, Greca N 1989 Characterization of non-motile neutrophil subpopulations in neonates and adults. Pediatr Res 25:519–524
22. Hilmo A, Howard TH 1987 F-actin content of neonate and adult neutrophils. Blood 69:945–949
23. Harris MC, Shalit M, Southwick FS 1992 Diminished actin polymerization by neutrophils from newborn infants. Pediatr Res 33:27–31
24. Hill HR, Augustine NH, Jaffe HS 1991 Human recombinant interferon gamma enhances neonatal polymorphonuclear leukocyte activation and movement, and increases free intracellular calcium. J Exp Med 173:767–770
25. Strieter RM, Kasahara K, Allen R, Showell HJ, Standiford TJ, Kunkel SL 1990 Human neutrophils exhibit disparate chemotactic factor gene expression. Biochem Biophys Res Commun 173:725–730