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*Vet Pathol* 1996 33: 639

DOI: 10.1177/030098589603300602

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What is This?
Passage of CD18- and CD18+ Bovine Neutrophils into Pulmonary Alveoli during Acute Pasteurella haemolytica Pneumonia

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Abstract. CD18 is a subunit for three \( \beta_2 \) integrin molecules (Mac-1, \( \beta_1 50,95 \), LFA-1), which are expressed on the plasma membrane of neutrophils. These molecules mediate passage of neutrophils into sites of infection. In children and animals that lack CD18 expression, neutrophil infiltration is impaired in most tissues. However, in lung, CD18- neutrophils have been identified in the airway spaces during spontaneous episodes of pneumonia. To determine whether CD18 is vital for passage through the pulmonary alveolar wall, lung lobes of cattle with neutrophils that were deficient in CD18 expression (CD18-) and cattle with normal CD18 expression (CD18+) were inoculated with Pasteurella haemolytica by fiberoptic bronchoscopy; control lobes were inoculated with pyrogen-free saline (PFS). Neutrophil passage into alveolar lumina at 4 and 6 hours postinoculation was measured by computerized image analysis. Blood levels of neutrophils for CD18- cattle ranged from 12- to 26-fold higher than for CD18+ cattle prior to inoculation, and counts in both groups rose slightly postinoculation. In P. haemolytica-inoculated lobes, total numbers of neutrophils in alveolar lumina of the two groups were similar. An increase in the number of neutrophils in the alveolar wall was fourfold greater in CD18- cattle than in CD18+ cattle. In PFS-inoculated lobes, the number of neutrophils in the alveolar wall was sixfold higher in CD18 cattle than in CD18+ cattle. This work shows that by 4 and 6 hours, CD18- neutrophils enter the alveolar lumen at a rate similar to that in CD18+ cattle. Higher numbers of CD18- neutrophils are present in the alveolar wall of control (PFS) and bacteria-inoculated lobes. Thus, the CD18- cells are increased in the walls of alveoli and numbers of neutrophils that enter the alveolar lumen are similar in CD18+ and CD18- cattle.

Key words: Inflammation; leukocyte adhesion deficiency; respiratory tract.

Neutrophils are essential in preventing colonization of the respiratory tract by microbial pathogens. Invading bacteria, viruses, and other agents stimulate neutrophils to pass across the vascular wall and respiratory interstitial tissue to enter the airway lumen. Integral to this process is CD18, a subunit for the three \( \beta_2 \) integrin molecules (Mac-1, \( \beta_1 50,95 \), LFA-1) expressed on the plasma membrane of activated neutrophils. The \( \beta_2 \) integrins mediate stable adherence to endothelial cells and have a role in adherence to the interstitial tissue matrix.

Deficiency of \( \beta_1 \) integrins has been characterized in children with leukocyte adhesion deficiency (LAD), cattle with bovine LAD (BLAD), and Irish Setter dogs. Cattle have an autosomal recessive point mutation in the \( \beta_2 \) subunit, CD18, of the \( \beta_2 \) integrin heterodimer. This mutation results in <1% normal expression of all three (Mac-1, \( \beta_1 50,95 \), LFA-1) \( \beta_2 \) integrins. Affected cattle develop a variety of pathologic changes because of impaired transmigration of neutrophils across vascular walls into sites of infection. Mucosal infections in these animals have few neutrophilic infiltrates. Paradoxically, in pneumonic lungs from cattle with BLAD, prominent neutrophilic infiltrates are present in the alveolar lumina, bronchi, and bronchioles. Neutrophilic infiltrates are also present in children with LAD that have died with pneumonia. Infiltrates of neutrophils within the pulmonary parenchyma of BLAD cattle and LAD children contrast sharply with the minimal infiltrates of neutrophils in mucosal lesions of the alimentary tract, skin, and other tissues.

It is difficult to directly compare the degree and rate of infiltration by neutrophils in pneumonic lungs of normal cattle and of cattle with BLAD because 1) the pneumonias are caused by a diverse set of pathogens...
and 2) lung tissue is examined during various stages of the inflammatory process. In this study, we identified three cattle homozygous for BLAD that were in relatively good health and free of respiratory infections. To determine if CD18 expression is necessary for neutrophil passage into the alveolar lumen during acute (4-6 hour) pneumonia, we compared neutrophil infiltration into the pulmonary alveolar lumina of these cattle with BLAD to that in age- and breed-matched control cattle with normal (100%) expression of CD18 by 1) administering identical interbronchial inoculations of Pasteurella haemolytica, a common respiratory pathogen of cattle, 2) collecting lung tissue from both sets of cattle during the acute stage of inflammation (4 or 6 hours postinoculation), and 3) measuring the numbers of neutrophils within the alveolar septa and alveolar lumina by morphometric analysis. This study is the first in which neutrophil infiltration into the alveolar lumen has been measured in vivo in an animal species with LAD. A mouse model with a gene mutation for CD18 retained some CD18 expression (2-16%) because of a cryptic promoter in the plasmid construct. However, in these BLAD-homozygous cattle, CD18 expression is consistently <1%.

Materials and Methods

Experimental design

Three cattle homozygous for CD18- defect and four age-matched CD18+ cattle were inoculated with Pasteurella haemolytica by fiberoptic bronchoscopy into the right cranial lung lobe. The left cranial lobe received pyrogen-free saline (PFS) and served as a control. One CD18- and one CD18+ animal were euthanatized at 4 hours postinoculation (PI), and two CD18- and three CD18+ cattle were euthanatized at 6 hours PI. Total blood leukocyte counts were measured pre- and postinoculation. At necropsy, lung tissue was collected for culture and histomorphometric analysis. Lung tissue was stained for cytokeratin and bovine macrophages (CD68 antigen). The following morphometric parameters were measured in lung alveoli: 1) numbers of macrophages and neutrophils in the alveolar septa and in the alveolar lumina, 2) the area of the alveolar septum and area of the alveolar lumen, and 3) area of the entire alveolar unit.

Animals and fiberoptic bronchoscopy

Three CD18- (<1% CD18 expression) Holstein cattle homozygous for the CD18 mutation, as determined by restriction endonuclease analysis of DNA amplified by polymerase chain reaction and by flow cytometric analysis, were identified and cared for at the National Animal Disease Center (NADC). The three cattle were a 3-year-old male (No. 2), a 3-month-old male (No. 5), and a 1-month-old male (No. 7). Four age- and breed-matched cattle that were similarly tested were homozygous for the normal CD18 genotype: a 3-year-old female (No. 1), a 3-month-old male (No. 3), a 3-month-old female (No. 4), and a 1-month-old male (No. 6), which were present in the NADC dairy herd. These cattle showed no clinical signs of respiratory disease. One CD18- animal and an age-matched CD18+ counterpart were inoculated with P. haemolytica by fiberoptic bronchoscopy into the right cranial lung lobe. PFS was instilled into the left cranial lobe. This procedure was repeated for three groups of cattle 3 years, 3 months, and 30 days of age. In one group (3 months of age), three calves were inoculated because an extra control animal was included (one CD18- [animal No. 5] and two CD18+ [animal Nos. 3, 4]). Cattle were euthanatized, and lung tissue was collected at either 4 (animal No. 1 [CD18-] and animal No. 2 [CD18+]) or 6 (animal Nos. 3, 4, 6 [CD18+] and 5, 7 [CD18+] hours PI. At necropsy, gross lesions were only present at the site of the inoculation. Microbiologic cultures yielded pure colonies of P. haemolytica. Microscopically, lung tissues lacked lesions typical of common bovine respiratory pathogens (e.g., Mycoplasma sp., parainfluenza 3, bovine respiratory syncytial virus, infectious bovine rhinotracheitis).

All animals were treated and cared for in accordance with the NADC and the American Association of Laboratory Animal Care (AALAC) guidelines. The NADC is an AALAC-accredited facility. The experimental protocol was approved by the NADC Animal Care and Use Committee (application no. 1407).

Cattle were sedated with intravenous xylazine at 0.1 mg/kg. Blood (10 ml) was collected from animals prior to inoculations and just prior to euthanasia for cell counts performed with a cell counter (Celltrak 2, Nova, Watham, MA). A bronchoscope was passed into the nasal orifice, along the ventral nasal meatus, through the larynx, and into the trachea. The right cranial lobe was identified by visual inspection. A sterile plastic canula was inserted into a port on the bronchoscope and used to instill inoculae into the desired bronchus. The plastic canula was removed after each instillation and replaced by a new sterile canula. A volume of 18 ml PFS was instilled into the first bronchial opening of the left cranial lobe, and 8 ml of 1 x 10^7 colony-forming units/ml P. haemolytica in PFS followed by 10 ml of sterile PFS. Calves were revived after the procedure with intravenous tolazoline (0.5-1.0 mg/kg, to effect).

At 4 or 6 hours PI, cattle were sedated with intravenous xylazine (0.1 mg/kg), euthanatized with an overdose of pentobarbital, and necropsied. Focally extensive areas of hemorrhage, interlobular edema, and consolidation were present in inoculated areas of lung. These areas were similar among cattle and were 10-15 x 10-15 x 20 cm. Tissue samples of left and right cranial lobes were collected at predetermined sites: 1) ventral border of the inoculation site, 2) center of the inoculation site, 3) dorsal border of the inoculation site, and 4) random areas throughout the lesion. In PFS-inoculated lobes, the mainstem bronchus was opened to the point of the first bronchial opening. Tissue sections were taken from this area to two sites located 4 and 8 cm distally. Tissue samples for immunocytochemical staining and morphometric analysis were immersion-fixed in lightly neutral buffered 10% zinc formalin.

Lung tissue for immunocytochemistry and morphometry were processed routinely, embedded in paraffin, and sectioned (5 µm) onto ProbeOn Plus (Fisher Scientific, Pittsburgh, PA) microscope slides. Sections were stained with a
double-labeling technique, incorporating a stain to label macrophages (anti-CD68) and a stain to label alveolar lining cells (anti-cytokeratin). The cytokeratin stain outlined the borders of alveolar lumina (and epithelium of bronchi and bronchioles), allowing delineation of the border of alveolar septa and lumina. This technique clearly defined the boundaries of the alveolar wall and allowed accurate counts of intraseptal and intraluminal neutrophils.

The CD68/cytokeratin double-labeling procedure has been previously described. Slides were incubated with mouse anti-CD68 primary antibody (EBM11, Dako Corp., Carpinetia, CA) at 1:25 dilution (37°C overnight in a humid chamber. After washings, secondary biotinylated goat anti-mouse was followed by an avidin-peroxidase solution. The chromogen for CD68 antibody was a metal-enhanced 3,3'-diaminobenzidine solution (Immunopure, Pierce Chemical Co., Rockford, IL) that created a brown precipitate. Slides lacked primary antibody (EBM11/MNF116) or the primary antibodies were substituted with an irrelevant antibody (mouse IgG anti-T cell).

Morphometric studies

Morphometric analyses followed a previously described protocol with slight modifications. Two tissue sections from each of the three sampling sites of each lung lobe were used for morphometric analysis (a minimum of six slide sections per lung lobe per animal). A minimum of 10 alveoli from each slide section were measured from P. haemolytica-inoculated lung lobes and a minimum of four alveoli were measured from PFS-inoculated (control) lobes. Counts and measurements were made from two alveoli in each of five fields located along a diagonal line across the section (minimal of 10 alveoli/section). Measurements of alveoli included 1) the number of neutrophils and macrophages within the septal wall (intravascular and intraseptal combined), 2) the number of neutrophils and macrophages in alveolar lumina (complete transmigration of cells across the blood-air barrier), 3) the area of the alveolar septum, 4) the area of the alveolar lumen, and 5) total area of the alveolar unit.

Counts were made on an image analysis system. Images of optical fields containing two alveoli were made with a Zeiss Axioplan universal microscope equipped with an Optronics CCD video camera system. All alveolar images were viewed through a Zeiss Plan-NEOFLOAT 40x objective and transmitted to a Sony PVM 1343 MD color monitor. Image processing of selected fields, including enhancement, manual tracing, point counts, and area of calculation, were performed with Vidas 2.1 software (Kontron Elektronik). Images were 512 x 480 pixels. Manual tracings (of alveolar lumen and septal outlines) and point counts (of neutrophils and macrophages) were accomplished interactively using a mouse on a digitizer tablet. The internal scaling feature of the software was calibrated to measure in micrometers for tracings. Alveolar lumina were identified by the red staining in the plasma membrane of type I pneumocytes (anti-CK). Macrophages were identified and differentiated from other cell types by the brown cytoplasm color (anti-CD68). Neutrophils were identified by the typical morphology of segmented and band cells. Measurements included 60 alveoli/entire lung lobe/animal from inoculated lobes. For measurements (tracing and point counts), images were available for viewing with the light microscope, the computerized color image monitor, or the computerized black and white monitor. Thus, if structures such as alveolar lumen outlines or cell types were in question on black and white images, then structures could be inspected on the color image or with the light microscope. All tracings and measurements were made by a single pathologist (M. R. Ackermann).

Lung tissue from control lobes of the CD18- cattle was examined for the number of neutrophils within alveolar septa, and those numbers were compared with those from breeder- and age-matched control cattle. Other parameters, such as the number of neutrophils within the alveolar lumen or luminal area, were not measured because the PFS did not induce neutrophilic infiltrates.

Statistical analysis

Morphometric data from light microscopy were analyzed by the general linear models procedure of SAS (Statistical Analysis System, User's Guide: Statistics, version 5 edition, SAS Institute, Cary, NC). The statistical model used in the analysis of variance included the fixed effects of treatment (CD18 group), time of necropsy (4 or 6 hours), and a time × treatment interaction term. Significance was limited to P < 0.05.

Results

Blood neutrophil counts

As previously reported, flow cytometric analysis demonstrated <1% expression of CD18 on neutrophils from CD18- cattle. Leukocyte counts were markedly elevated beyond normal ranges for all CD18- cattle prior to inoculation (145,000, 129,600, 147,000) and rose PI (179,000, 168,000, 158,000, respectively). Blood counts of CD18+ cattle were within normal range (5,600, 6,700, 10,800, 9,300) for cattle prior to inoculation. Counts PI decreased in two cattle and rose in two (5,200, 7,200, 6,000, 9,400, respectively).

Morphometric studies

Cytokeratin immunocytochemistry distinctly outlined the walls of alveolar lumina (Fig. 1) because of the immunoreactivity of the antibody to the plasma membrane and cytoplasm of type I pneumocytes. Type II pneumocytes and bronchiolar and bronchial lining cells were also immunoreactive. Anti-CD68 clearly delineated intraluminal and intraseptal (intravascular) macrophages and distinguished macrophages from neutrophils and degenerate neutrophils typical of bovine pulmonary pasteurellosis (Fig. 2). The staining
was visible on computerized color images and variably visible on computerized black and white images (Fig. 3).

In PFS-inoculated (control) lobes, sixfold greater numbers of neutrophils were present in the septa of alveoli in CD18− cattle. This high number of septal neutrophils was statistically significant ($P = 0.0046$) when compared with PFS-inoculated lobes of CD+ cattle and was associated with the typical high level of neutrophils within the blood of BLAD cattle. PFS-inoculated (control) lobes also had increased numbers of cells that were immunoreactive for CD68 (macrophages) in the alveolar septa ($1.7 \pm 0.2$/alveolus) of CD18− cattle when compared with CD18+ cattle ($0.8 \pm 0.4$).

At 4 and 6 hours PI (data combined) with *P. haemolytica*, CD18− cattle had a significant increase (nearly fourfold; $P = 0.0004$) in the numbers of neutrophils ($7.85 \pm 1.2$/alveolus) in the septa of alveoli when compared with CD18+ cattle ($2.1 \pm 0.7$/alveolus; Table 1). Alveolar septa were generally thickened in CD18− cattle; however, this trend was not statistically significant. There were no significant differences in alveolar lumen areas, septum areas, and numbers of neutrophils within alveolar lumina (Table 1). No significant difference in neutrophil numbers, areas, and other parameters were present when the 4 hours PI subset of the data was analyzed separately. However, the CD18− animal had roughly 3 times the number of neutrophils within the alveolar septa and fewer intraluminal neutrophils when compared with its CD18+ counterpart. CD18− cattle had a trend of increased numbers of alveolar macrophages in both PFS- and *P. haemolytica*-inoculated lobes, but this increase was not statistically significant.

### Discussion

The similar numbers of neutrophils in alveolar lumina of CD18− and CD18+ cattle suggests that CD18 is not vital for passage of neutrophils into the alveolar lumen. This conclusion is supported by the infiltrates of neutrophils seen in BLAD (CD18−) cattle and human LAD patients and increased numbers of young CD18− cattle that have numbers of blood neutrophils similar to those of CD18+ cattle, are needed to confirm these findings and assess infiltration at earlier times (<4 hours) and in other intrapulmonary locations (e.g., bronchial lumina). Increased numbers of CD18− neutrophils within the septa of infected lobes and control lobes was associated with the high level of blood neutrophils in these cattle. In conventionally reared CD18−

### Table 1. Morphometric parameters ($\bar{X} \pm \text{SEM}$) from pyrogen-free saline (PFS)- and *P. haemolytica* (Ph)-inoculated lungs from CD18− and CD18+ cattle 4 and 6 hours postinoculation.

<table>
<thead>
<tr>
<th>Status</th>
<th>Alveolar Lumen Area ($\mu m^2$)</th>
<th>Alveolar Septum Area ($\mu m^2$)</th>
<th>PMN*/Alveolar Lumen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ph</td>
<td>PFS</td>
<td>Ph</td>
</tr>
<tr>
<td>CD18+</td>
<td>2,947 ± 790</td>
<td>2,542 ± 567</td>
<td>2,065 ± 461</td>
</tr>
<tr>
<td>CD18−</td>
<td>3,388 ± 567</td>
<td>2,915 ± 722</td>
<td>2,251 ± 710</td>
</tr>
</tbody>
</table>

* Polymorphonuclear cells (neutrophil).
† Macrophage.
‡ Significance at $P < 0.05$. 

![Fig. 1. Lung; cow No. 3. Type 1 pneumocytes immunoreactive for cytokeratin. Intraluminal macrophages (arrows) are immunoreactive for EBM11 (anti-CD68). Alkaline phosphatase stain, hematoxylin counterstain. HE. Bar = 25 pm.](image1)

![Fig. 2. Lung; cow No. 5. Neutrophils (arrows) within the alveolar septal membrane of CD18− animal. HE. Bar = 25 pm.](image2)
Fig. 3. Lung; cow No. 2. Computer images of an alveolus captured electronically for morphometric analysis. Fig. 3A. The lung alveoli are labeled with cytokeratin (red), which binds to the plasma membrane of alveolar epithelial cells and outlines the alveolar lumina. Within the lumen are numerous neutrophils, and a single cell within the lumen is red (arrowhead); this is a sloughed (detached) type II cell or bronchiolar epithelial cell. Also within the lumen are two faint-brown macrophages (arrows) that are immunoreactive to EMB11 antibody, which is a marker for bovine macrophages. This color image is displayed on a computer screen while the image is projected on a second computer monitor in black and white that is used for tracings and point counts. At the same time, the alveolus can be examined in a light microscope (which is sending the light image to the computer electronically) for optimal resolution and clarity. Fig. 3B. Black-and-white image of the alveoli in which the alveolus of interest is traced along the outer and inner edges of the alveolar septum. Surface area of entire alveolus (outer tracing), alveolar lumen (inner tracing), and the septum are measured by the software. Fig. 3C. Isolated alveolus in which individual neutrophils are marked by hand for counting by the software. This particular alveolus lacks intraseptal neutrophils and macrophages. Fig. 3D. Alveolar lumen area (hollow center) and septal area are defined for measurement by the software. 40 x, cytokeratin/macroage (EBM11) double stain and hematoxylin counterstain.

(BLAD) cattle, blood neutrophil counts often are >80,000 cells/μl (normal = 4,000/μl), and cattle in these studies had counts >100,000/μl. This study is limited, however, by the availability of healthy CD18- cattle.

The alveolar lumen is the major site of passage of neutrophils into the pulmonary air spaces, and passage of neutrophils across the alveolar septum into the alveolar lumen is a complicated process. Successful passage requires attachment of neutrophils to endothelial cells of septal capillaries, passage through the interstitial tissue of the septum, and passage across the epithelium. There are several possible mechanisms of septal passage by CD18- neutrophils, e.g., migration through porelike fenestrae, CD18-independent adherence, and septal degradative processes.

Recent work in a rabbit model has shown that neutrophils can pass along channels formed by small fenestrae in the basement membranes of pulmonary capillaries and the septal epithelium. These fenestrae are bridged by fibroblasts, which serve as a stationary cell

<table>
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<tr>
<th>PMN in Alveolar Septum</th>
<th>Mø†/Alveolar Septum</th>
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<tbody>
<tr>
<td>Ph</td>
<td>PFS</td>
</tr>
<tr>
<td></td>
<td>Ph</td>
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<tr>
<td>2.1 ± 0.7</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>7.8 ± 1.1†</td>
<td>3.0 ± 1.2†</td>
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Table 1. Extended.
type for attachment by migrating neutrophils. The high level of circulating CD18 - neutrophils provides an abundance of cells in the pulmonary microvasculature, and these cells are in close proximity for entrance into such fenestrae in the presence of a chemotactic substance (P. haemolytica). Additional studies will be needed to differentiate between intraseptal neutrophils that are present within septal capillaries and neutrophils within the scant interstitium of the alveolar septum. This information will determine if passage is impaired at endothelial cell locations or within the septal wall. Additional studies should utilize young cattle with BLAD that have similar numbers of blood neutrophils compared to CD18+ cattle.

CD18-independent molecules also mediate adherence and passage into the airway lumen. Recent work has shown that a β1 integrin (β1α6) mediates adherence of neutrophils to interstitial tissue. Other CD18-independent molecules, such as L-selectin and Sialyl Lewis X (SLeX), are especially important for initial adherence of neutrophils to vascular endothelial cells. Adherence to endothelial cells by selectins may be especially efficient in the lung because the flow pressures are low in the pulmonary capillaries. After this initial adherence, molecules such as the β1 integrins in conjunction with β2 integrins may mediate passage of neutrophils across the alveolar septum. Recent work has also shown that neutrophils may have a novel CD18-independent migration mechanism that is activated by C5a in conjunction with ligands (i.e., E-selectin) expressed on endothelial cells activated by cytokines.

In the lung, CD18-mediated adherence is dependent on the duration of the inflammatory process and the type of stimulus. In a mouse model, increased ICAM-1 expression is associated with CD18-dependent infiltration, and ICAM-1 is not upregulated during CD18-independent infiltration. This study was designed to elicit a CD18-dependent process by inoculating the cattle with a gram-negative bacterium (Pasteurella haemolytica, A1) and examining lung tissue during the acute stages of the inflammatory process. In this way, the greatest differences between CD18+ and CD18- adherence could be assessed.

Neutrophils within the alveolar septum may damage the air–blood barrier, allowing enhanced infiltration of neutrophils regardless of CD18 expression. Degradative proteases, arachidonic acid metabolites, and toxic oxygen metabolites released by neutrophils clearly damage the pulmonary parenchyma. This damage may be enhanced in CD18− (BLAD) cattle because they typically have high levels of circulating neutrophils in an activated state, as evidenced by low L-selectin expression (M. E. Kehrli, Jr., personal observation). High numbers of CD18− neutrophils within the large blood volume (and low blood pressure environment) of the pulmonary vasculature may release destructive enzymes that damage the septa. Also, P. haemolytica and its products, namely lipopolysaccharide, can damage the lung parenchyma. Additional studies in the BLAD model should be aimed toward examination earlier in the infection (0–4 hours postinoculation), when there is less chance of membrane and interstitial tissue degradation. Inoculation of less damaging chemotactic compounds, such as platelet activating factor or interleukin-8, will probably also stimulate CD18 expression and infiltration but will minimize possible degradation of the air–blood barrier. Although in this study we focused on the alveolus, neutrophils enter the lungs at other sites such as the larger pulmonary blood vessels and the mucosa of bronchi and bronchioles. All of these structures have walls thicker than those of alveoli, and passage may be impeded to a greater degree. The BLAD model used in this study has distinct advantages over strategies that utilize antibodies directed against CD18 and its receptors. Transfusion of CD18− cells from cattle or human beings with LAD into a recipient host, or drugs such as dexamethasone and NPC15669, which decrease the expression of adherence molecules. Much has been learned from these experiments, but inherent problems are obvious. For example, it cannot be assured that antibodies completely inhibit CD18 function when in the body, and the antibody may bind nonspecific sites; antibodies that bind Fcγ receptors can activate neutrophil adhesion; antibodies have limited half-lives and their stability deteriorates with time; transfused cells have a short circulation time and the transfusion process may activate the cells; and drugs do not completely block molecular expression and often have broad-based effects. In contrast, in the BLAD model there is consistent lack of CD18 expression.

Acknowledgements

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. The authors thank Miss K. Driftmier for technical assistance.

References


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