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Neutrophil adhesion molecules in term and premature infants: normal or enhanced leucocyte integrins but defective L-selectin expression and shedding

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SUMMARY

The functional deficits of neonatal neutrophils are well documented and are thought to contribute to the increased susceptibility of newborn infants to infection. We measured the adhesion molecules L-selectin, CD11a/CD18 and CD11b/CD18 on neutrophils from the cord blood of term ($n = 22$) and premature ($n = 32$) infants using a whole blood method with flow cytometry and quantitative bead standards to enumerate cell surface receptors. We also assayed plasma for the shed form of L-selectin (sL-selectin). Our results suggested that L-selectin expression on term infant neutrophils is lower than that on adult neutrophils (unstimulated and stimulated, both $P < 0{\cdot}001$), but that stimulated premature infant cells express higher L-selectin than term infants ($P < 0{\cdot}05$); it is possible that this deficiency is caused by physiological changes occurring around the normal time of parturition. We observed reduced sL-selectin in term infants ($P < 0{\cdot}001$) compared with adults, and even lower concentrations in premature infants ($P < 0{\cdot}001$). The sL-selectin concentrations in plasma may be a reflection of granulopoiesis, which may be reduced in premature infants. Our results showed increased resting neonatal neutrophil expression of CD11b/CD18 compared with adults, and the absence of any neonatal deficit of the ability to up-regulate CD11b/CD18 expression on stimulation. These findings are contrary to previous reports. Further studies suggested that the isolation procedures used in previous reports reduces the capability of the cells to respond to a formyl methionine leucine phenylalanine (fMLP) stimulus. This effect is more marked in neonatal neutrophils, suggesting that the previously reported deficiency is in fact due to the isolation techniques used rather than the cells' innate ability to up-regulate CD11b/CD18 expression. The results of our study lead us to propose that the adhesive function of neonatal neutrophils may be less defective than previously thought.

Keywords neutrophil neonatal integrins L-selectin sL-selectin

INTRODUCTION

Neutrophils defend the host against bacterial invasion as part of the immune system's primary defence mechanism, providing protection in a rapid, relatively non-specific manner. In order to achieve this, neutrophils must be able both to circulate freely in the blood and to migrate as adherent cells into the extravascular tissues to phagocytose and kill invading microorganisms.These migratory properties of the neutrophil are made possible by the presence of cell surface adhesion molecules. The adhesion molecules which mediate the cell–cell and cell–matrix interactions necessary for adhesion and extravasation include members of the two major families, the selectins and the integrins.

The selectins are a family of adhesion molecules which mediate the initial stages of neutrophil adhesion to endothelium [1]. They are characterized by an NH_2-terminal Ca^{2+}-dependent lectin domain, an epidermal growth factor (EGF)-like domain followed by repeated C3/C4 binding protein-like domains, a transmembrane region, and a cytoplasmic tail [2]. L (leucocyte)-selectin is constitutively functional on resting neutrophils [3]. The lectin domain of the L-selectin molecule appears to mediate neutrophil attachment to endothelium at sites of inflammation [4]. Endothelial cell surface expression of the ligand(s) for L-selectin is increased by inflammatory cytokines [4]. Interactions of L-selectin and its ligand appear to mediate rapid binding of the rolling neutrophil along endothelium under conditions of flow [5]. The speed of the neutrophil is greatly reduced by this rolling. The rolling interaction and subsequent deceleration of the neutrophil appear to be prerequisites for the chemoattractant-stimulated stable binding of neutrophils to the endothelium mediated by the β_2 integrins CD11a/CD18 and CD11b/CD18 [1]. Blocking MoAbs to the β_2 chain have no effect on the initial rolling of the neutrophil, but they completely prevent the stable

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attachment of rolling neutrophils (in the presence of such antibodies, neutrophils are released to return into the circulation) [3]. Activated neutrophils have populations of constitutively expressed and newly inserted CD11a/CD18 and CD11b/CD18 on their surface, and each population is capable of a rapid up-regulation of avidity, but the control of adhesion and the mechanisms that regulate integrin avidity are poorly understood.

L-selectin is shed from the neutrophil surface after cellular activation. It is thought that protease cleavage of the receptor near the membrane releases a nearly intact extracellular domain into the extracellular surroundings. Although the mechanism of shedding is unknown, either specific activation of a membrane-bound protease may result in enzymatic cleavage, or alternatively, activation-induced changes in the conformation of the L-selectin molecule may expose sites which are then susceptible to cleavage by soluble proteases [6]. Schleiffenbaum *et al.* have shown that the shed form of L-selectin (sL-selectin) is functionally active in that it inhibits the binding of lymphocytes to tumour necrosis factor (TNF)-activated endothelium at physiological concentrations [7]. sL-selectin may be generated by the continuous shedding of L-selectin from the surface of leucocytes *in vivo*, since the amounts of sL-selectin found in plasma are far higher than the quantity of L-selectin that could derive from maximal shedding by circulating leucocytes at one time [7]. sL-selectin concentrations might be useful as markers of the *in vivo* activation of leucocytes in inflammation, since markedly elevated levels have been found in the sera of patients with sepsis [8].

Bacterial infections are a major cause of morbidity and mortality in neonates. A number of abnormalities have been described in the host defence mechanisms of the newborn infant, including neutrophil function. Studies of fetal and neonatal neutrophils (both term and premature) have consistently documented diminished chemotaxis [9–13] and adherence [14–16], deficiencies in the expression of adhesion molecules and complement receptors [17–22].

Török *et al.* and Anderson *et al.* have reported diminished levels of L-selectin on resting term infant neutrophils compared with adults, and minimal down-regulation upon stimulation with formyl methionine leucine phenylalanine (fMLP) [10,19]. While Smith & Tabsh found that resting and stimulated fetal neutrophils express normal adult levels of L-selectin [21], Koenig *et al.* have suggested that there is less L-selectin loss from stimulated fetal cells [23]. The neutrophil expression of CD11a/CD18 on term infant cells has been found to be no different from that on adult neutrophils [18]. In contrast, although this group demonstrated a similar total cellular content of CD11b/CD18 to that of adult cells [17], and another recent report suggests slightly reduced content in neonatal neutrophils [24], several groups have published evidence that both term and premature neonates have a greatly reduced capacity to up-regulate their CD11b/CD18 expression [10,15,17,20]. However, the clinical consequences of altered neutrophil adhesion molecule expression and plasma sL-selectin remain unclear.

All previous studies have evaluated small numbers of individuals and have involved leucocyte preparatory steps before staining. In this study we measured the expression of the neutrophil adhesion molecules L-selectin, CD11a/CD18 and CD11b/CD18 and plasma sL-selectin levels in large groups of normal healthy adults, and cord blood from term infants and premature (< 37/40) infants. Cellular expression was measured using an experimental technique designed to minimize possible handling artefacts, and which allows receptor expression to be quantified in absolute numbers. We also compared the results using our methodology with those we obtained with an approach commonly used in previous reports.

SUBJECTS AND METHODS

Subjects

Venous blood samples were taken at the time of birth from the umbilical veins of term infants (n = 22, gestational age range 37–42 weeks) delivered by Caesarian section, and premature infants (n = 32, gestational age range 27–37 weeks) born either vaginally (n = 16) or by Caesarian section (n = 16). Control samples were drawn from 33 healthy adult volunteers by venipuncture and processed in parallel with neonatal samples. All samples were processed within 1 h of being taken. The study was approved by the South Sheffield ethical committee.

Stimulation of neutrophils with fMLP

The low molecular weight chemotactic factor fMLP (Sigma Chemical Co., Poole, UK) was used to modulate neutrophil adhesion molecule expression *in vitro*. fMLP was stored as a stock solution of 1 mM in DMSO (Sigma) and further diluted in Hanks' balanced salt solution (HBSS; without calcium, magnesium or phenol red; GIBCO Life Technologies, Paisley, UK) before use. In dose-response experiments using adult, term and premature infant neutrophils, incubation with 100 nM fMLP for 10 min at room temperature was shown to induce maximal changes in adhesion molecule expression in all cases (data not shown). Thus 100 nM fMLP was used under these conditions in all the studies presented here.

Direct immunofluorescence staining

Blood samples were anticoagulated with preservative-free heparin (20 U/ml). Aliquots (25 μl) of unstimulated or chemotactically stimulated whole blood were stained with saturating concentrations of MoAb for 10 min at room temperature. Erythrocytes were then lysed with FACS lysing solution (Becton Dickinson, Oxford, UK) for 10 min in the dark. After centrifugation and removal of the supernatant, samples were washed once with HBSS and fixed in a 0·4% solution of formaldehyde in PBS and 2% glucose and held at 4°C in the dark for at least 30 min until analysis.

Monoclonal antibodies

Monoclonal antibodies used in this study were: FITC-conjugated, anti-human antibody to an irrelevant epitope X927 (Dako, High Wycombe, UK); FITC-conjugated mouse anti-human antibody to CD11a 25-3-1 (Immunotech, Marseille, France); FITC-conjugated mouse anti-human antibody to CD11b CB145 (Cymbus Bioscience Ltd., Southampton, UK); and PE-conjugated mouse anti-human antibody to L-selectin TQ1 (Coulter Electronics, Hialeah, FL).

Isolation of neutrophils

In some experiments, neutrophils were isolated from whole blood as previously described [14]. Briefly, blood samples were

anticoagulated with EDTA and layered over an equal volume of a Ficoll–Hypaque mixture (24 parts Ficoll Type 400; Sigma; to 10 parts Hypaque (sodium diatriozate); Sigma). After centrifugation at 300 *g* for 30 min at 4°C, the neutrophil/erythrocyte pellet was removed and resuspended in PBS + 2% glucose. Neutrophils were isolated by Dextran sedimentation, washed twice and resuspended in PBS + 2% glucose. Erythrocyte contamination was eliminated by hypotonic lysis of suspensions with ice-cold distilled water. Final cell preparations were adjusted to the concentration of neutrophils as previously determined from the whole blood. To minimize degranulation or spontaneous up-regulation of CD11b/CD18 surface expression during the isolation of neutrophils, anticoagulated blood samples were processed immediately at 4°C, and all purification procedures were performed at 4°C. fMLP was added to isolated neutrophils to a final concentration of 100 nM and incubated for 10 min at room temperature. Aliquots (25 μl) of unstimulated or chemotactically stimulated neutrophils were stained with saturating concentrations of MoAb for 10 min at room temperature. Any unbound MoAb was removed by washing in HBSS. Samples were fixed in a 0·4% solution of formaldehyde in PBS and 2% glucose and held at 4°C in the dark for at least 30 min until analysis.

Flow cytometric analysis

Analysis of granulocyte populations and measurement of adhesion molecule expression were performed on a FACScan flow cytometer (Becton Dickinson). Green and red amplifier gains were calibrated before each experiment with Calibrite beads (Becton Dickinson) to ensure that relative fluorescence values were comparable between experiments; 2000 events were recorded from each sample and analysed using Lysis II analysis software (Becton Dickinson). Granulocytes were gated by their characteristic forward and orthogonal light scattering characteristics. The median fluorescence channel number was computed for each gated population and converted mathematically into a relative fluorescence value corresponding to the average level of expression of the epitope on the cells' surface.

Quantification of cell surface binding sites

Calibration fluorescence standards, with four FITC-labelled standards of varying intensities and four PE-labelled standards of varying intensities (Quantum Beads; Flow Cytometry Corp., San Juan, PR) were used to calibrate the flow cytometer. Simply Cellular beads (Quantum Beads) are uniform cell-sized beads which have goat anti-mouse antibody covalently bound to their surface with a known average number of binding sites per bead. These are used to measure the apparent fluorochrome:protein ratio for each antibody used as previously described [25].

The number of adhesion molecules is expressed as calculated from the apparent number of receptors for each antibody used minus the apparent number of receptors for the negative control antibody, thus eliminating any error due to cellular autofluorescence. Where this sum produced a negative value, it was corrected to zero.

sL-selectin ELISA

Blood samples were taken onto EDTA. After centrifugation at 4°C, aliquots of plasma were immediately frozen and stored at −70°C. These samples were assayed for sL-selectin using a quantitative ELISA for the detection of soluble human L-selectin (Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. Briefly, sL-selectin present in the plasma sample binds to anti-sL-selectin MoAb adsorbed onto the microwells of the ELISA plate. Another anti-sL-selectin antibody which has been horseradish peroxidase (HRP)-conjugated is added and binds a different epitope of the sL-selectin molecule. Any unbound enzyme-conjugated antibody is removed during washing, and a substrate reactive with HRP is added. A coloured product is formed in proportion to the amount of sL-selectin present in the plasma sample, which can be calculated using a standard curve. The lower limit of detection of the assay, defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium, was 0·3 ng/ml.

Statistical analysis

Differences were shown using Kruskal–Wallis one-way analysis of variance, and the Mann–Whitney *U*-test was applied to prove that these differences were significant.

RESULTS

L-selectin

Unlike adults, in both infant groups there were individuals whose neutrophils had no detectable expression of L-selectin (Fig. 1). As a group, term neonatal neutrophil expression of L-selectin was lower than in adults both in unstimulated and stimulated (both $P < 0{\cdot}001$). However, stimulated premature infants' cells expressed L-selectin at significantly higher average levels than term infants ($P < 0{\cdot}05$), so that levels were not significantly different from adults.

The levels of sL-selectin were measured in the plasma of 10 normal healthy adults, 14 term infants and 16 premature

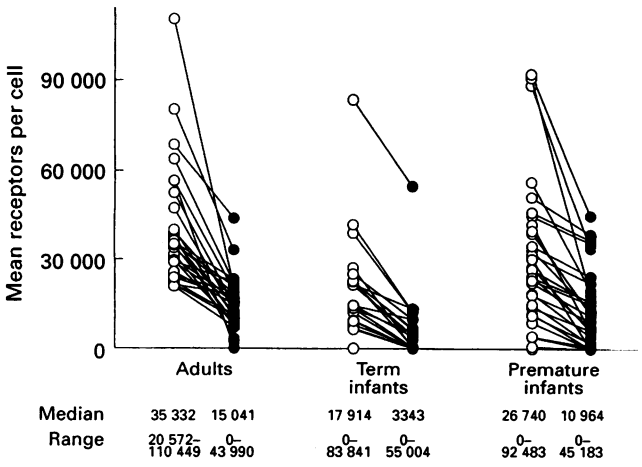

Fig. 1. Expression of L-selectin on adult ($n = 33$) and term ($n = 22$) and premature ($n = 32$) infant neutrophils. Neutrophils were unstimulated (○) or stimulated (●) with 100 nM formyl methionine leucine phenylalanine (fMLP). There were individuals in both neonatal groups whose neutrophils had no detectable L-selectin expression. When compared with adult controls, L-selectin expression was markedly lower on both unstimulated and stimulated term infant neutrophils (both $P < 0{\cdot}001$). Stimulated premature infant neutrophils had significantly higher L-selectin expression than that seen on term infant cells ($P < 0{\cdot}05$).

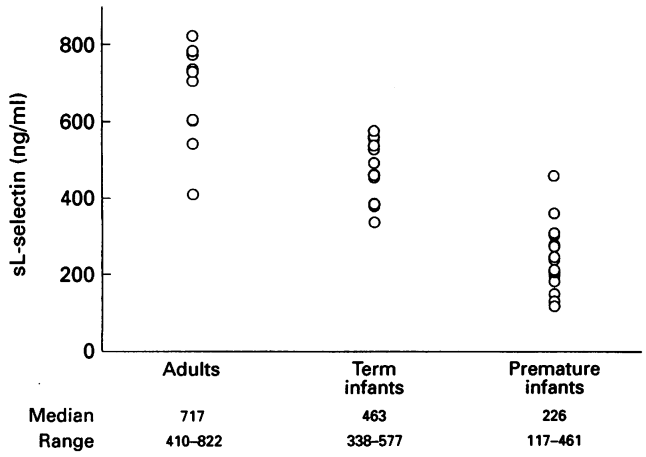

Fig. 2. Concentrations of the shed form of L-selectin (sL-selectin) in the plasma of adults (n = 10) and term (n = 14) and premature (n = 16) infants. We observed significantly lower sL-selectin in the plasma of term infants compared with adult plasma ($P < 0.001$), and even lower concentrations still in the plasma of premature infants ($P < 0.001$).

infants. sL-selectin was found in the plasma of all subjects studied (Fig. 2). The level of sL-selectin in normal adult plasma was significantly greater than that found in term infant plasma ($P < 0.001$), and the sL-selectin in premature infant plasma was significantly lower than that in both adult ($P < 0.001$) and term ($P < 0.001$) infant plasma.

CD11a/CD18

Unlike the other adhesion molecules studied, the expression of CD11a/CD18 was similar in all three groups, with a wide range of mean numbers of receptors per cell between individuals (Fig. 3). There was a small detectable increase in expression in nearly all cases in response to stimulation with fMLP.

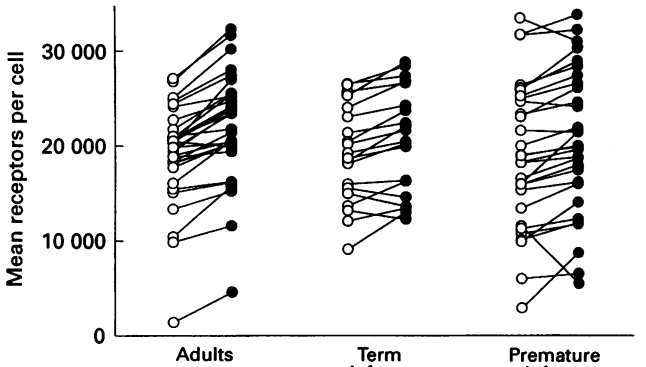

Fig. 3. Expression of the integrin CD11a/CD18 on the surface of neutrophils of adults (n = 33) and term (n = 22) and premature (n = 32) infants. Neutrophils were unstimulated (○) or stimulated (●) with 100 nM formyl methionine leucine phenylalanine (fMLP), and then stained with monoclonal antibody as described in Materials and Methods. CD11a/CD18 expression was similar in all three groups, with a small detectable increase in expression with the addition of fMLP in nearly all patients studied.

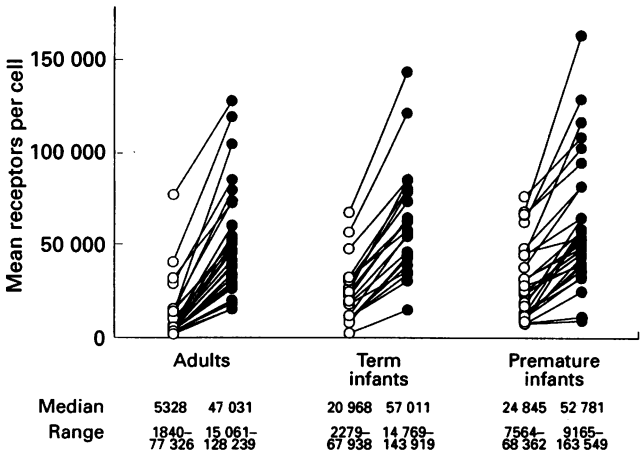

Fig. 4. Expression of CD11b/CD18 on the surface of neutrophils from adults (n = 33) and term (n = 22) and premature (n = 32) infants. Neutrophils were unstimulated (○) or stimulated (●) with 100 nM formyl methionine leucine phenylalanine (fMLP). There were individuals in all three groups whose neutrophils expressed almost undetectable CD11b/CD18. Both term and premature infant neutrophils expressed significantly more CD11b/CD18 than adult controls (both $P < 0.001$) in the unstimulated state. When stimulated with fMLP, all three groups were able to express a similar mean number of receptors ($P \geq 0.05$), contrary to previous reports.

CD11b/CD18

In all three groups, there were individuals whose neutrophils expressed almost undetectable levels of CD11b/CD18 at rest (Fig. 4). However, on average, both term and premature infants' neutrophils expressed significantly more CD11b/CD18 than adults' when unstimulated (both $P < 0.001$). Furthermore, contrary to previous reports [10,19,20,24,26], there were no differences in expression between either neonatal group and adults following fMLP stimulation ($P \geq 0.05$).

Adhesion molecule expression on Ficoll–Hypaque-separated neutrophils

Our data, using a whole blood method, differed from previous reports in that it did not demonstrate a deficiency in expression and up-regulation of CD11b/CD18 in either term or premature neonatal neutrophils. In order to elucidate whether the reason for these conflicting data was methodological or due to differences between the patient groups studied, we compared methods. We isolated neutrophils from whole blood as previously reported [14,17–20,24] using Ficoll–Hypaque gradient centrifugation, Dextran sedimentation, and hypotonic lysis to remove contaminating erythrocytes. We compared neutrophil adhesion molecule expression and modulation by 100 nM fMLP in these isolated cells with leucocytes in whole blood from six adults, three term infants and three premature infants.

L-selectin expression. Isolation of neutrophils from whole blood resulted in reduced L-selectin expression of both unstimulated and stimulated cells in all three groups (Table 1). Consequently, although addition of fMLP reduced the L-selectin expression of all neutrophils further, the apparent decrease in expression was significantly less marked in the separated neutrophils (adult $P < 0.05$, and neonatal $P < 0.005$).

Table 1. Median (range) expression of L-selectin and CD11a/CD18 on Ficoll-Hypaque separated neutrophils and those prepared using the whole blood method. The isolation procedure reduced L-selectin expression in both groups and significantly reduced the down-regulation on adult and neonatal neutrophils in response to formyl methionine leucine phenylalanine (fMLP) ($P < 0.05$ and $P < 0.005$, respectively). It did not significantly or consistently affect CD11a/CD18 expression

		Adult ($n = 6$)		Neonate ($n = 6$)	
		-fMLP	+fMLP	-fMLP	+fMLP
L-selectin					
Whole blood	median	77 517	17 848	36 667	12 793
	range	(33 796–110 449)	(0–33 996)	(9077–88 736)	(0–35 822)
Isolated cells	median	18 112	8274	18 328	13 003
	range	(4548–54 539)	(0–30 060)	(0–42 725)	(0–34 797)
CD11a/CD18					
Whole blood	median	15 272	17 949	17 030	16 787
	range	(1369–20 476)	(4592–20 536)	(10 097–23 367)	(11 916–23 229)
Isolated cells	median	20 436	18 658	17 361	19 335
	range	(14 646–22 773)	(16 461–23 188)	(2961–21 162)	(3949–19 925)

CD11a/CD18 expression. The CD11a/CD18 expression of neutrophils was altered by isolation from whole blood in most cases in both adults and neonates. However, the changes induced showed no consistent pattern (Table 1).

CD11b/CD18 expression. Ficoll–Hypaque isolation produced two important changes in neutrophil CD11b/CD18 expression from the neonatal group. First, the apparent level of expression in unstimulated cells was reduced in isolated cells relative to cells in whole blood in five out of six cases (Fig. 5). This was not seen in adult neutrophils. Second, the up-regulation of CD11b/CD18 in response to 100 nM fMLP was almost completely abrogated by the isolation procedure, whereas a marked up-regulation was seen in whole blood controls (adult $P < 0.005$, neonatal $P < 0.01$) (Fig. 5). This effect of Ficoll separation appeared to be more complete in neonates than in adults.

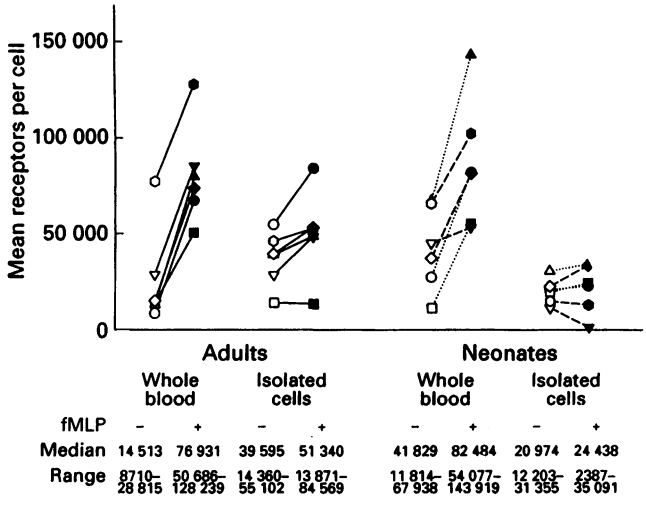

Fig. 5. Expression of CD11b/CD18 on Ficoll–Hypaque-separated neutrophils from adult ($n = 6$) and term ($n = 3$) and premature ($n = 3$) infants. Comparing the expression of CD11b/CD18 on neutrophils prepared using a whole blood method with those separated from whole blood using Ficoll–Hypaque gradient centrifugation, Dextran sedimentation and hypotonic lysis, as described in Subjects and Methods. All neutrophils were either unstimulated (all open symbols) or stimulated (all closed symbols) by the addition of 100 nM formyl methionine leucine phenylalanine (fMLP). The CD11b/CD18 expression of unstimulated neonatal, but not adult, neutrophils was reduced in isolated cells compared with cells in whole blood. The fMLP-stimulated CD11b/CD18 up-regulation response of both groups of neonatal neutrophils (term infants, ·····; premature infants, -----) and adults (——) was reduced by isolation (adult $P < 0.005$, neonatal $P < 0.01$). A marked up-regulation of CD11b/CD18 expression was observed in all whole blood samples on stimulation with fMLP.

DISCUSSION

Defects in the microbicidal function of fetal, term infant and premature infant neutrophils have been well documented, and are thought to contribute to the susceptibility of the neonate to bacterial infection. Since leucocyte adhesion to endothelium is a prerequisite for phagocyte antimicrobial action, abnormalities of adhesion molecule expression and function may be similarly important.

Our results suggest that expression of L-selectin is lower on term infant neutrophils than adults, but that this deficiency is not apparent in premature infants (Fig. 1). Previous reports have shown similarly low expression in term infants [10,19,26]. Our data raise the possibility that the L-selectin deficiency shown in term neonates is caused by physiological changes occurring around the normal time of parturition. This possibility is supported by the recent demonstration of expression of L-selectin on granulocytes of 23–25 week fetuses at levels similar to those seen in adults [21].

Our data on sL-selectin show a pattern which contrasts sharply with that on cellular L-selectin. Again, term infants had plasma concentrations which were significantly lower than in adults ($P < 0.001$). However, in premature infants the concentrations were significantly lower still ($P < 0.001$). It has been shown that sL-selectin markedly inhibits leucocyte adhesion to activated endothelium *in vitro* [7]. Appreciable inhibition was demonstrated over the concentration range observed in the patients in our study. Thus, the differences we observed

may have important functional significance *in vivo*: low levels of circulating sL-selectin may result in increased leucocyte adhesion under inflammatory conditions.

It appears paradoxical that term infants have significantly lower neutrophil expression of L-selectin than premature infants, but significantly higher plasma concentration of sL-selectin. sL-selectin is thought to be a shed form of the leucocyte surface protein. The mean number of L-selectin molecules shed by term neonatal neutrophils upon maximal stimulation with fMLP in our study (14333), the mean neutrophil count ($6{\cdot}22 \times 10^6/l$), the molecular weight of the soluble form of L-selectin (62 kD) [7], and the median plasma concentration of sL-selectin (463 ng/ml) are known. Using these values and Avogadro's number, it is possible to predict that simultaneous maximal shedding of all L-selectin from circulating neutrophils would only account for <0·001% of the total circulating soluble form of the protein in the plasma. Lymphocyte and monocyte L-selectin could not add significantly to this (<0·001%). Thus, the soluble protein is likely to be the stable product of continuous L-selectin shedding. This process apparently reflects neither the level of expression on circulating leucocytes at any one time, nor their potential to shed in response to maximal chemotactic stimulation *in vitro*. Concentrations of sL-selectin may, on the other hand, reflect rates of granulopoiesis [27], which may be reduced in premature infants and increased in adults with sepsis who have higher plasma levels [8].

Our findings of increased CD11b/CD18 expression on resting neonatal neutrophils relative to adult controls, and the absence of any neonatal defect in CD11b/CD18 up-regulation on stimulation, appear to contradict a number of previous reports. Surprised by this, we performed further studies to examine whether this might be due to the methodology used. Our results suggest that the isolation procedure reduces the capability of the cells to respond to an fMLP stimulus with an increase in CD11b/CD18 expression, but this effect is particularly marked in neonatal neutrophils (Fig. 5). Thus, a difference does exist between adults and neonates, but this is in their resistance to the modifying effects of isolation rather than their innate ability to express integrins. Further studies of neutrophils adherent to endothelium will help elucidate whether significant differences exist under conditions in which leucocyte integrins exercise their adhesive function. However, the results of this study indicate that the effects of isolation may be important in studies where neonatal and adult neutrophils are compared, and demonstrate that the circulating granulocytes of normal individuals commonly express very low levels of CD11b/CD18 until stimulated or manipulated.

Most previous reports of neonatal non-specific immunity and, in particular, neutrophil function have demonstrated deficiencies which have generally been postulated as factors in explaining their peculiar susceptibility to infection. While important defects undoubtedly exist, particularly in granulopoiesis [27], septicaemic infants also suffer the vascular and organ injury seen in older patients with overwhelming infection, despite treatment with high doses of specific antimicrobials. Such pathology may, at least in part, be the result of neutrophil-mediated endothelial injury. This study provides evidence that the adhesive function of term and premature infants' neutrophils may be less defective than previously supposed. If future studies demonstrate that neonatal neutrophils can both adhere to and damage microvascular endothelium significantly in sepsis, this may have implications for the design of new interventions for the prevention and treatment of infection in the newborn.

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