CD18-Dependent and L-Selectin-Dependent Neutrophil Emigration Is Diminished in Neonatal Rabbits

By James D. Fortenberry, Joanne R. Marolda, Donald C. Anderson, C. Wayne Smith, and M. Michele Mariscalco

Human neonatal neutrophils manifest decreases in mobility, adherence, and emigration compared with adult neutrophils that may contribute to the increased susceptibility of neonates to infection. In a developmental rabbit model, we show a reduced ability of neutrophils from 1-day-old rabbit pups to emigrate to inflamed peritoneum (3.7 ± 0.35 × 10⁶ neutrophils/mL peritoneal exudate) compared with 14-day-old (8.5 ± 0.7 × 10⁶/mL) and adult rabbits (9.4 ± 1.4 × 10⁶/mL, P < .05) despite significantly increased blood neutrophil counts. Because the reductions in functional Mac-1 (CD11b/CD18) as well as the amount of surface L-selectin are hypothesized to be primarily responsible for the differences in human neonatal neutrophil mobility, we examined CD11b/CD18 and L-selectin in our model. Using flow cytometric analysis we found that similar to human neonates, neutrophils from 1-day-old rabbit pups had 57% of adult rabbit levels of L-selectin and, in contrast with adults, failed to show significant decreases in L-selectin after chemotactic stimulation. In addition, neutrophils from 1-day-old pups compared with adults showed a significantly diminished capacity to upregulate CD11b/CD18 after chemotactic stimulation in vitro, or after emigration to the inflamed peritoneum. Systemic administration of anti-L-selectin monoclonal antibody (MoAb) resulted in significant reduction in peritoneal neutrophils in adult (47%, P < .05) and 14-day-old rabbits (47%, P < .05), but was without effect in 1-day-old rabbits. Administration of anti-CD18 MoAb resulted in significant reduction in peritoneal neutrophil accumulation in all age groups though less in 1 day and 14 day (58% and 65%, respectively) than in adults (91%, P < .05). Only in the 14-day-old rabbits was there an additive effect of anti-L-selectin and anti-CD18 MoAbs compared with anti-CD18 alone (84% vs 65%, P < .05). The findings in this in vivo rabbit model support the hypothesis that the previously described in vitro defects in human neonatal L-selectin and CD11b/CD18 may be major contributors to human neonatal inflammatory deficits.

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lous Amoebocyte Assay, Cape Cod Associates, MA). The anti-CD11b MoAb, SG12/8 (IgG1), was produced by immunization of mice with membrane preparations from rabbit neutrophils stimulated with chemotactic peptide 100 nmol/L f-met-leu-phe (Sigma Chemical Co, St Louis, MO) as described.24 Using flow cytometry (see below) MoAb SG12/8 showed low levels of binding to unstimulated rabbit neutrophils.25 With chemotactic factor stimulation, SG12/8 binding increased twofold to threefold. SG 12/8 showed minimal binding to rabbit lymphocytes. Binding characteristics of SG12/8 to rabbit neutrophils and lymphocytes were identical to that of MoAb M1/70, a rat-antimouse CD11b (IgG2a) that has been shown to crossreact with rabbit neutrophils.26 Iodine-labeled rabbit neutrophil extracts were immunoprecipitated with SG12/8 or M1/70, then analyzed on sodium dodecyl sulfate nonreducing polyacrylamide gel electrophoresis gels. Autoradiographs showed identical bands at 165 kD and 90 kD for both SG12/8- and M1/70-precipitated extracts. These protein bands approximately correspond to the molecular weight of human CD11b and CD18, respectively. SG12/8 is able to inhibit the chemiluminescence response of neutrophils ingesting C3bi-coated zymosan. Mac-1-dependent function.27

Immunofluorescence flow cytometry. The quantification of MoAb binding to neutrophils involved the use of fluorescein isothiocyanate (FITC) or biotinylated antibodies. F(ab')2 goat-antimouse second antibody attached to FITC (Zymed Laboratories, South San Francisco, CA) was used for indirect detection of the primary MoAb binding. Anti-L-selectin, DREG-200, and anti-CD18, R15.7, were also biotinylated and binding of these MoAbs to neutrophils was detected using streptavidin-phycerythrin (Tago Inc, Burlingame, CA). Analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA) and results were expressed as mean relative linear fluorescence.18 Neutrophils were identified by forward and side scatter in both whole blood samples and in peritoneal exudate samples.

Blood from neonatal and adult rabbits was collected in heparinized syringes and kept at 25°C until staining was performed (less than 1 hour). To study the change in surface antigen recognition with chemotactic factor stimulation, whole blood was incubated with either human CD11b and CD18, respectively. Indomethacin was used to determine the relative contributions of CD18 and L-selectin to the inflammatory response in pups and adult rabbits. At T-0,1 mg/kg of either anti-CD18 and/or anti-L-selectin MoAbs or the equivalent volume of DPBS was given either intravenously (adult) or intracardially (pups). Peritoneal injection of TG was performed 30 minutes later at T6. Rabbits were maintained for 6 hours, at which time PEx fluid volume was collected and the animals weight, the PEx neutrophils/ml were calculated assuming complete extraction of all PEx. Aliquots of PEx also were washed with Ca/Mg-free DPBS, then reacted with MoAbs and stained with FITC-F(ab')2; antihuman Ig to determine expression of surface Ag.

In vivo effects of anti-CD18 and anti-L-selectin MoAbs. Anti-CD18 and anti-L-selectin MoAbs were administered systemically to determine the relative contributions of CD18 and L-selectin to the inflammatory response in pups and adult rabbits. At T-0,1 mg/kg of either anti-CD18 and/or anti-L-selectin MoAbs or the equivalent volume of DPBS was given either intravenously (adult) or intracardially (pups). Peritoneal injection of TG was performed 30 minutes later at T6. Blood was obtained before injection of MoAb(T-0.5), at the time of ip TG administration (T0), and 6 hours later (T6). This blood was collected from leukocyte count, for binding of administered MoAb to circulating neutrophils by reacting whole blood with FITC-F(ab')2; antihuman Ig, and for neutrophil L-selectin and CD18 expression by reacting whole blood with biotinylated MoAbs. Sera at these time points were also evaluated for excess functional MoAb. This was done by incubating sera with neutrophils from an adult donor rabbit and staining with FITC-F(ab')2; antihuman Ig.

Data calculation and presentation. Results are presented as means ± SEM. Statistical comparisons among age groups for PEx neutrophil accumulation were made using multiple analysis of variance and Student-Neuman-Keuls test for significance. Comparisons between adults and 1-day-old rabbits were made by Student unpaired t-test.

RESULTS

Age-related differences in L-selectin and CD18. Neutrophils from neonatal (1-day-old) rabbits were evaluated in vitro to determine if the abnormality in surface levels of L-selectin seen in human neonates was present in rabbits. When compared to cells from adult and 14-day-old animals, neonatal neutrophils exhibited significantly reduced amounts of L-selectin (Table 1). In contrast to human neonates where L-selectin levels are still significantly reduced at 48 hours after birth,4 L-selectin reached adult levels by day 2 in rabbits. The effect of chemotactic stimulation in vitro on surface L-selectin was also evaluated, and much like human neutrophils was calculated using the kd for Fura-2 and maximum and minimum fluorescence values as described.27

Animal protocols. Blood was obtained from unsedated adult rabbits by ear arterial puncture. Blood from neonatal rabbits was obtained via intracardiac puncture. Leukocyte counts were performed using a Coulter counter (Coulter, Hialeah, FL), and manual differential counts were performed.

Stable peritonitis was induced in both adults and neonates by injection of 3% thiglycollate (TG) (Sigma), previously shown to produce marked acute neutrophil migration to the peritoneum.28 After sediment with intramuscular ketamine (25 mg/kg) and xylazine (10 mg/kg), an 18-gauge or 22-gauge catheter was placed in the peritoneum of adult and newborn rabbits respectively. TG (50 ml/kg) was slowly injected after confirming intraperitoneal (ip) placement of the catheter. At 2, 4, or 6 hours after ip TG, the animal was euthanized by intracardiac pentobarbital (50 mg/kg). The abdomen was massaged and an aliquot of the peritoneal exudate fluid removed and maintained in heparin and citrate-phosphate solution. Leukocyte counts were obtained by hemacytometer reading, and differential counts were performed manually. These counts are expressed as total neutrophils/ml of peritoneal exudate fluid (PEX). Based upon the total PEX fluid volume collected and the animals weight, the PEx neutrophils/ml were calculated assuming complete extraction of all PEx. Aliquots of PEx also were washed with Ca/Mg-free DPBS, then reacted with MoAbs and stained with FITC-F(ab')2; antihuman Ig to determine expression of surface Ag.
phils, adult rabbit neutrophils showed a slight increase in L-selectin within 2 minutes after the addition of ZARS followed by the downregulation of L-selectin within 10 minutes (Fig 1). However, compared with human neutrophils that demonstrate almost complete loss of L-selectin after chemotactic factor stimulation, L-selectin levels on stimulated adult rabbit neutrophils are decreased by only 50%. Though neonatal rabbit neutrophils also showed a decrease in L-selectin levels under the same conditions, these changes were not significant compared with baseline (Fig 1).

Surface levels of CD11b/CD18 on rabbit neutrophils were also evaluated because we have previously found that human neonatal cells show reduced upregulation of CD11b/CD18 after chemotactic stimulation. CD18 was significantly lower on neonatal neutrophils than on adult cells (Table 1) and as in humans, upregulation of CD11b/CD18 after stimulation with either ZARS or FMLP (data not shown) was significantly less in neonatal cells than with adult rabbit neutrophils (Fig 2). By 14 days of age, neutrophil surface expression of CD18 was equal to that of adult neutrophils (Table 1).

Neutrophil accumulation in vivo. Over a 6-hour observation period, peritoneal accumulation of neutrophils in response to intraperitoneal thioglycollate injection was greater in 14-day-old and in adult rabbits than in 1 day olds (Fig 3). Within the first hour, there was a significant increase in peritoneal exudate neutrophils in the adults compared with the 1-day olds (0.23 ± 0.03 neutrophils × 10⁶/mL v 0.022 ± 0.001 × 10⁶/mL, P < .05). By 6 hours there were significantly more neutrophils in the peritoneal exudate in both the 14 day old and adult rabbits whether neutrophil numbers were calculated per volume of exudate fluid (Fig 3), or per gram body weight (neonate, 2.7 ± 1.3 × 10⁶ neutrophil/gm, n = 19; adult, 5.0 ± 2.1 × 10⁶/gm, n = 15, P < .05). At baseline, peripheral blood neutrophil counts were significantly higher in the neonate than in either 14-day-old or adult animals (neonate, 12.4 ± 1.8 × 10⁶/mL; 14 day old, 3.1 ± 0.6 × 10⁶/mL; adult, 3.3 ± 0.3 × 10⁶/mL, P < .05). After the induction of peritonitis, blood neutrophil counts in each age group remained constant over the 6-hour study period. Thus, though the neonatal rabbit has an increased circulating pool of neutrophils, the ability of the neonatal neutrophils to emigrate to the inflamed peritoneum is markedly impaired. A systemic effect of the thioglycollate injection was not found in the neonate, 14-day-old, or adult animals. By 6 hours, blood neutrophil L-selectin levels showed decreases of 3% in neonates, (n = 33), 25% in 14 day olds,

Table 1. Age-Related Changes in Whole Blood Neutrophil L-Selectin, CD18, and CD11b

<table>
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<th>L-Selectin</th>
<th>CD18</th>
<th>CD11b</th>
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<tr>
<td>0-24 h</td>
<td>174 ± 10 (15)*</td>
<td>124 ± 11 (9)</td>
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<tr>
<td>24-48 h</td>
<td>205 ± 8 (11)*</td>
<td>97 ± 4 (11)</td>
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<tr>
<td>7 d</td>
<td>101 ± 7 (7)*</td>
<td>143 ± 18 (7)*</td>
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<tr>
<td>14 d</td>
<td>264 ± 40 (7)*</td>
<td>250 ± 36 (8)</td>
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<tr>
<td>Adult</td>
<td>239 ± 33 (13)</td>
<td>153 ± 17 (15)</td>
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MoAbs used were DREG-200 (anti-L-selectin), R 15.7 (anti-CD18), and SG 12/8 (anti-CD11b). Results are expressed as the averages of the mean fluorescence intensity ± SEM for each surface antigen; ( ) number of animals in each group.

* P < .05 compared with adult and 14-day-old neutrophils.

Fig 2. Effect of chemotactic stimulation on expression of CD11b and CD18 on neonatal (1-day-old) and adult neutrophils. Whole blood from adult (n = 13) and neonatal (n = 15) rabbits was either maintained at 25°C (PBS control) to eliminate the effect of temperature change on neutrophil CD11b, or stimulated with ZARS (1%, 15 minutes, 37°C), then reacted with anti-CD18 MoAb and anti-CD11b MoAb before staining with FITC-labeled antimouse Ig. Surface expression of CD11b and CD18 shown as the averages of the mean fluorescence intensity ± SEM * P < .01 compared with unstimulated age-matched control. ** P < .05 compared with neonate under same stimulus conditions.
Fig 3. Effect of age on development of sterile peritonitis. Three percent thioglycollate was injected into the peritoneal cavity of neonatal, 14-day-old, and adult rabbits; peritoneal exudate fluid and peripheral blood were obtained. Accumulation of neutrophils in exudate fluid (mean number neutrophils/mL fluid ± SEM) is expressed as a function of age of animal and time after ip instillation. *P < .01 for neonate (●) versus adult (■). **P < .01 neonate versus 14-day-old animals (+).

(n = 8), and 22% in adults, (n = 6). None of the apparent changes were statistically significant. Similarly, no significant change in blood neutrophil CD18 could be shown after the induction of peritonitis.

Exudate neutrophils were evaluated for surface levels of L-selectin and CD11b/CD18. In both adult and neonatal animals, L-selectin levels were markedly reduced on exudate cells (93% and 81%, respectively) compared with neutrophils in blood (Fig 4). Thus the loss of L-selectin from exudate neutrophils is more profound than in blood neutrophils chemotactically stimulated in vitro (Fig 1). CD11b/CD18 on exudate cells was elevated compared with blood

Fig 5. Age-dependent expression of CD11b on peritoneal exudate neutrophils compared with blood neutrophils. Blood was collected before ip thioglycollate, and peritoneal exudate fluid was collected 6 hours after ip thioglycollate (T6) from neonatal (n = 10), 14-day-old (n = 4), and adult (n = 6) rabbits. Cells were incubated with anti-CD11b MoAb (SG12/8) then stained with FITC-labeled antimouse Ig. CD11b expression on blood and peritoneal exudate neutrophils is expressed as the average mean fluorescence intensity ± SEM. *P < .05 compared with 1-day-old exudate neutrophils. **P < .05 compared with adult exudate neutrophils.
neutrophils in neonatal, 14-day-old, and adult animals, but the extent of upregulation was significantly greater in adult and 14-day-old rabbits (Fig 5).

Effects of administration of anti-CD18 and anti-L-selectin MoAbs. Administration of anti-CD18 MoAb, R15.7, was initially evaluated for possible effects on circulating neutrophil counts or the surface levels of neutrophil CD18, CD11b, or L-selectin. Circulating neutrophil counts did not significantly change after antibody administration in any age group, and surface glycoprotein levels were also unchanged (data not shown). Complete saturation of neutrophil CD18 binding sites was confirmed by flow cytometry in all animals by showing that the level of anti-CD18 MoAb on either blood or emigrated peritoneal neutrophils from treated animals was not increased by addition of more anti-CD18 MoAb to the sample in vitro. Excess functional anti-CD18 MoAb in treated animals' sera was confirmed by incubating isolated neutrophils from untreated adult rabbits in sera from treated animals, and then determining the presence of bound anti-CD18 MoAb using flow cytometry.

Such studies were also performed on animals receiving the anti-L-selectin MoAb, DREG-200. Unlike other studies that showed neutropenia in animals receiving anti-L-selectin MoAb,26 there was no alteration in circulating neutrophil counts (Fig 6A) or cell surface glycoprotein levels with the administration of anti-L-selectin MoAb. Functional anti-L-selectin MoAb was found in serum samples of treated animals.

We wished to investigate if the binding of the anti-L-selectin MoAb to rabbit L-selectin itself causes neutrophil activation. In a separate group of experiments, addition of saturating and supersaturating concentrations of anti-L-selectin MoAb to a suspension of adult rabbit neutrophils did not cause an increase in cytosolic calcium. In addition, the binding of anti-L-selectin MoAb to rabbit neutrophils had no demonstrable effects on FMLP-induced increase in cytosolic calcium (data not shown).

Effect of MoAbs on peritoneal neutrophil emigration. Peritoneal accumulation of neutrophils was significantly reduced in all three age groups after administration of 1 mg/kg anti-CD18 MoAb (Fig 7). However some potentially important differences were seen between the groups. The inhibitory effect of the MoAb was significantly less ($P < .05$) in the neonates and 14 day olds (58% and 65% reduction, respectively) than in the adults (91% reduction). In adult rabbits with peritonitis, the circulating neutrophil counts were significantly elevated at 6 hours in animals receiving anti-CD18 (without MoAb administration, $4.6 \pm 0.80 \times 10^6$ neutrophils/mL; with administration, $8.6 \pm 1.1 \times 10^6$/mL, $P < .01$). This effect of antibody administration was not seen in neonates or 14-day-old rabbits with peritonitis (Fig 6B).

The effect of 1 mg/kg anti-L-selectin MoAb administration also varied in each age group. Peritoneal accumulation of neutrophils in both adult and 14-day-old rabbits was significantly diminished, but in neonates, no significant effect could be shown (Fig 7). Unlike the administration of anti-CD18, anti-L-selectin had no significant effect on circulating neutrophil counts in any age group. Simultaneous administration of both MoAbs (each at 1 mg/kg) also did not show any effect of anti-L-selectin in neonates because the reduction in exudate neutrophils was not greater with combined treatment than with anti-CD18 alone. In contrast, combined antibody treatment produced significant additional reduction in peritoneal emigration in the 14-day-old rabbits when compared with anti-CD18 MoAb alone. In adult animals, the effect of anti-CD18 was so great that additional inhibitory effects of anti-L-selectin could not be shown (Fig 7).

As noted above, combined antibody administration in neonates did not completely inhibit the peritoneal emigration of neutrophils. The extent of neutrophil accumulation in neonates after combined antibody treatment was significantly greater ($P < .05$) than that in adult rabbits after combined antibody treatment.
MoAb as L-selectin reduction for anti-CD18), migration (50% reduction for anti-L-selectin MoAb and 91% bits is supported by the studies in which systemic administration of either anti-L-selectin or CD18 MoAb results in either a negligible or greatly diminished effect, respectively, when compared with adult animals. Although the effect may be explained by the neonatal neutrophils initially escaping the sites of inflammation, in adult rabbits, neonatal neutrophils have significantly increased levels of L-selectin. Current published evidence, both in vitro and in vivo, supports the hypothesis that L-selectin plays an important role in the process of margination at sites of inflammation, and that margination (the initial rolling adhesion seen before localization at the inflammatory site) is necessary for effective extravasation of neutrophils. In the present studies, we show that as in the human neonate, neonatal rabbits have a decreased amount of L-selectin on the neutrophil surface compared with neutrophils from both adult and 14-day-old rabbits. In addition, the neonatal rabbits failed to respond to systemic administration of anti-L-selectin MoAb. This anti-L-selectin MoAb has been shown to inhibit neutrophil rolling in mesenteric venules of adult rabbits and in the current study, significantly reduced peritoneal neutrophil infiltration in adult and 14-day-old rabbits. Therefore, one reasonable interpretation of these results is that the neutrophil emigration in neonatal rabbits that occurs does not depend on L-selectin.

Our current evidence from studies in vitro is that some L-selectin–independent margination of neutrophils on endothelial monolayers under conditions of flow is dependent on E-selectin and P-selectin. We have also found that human neonatal neutrophils have a high level of binding of MoAb CSLEX-1 indicating that there may be sufficient surface levels of sLeX to support E- or P-selectin–dependent rolling. Though the current studies do not deal with the other selectin–dependent mechanisms, it may be that the level of margination that is evident in the neonatal rabbit is made possible through the functions of E-selectin or P-selectin. There are studies in vivo that both support and refute these hypotheses in the adult models of peritonitis.

Blockade of E-selectin by MoAb in a rat model of glycogen-induced peritonitis resulted in a 70% decrease in neutrophil accumulation. MoAb PB1.3, an antihuman P-selectin antibody that cross-reacts with rabbit, has been shown to abrogate the injury in an adult model of ear ischemia/reperfusion. However, PB1.3 was unable to effect neutrophil accumulation after intraperitoneal installation of Escherichia coli in adult rabbits. Using both P-selectin–deficient and wild-type mice, Mayadas et al were able to show a decrease in neutrophil accumulation after the first 1 to 2 hours after intraperitoneal thioglycollate in the P-selectin–deficient mice. This effect had diminished substantially by 4 hours. Thus the involvement of P-selectin in peritoneal neutrophil emigration in adult animals is problematic. Nonetheless our data show that the developing rabbit uses mechanisms for neutrophil emigration differently than the adult. Therefore, E-selectin, P-selectin, or other unknown mechanisms may be of increased importance in the neonate. This is the focus of studies now in progress.

As we and others have described, the in vitro stimulation of rabbit neutrophils by chemotactic factors results in only a 50% loss of L-selectin (Fig 1) as compared with 85% to 95% loss by human neutrophils. Adult and neonatal

**DISCUSSION**

In the present study, neonatal rabbit neutrophils show a reduced ability to emigrate to the inflamed peritoneum compared with neutrophils from 14-day-old and adult rabbits. At least part of this reduction can be explained by low levels of neutrophil L-selectin and a diminished capacity to upregulate CD1lb/CD18 after either chemotactic stimulation or after emigration to the inflamed peritoneum. That these findings contribute to diminished emigration in the neonatal rabbits is supported by the studies in which systemic administration of either anti-L-selectin or CD18 MoAb results in either a negligible or greatly diminished effect, respectively, compared with adult animals. Although the effect may be explained by the neonatal neutrophils initially escaping their microvascular units, it is unlikely based upon the following observations: (1) equivalent doses of blocking MoAbs in adult rabbits manifested marked effects on emigration (50% reduction for anti-L-selectin MoAb and 91% reduction for anti-CD18), (2) these MoAb doses in all-age animals resulted in saturation of blood neutrophil CD18 and L-selectin binding sites both at T0 and T6, as well as the CD18 binding sites of the neutrophils that emigrated to the peritoneum (this could not be shown with anti-L-selectin MoAb as L-selectin is lost from peritoneal neutrophils), and (3) functional MoAb was present in serum samples.

The results in the present report are consistent with earlier studies showing that extravasation of neutrophils at sites of inflammation in neonates is significantly reduced when compared with older animals. Our results are also consistent with earlier studies in vitro showing that diminished adhesive functions may contribute to reduced emigration. One possible explanation derives from the fact that when compared with adults, neonatal neutrophils have significantly reduced levels of L-selectin. Current published evidence, both in vitro and in vivo, supports the hypothesis that L-selectin plays an important role in the process of margination at sites of inflammation, and that margination (the initial rolling adhesion seen before localization at the inflammatory site) is necessary for effective extravasation of neutrophils. In the present studies, we show that in the human neonate, neonatal rabbits have a decreased amount of L-selectin on the neutrophil surface compared with neutrophils from both adult and 14-day-old rabbits. In addition, the neonatal rabbits failed to respond to systemic administration of anti-L-selectin MoAb. This anti-L-selectin MoAb has been shown to inhibit neutrophil rolling in mesenteric venules of adult rabbits and in the current study, significantly reduced peritoneal neutrophil infiltration in adult and 14-day-old rabbits. Therefore, one reasonable interpretation of these results is that the neutrophil emigration in neonatal rabbits that occurs does not depend on L-selectin.

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As we and others have described, the in vitro stimulation of rabbit neutrophils by chemotactic factors results in only a 50% loss of L-selectin (Fig 1) as compared with 85% to 95% loss by human neutrophils. Adult and neonatal
neutrophils recovered from the peritoneal exudate show complete shedding of L-selectin (Fig 4). L-selectin shedding has been hypothesized to occur during firm neutrophil attachment to the endothelium and transvascular emigration. Using tissue sections of pneumonic rabbit lungs, it has been shown that neutrophils show a decreasing amount of L-selectin as they emigrate through the interstitium to the alveolar air space. Alveolar neutrophils have no demonstrable L-selectin (Alan Burns, personal communication, December 1993). An alternative explanation for complete loss of L-selectin by exudate neutrophils is enzymatic cleavage by secreted leukocyte proteases in the peritoneal exudate itself.

In addition to the apparent deficiency in L-selectin–based adhesion, human neonatal neutrophils also exhibit reduced functions of CD11b/CD18, evidenced by reduced reduction to endothelial monolayers and protein-coated glass after chemokinetic stimulation. Also there is reduced mobilization of CD11b/CD18 from intracellular store.s and lower cellular content of CD11b/CD18 than adult neutrophils. Current evidence indicates that CD18 integrins are necessary for effective localization at inflammatory sites in animal models (as shown by the anti-inflammatory effects of anti-CD18 and anti-CD11b MoAbs), and in humans and bovine with CD18 deficiency where neutrophil emigration at sites of infection is profoundly reduced.

Our earlier studies of human neonatal neutrophils in vitro showed some ability of the cells to migrate through confluent endothelial cell monolayers, but in contrast to adult neutrophils, only anti-CD11a MoAbs were significantly active in blocking this migration. These results are consistent with the hypothesis that adult neutrophils use both LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) in the process of transendothelial migration, but neonatal neutrophils appear to be heavily dependent on LFA-1 without significant contribution of Mac-1. The current results show that anti-CD18 has significantly less inhibitory effect in neonates than in older rabbits, supporting the idea that some CD18-dependent functions are also decreased in neonatal rabbits.

The finding that CD11b/CD18 upregulation is significantly less in the neonatal rabbit, either after stimulation in vitro or emigration into the peritoneal cavity, is also consistent with the findings in human neonates, but our current results do not distinguish whether there is reduced translocation from intracellular stores or reduced cellular levels of CD11b/CD18 in lapine neonatal neutrophils. The quantitative increases in Mac-1 on the cell surface are of unknown significance in vivo, but we have recently provided evidence that this upregulation may be important in cell locomotion, providing a renewable source of Mac-1 for adherence-dependent chemokinetic or chemotactic migration.

It is intriguing that 42% and 35% of the neutrophil transmigration in neonatal and 14-day-old rabbits, respectively, was unable to be blocked by anti-CD18 MoAb compared with only 9% in adult rabbits (Fig 7). Others have confirmed that CD18-independent mechanisms are involved in neutrophil emigration in various adult animal models and in the lungs of patients with CD18-deficiency and acute respiratory failure. Neutrophil emigration into the normal rabbit peritoneum in response to a 4-hour instillation of Streptococcus pneumoniae is inhibited by greater than 85% by anti-CD18 MoAb, but is inhibited less than 50% in macrophage-enriched peritoneum. In animals treated with the combination of anti-CD18 and anti-VLA-4 MoAbs, neutrophil emigration was completely inhibited 4 hours after the instillation of protease peptone. This treatment also prevented the increase in peritoneal macrophages. Thus it appears that in adult rabbits there is at least one mechanism other than CD18 responsible for neutrophil emigration. This mechanism appears to be functionally linked to the presence or accumulation of peritoneal macrophages.

Therefore, it is possible that the decrease in emigration in the neonate shown in our model could in part be caused by either a decrease in number, recruitability, or function of resident peritoneal macrophages or other cells, such as mast cells. A number of deficiencies have been identified in neonatal monocyte/macrophage function. The current results show that anti-CD18 MoAb had no effect on neutrophil counts. The neutrophilia can be explained by an increased bone marrow release in response to an inflammatory focus associated with an inability to emigrate. Because the anti-L-selectin MoAb had a modest effect on inhibiting neutrophil emigration, it had no significant effect on circulating neutrophil counts.

Thus, there is evidence that three adhesive mechanisms may be deficient in neonatal neutrophils compared with adults—reduced levels of L-selectin, reduced Mac-1–dependent adhesion after chemokinetic stimulation, and reduced ability to increase the amount of Mac-1 on the cell surface after chemokinetic stimulation. In the current studies, we provide evidence that adhesive pathways involving both L-selectin and CD18 integrins are reduced in neonatal rabbits. We also show that these reductions are reversed in the 14-day-old rabbits, as is the ability to emigrate to the inflamed peritoneum. Whether the apparent deficits in the neonate result from developmental delays, as our data suggests, remains to be defined, but current evidence is most consistent with the idea that some deficits are acquired shortly before birth. L-selectin levels on human fetal neutrophils appear to be equivalent to those of adults, raising the possibility that stimulating factors in neonatal plasma may be responsible for the reduced L-selectin in the neonatal period. One possibility arises from the finding that granulocyte-macrophage colony-stimulating factor (GM-CSF) is elevated in cord blood. This factor is known to activate shedding of L-selectin from adult neutrophils in vitro, and in adults, systemic administration of GM-CSF reduces the emigration of neutrophils into skin windows.
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