Shedding of L-selectin and PECAM-1 and upregulation of Mac-1 and ICAM-1 on neutrophils in RSV bronchiolitis

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Wang, Shan-Ze, Peter K. Smith, Melanie Lovejoy, Jeffrey J. Bowden, John H. Alpers, and Kevin D. Forsyth. Shedding of L-selectin and PECAM-1 and upregulation of Mac-1 and ICAM-1 on neutrophils in RSV bronchiolitis. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L983–L989, 1998.—Bronchiolitis is characterized histologically by epithelial necrosis and peribronchial infiltration of leukocytes, with a high percentage of neutrophils in the airways. We investigated the expression of adhesion molecules (CD11a, CD11b, CD18, CD31, CD54, and CD62L) on neutrophils from nasopharyngeal aspirates (NPAs) and peripheral blood (PB) of infants with respiratory syncytial virus (RSV)-induced bronchiolitis. The expression of CD31 and CD62L on neutrophils from NPAs is decreased and the expression of CD11b, CD18, and CD54 on neutrophils from PB of RSV-infected infants is increased compared with cells from PB of control infants. The expression of CD11b and CD54 on neutrophils from PB of RSV-infected infants is also increased compared with cells from PB of control infants. Shedding of CD31 and CD62L on neutrophils in RSV infection may contribute to the neutrophil emigration from blood to airways; the upregulation of Mac-1 (CD11b/CD18) and CD54 on neutrophils may help explain the high percentage of neutrophils in the airways of RSV bronchiolitis; and the upregulation of Mac-1 may be involved in the increased neutrophil-airway epithelial adhesion in RSV infection.

RESPIRATORY SYNCYTIAL VIRUS (RSV) is the most frequent cause of bronchiolitis and pneumonia in infants requiring hospitalization (9). Bronchiolitis is characterized histologically by epithelial necrosis and peribronchial infiltration with leukocytes, especially a high percentage of neutrophils or polymorphonuclear leukocytes (PMNs) in the lung tissues (4) and airway lumen (7). Neutrophils and their products are likely to have an important role in the various clinical and pathological changes occurring in RSV infection of the airways. However, the mechanisms of migration of neutrophils into the respiratory tissues and the airway lumen in RSV bronchiolitis remain unclear.

For circulating neutrophils to reach the respiratory tissues and airway lumen, they must first migrate through the vascular endothelium, enter the respiratory tissues, and finally pass through the airway epithelium and enter the airway lumen. Although the process of neutrophil migration from the endothelium into the tissues has been well described, neutrophil migration across the respiratory epithelium into the airway lu-
Table 1. Monoclonal antibodies used in this study

<table>
<thead>
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<th>Antibody</th>
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<th>Ligand</th>
<th>Comments</th>
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<tr>
<td>7E4</td>
<td>IgG1</td>
<td>CD18</td>
<td>Recognizes structural epitope on common β2-chain of integrins</td>
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<td>Recognizes PECAM-1</td>
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<td>X63</td>
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LFA-1, leukocyte function-associated antigen-1; PECAM-1, platelet endothelial cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1; ATCC, American Type Culture Collection.

admitted for acute bronchiolitis to Flinders Medical Centre (Bedford Park, Australia) were enrolled. The RSV antigens were detected from an aliquot of nasopharyngeal aspirates (NPAs) by ELISA. The infants were healthy until the time of infection, and all had a severe infection, with coughing, wheezing, chest overinflation, and tachypnea.

A healthy control group of nine infants between 1 and 12 mo of age (median 5.2 mo) who required blood tests before minor surgery was selected. Another healthy control group of seven adult volunteers between 27 and 38 yr of age (median 34 yr) was also studied.

Informed consent for this study was given by the parents of the RSV-infected and control infants. The study was approved by the Clinical Investigations (Ethics) Committee at Flinders Medical Centre.

Collection and separation of NPAs. For the nasal wash, the children were placed in the supine position, and one or two drops of sterile normal saline were placed in each nostril. The nasal airway was suctioned with a fine catheter. Secretions and lavage fluid were collected into a trap that was connected to a suction tube. About 0.5 ml of NPA was collected from both nostrils and diluted with saline to a volume of 4 ml. A 2-ml aliquot of the diluted NPA was used for detecting viruses (RSV, influenza virus A and B, adenovirus, and para-influenza viruses 1–3). The other 2-ml aliquot of diluted NPA was put on ice immediately for isolation of neutrophils.

To further dissolve the mucus, 50 µl of N-acetyl-L-cysteine (2 g in 10 ml; David Bull Laboratories) were added to the 2 ml of NPA. The NPA was mixed gently with a 5-ml syringe or a pipette. The PA cells were collected with a hemocytometer, and an aliquot was smeared onto slides, dried, fixed, and stained for morphological assessment of cells in the fluid. The suspended NPA was spun at 1,500 rpm for 5 min at 4°C. The supernatant was collected and stored at −80°C for later cytokine analysis. The cell pellet was resuspended in 5 ml of Dulbecco’s modified Eagle’s medium (DMEM; Gibco BRL, Life Technologies) with high glucose, L-glutamine, and 5% fetal calf serum (FCS) for isolating neutrophils. The process used for isolation of neutrophils from NPAs is similar to that for isolation of neutrophils from peripheral blood (PB).

Isolation of neutrophils. Immediately before each experiment, RSV-positive NPA neutrophils (n = 19 infants), RSV-negative NPA neutrophils (n = 12 infants), and PB neutrophils from infants infected with RSV (n = 11), healthy control infants (n = 9), and healthy adult volunteers (n = 7) were isolated by Lymphoprep (Nycomed Pharma, Oslo, Norway) and 3% dextran (Pharmacia Biotech) sedimentation techniques (20). Residual erythrocytes in the granulocyte-rich fraction were eliminated by hypotonic lysis in 0.2% sodium chloride two times for 20 s each. This resulted in a cell fraction containing >97% PMNs, with >97% viability as determined by trypan blue exclusion. The main contaminating cells in the neutrophil preparations were eosinophils. Eosinophils account for <3% in these preparations as determined with an automated Coulter STKS Counter (Coulter Electronics, Hialeah, FL) and examination of Jenner-Giemsa-stained slides for NPA samples. Neutrophils were suspended in DMEM at a concentration of 1 × 10⁶/ml and were put on ice for flow cytometric analysis.

Duplicate controls were undertaken on the effect of N-acetyl-L-cysteine, which was used in the process of isolating neutrophils from NPAs. No effect was observed on the expression of adhesion molecules on neutrophils from PB of healthy control subjects.

In an attempt to obtain NPA neutrophils from age-matched control infants, nasal washing was also undertaken on four healthy infants. Insufficient neutrophils were obtained for analysis by NPA from these infants.

Flow cytometric analysis. Neutrophils (5 × 10⁶ in 50 µl) were incubated on ice for 30 min with an excess concentration of various MAbs (CD11a, CD18, CD31, CD54, and CD62L, with X63 used as a negative control). The cells were washed twice with phosphate-buffered saline (PBS) plus sodium azide and labeled with Silenus anti-mouse F(ab)² fragment conjugated to FITC diluted 1:100. The cells were incubated on ice for another 30 min. After two washes with PBS, the cells were incubated for 10 min on ice with 5 µl of mouse IgG to block nonspecific binding. Then, 5 µl of the MAB against CD11b conjugated with phycoerythrin was added to the cells and incubated on ice for another 30 min to discriminate PMNs from epithelial cells, which were mixed in NPA samples. After being washed in PBS, the cells were analyzed by fluorescence-activated cell sorter flow cytometry (FACScan, CellQuest, Becton Dickinson) with standard settings. Ten thousand cells were analyzed from each sample. The mean fluorescence intensity (MFI) on cells in the gated neutrophil area was measured by flow cytometry as a marker of cell surface expression of adhesion molecules.

Statistical analysis. Data are given as means ± SD. Differences in results between groups were examined with one-way analysis of variance. P values were corrected with the Bonferroni method to adjust for the number of comparisons. Differences were considered to be significant at P < 0.05.

RESULTS

Expression of L-selectin on neutrophils in RSV bronchiolitis. The expression of L-selectin (CD62L) on neutrophils obtained from NPAs decreased compared with cells from PB of RSV-positive infants and control infants. The MFI for CD62L on neutrophils from RSV-positive NPAs (34.8 ± 22.5) markedly decreased compared with cells obtained from PB of RSV-positive infants and control infants (320.6 ± 142.4, P < 0.01 and 341.5 ± 74, P < 0.01, respectively). The positive percentage of CD62L on neutrophils from RSV-positive
NPAs (8.9 ± 7.0%) also markedly decreased compared with cells from PB of RSV-positive infants and control infants (98 ± 1.8%, P < 0.001 and 98.6 ± 1.2%, P < 0.001, respectively). There was no significant change for both MFI and positive percentage among neutrophils from PB of RSV-positive infants, control infants, and control adults (see Figs. 1 and 2).

Expression of PECAM-1 on neutrophils in RSV bronchiolitis. The expression of PECAM-1 (CD31) on neutrophils obtained from NPAs also decreased compared with cells from PB of RSV-positive infants and control infants (98.6 ± 1.8%, P < 0.001 and 98.6 ± 1.2%, P < 0.001, respectively). There was no significant change for both MFI and positive percentage among neutrophils from PB of RSV-positive infants, control infants, and control adults (see Figs. 1 and 2).

Expression of Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18) on neutrophils in RSV bronchiolitis. The MFI of CD11b on neutrophils obtained from RSV-positive NPAs increased compared with cells from PB. The MFI of CD11b on neutrophils from RSV-positive NPAs (1,683 ± 668) significantly increased compared with cells from PB of RSV-positive infants and control infants (818 ± 468, P < 0.01 and 841 ± 217, P < 0.01, respectively). There was no significant change in MFI among neutrophils from PB of RSV-positive infants, control infants, and control adults (Fig. 3). CD11b was expressed on all neutrophils.

No significant change was observed for CD11a between NPAs and PB among groups (Fig. 3).

The MFI of CD18 on neutrophils obtained from RSV-positive NPAs increased compared with cells from PB, and the MFI of CD18 on neutrophils from PB of RSV-positive infants was higher than that from PB of control infants. The MFI of CD18 on neutrophils from RSV-positive NPAs (517 ± 220) significantly increased compared with cells from PB of RSV-positive infants and control infants (260 ± 85, P < 0.01 and 147 ± 26, P < 0.01, respectively). The MFI of CD18 on neutrophils obtained from NPAs also markedly decreased compared with cells from PB of RSV-positive infants and control infants (98 ± 1.8%, P < 0.001 and 98.6 ± 1.2%, P < 0.001, respectively). There was no significant change for both MFI and positive percentage among neutrophils from PB of RSV-positive infants, control infants, and control adults (see Figs. 1 and 2).
phils from PB of RSV-positive infants was also higher than that from PB of control infants (P < 0.05). There was no significant change in MFI between neutrophils from PB of control infants and control adults (Fig. 4). All neutrophils are positive for CD18. There was no significant difference in the expression percentage among groups.

Expression of ICAM-1 on neutrophils in RSV bronchiolitis. The MFI of ICAM-1 (CD54) on neutrophils from RSV-positive NPAs increased compared with cells from PB, and the MFI of CD54 on neutrophils from PB of RSV-positive infants was higher than that from PB of control infants. The MFI of CD54 on neutrophils from RSV-positive NPA (50.7 ± 28.5) significantly increased compared with cells from PB of RSV-positive infants and control infants (19.2 ± 7.4, P < 0.01 and 11.4 ± 4.5, P < 0.01, respectively). The MFI of CD54 on neutrophils from PB of RSV-positive infants was also higher than that from PB of control infants (P < 0.05). There was no significant change in MFI between neutrophils from PB of control infants and control adults (Fig. 5). In regard to the fluorescent-intensity histograms, all neutrophils were positive for ICAM-1 at low intensity. Post-RSV infection, all cells remained positive, but the intensity of ICAM-1 increased (Fig. 6).

Comparison of adhesion molecule expression on neutrophils from RSV-positive and RSV-negative bronchiolitis. There was no significant difference in adhesion molecule expression on neutrophils between RSV-positive NPA and RSV-negative NPA groups (Figs. 7 and 8).

DISCUSSION

Our results show that both L-selectin and PECAM-1 on neutrophils from NPAs of RSV-positive infants are downregulated compared with cells from PB and that the expression of CD11b, CD18, and CD54 on neutrophils from RSV-positive NPA is upregulated compared with cells from PB of RSV-positive infants. Furthermore, the expression of CD18 and CD54 on neutrophils from PB of RSV-positive infants is increased compared with cells from PB of normal control infants. However, there is no significant difference in adhesion molecule expression between RSV-positive and RSV-negative NPA groups. Therefore, this study does not imply that the cell adhesive mechanisms for RSV are different from other inflammatory conditions. These changes in adhesion molecules seem to occur in response to inflammation regardless of stimulus.

Our results show that L-selectin on neutrophils from NPAs of RSV-positive infants is shed compared with
cells from PB. The process of neutrophil migration can be divided into four steps: rolling, adhesion, extravasation, and migration (15). L-selectin constitutively expressed on mature neutrophils plays a key role in establishing the weak adhesive interaction associated with leukocyte rolling (16). However, within minutes of neutrophil exposure to low levels of chemotactic factors in vitro, L-selectin is downregulated from the cell surface (12). More recently, Georas et al. (8) reported that L-selectin expression was significantly decreased on neutrophils and eosinophils from bronchoalveolar lavage after antigen challenge compared with cells from PB in allergic subjects. In contrast to L-selectin, CD11b expression was increased on bronchoalveolar lavage cells compared with PB cells (8). Interestingly, the rapid shedding of L-selectin in vitro follows the kinetics of Mac-1 upregulation from intracellular stores (12, 13). Our data on neutrophils that are recovered from the upper airways of infants infected with RSV support the observations of Georas et al. (8) and suggest that this inverse regulation of adhesion molecules occurs in vivo as well. These observations indicate that RSV infection or other stimuli provide a critical trigger that turns off one adhesion mechanism (L-selectin) and simultaneously induces another (Mac-1).

Although the physiological relevance of L-selectin shedding remains unclear, the downregulation of L-selectin may facilitate the transition from neutrophil adherence to extravasation (11). It is also possible that L-selectin shedding may limit distant site sequestration of leukocytes that were activated but managed to escape an inflammatory site (15). Some evidence suggests that cells that become firmly adherent are recruited almost exclusively from the rolling cell population (17). The prerequisite of leukocyte rolling in establishing firm adhesion may explain why leukocyte emigration can be inhibited by administration of antibodies that block either the weak adhesive interaction associated with leukocyte rolling or the stronger adhesive interaction associated with firm adhesion (15).

Our data show that PECAM-1 on neutrophils from NPA of RSV-positive infants is downregulated compared with cells from PB. In normal PB, PECAM-1 is present on virtually all platelets, monocytes, and neutrophils and ~50% of lymphocytes (30). The functions of PECAM-1 on neutrophils are poorly understood. An in vitro study showed that CD31 MAbs could partially block transmigration of neutrophils across tumor necrosis factor (TNF)-α-activated human umbilical vein endothelial cell monolayers and that a polyclonal CD31 antibody or soluble recombinant PECAM-1 could block ~80% of neutrophil transmigration (21). Animal studies (3, 28) showed that CD31 antibodies reduced neutrophil recruitment to the sites of inflammation in vivo. Therefore, the extravasation and migration steps of neutrophils are thought to be at least partially mediated by the adhesive and chemotactic functions of PECAM-1 (3, 28, 30).

Studies on changes in PECAM-1 expression on activation are not conclusive. PECAM-1 is downregulated on neutrophils after their stimulation with the chemotactic peptide formyl-Met-Leu-Phe in vitro (26). Our data show that PECAM-1 on neutrophils from the upper airways of infants with RSV bronchiolitis is downregulated in vivo as well. Our observations support the above in vitro studies. To our knowledge, this is the first report on the shedding of PECAM-1 on neutrophils in viral infection in vivo.

The downregulated expression of PECAM-1 on neutrophils may be due to the engagement with its ligand or dimerization by itself. Unpublished data quoted by Muller et al. (21) suggests that ligation of PECAM-1 on monocytes and neutrophils with CD31 activates β2-integrins in an adhesion cascade. Such mechanisms could be important in two respects: 1) the triggering of integrin-mediated adhesion to endothelial cells and 2) the enhanced adhesion of hemopoietic progenitor cells.
Neutrophil recruitment to sites of inflammation or injury is a vital part of the normal inflammatory response. However, neutrophils are a potent destructive force capable of generating oxygen radicals, releasing proteases, and producing proinflammatory factors. A neutrophil component in vascular and tissue injury has been implicated in the pathogenesis of a wide variety of inflammatory diseases (13). A recent study by Wang et al. (29) also showed that neutrophils can augment the epithelial damage and detachment induced by RSV infection. Neutrophil-mediated cytotoxicity is most efficient under conditions of cell-cell adhesion (14). In support of this contention is the observation that an antibody to Ma-1 could reduce phorbol 12-myristate 13-acetate-activated neutrophil adherence to epithelial cells and limit damage to epithelial cells (23). These observations indicate that the upregulation of Mac-1 on neutrophils in RSV infection does increase neutrophil adhesion to epithelial cells, contribute to neutrophil-mediated cytotoxicity to epithelial cells, and augment RSV pathogenesis.

Our studies also show that the expression of Mac-1 (CD11b/CD18) on neutrophils from RSV-positive NPAs is upregulated compared with cells from RSV-positive PB. Furthermore, the expression of CD18 on neutrophils from RSV-positive PB is increased compared with cells from control PB, although the expression (as measured by MFI) of CD11a and CD11b on neutrophils from RSV-positive PB is not significantly increased compared with cells from control PB. These data suggest that, in addition to NPA neutrophil activation in hospitalized RSV bronchiolitic patients, there is systemic PB neutrophil activation. The upregulation of CD18 or Mac-1 on neutrophils has important effects on neutrophil adhesion to endothelial cells, extravasation through the endothelium, and migration to infected sites (13). It has been shown that neutrophil migration across the endothelial monolayer is totally blocked by anti-CD18 MAbs in vitro (24). The in vivo studies also demonstrated that anti-CD18 MAbs could block neutrophil migration to the sites of inflammation in the lung and other tissues (5, 13). Therefore, the upregulation of CD18 or Mac-1 on neutrophils in PB and NPAs from infants with RSV bronchiolitis could help explain the high percentage of neutrophils recovered by nasopharyngeal aspiration in the upper airway (93%) and by bronchial lavage in the lower airway (76%) in infants with RSV infection (7) and the significantly increased bronchiolar neutrophil infiltrates in RSV-inoculated guinea pigs (4).

Furthermore, a recent study by Stark et al. (25) showed that RSV infection enhances neutrophil adhesion to cultured A549 respiratory epithelial cells. Interestingly, this increased neutrophil adhesion to RSV-infected epithelial cells could be completely blocked with anti-CD18 antibodies, but anti-ICAM-1 MAb inhibited neutrophil adhesion to the epithelial cells by only 30% (25). It is also reported that neutrophil adhesion to human epithelial cells exposed in vitro to ozone is CD18 dependent and ICAM-1 independent (27). Because anti-CD18 MAb totally inhibited adhesion of both neutrophils and eosinophils to epithelial cells, β2-integrins are likely the only granulocyte-receptor family involved in adhesion to RSV-infected A549 cells (25); however, it has been postulated that a non-ICAM-1 ligand for neutrophil CD18 is present on epithelial cell cultures (25, 27).

Neutrophil recruitment to sites of inflammation or injury is a vital part of the normal inflammatory response. However, neutrophils are a potent destructive force capable of generating oxygen radicals, releasing proteases, and producing proinflammatory factors. A neutrophil component in vascular and tissue injury has been implicated in the pathogenesis of a wide variety of inflammatory diseases (13). A recent study by Wang et al. (29) also showed that neutrophils can augment the epithelial damage and detachment induced by RSV infection. Neutrophil-mediated cytotoxicity is most efficient under conditions of cell-cell adhesion (14). In support of this contention is the observation that an antibody to Mac-1 could reduce phorbol 12-myristate 13-acetate-activated neutrophil adherence to epithelial cells and limit damage to epithelial cells (23). These observations indicate that the upregulation of Mac-1 on neutrophils in RSV infection does increase neutrophil adhesion to epithelial cells, contribute to neutrophil-mediated cytotoxicity to epithelial cells, and augment RSV pathogenesis.

Our studies also show that CD54 on neutrophils from NPA of infants with RSV bronchiolitis is upregulated compared with cells from PB of RSV-positive infants. Furthermore, the expression of CD54 on neutrophils from PB of RSV-positive infants is also increased compared with cells from PB of normal control subjects. ICAM-1 is expressed basally only at low levels on vascular endothelial cells and lymphocytes and is expressed at moderate levels on monocytes (6). Most recently, it was reported that neutrophils express ICAM-1 on their surface; it is upregulated by in vitro stimulation with TNF, granulocyte-macrophage colony-stimulating factor, and Staphylococcus aureus; and the basal expression of ICAM-1 on neutrophils of septic patients is higher than that in the case of normal blood donors (18). We show that the expression of ICAM-1 on neutrophils from PB of normal control subjects is at a very low level but is upregulated on neutrophils from both PB and NPAs of infants with RSV bronchiolitis. Our data confirm the above study that neutrophils basally express ICAM-1 at a very low level and that the expression of ICAM-1 on neutrophils can be upregulated in response to inflammation or infection. The upregulation of ICAM-1 on neutrophils in RSV bronchiolitis may be due to the production of TNF in RSV infection (18, 19). On the basis of an extensive review of the literature, this is the first report on the upregulation of ICAM-1 on neutrophils in viral infection in vivo.

The effect of ICAM-1 on neutrophils is unclear. It might also be involved in neutrophil migration. The only known ligands for ICAM-1 are LFA-1 and Mac-1, which are expressed on leukocytes. The upregulation of ICAM-1 on neutrophils may allow ICAM-1-positive neutrophils to physically interact with LFA-1- or Mac-1-expressing inflammatory cells (18). Because there are no known ligands for ICAM-1 on endothelial or epithelial cells, it is unknown whether the upregulation of ICAM-1 on neutrophils could contribute to neutrophil adhesion to endothelial or epithelial cells.

It should be noted that because NPA neutrophils are presumably bound to mucus, the changes in adhesion molecules on NPA neutrophils might also be related to the process of mucociliary clearance from the lower airway to the nasopharynx, although there are no data to confirm this possibility.

This study has not determined the mechanisms underlying neutrophil migration. No doubt, chemokines such as IL-8 and neutrophil-activating peptide-2 are important promoters of neutrophil adhesion mol-
ecule alterations and neutrophil migration into the airways (2).

In summary, RSV infection induces shedding of L-selectin and PECAM-1 and upregulation of Mac-1 and ICAM-1 on neutrophils, which may play a key role in neutrophil migration to the inflammatory sites in the airways and in the increased neutrophil-epithelial cell adhesion in RSV infection and contribute to the pathogenesis of RSV disease.

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REFERENCES


