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Tissue Distribution, Intracellular Localization, and *In Vitro* Expression of Bovine Macrophage Scavenger Receptors

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Macrophage scavenger receptors are trimeric membrane proteins implicated in the pathologic deposition of cholesterol in atherogenesis. The authors have studied the tissue distribution and intracellular localization of bovine scavenger receptors using monoclonal antibovine receptor antibody IgG-D2. The receptor proteins were detectable in macrophages of various organs and tissues, particularly Kupffer cells, alveolar macrophages, and macrophages in the spleen and lymph nodes. In the brain, perivascular macrophages were immunoreactive with IgG-D2. Fibroblasts, endothelial cells, smooth muscle cells, and dendritic cells such as epidermal Langerhans cells, interdigitating cells, or follicular dendritic cells, however, showed no immunoreactivity to IgG-D2. Immunoelectron microscopy showed localization of reaction products for these receptors on the cell surface, vesicles, and endosomes of macrophages. Transient expression of bovine scavenger receptors on cultured cells shows that scavenger receptors are mainly expressed in the endoplasmic reticulum, nuclear envelope, and Golgi apparatus of nonmacrophage cells and moved to the cell surface and endosomes of macrophagelike cells. These results indicate that efficient intracellular transport of scavenger receptors in macrophages is mediated by a macrophage-specific transport system. (Am J Pathol 1991, 139:1411–1423)

Macrophage scavenger receptors are trimeric membrane glycoproteins implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis.[1–5] The molecular cloning of bovine,[6,7] murine,[8] and human[9] scavenger receptors has disclosed several unexpected features. In both cases, two types of receptor subunits exist, and these receptor proteins contain two extracellular domains that are predicted to form a long triple-stranded alpha-helical coiled coil and collagenlike triple helix.[6–9] These receptors mediate the endocytosis of a diverse group of macromolecules, including modified low-density lipoproteins (LDL).[1–9] Recent human immunohistochemical studies[9] have indicated that the receptors are richly present in atheromatous plagues, especially in foam cells. When macrophages are incubated with LDL modified with acetic anhydride (acetylated LDL; acLDL),[1] it is internalized through coated pits and transferred into endosomes and further into lysosomes.[10–16] Little is known, however, about the physiologic role, tissue distribution, and intracellular localization of receptors themselves.

In this study, we used monoclonal antibody against bovine scavenger receptors, IgG-D2, and performed the immunohistochemical and immunoelectron microscopic studies of bovine scavenger receptors *in vivo* and *in vitro* to examine tissue distribution, intracellular localization, and expression of bovine scavenger receptors.

Materials and Methods

Animals and Tissues

Tissue specimens were obtained from various bovine organs and tissues: lungs, heart, liver, spleen, kidneys, esophagus, stomach, small intestine, lymph nodes, pancreas, adrenal glands, skin, and brain. To evaluate interspecies cross-reactivity of the antibodies, liver, lungs, and lymph nodes were obtained from horses, pigs, goats, New Zealand white rabbits, guinea pigs, Golden

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hamsters, Wistar rats, and C3H mice. Surgical specimens were obtained from similar tissues of several patients with nonmalignant diseases.

Culture

Bovine alveolar macrophages were obtained by bronchoalveolar lavage. For 1 hour, 1×10^5 cells were cultured in plastic dishes (35 mm; Nunclon, Roskilde, Denmark) with RPMI without fetal calf serum. Adherent cells were further cultured with a medium containing 50 μg/ml acLDL at 4°C for 1 hour, and washed in ice-cold medium to remove unbound ligands. After shifting temperature to 37°C, cells were cultured in RPMI1640 without fetal calf serum for 1, 5, 10, 20, and 30 minutes in the medium without acLDL.

For collection of Kupffer cells, bovine liver tissues were mashed with a steel grater, digested by 1000 U/ml of Dispase (Gohdoh-Shusei Co., Tokyo, Japan) for 1 hour, and filtrated through nylon stocking meshes to obtain cell suspensions. The cell suspensions were centrifuged using Lymphoprep (Ohtsuka, Tokyo, Japan) to obtain a Kupffer-cell–rich fraction and cultured for 1 hour to collect adherent Kupffer cells.

Blood was sampled from the jugular vein of the animals, and monocytes were isolated by gradient centrifugation using Lymphoprep and cultured in dishes from 1 to 7 days in RPMI 1640 containing 10% fetal calf serum.

DNA Transfection

COS cells were transfected with bovine macrophage scavenger receptor type I expression vector (pXSR7)[6] by the DEAE-dextran method[17] and were cultivated in Dulbecco's minimum essential medium (DMEM) with 10% fetal calf serum for 2 days. Human erythroleukemic cell line cells (HEL cells) were transfected with pXSR7 or an empty vector (pCDNA1) by electroporation.[18] After electroporation, HEL cells were either cultivated in RPMI medium containing 10% fetal calf serum in the presence of 200 nmol/l phorbol 12-myristate 13-acetate (PMA) for 2 days to force differentiation into macrophagelike cells or in the absence of PMA, to keep them in a nonmacrophage status.

Monoclonal and Polyclonal Antibodies

A hybridoma cell line (D2) secreting monoclonal antibodies against bovine scavenger receptor (IgG-D2) was established, as described previously,[5] and was cultured in RPMI1640 with 5% fetal calf serum. The supernatant was harvested and changed every 3 days. Cell density was approximately $5 \times 10^7/mm^3$. Immunoprecipitation and immunoblotting of bovine scavenger receptors were performed using partially purified bovine lung scavenger receptors, also as previously described.[5]

To identify macrophages, mouse anti-human macrophage monoclonal antibody, EBM11 (Dako, Glostrup, Denmark) was used at a dilution of 1:100, because it is known to cross-react with bovine macrophages.[19]

Immunohistochemistry

The bovine tissue specimens were fixed for 4 hours with 2% periodate-lysine-paraformaldehyde solution.[20] After washing in phosphate-buffered saline (PBS) containing a graded series of sucrose (10%, 15%, 20%), the tissues were rinsed in PBS containing 20% sucrose and 5% glycerol for 1 hour. The tissues then were embedded in OCT compound (Miles, Elkhart, IN) and frozen in dry ice–acetone. Cryostat sections were cut at 6 to 8 μ and dried in air. After inhibition of endogenous peroxidase activity by the method of Isobe et al,[21] the specimens were incubated for 1 hour with mouse anti-bovine macrophage scavenger receptor monoclonal antibody IgG-D2 as the culture supernatant diluted 1:10, and mouse anti-human macrophage monoclonal antibody EBM11 at a dilution of 1:100. After washing in PBS, they were covered with species-specific goat anti-mouse Ig [$F(ab')_2$] conjugated with peroxidase (Amersham, Amersham, UK) diluted 1:100 for 1 hour. Peroxidase activity was visualized using 3-3′-diaminobenzidine (DAB) as substrate. For control, tissue slides were incubated with nonimmunized mouse serum or PBS instead of primary antibody, and afterwards processed by the same procedure as described above. Tissues of other species were processed and stained in the same manner as for bovine tissues.

Immunoelectron Microscopy

Bovine alveolar macrophages, Kupffer cells, monocytes, human erythroleukemia (HEL) cells and COS cells in culture were fixed for 10 minutes in 0.1% glutaraldehyde in 0.1 mol/l cacodylate buffer, pH 7.4. After inhibition of endogenous peroxidase, immunostaining was performed as described above using IgG-D2 as the culture supernatant without dilution. After demonstration of peroxidase activity with DAB, the cells were postfixed with 1% osmium tetroxide for 60 minutes. The cells then were dehydrated through a graded series of ethanols and detached from the dishes by adding propylene oxide. The cell pellets were embedded in epoxy resin and sec-

tioned. The ultrathin sections were cut by a Ultrotome NOVA (LKB, Uppsala, Sweden) and observed in JEM 2000EX (JEOL, Tokyo, Japan) after staining with lead citrate or without staining.

IgG-D2–immunoreactive coated pits, vesicles, and vacuoles, which were sectioned approximately in the center of the cells, were counted in 100 alveolar macrophages.

Enzyme Cytochemistry

For cytochemical detection of peroxidase activity, the method of Graham and Karnovsky was performed.[22] In cultured cells, the method of Barka and Anderson for acid phosphatase was employed to detect lysosomal compartments.[23]

Ligand Dissociation

To confirm the ligand dissociation at acidic pH, the acid-mediated release of ^{121}I-labeled acLDL at 4°C was determined as reported previously.[24] Alveolar macrophages were incubated at 4°C with ^{125}I-labeled acLDL (5 μg/ml in prechilled Eagles minimum essential medium without bicarbonate containing 10% fetal calf serum and 20 mmol/l [millimolar] HEPES at pH 7.4) for 2 hours. Each dish was then rapidly washed five times with ice-cold wash buffer (50 mmol/l TRIS-HCl, 150 mmol/l NaCl, 2 mmol/l $CaCl_2$, and 0.2% bovine serum albumin at pH 7.4). The dishes then received the buffer containing either 50 mmol/l TRIS-maleate (pH 7, 6, 5.5) or TRIS-succinate (pH 5, 4.5) at the indicated pH plus 50 mmol/l NaCl, 2 mmol/l $CaCl_2$, and 5% bovine serum albumin. After incubation for either 5 minutes at 37°C or 15 minutes at 4°C, the amount of ^{125}I-labeled acLDL released into the buffer was determined. The cells were dissolved in 0.1 mol/l NaOH and the amount of protein was determined.

Results

Recognition of Bovine Scavenger Receptors by the Monoclonal Antibody, IgG-D2

IgG-D2 recognized a 220-kd trimeric bovine receptor protein on immunoblotting, but it could not recognize dimeric or monomeric receptor proteins (Figure 1). The results of immunoprecipitation of ^{125}I-labeled specimens by IgG-D2 confirmed the recognition of bovine receptors by IgG-D2. Recently Doi et al confirmed that this antibody

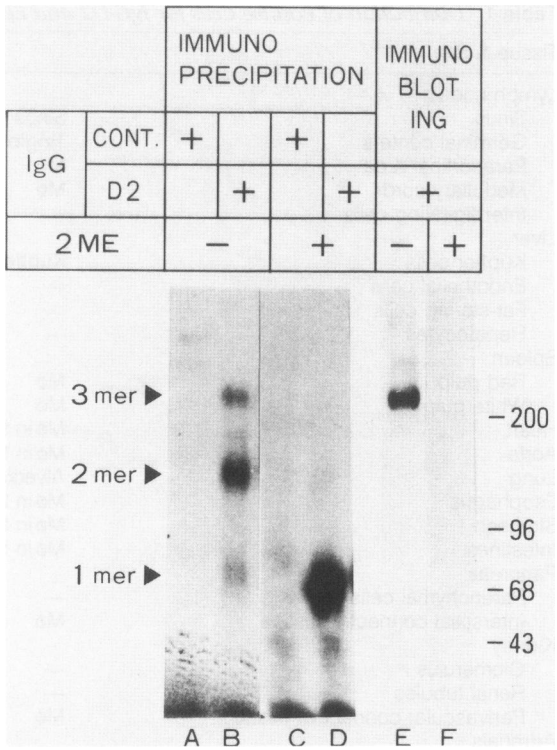

Figure 1. *Immunoprecipitation and immunoblotting of bovine scavenger receptors. Bovine lung macrophage scavenger receptors were partially purified using a combination of maleyl-BSA affinity chromatography and hydroxylapatite chromatography by the method of Kodama et al.[5] For immunoprecipitation, aliquots of iodinated partially purified receptor (10-6 cpm) were incubated with 10 μg of monoclonal antibody IgG-D2 (lanes B and D) or control antibody (lanes A and C), then the antibody was precipitated with protein A sepharose. The precipitate was washed four times, dissolved in sample buffer (10% SDS, 10% glycerol, 12.5mM Tris-HCl pH6.8 with or without 5% 2-mercaptoethanol, 2ME as indicated), was subjected to SDS polyacrylamide gradient (3–10%) gel electrophoresis, and was visualized by autoradiography.[5] For immunoblotting, aliquots of partially purified receptors (20 μg per lane) were subjected to SDS polyacrylamide gel electrophoresis with (lane F) or without 5% 2ME (lane E). The proteins on the gel were electroblotted onto cellurose nitrate paper, and were incubated with either monoclonal antibody IgG-D2 (5 μg/ml). The bound IgG was visualized using goat anti-mouse IgG conjugated with peroxidase.[5] Arrows indicate the position of the trimeric, dimeric and monomeric bovine receptor bands.*

recognizes the truncated receptor, which lacks the collagenlike domain and the C-terminal type specific domain, and can bind IgG-D2 (unpublished observation). IgG-D2 and EBM11 were not reactive with macrophages of mice, rats, guinea pigs, hamsters, rabbits, goat, horses, and pigs. In humans, EBM11 recognized macrophages, but IgG-D2 did not.

Tissue and Cell Type Specificity of Bovine Macrophage Scavenger Receptors

Table 1 summarizes the distribution of positive cells for each monoclonal antibody. IgG-D2 reacted with Kupffer

Table 1. *Distribution of Positive Cells for IgG-D2 and EBM11*

Tissue & Site	IgG-D2$^+$ cell	EBM11$^+$ cell
Lymph nodes		
Sinus	Sinus Mø*	Sinus Mø
Germinal centers	Tingible body Mø	Tingible body Mø
Paracortical area	Mø	Mø
Medullary cords	Mø	Mø
Interdigitating cells	—	—
Liver		
Kupffer cells	Kupffer cells	Kupffer cells
Endothelial cells	—	—
Fat-storing cells	—	—
Hepatocytes	—	—
Spleen		
Red pulp	Mø	Mø
White pulp	Mø	Mø
Heart	Mø in the connective tissue	Mø in the connective tissue
Aorta	Mø in the adventitia	Mø in the adventitia
Lung	Alveolar Mø	Alveolar Mø
Esophagus	Mø in the submucosa	Mø in the submucosa
Stomach	Mø in the lamina propria	Mø in the lamina propria
Intestines	Mø in the lamina propria	Mø in the lamina propria
Pancreas		
Parenchymal cells	—	—
Interstitial connective tissue	Mø	Mø
Kidney		
Glomerulus	—	—
Renal tubules	—	—
Perivascular connective tissue	Mø	Mø
Adrenals		
Interstitial connective tissue	Mø	Mø
Skin		
Epidermis	—	—
Dermis	Histiocytes	Histiocytes
Uterus		
Endometrium	Mø	Mø
Myometrium	Mø	Mø
Ovary	Mø	Mø
Brain		
Cerebrum	Perivascular Mø	Perivascular Mø
Cerebellum	Perivascular Mø	Perivascular Mø
Subarachnoid space	Mø	Mø
Spinal cord	—	—
Peripheral nerve	Mø in the perineurium	Mø in the perineurium

* Mø = Macrophages, — = not immunoreactive.

cells in the liver (Figure 2a), alveolar macrophages in the lungs (Figure 3a), macrophages in the red pulp of the spleen, in the lymphatic sinuses of lymph nodes (Figure 4a), and in other tissues. Their distribution was very similar to that of EBM11-positive macrophages in these tissues (Figures 2b, 3b, 4b, and 5b). In the brain, perivascular macrophages were also reactive to IgG-D2 (Figure 5a), but neuronal cells or astroglial cells were unreactive. In the subarachnoid space, macrophages were also immunoreactive. Among tissue macrophages in bovine visceral organs and tissues, Kupffer cells, alveolar macrophages, and round macrophages in the red pulp of the spleen, endometrium, and ovaries showed intense immunoreactivity to IgG-D2. The sinusoidal endothelial cells, fat-storing cells, and hepatocytes in the liver, Langerhans cells in the epidermis, and microglia in the brain, however, did not react to the monoclonal antibody. In the paracortical area of the lymph nodes, periarteriolar lymphatic sheaths of the spleen, and thymic medulla, interdigitating cells showed no immunoreactivity for IgG-D2 as did marginal zone metallophils and marginal zone macrophages in the spleen. In all bovine tissues, including the aorta, we were unable to find any atherosclerotic lesions, but only a few IgG-D2–immunoreactive cells were found in the adventitia of the aorta. In the aorta, arteries, and veins, the endothelial cells and medial smooth muscle cells were unreactive with IgG-D2. In the heart, there were no IgG-D2–immunoreactive cells in the endocardium, subendocardial layer, myocardium, and subpericardium except for a few cells in the myocardial interstitium and subpericardium. In the subcutaneous connective tissue, interstitial tissues, and subserosal layer

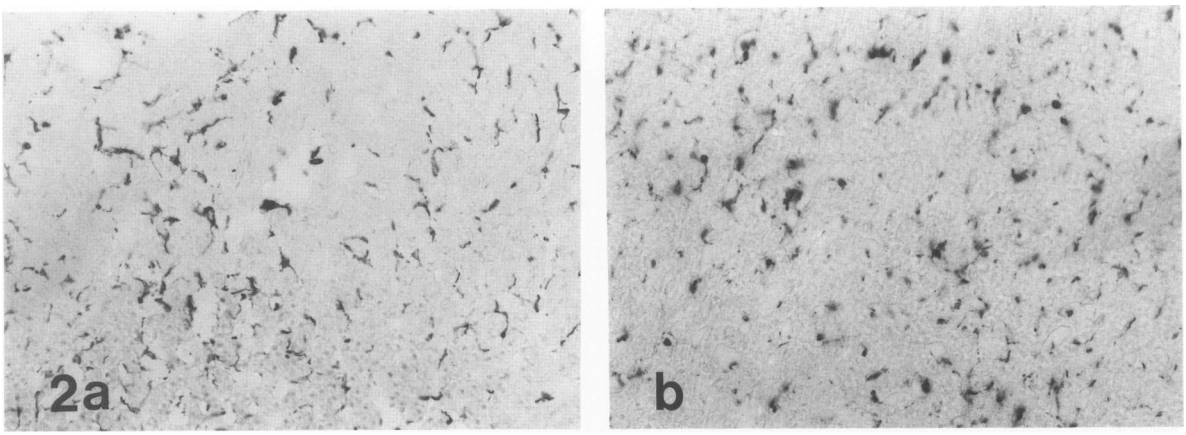

Figure 2. *Immunohistochemical staining of bovine tissues and cells using IgG-D2* **(a)** *and EBM11* **(b)**. *The distribution of IgG-D2-positive cells was very similar to that of EBM11-positive macrophages in the tissues. Kupffer cells in the liver were reactive to each monoclonal antibody, ×200.*

of various visceral organs, macrophages were reactive to IgG-D2, but fibroblasts were not.

In tissues incubated with nonimmunized mouse serum or PBS instead of primary antibodies, no positive reactivity was observed in any cell types.

Intracellular Localization of Bovine Macrophage Scavenger Receptors

In alveolar macrophages and Küpffer cells, reaction products for IgG-D2 were localized on the cell surface membrane and occasionally on the membrane of endocytic vesicles and vacuoles with or without multivesicular profiles (Figures 6 and 7). Vesicles and vacuoles immunoreactive for IgG-D2 were negative for acid phosphatase activity (Figure 8a), indicating that they were not lysosomal compartment.

Monocytes possessed no scavenger receptors, but when they differentiated into macrophages after 5 days in culture, IgG-D2 immunoreactivity was expressed on the cell surface membrane (Figure 9a, b). These results indicate that scavenger receptors are a macrophage-specific membrane-associated protein.

Scavenger Receptors and Receptor-mediated Endocytosis of acLDL

To examine the changes in intracellular localization of scavenger receptors during receptor-mediated endocytosis of acLDL, we incubated these cells with acLDL at 4°C for 1 hour. Alveolar macrophages incubated at 4°C showed IgG-D2 immunoreactivity on the cell membrane, particularly in coated pits (Figure 10). After temperature shift to 37°C, acLDL are known to be rapidly internalized. The number of small vesicles positive for IgG-D2 increased with time. Meanwhile, IgG-D2 immunoreactivity on the membrane of larger vacuoles increased. Five minutes later, the number of coated pits, vesicles, and vac-

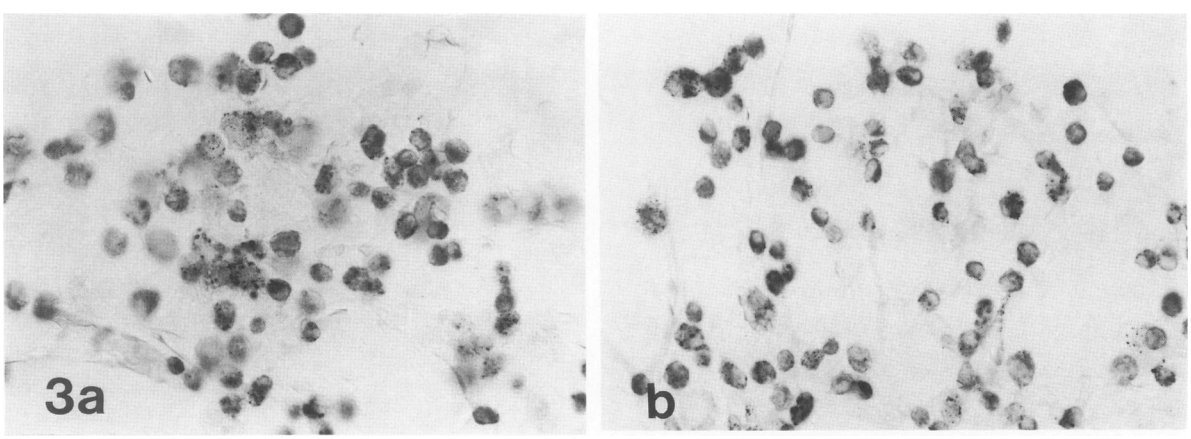

Figure 3. *Alveolar macrophages obtained by alveolar lavage were positive for each monoclonal antibody, ×200.*

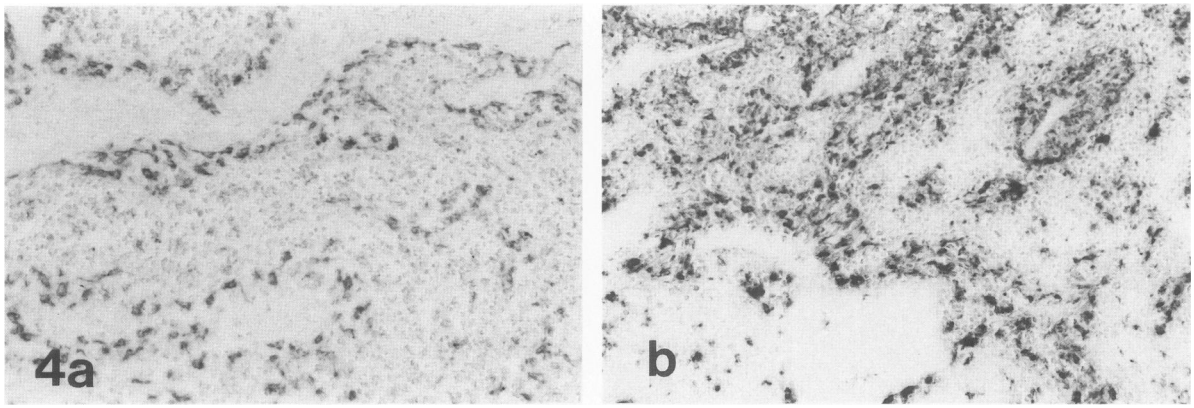

Figure 4. *Macrophages in the lymphatic sinus were reactive to both antibody, ×200.*

uoles showing immunoreactivities for IgG-D2 increased, and vacuoles containing curled structures or showing multivesicular profiles were observed (Figure 11). Almost all the vacuoles and vesicles were negative for acid phosphatase. These vacuoles were larger than endosomes of nonmacrophage cells (ordinarily 100 to 400 nm), and half of them measured 500 to 1000 nm in diameter, some being over 1000 nm and about 2000 nm at largest. Ten minutes after temperature shift to 37°C, the number of IgG-D2–immunoreactive vacuoles reached a peak (Figures 12 and 13). Twenty minutes later, the number of IgG-D2–immunoreactive vacuoles with or without multivesicular profiles decreased, followed by a reduction in the intensity of immunoreactivity, and the numbers of immunoreactive vesicles and vacuoles returned to that before incubation. A few endosomes were observed to be positive for acid phosphatase (Figure 8b), suggesting the fusion of endosomes with lysosomes. These results indicate that the scavenger receptors are located on the cell surface at 4°C and are bound to acLDL. In addition, after temperature shift to 37°C, the receptors bound to acLDL were internalized through coated pits and small vesicles, and then transferred to endosomes. The reduction of IgG-D2 immunoreactivity in the endosomes after 20 minutes suggests ligand dissociation and receptor recycling.

Acid-dependent release at 37°C or 4°C of receptor-bound ^{125}I-labeled acLDL from the surface of alveolar macrophages can be seen in Figure 14. At pH 7, there was some dissociation from the macrophage cell surface. When the pH was reduced to 6.0 and 5.5, significant increase in ligand dissociation occurred either at 37°C or 4°C. These results support the hypothesis that scavenger receptors dissociate their ligands at acidic condition.

Stable Expression of Bovine Receptors on COS Cells and HEL Cells

COS cells transfected with pXSR7 showed marked immunoreactivity against IgG-D2 in the nuclear envelope, endoplasmic reticulum, and Golgi apparatus, but not on the cell surface (Figure 15). COS cells transfected with the empty vector indicated no immunoreactivity in any intracellular organelles or on the cell surfaces. The intracellular localization pattern of scavenger receptors in the COS cells transfected with pXSR7 was quite different

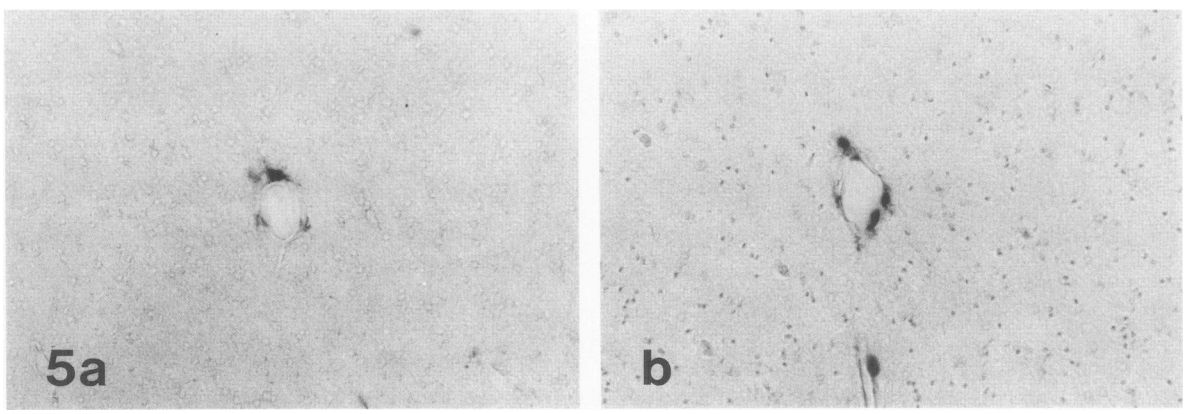

Figure 5. *Perivascular macrophages in the cerebrum were reactive against both monoclonal antibodies, ×200.*

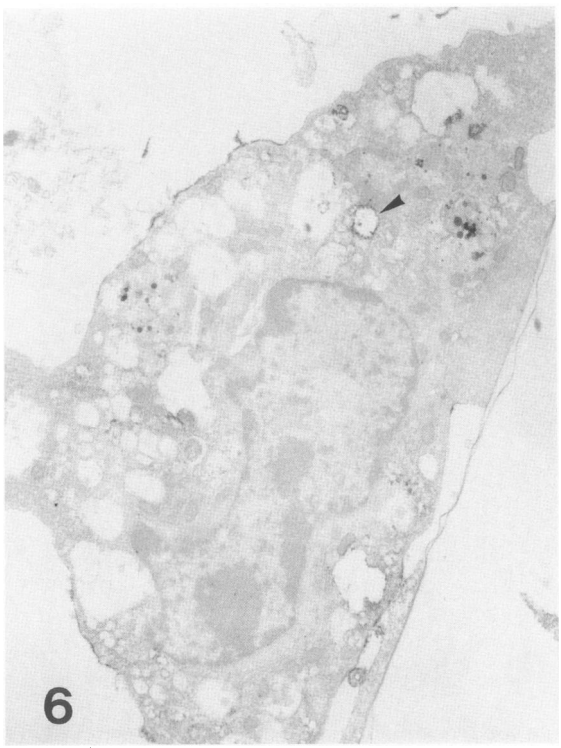

Figure 6. *Alveolar macrophages showed a positive reaction for IgG-D2 on the cell membrane and on the membrane of a few endosomes (arrowheads). Immunoelectron microscopy using IgG-D2, ×6,500.*

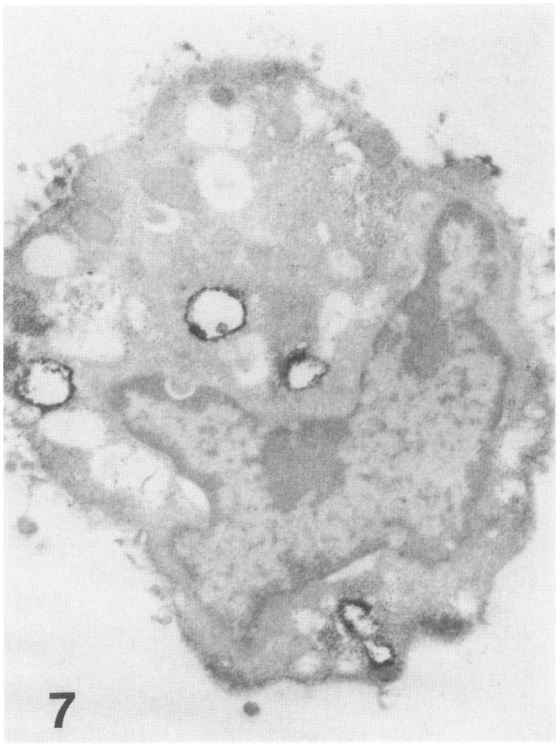

Figure 7. *Kupffer cells show several IgG-D2-positive endosomes. Immunoelectron microscopy using IgG-D2, ×8,000.*

from that shown in macrophages. To study the mechanism by which scavenger receptors are transported in macrophages, we compared the localization of expressed receptors in HEL cells cultivated with or without PMA. In the absence of PMA, the HEL cells transfected with pXSR7 did not differentiate into macrophages and showed no expression of receptors in the nuclear envelope, endoplasmic reticulum, Golgi apparatus, or on the cell surface (Figure 16a). Conversely pXSR7-transfected HEL cells cultivated in the presence of PMA transformed into macrophages and possessed scavenger receptors mainly on the cell surface and endocytic vesicles (Figure 16b). The HEL cells transfected with the empty vector (pCDNA1) did not show immunoreactivity, indicating that IgG-D2 is specific to bovine receptors and cannot recognize human scavenger receptors.

Discussion

In this study we used a monoclonal antibody against the bovine scavenger receptors, IgG-D2, which recognizes the 220-kd trimeric receptor protein. IgG-D2 can not bind to monomeric or dimeric receptor subunits or scavenger receptors of other animal species and humans. By means of IgG-D2 in combination with macrophage-specific monoclonal antibody EBM11, scavenger receptors were detected on macrophages of the various bovine organs and tissues ubiquitously. IgG-D2 immunoreactivity was prominent on alveolar macrophages, Küpffer cells, and macrophages in the spleen and lymph nodes, agreeing with the results of a previous immunohistochemical study with IgG-D1 on immunoblotting.[5] These cells are highly active on the clearance of foreign bodies, noxious materials, and denatured or modified self-components, suggesting that scavenger receptors may play a role in scavenging these materials *in vivo*. The scavenger receptors were also richly present in the macrophages of the adrenal glands, an organ that plays an important role in cholesterol and related lipid metabolism. In the present study, scavenger receptors were detected in perivascular macrophages of the bovine brain, in agreement with the results of a study showing that two types of scavenger receptor mRNAs are expressed in human brain.[8] The physiologic role of the receptors in the brain is totally unknown, however. In contrast, epidermal Langerhans cells, interdigitating cells in the T-zone of lymphoid tissues, and follicular dendritic cells in the lymph follicles possessed no scavenger receptors, indicating that they are functionally different cell populations from macrophages and have no scavenger cell function.

In animal experiments, injected fluorescent-labeled acLDL were efficiently taken up by sinusoidal endothelial cells of the liver,[25–27] and an immunohistochemical ap-

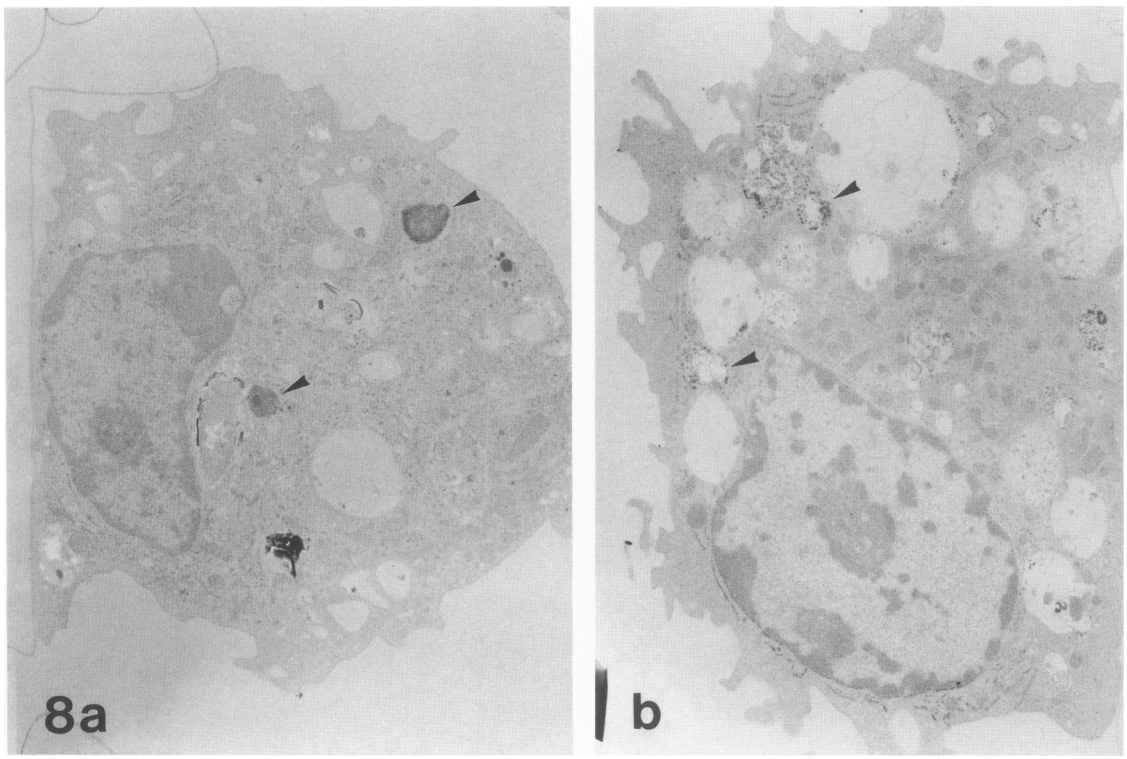

Figure 8. a: *Alveolar macrophages possess a few small lysosomes positive for acid phosphatase (arrowheads). Acid phosphatase reactivity is not demonstrated in endosomal vesicles and vacuoles. Electron microscopical cytochemistry for acid phosphatase by Barka-Anderson method,[23] ×7,500.* **b:** *A few alveolar macrophages showed positive reaction for acid phosphatase in vesicles or vacuoles (arrowheads) 1 hour after incubation in the medium containing acLDL at 4°C and 20 minutes after a temperature shift to 37°C. Electron microscopical cytochemistry for acid phosphatase by Barka-Anderson method,[23] ×7,500.*

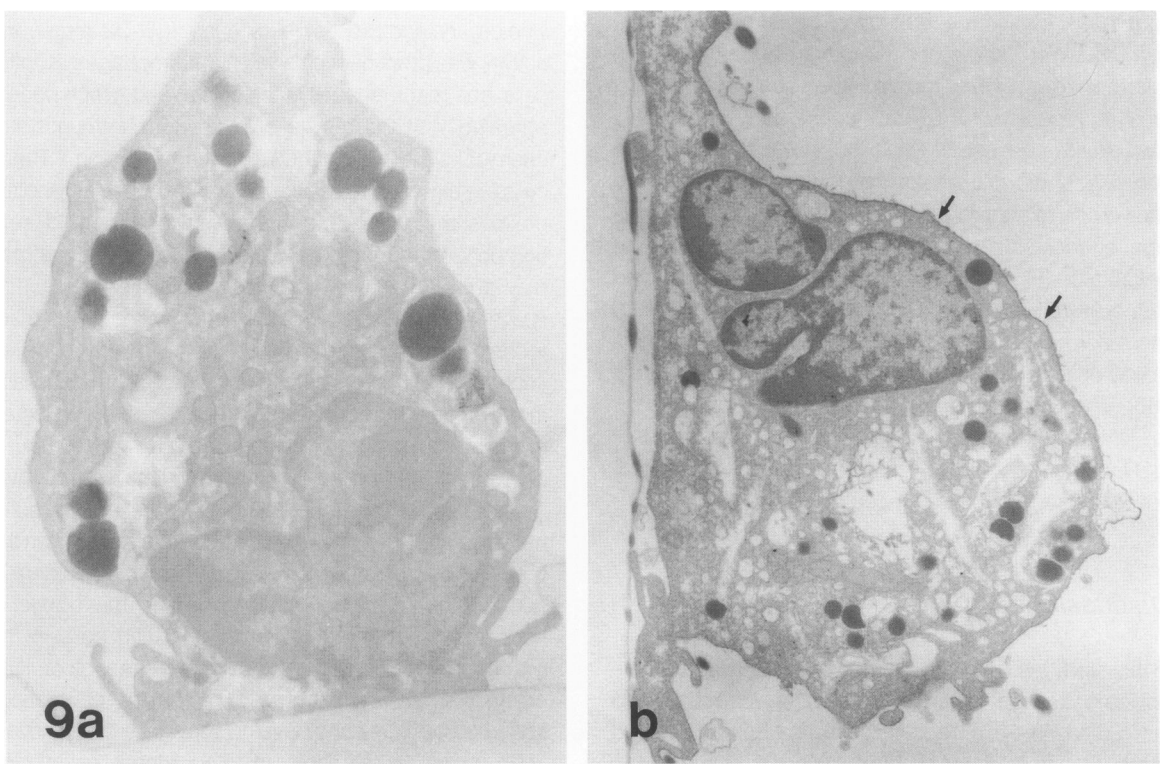

Figure 9. a: *Blood monocytes cultured for 2 days do not show any reaction products on the cell membrane. Immunoelectron microscopy using IgG-D2, ×7,000.* **b:** *Monocytes cultured for 5 days show reaction products on the cell membrane (arrows). Immunoelectron microscopy using IgG-D2, ×7,000.*

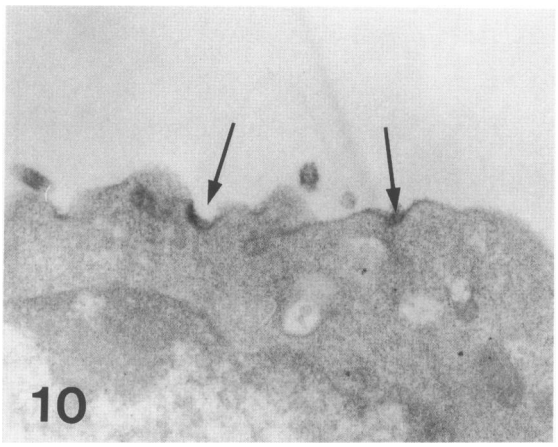

Figure 10. *Alveolar macrophages in the endocytic process of acLDL. Immunoelectron microscopy using IgG-D2. Alveolar macrophages incubated for 2 hours with acLDL at 4°C. Accumulation of the receptor protein is observed on the cell membrane, especially the coated pits (arrows), ×15,000.*

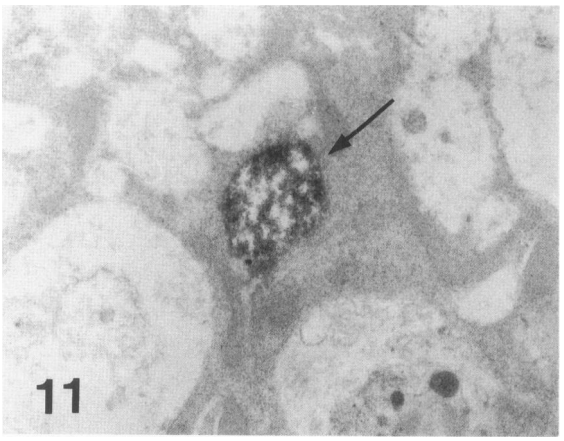

Figure 11. *Alveolar macrophages incubated for 5 minutes after temperature shift to 37°C. The multivesicular body is reactive for IgG-D2, ×20,000.*

proach with another monoclonal antibody against bovine scavenger receptors, IgG-D1, indicated the presence of immunoreactive materials in the liver sinusoidal endothelial cells.[5] We could detect no significant IgG-D2 immunoreactivity in the sinusoidal endothelial cells of the liver, however. These results suggest the possibility that the sinusoidal endothelial cells possess different receptors for acLDL, immunologically closely related to the type I and II scavenger receptors.

Immunoelectron microscopic studies of bovine macrophages with IgG-D2 showed that scavenger receptors are localized on the cell surface membrane and occasionally on the membranes of vesicles and endosomal vacuoles, indicating that the scavenger receptors are membrane-associated receptors. However IgG-D2 was not detected on monocytes but expressed after they had

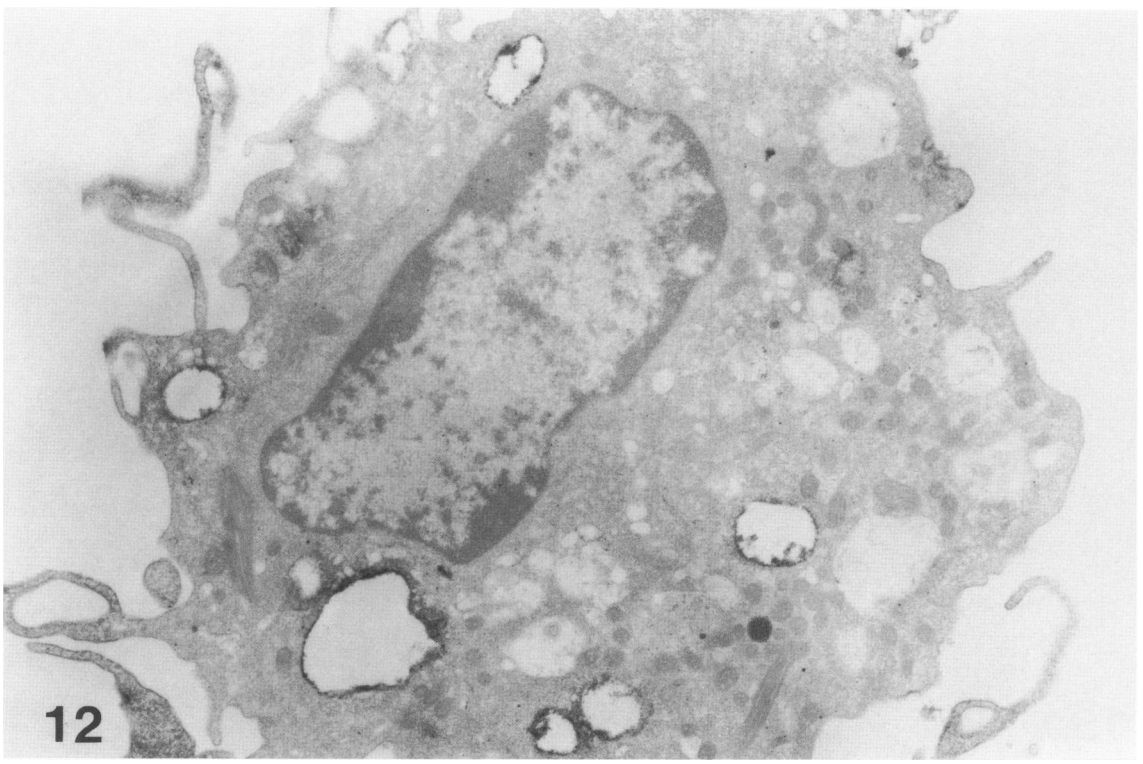

Figure 12. *Alveolar macrophages incubated for 10 minutes after temperature shift to 37°C. The cells contain several IgG-D2-positive endocytic vacuoles (endosomes), ×11,000.*

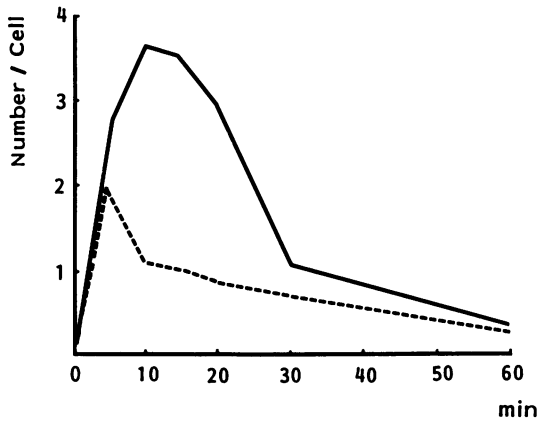

Figure 13. *Number of IgG-D2-positive coated pits (---) and endosomes (—) in an alveolar macrophage in the endocytic process of acLDL. Alveolar macrophages were incubated for 2 hours in a medium containing 50 μg/ml of acLDL at 4°C and further incubated in a medium without acLDL at 37°C. Only distinct immunoreactivity was judged to be positive. Data are representative of 100 macrophages.*

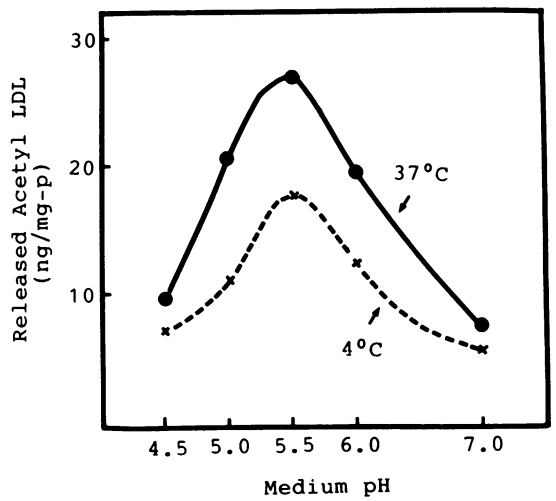

Figure 14. *Acetyl-LDL dissociation at acidic pH from bovine alveolar macrophages. To confirm the ligand dissociation at acidic pH, cultured bovine alveolar macrophages were incubated with ^{125}I-acLDL (5 μg/ml at pH7.4) for 2 hours at 4°C. Each dishes were washed five times with ice-cold wash buffer. The dishes then received the buffer containing either 50mM Tris-maleate (pH 7, 6, 5.5) or Tris-succinate (pH 5, 4.5) at the indicated pH plus 50mM NaCl, 2mM $CaCl_2$ and 5% bovine serum albumin. After incubation for either 5 minutes at 37°C or 4°C, the amount of ^{125}I-acLDL released into the buffer was determined.*

differentiated into macrophages *in vitro*. In our further study on HEL cells transfected with the bovine scavenger receptor type 1 expression vector (pXSR7), IgG-D2 immunoreactivity was first detected on the cell membrane, when they were differentiated into macrophagelike cells. These results imply that scavenger receptors are expressed on the cell membrane of macrophages in the terminal stage of mononuclear phagocyte differentiation.

The internalization process of acLDL through coated pits and its lysosome-directed pathway through endosomes have been reported in previous studies.[10–16] With the aid of IgG-D2, we demonstrated the presence of

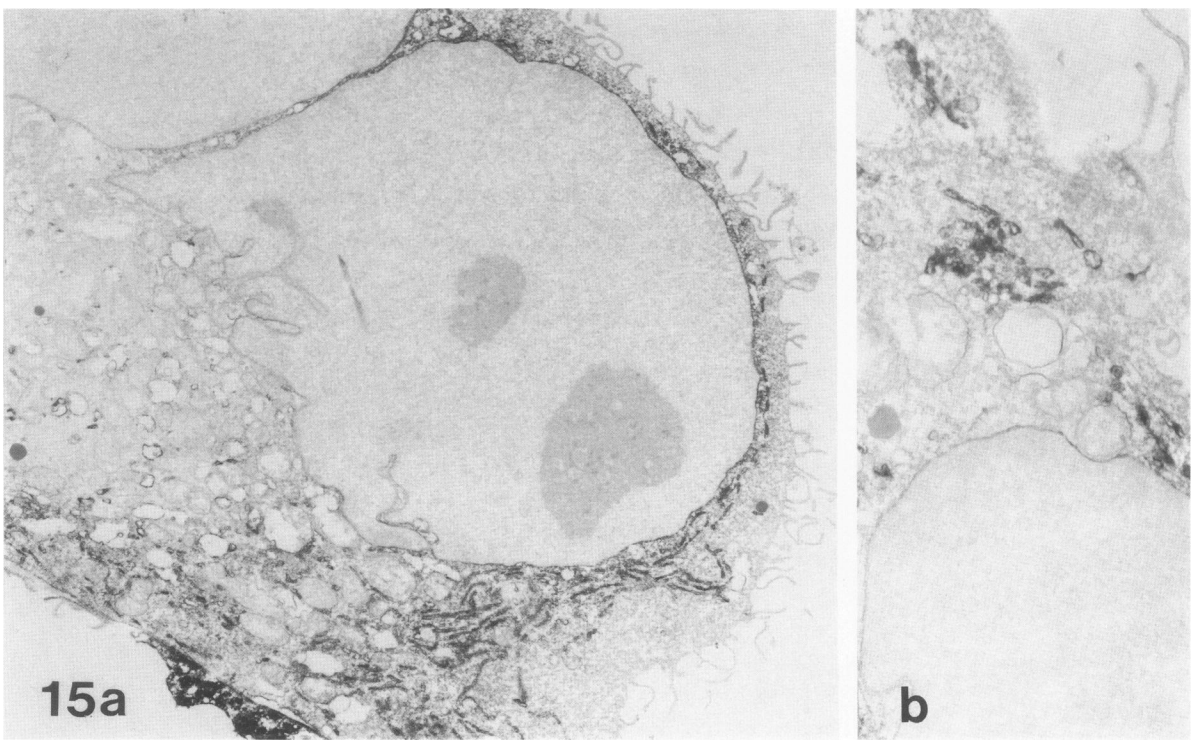

Figure 15. *COS cell transfected with the bovine scavenger receptor type I expression vector (pXSR7). Reaction products are present in the nuclear envelope, rER (a, ×3,000) and Golgi apparatus (b, ×10,000), but not on the cell surface. Immunoelectron microscopy using IgG-D2.*

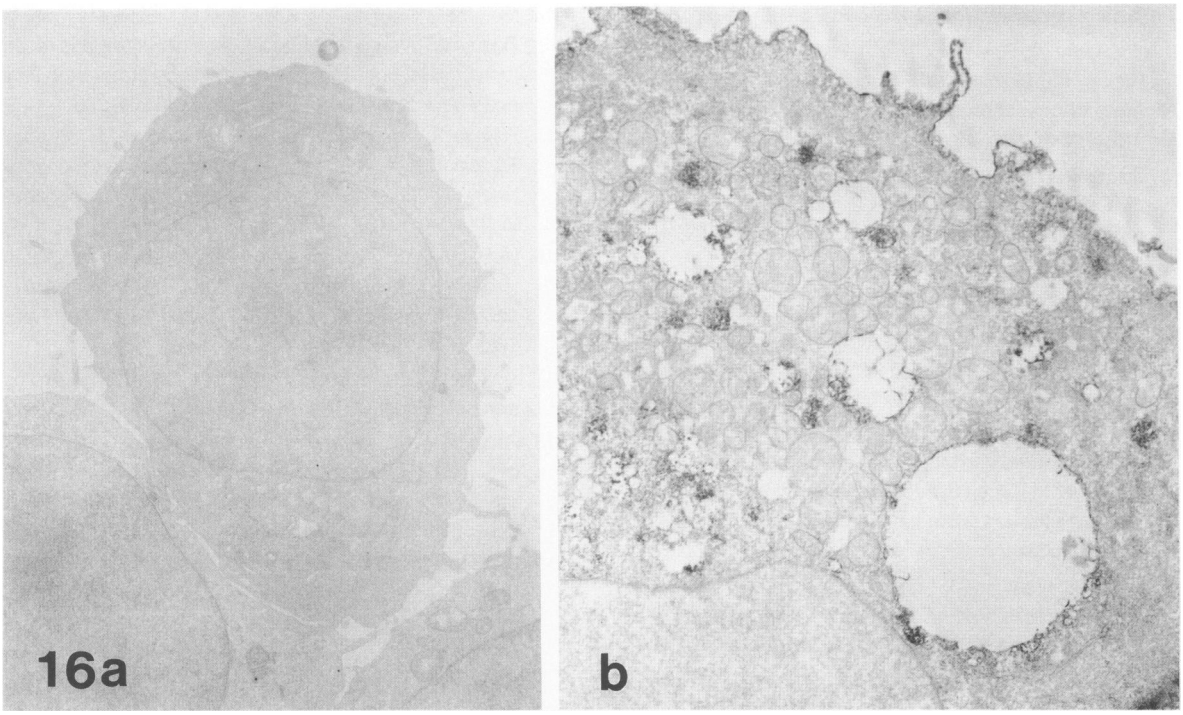

Figure 16. a: *HEL cell transfected with pXSR7. They were incubated for 2 days in a medium without phorbol myristate acetate. Reaction products for IgG-D2 are not found in any organelles, ×3,000.* **b**: *HEL cells transfected with pXSR7 were incubated for 2 days in a medium containing phorbol myristate acetate. Transformation into a macrophage shows localization of scavenger receptor proteins mainly on the membrane of the cell surface and most endosomes, ×6,000.*

scavenger receptors on the cell membrane and in coated pits of bovine macrophages at 4°C and in vesicles and vacuoles after temperature shift to 37°C. Because almost all the vesicles and vacuoles were negative for acid phosphatase, it is indicated that they are endosomal but not lysosomal compartments. Interestingly variation in size of the endosomes in alveolar macrophages was more marked, and half of them were larger than those of fibroblasts (150–400 nm),[28,29] peritoneal macrophages (200–300 nm),[30] neutrophils and monocytes (150–400 nm),[31] hepatocytes (200–500 nm),[32] and CHO cells (200–500 nm).[33] Disappearance patterns of the immunoreactivity for IgG-D2 within phagocytic vacuoles and vesicles, the absence of IgG-D2 immunoreactivity within secondary lysosomes, and the results of the ligand dissociation experiment at acidic pH suggest that the scavenger receptors are dissociated from acLDL in the late endosomal compartment and recycled to the cell surface. Further studies are required, however, to clarify the precise route of the recycling of the scavenger receptors.

In the present *in vitro* study, intracellular expression of bovine scavenger receptors in bovine alveolar macrophages, COS cells transfected with pXSR7 or empty vector, and similarly transfected HEL cells was compared. In the pXSR7-transfected COS cells, transient expressions of the scavenger receptors were demonstrated in the nuclear envelope, endoplasmic reticulum, and Golgi apparatus, but not on the cell membrane. When the transfected HEL cells were transformed into macrophagelike cells by phorbol ester treatment, however, scavenger receptors were demonstrated on the cell surface. Taken together with the fact that scavenger receptors first emerge on the cell surface when monocytes are differentiated into macrophages, these results suggest that the efficient intracellular transport of scavenger receptors to the cell surface occurs only in macrophages. Further studies regarding the mechanism of such macrophage-specific scavenger receptor transport will provide us important information about the receptor-mediated endocytosis in macrophages.

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