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What is This?
EXPERIMENTAL DISEASE

Distribution of Anti-CD68 (EBM11) Immunoreactivity in Formalin-fixed, Paraffin-Embedded Bovine Tissues

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Abstract. A commercially acquired anti-human macrophage antibody (anti-CD68; EBM11) was used in an immunocytochemical technique to detect macrophages in formalin-fixed, paraffin-embedded tissues from cattle, pigs, humans, rats, turkeys, dogs, and cats. In healthy cattle, the antibody labeled alveolar macrophages, pulmonary intravascular cells (presumably intravascular macrophages), and macrophage-like cells in other tissues. In bovine lungs infected with Pasteurella haemolytica, EBM11 antibody labeled 95% of alveolar macrophages and macrophages within alveolar septa but only 0–2% of streaming or “oat” leukocytes. Alveolar macrophages were also stained by EBM11 in pigs but not in rats, turkeys, dogs, and cats. The antibody also stained macrophage aggregates in the mesenteric lymph nodes and intestinal lamina propria of Mycobacterium paratuberculosis-infected cattle. This study shows that the anti-CD68 (EBM11) antibody is a useful marker of macrophages in normal bovine tissues or tissues from areas of acute or chronic inflammation that have been routinely processed. The study also adds strength to the growing evidence suggesting that streaming leukocytes seen in pneumonic pasteurellosis are neutrophils.

Key words: Bovine lung; CD68; EBM11; immunocytochemistry; intravascular macrophages; Pasteurella haemolytica.

The macrophage is a multifunctional cell essential for immunity against pathogens and release of physiologic levels of cytokines. There are many subtypes and differentiated forms: 1) circulating monocytes, which can enter sites of inflammation, 2) fixed macrophages, such as alveolar macrophages, intravascular macrophages, Kupffer cells, etc., and 3) epithelioid and multinucleate macrophages in chronic inflammation.

The need exists in both veterinary diagnostic and experimental pathology to develop techniques that identify macrophages in animal tissues. Macrophages in tissue sections can be labeled with enzyme histochemical stains, such as nonspecific esterase, and with immunocytochemical markers to epitopes, such as Mac-1 (CD11b/CD18), Fc receptor, CD13, CD14, CD33, CD64, CD68 (KP1), and α1-antitrypsin. However, these techniques have inherent limitations and problems, i.e., a requirement for frozen sections, cross-reactivity, poor specificity, or weak staining.

In the past few years, antibodies directed against different portions of the CD68 molecule have been used successfully to identify macrophages in formalin-fixed, paraffin-embedded tissues from human beings. CD68 is a 110-molecular weight glycoprotein associated with lysosomes in macrophages, myeloid cells, and certain neoplastic mononuclear cells. One of these antibodies, EBM11, has been used to label macrophages in frozen sections of human, bovine, and macaque tissues. EBM11 has also been used to detect CD68 antigen in protease XIV-treated, formalin-fixed, paraffin-embedded tissues from human beings. Other antibodies immunoreactive with CD68 of macrophages in routinely processes human tissues include Ki-M1P, KP1, and more recently PG-M1.

In this study, we report the distribution of immunoreactivity by an anti-human CD68 antibody (EBM11) in formalin-fixed, paraffin-embedded and trypsin-digested tissues from cattle. To test EBM11 immunoreactivity to macrophages in routinely processed tissues from various other species, easily recognizable alveolar macrophages in lung tissue were used as a screening guide. Bovine tissues that contained acute and chronic inflammation were also tested: lungs with acute Pasteurella haemolytica pneumonia and ileum and mesenteric lymph node infected with Mycobacterium paratuberculosis.
### Materials and Methods

**Tissues**

*Alveolar macrophages of the lung.* Multiple tissue sections from formalin-fixed (10% neutral-buffered formalin), paraffin-embedded tissues from several animal species were obtained. These included normal lungs from four cattle, two pigs, three rats, two turkeys, one dog, and one cat and lung tissues from one dog and one cat each with chronic passive pulmonary congestion and moderate intraalveolar histiocytosis.

*Other tissues.* Because immunoreactivity to alveolar macrophages was present in sections of tissues from cattle and pigs (Table 1), additional selected tissues were examined from these two species. Tissues from cattle included tonsils (n = 6), spleen (n = 6), liver (n = 6), ileum (n = 6), mesenteric lymph node (n = 4), thymus (n = 4), kidney (n = 1), and bone marrow (n = 1); selected porcine tissues included spleen, liver, lymph node, and bone marrow. Human tissues were used as a positive control and included tonsil (n = 1) removed from a human female suffering from recurrent tonsillitis and a granuloma (n = 1) from an adult male. To test immunoreactivity in areas of inflammation, lung tissue from cattle (n = 7) with spontaneous *Pasteurella haemolytica* pneumonia and pigs (n = 2) with experimental *Actinobacillus pleuropneumoniae* infection (4.2 × 10^6 cfu/ml administered intravenously) were examined. Also included were ileum (n = 4) and mesenteric lymph node (n = 4) from cattle with granulomatous ileitis and lymphadenitis caused by experimental infection with *Mycobacterium paratuberculosis* (archival tissues provided by Diana Whipple, National Animal Disease Center, Ames, IA).

Pig, rat, and turkey tissues were fixed immediately after the animals were killed. The human tonsil and granuloma were from surgical procedures and were fixed immediately after removal. Tissues from the cattle, dogs, and cats were collected 1 to several (up to 10) hours after death. Fixed tissues were left in formalin for less than 7 days for all tissues except for sections of ileum and mesenteric lymph nodes from cattle infected with *M. paratuberculosis*, which were stored in formalin for >45 days. Following routine processing and paraffin embedment, tissue sections were cut at 3 µm and affixed to glass slides precoated with poly-L-lysine.

**Antibody and technique**

The monoclonal antibody EBM11 (anti-CD68) (Dako, Carpinteria, CA) was used in an immunocytochemical technique, which included trypsinization to unmask tissue antigens. Sections were deparaffinized with xylene, rehydrated with graded alcohols, and immersed in distilled water then incubated with 0.1% trypsin solution in Tris buffer (pH 7.6) containing 0.1% calcium chloride. The primary antibody, monoclonal mouse anti-CD68 (EBM11), was used at a 1:25 dilution and incubated overnight at 4°C. The remaining immunocytochemical reagents were also commercially acquired and contained peroxidase-labeled goat anti-mouse IgG and 3,3′-diaminobenzidine substrate (VectaStain ABC Kit, Vector Laboratories, Burlingame, CA). Control sections lacked primary antibody (EBM11) or the primary antibody was substituted with an irrelevant antibody (mouse IgG antihuman T cell). Sections were counterstained with Harris hematoxylin. In sections of pneumatic lungs, bronchial and bronchiolar epithelial cells were differentiated from other cell types with cytokeratin immunocytochemistry. Pancytokeratin rabbit anti-cow Igs (Dako, Carpinteria, CA) was used as the primary antibody (1:400 dilution) in the above protocol that included trypsinization and a secondary biotinylated goat anti-rabbit IgG.

Labeling intensity of cells was scored on a subjective scale of 0 to ++++, where 0 was no staining, + was faint (minimally detectable staining), ++ was light brown, and ++++ was dark brown (Tables 1–3). All scores were determined by one pathologist (MRA).

In sections of lungs with acute pneumonia, the percentages of three cell types that appeared as 1) alveolar macrophages, 2) streaming or “oat” cells, or 3) degenerate cells with a large (>3 µm), pale basophilic nucleus were determined. The percentage of alveolar macrophages stained were determined by counting the number of stained alveolar macrophages out of a total of 100 macrophages (no more than 25 cells per 250 × field were counted) with typical alveolar macrophage structure. The percentages of oat cells and degenerate cells with round basophilic nuclei that stained were determined by counting the number of positively stained cells out of total of 250 leukocytes (from at least five different 250 × fields).

Lungs from cattle and swine with similar lesions of acute *P. haemolytica* or *A. pleuropneumoniae* pneumonia were chosen for study. These lungs had an accentuated lobular pattern due to marked thickening of alveolar septa by fibrin, edema, dilated lymphatic vessels, and infiltrates of neutrophils and lesser numbers of macrophages. There were multifocal extensive infiltrates of neutrophils and macrophages into alveolar lumina admixed with fibrin and seroproteinaceous fluid and multifocal coalescing areas of necrosis often demarcated by zones of neutrophils and macrophages of variable thickness. Each lung had multiple areas with streaming cells, which were elongated and curvilinear with a thin, slender deeply basophilic nucleus. Adjacent to these streaming cells were very small numbers of a second type of degenerate cell that was polygonal to round (5–10 µm) with pale cytoplasm and a single oval to round, relatively large (4–5 µm in diameter) nucleus with lightly basophilic, evenly dispersed chromatin. These cells were also present in dilated blood vessels and lymphatic vessels.

Sections of ileum and mesenteric lymph node infected with *M. paratuberculosis* were characterized by typical lesions present in Johne's disease. Sections of ileum contained moderate infiltrates of macrophages, epithelioid macrophages, and multinucleate macrophages within the lamina propria of the upper ⅔ of villi. These areas of lamina propria were moderately expanded by the infiltrates and the villi were widened and club shaped. Lymph nodes contained moderate, multifocal infiltrates of macrophages, epithelioid macrophages, and multinucleate cells in the medullary cords and, to a lesser degree, in follicular and perifollicular areas.

The human tonsil was characterized by a diffuse germinal center and perifollicular lymphocytic hyperplasia. Sections of granuloma had numerous macrophages and epithelioid macrophages surrounding a small core (100 µm in diameter).
of necrotic cell debris and a peripheral zone of lymphocytes and plasma cells.

Apparent molecular weight determination of EBM11 antigen in cultured cells

Cultured bovine peritoneal macrophages (7 x 10^5 cells; contributed by Dr. Judith Stabel, National Animal Disease Center, Ames, IA) and murine J774.16 macrophage-like cells (8 x 10^5 cells; American Type Culture Collection, Rockville, MD) were lysed with lysis buffer (2% Triton X-100 in phosphate-buffered saline, pH 8.0, containing 1 mM ethylenediaminetetraacetic acid and 1 μM phenylmethylsulfonyl fluoride) and centrifuged. Supernatants were electrophoresed under reducing conditions on a sodium dodecyl sulfate-polyacrylamide gel (12%). Separated proteins were transferred to nitrocellulose (Schleicher and Schuell, Keene, NH)²⁰ and allowed to react overnight (4°C) with either EBM11 antibody (1:25 dilution) or an irrelevant monoclonal anti-

Fig. 1. Lung; bovine. Alveolar macrophages and intravascular cells (arrows) are stained (brown) using the EBM11 (anti-CD68) antibody. Harris hematoxylin counterstain. Bar = 25 μm.

Fig. 2. Lung; bovine. Pulmonary alveolar lumen contains numerous neutrophils; macrophage are stained with EBM11. Note streaming (oat) cells within the lumen that are not stained. Harris hematoxylin counterstain. Bar = 50 μm.

Fig. 3. Lung; bovine. The cytoplasm of several degenerate cells with large, round, pale basophilic nuclei are stained (brown). Harris hematoxylin counterstain. Bar = 25 μm.

Fig. 4. Lung; bovine. The cells lining a small bronchiole and hypertrophied type II cells within an alveolar lumen are stained (brown) using the anti-cytokeratin antibody. Harris hematoxylin counterstain. Bar = 50 μm.
body of the same isotype and concentration. Immunoblots were then incubated overnight (4 C) with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Fischer, Orangeburg, NY). Conjugated antibodies were seen with a substrate solution containing 1 mg naphthol AS-MX phosphate and 2 mg Fast Red TR salt per milliliter (Sigma, St. Louis, MO). Gels were stained for total protein with Coomassie blue.

Flow cytometric analysis of bovine leukocytes using EBM11

To determine if CD68 is expressed on the surface of bovine monocytes, EBM11 was applied to buffy coat cells and compared with a known mouse anti-bovine monocyte/granulocyte IgG1 (DH59B; VMRD, Pullman, WA) using flow cytometric analysis. Buffy coat cells were isolated by centrifugation and resuspended in Hanks buffered saline solution with 0.5% bovine serum albumin. Nonpermeabilized buffy coat cells (1 x 10^6) were then incubated with various concentrations and combinations of antibodies. Positive control wells were treated with the primary antibody (DH59B) at 1:100 dilution followed by fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse IgG Fab fragment (Cappel Laboratories, Cochranville, PA) at 1:400 dilution. The cells were scanned from the bottom of the wells, resuspended in 1% paraformaldehyde, and placed in silicon-coated microcentrifuge tubes. Negative control wells included bovine buffy coat cells lacking antibodies, buffy coat cells plus the primary antibody (DH59B) only (1:100), buffy coat cells plus the secondary antibody (FITC-labeled anti-mouse IgG) only (1:400), and buffy coat cells plus EBM11 only (1:10). Wells testing the affinity of EBM11 to bovine monocytes containedbuffy coat cells plus concentrations of EBM11 at 1:10, 1:100, or 1:1,000 substituted for the primary antibody, followed by the secondary antibody. All samples were assayed in triplicate by flow cytometry (EPICS Profile Analyzer, Coulter; Dr. Kristi R. Harkins, Iowa State University Cell Facility, Ames, IA).

Results

In lungs from cattle, multifocal cells within the capillaries of alveolar septa were intensely (++) stained (Table 1). Most of the capillaries contained one to two stained cells; similar staining was not seen in either medium or large pulmonary veins and arteries. Intravascular cells were not stained in porcine lung tissue. Staining of alveolar macrophages or intravascular cells was not seen in the lungs of rats, dogs, cats, or turkeys.

In lungs from cattle and pigs with acute pneumonia, macrophages at all locations demonstrated EBM11 IR: those in alveoli with abundant, highly vacuolated cytoplasm; intravascular cells (cattle only); small cells within alveolar septa; and those macrophages in areas of dense cellular infiltrates (Table 2). Only rare (up to 2%) streaming (oat) cells were immunoreactive, and the vast majority did not stain (Fig. 2, Table 2). Less than 3% of degenerate cells with the large, round, pale basophilic nucleus (second degenerate cell type) demonstrated EBM11 IR (Fig. 3, Table 2). Intravascular macrophages were stained but did not have a streaming cell appearance.

Anticytokeratin antibody stained epithelial cells lining bronchi and bronchioles and hypertrophied type II cells lining alveolar lumina (Fig. 4). In areas of lung with marked inflammation and type II cell hypertrophy, from ½ to ⅔ of the cells within alveolar lumina were stained with cytokeratin. Many of these cells were hypertrophied type II cells, but some cells were unattached and considered to be sloughed bronchial, bronchiolar, or type II cells. Streaming cells and the degenerate cells with large nuclei were not stained with cytokeratin.

Macrophages were intensely (++) and consistently labeled in the bovine tonsil, spleen, liver, ileum, mesenteric lymph node, and thymus (Table 3). In tonsil, tingible body macrophages within germinal centers were clearly labeled, as were individual perifollicular cells throughout the sections (Fig. 5), a similar staining pattern was present in sections of human tonsil (Fig. 6). In spleen, one to five cells within the center of germinal follicles were immunoreactive, as were individual multifocal macrophage-like cells in the remaining white pulp. The perifollicular cells in both tonsil and spleen were elongate to polygonal with abundant cytoplasm and a single oval to indented nucleus (1–2 μm in diameter). In liver, individual cells lining sinusoids (Kupffer cells) were stained throughout sections of all samples (Fig. 7). These cells were elongate or, on occasion, polygonal. Subjectively, these cells were less numerous in periportal regions and more commonly seen in perportal areas. In ileum, multifocal cells were labeled in the lamina propria and submucosa. In the Peyer’s patches, staining of elongate to polygonal cells in the follicular and perifollicular areas was similar to that seen in tonsil. In lymph node sec-
with abundant cytoplasm were stained in the cortex of thymus, macrophages (roughly 25 µm in diameter) were the average of the 25 cells.

* Ratio represents the number of animals in which macrophage-like cells were labeled with the given tissue over the total number of animals tested.
† Intensity determined using a subjective scale of 0 to +++, where 0 is no staining, + is minimally detectable staining, ++ is light brown, and +++ is dark staining (see Materials and Methods).

**Table 1.** Staining properties of alveolar macrophages and intravascular macrophages in the lungs of cattle, pigs, rats, turkeys, dogs, and cats using an anti-CD68 (EBM11) antibody.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Alveolar Macrophage*</th>
<th>Intravascular Cell*</th>
<th>Intensity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>4/4</td>
<td>4/4</td>
<td>+++</td>
</tr>
<tr>
<td>Pig</td>
<td>2/2</td>
<td>0/2</td>
<td>+</td>
</tr>
<tr>
<td>Rat</td>
<td>0/3</td>
<td>0/3</td>
<td>0</td>
</tr>
<tr>
<td>Turkey</td>
<td>0/2</td>
<td>0/2</td>
<td>0</td>
</tr>
<tr>
<td>Dog</td>
<td>0/2</td>
<td>0/2</td>
<td>0</td>
</tr>
<tr>
<td>Cat</td>
<td>0/2</td>
<td>0/2</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ratio represents the number of animals in which macrophage-like cells were labeled over the number of animals tested.
† Intensity determined using a subjective scale of 0 to +++, where 0 is no staining, + is minimally detectable staining, ++ is light brown, and +++ is dark staining (see Materials and Methods).

Table 2. Staining intensity* and percentage† of labeled macrophages, streaming (oat) cells, and degenerate leukocytes with a round, basophilic nucleus using the anti-macrophage antibody EBM11 in pneumonic lung tissue.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Alveolar Macrophages</th>
<th>Oat Cells</th>
<th>Degenerate Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+++</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+++</td>
<td>98</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>97</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+++</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>+++</td>
<td>97</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>+++</td>
<td>100</td>
<td>NP‡</td>
</tr>
<tr>
<td>7</td>
<td>+++</td>
<td>98</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+++</td>
<td>96</td>
<td>0</td>
</tr>
<tr>
<td>Pigs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>95</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>96</td>
<td>+</td>
</tr>
</tbody>
</table>

* Subjective scale: 0 = no staining; + = minimally detectable staining, ++ = light brown, +++ = dark staining. Five cells from five different sites in each lung section were scored. Numbers shown are the average of the 15 cells.  † Denotes the percent of cells stained by the anti-CD68 (EBM11) antibody. For alveolar macrophages, 100 cells with typical macrophage morphology were counted. For oat cells and degenerate cells with round nuclei, 250 cells in five sites from two lung sections were counted.  ‡ NP = not present in the lung sections.

In sections of ileum and lymph node from cattle infected with *Mycobacterium paratuberculosis*, aggregates of macrophages, epithelioid macrophages, and multinucleate macrophages were clearly distinguishable histologically in the lamina propria of villi and throughout the lymph node, Staining intensity in these cells (fixed >45 days) was light brown (+++), and individual resident macrophages of the lamina propria and Peyer's patches were not stained (Table 3). Attempts using a commercially acquired antigen retrieval kit for progesterone receptor (Bionex, San Ramon, CA) did not improve EBM11 IR in these tissues.

In the human tonsil, macrophages and dendritelike cells within the center of germinal centers were stained (+++), as were perifollicular cells (Fig. 6). In the granuloma, macrophages stained intensely (+++).
Fig. 5. Tonsil; bovine. Dendriticike cells within the germinal center of a tonsil follicle are labeled (brown). Harris hematoxylin counterstain. Bar = 50 μm.

Fig. 6. Tonsil; human. Several macrophages within the center of germinal centers are stained (arrows). Some of the macrophages contain ingested material. Harris hematoxylin counterstain. Bar = 50 μm.

Fig. 7. Liver; bovine. Individual cells lining sinusoids (Kupffer cells) are stained (arrows), as are several periportal cells. Harris hematoxylin counterstain. Bar = 50 μm.

Fig. 8. Lymph node; bovine. Macrophages within the medullary sinus and subtrabecular sinus are stained with EBM11. Harris hematoxylin counterstain. Bar = 50 μm.
Fig. 9. Macrophage whole cell lysates were electrophoresed on sodium dodecyl sulfate–polyacrylamide gels and either stained with Coomassie blue (A) or transferred to nitrocellulose membrane for immunoblot analysis (B). Fig. 9A. Lane 1, prestained standard (Bio Rad, Richmond, CA); lane 2, murine J774.16 macrophage lysate; lane 3, cultured bovine peritoneal macrophage lysate. Fig. 9B. Lane 1, prestained standard. Murine J774.16 cells (lanes 2–4) and bovine macrophages (lanes 5–7) were immunoblotted with either no antibody (lanes 2, 5), irrelevant monoclonal antibody of the same isotype and concentration (lanes 3, 6), or EBM11 antibody (lanes 4, 7).

Apparent molecular weight determination of EBM11 antigen in cultured cells

Whole cell lysate supernatants contained numerous proteins (Fig. 9A). In both cultured bovine peritoneal macrophage and murine macrophage-like cells, the anti-CD68 (EBM11) monoclonal antibody recognized only one protein with an apparent molecular weight near 51 kD under reducing conditions (Fig. 9B).

Flow cytometric analysis of bovine leukocytes using EBM11

No expression of fluorescence beyond background was detected by flow cytometric analysis in samples containing EBM11 or in negative control preparations. In these preparations, expression was <4.0% for monocytes (x = 1.9%), <1.1% for lymphocytes (x = 0.4%), and <2.5% for granulocytes (x = 1.5%). In contrast, using the anti-bovine monocyte antibody DH59B, immunofluorescence was detected in monocyte gated cells (x = 61.5% expression) and, to a much lesser degree, in granulocytic (x = 5.5% expression) and lymphocytic (x = 3.5%) gated cells.

Discussion

This study demonstrates that anti-CD68 (EBM11) is a reliable marker of alveolar macrophages in formalin-fixed (<7 days), paraffin-embedded tissues of cattle. It is also a marker of an intravascular cell type, presumably intravascular macrophages, in cattle.14 The decreased staining intensity and inability to label individual macrophages in tissues infected with Mycobacterium paratuberculosis somewhat limits the use of the technique for all bovine tissues. The decreased intensity may be due to prolonged fixation (>45 days) or other factors, such as macrophage differentiation. Further evaluation of the technique using tissues fixed for various lengths of time may clarify the effect of prolonged fixation. The inability of the antibody to label nonpermeabilized monocytes with flow cytometric analysis is consistent with results of previous studies,5 studies demonstrating CD68 within the plasma membrane of cytoplasmic granules18 and with CD68 expression being dependent upon the state of cellular differentiation.5

Ultrastructural studies using immunolabeling pro-
cedures are needed to determine if the pulmonary intravascular cells labeled by this technique are truly intravascular macrophages. Easy identification of intravascular macrophages would greatly enhance research efforts. These cells have a vital and beneficial function in clearance of hematogenous bacterial cells. Conversely, they may have a detrimental effect during clearance of hematogenous lipopolysaccharides due to their production of lipoxygenase products and, most likely, tumor necrosis factor and interleukin-1. The cells are key targets in Pasteurella haemolytica and Haemophilus somnus pneumonia in the bovine and are also central to pulmonary pathology studies that use ruminants as animal models for diseases, such as the adult respiratory distress syndrome (ARDS) and the generalized Schwartzman reaction. The inability of the antibody to stain porcine intravascular cells cannot be explained. Intravascular macrophages occupy 6% of pulmonary capillary lumina surface area in neonatal pigs and 25% in 1-month-old pigs. This study also demonstrates that the vast majority of degenerate streaming (oat) cells in the lungs of animals do not stain with antibodies specific to macrophages or epithelial cells. Thus, the streaming cells are probably neutrophils, and probably only rare streaming cells are macrophages. This interpretation is tentative because there may be partial degradation of CD68 in the degenerate cells; however, a similar conclusion was made in an ultrastructural study in pigs with experimental H. (Actinobacillus) pleuropneumoniae infection. Studies have also suggested that oat cells seen in pneumonic bovine lungs are neutrophils. Formation of the streaming or oat cell appearance is due to the leukotoxin of Pasteurella haemolytica. Leukotoxin has an affinity for the surface of leukocytes but has little effect on parenchymal cells of the lung. The A. pleuropneumoniae toxin has extensive homology with leukotoxin and may work in a similar manner. The toxins probably bind to the surface of macrophages, neutrophils, and platelets, cause pore formation, and induce degeneration of each cell type.

The streaming cells and degenerate cells with large, pale, basophilic nuclei probably are not sloughed epithelial cells because 1) anti-cytokeratin antibody stained epithelial cells and hypertrophied type II cells but not degenerate cells in the lumina of airways and 2) the epithelium of conducting airways remained largely intact. The small population of degenerate leukocytes with a large, round basophilic nucleus could not be conclusively categorized. The cytoplasm only rarely stained with EBM11, and the cytoplasm was not stained with anti-cytokeratin antibody. Our hypothesis is that these cells are immature neutrophils or macrophages that develop an enlarged, pale nucleus upon degeneration. In contrast, the mature segmented neutrophil becomes a typical oat cell and has a dense, deeply basophilic, and elongate nucleus following degeneration.

Previous work has demonstrated that EBM11 binds a 110-molecular weight protein in isolated bovine monocytes. Such a band was not present in the blots in the current study using the mouse macrophage cell line or transformed bovine peritoneal macrophages. The antigen may no longer be expressed in the transformed bovine cells or may be present in a truncated form that would give rise to the 51-molecular weight protein seen in this study. In any event, the bovine antigen is not destroyed during routine processing and is a useful marker for tissue macrophages. It is also useful for the identification of alveolar macrophages in routinely processed porcine lung. Although immunoreactivity was not present in sections of routinely processed lung from other species, immunoreactivity may be demonstrated in frozen sections or in tissues processed with fixatives other than formalin.


