Roles of a Macrophage Receptor with Collagenous Structure (MARCO) in Host Defense and Heterogeneity of Splenic Marginal Zone Macrophages*

Shigeo Ito1,2, Makoto Naito1, Yoshiaki Kobayashi1,2, Hisakazu Takatsuka1, Shuying Jiang1, Hiroyuki Usuda1, Hajime Umezu1, Go Hasegawa1, Masaaki Arakawa2, Leonard D. Shultz3, Outi Elomaa4 and Karl Tryggvason4

Second Departments of Pathology1 and of Internal Medicine2, Niigata University School of Medicine, Niigata, Japan; The Jackson Laboratory4, Bar Harbor, ME, USA; and Division of Matrix Biology1, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Sweden

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Summary. Class A type I and type II macrophage scavenger receptors (MSR-A) and a macrophage receptor with collagenous structure (MARCO) are trimeric membrane glycoproteins mediating the uptake of chemically modified low density lipoproteins. MSR-A is expressed constitutively in several tissue macrophages and in liver sinusoidal endothelial cells, whereas MARCO is expressed constitutively in splenic marginal zone macrophages and in macrophages and endothelial cells in the lymphatic medullary sinuses of lymph nodes. The administration of LPS, zymosan, BCG, or L. monocytogenes to mice resulted in marked and transient MARCO expression and in the upregulation of MSR-A expression in the liver and spleen. In osteopetrotic (op) mutant mice defective in the production of M-CSF, ER-TR9-positive marginal zone macrophages and MOMA-1-positive marginal metallophillic macrophages were absent, whereas MARCO-expressing marginal zone macrophages were present, indicating the heterogeneity of marginal zone macrophages. Intravenous administration of BCG resulted in marked accumulation of BCG bacilli in the both marginal zone macrophages and marginal metallophilic macrophages in littermate control mice. In contrast, BCG bacilli were incorporated almost exclusively by MARCO-expressing marginal zone macrophages in op/op mice. These results indicate that MARCO is not only expressed constitutively in specific macrophage subpopulations but is also induced by various bacterial antigens and plays a role in host defense against bacteria.

Macrophage scavenger receptors have been implicated in the deposition of cholesterol in arterial walls during atherogenesis through receptor-mediated endocytosis of chemically modified low density lipoproteins (LDL) (Kodama et al., 1990; Rohrer et al., 1990; Freeman et al., 1990; Matsusoto et al., 1990; Naito et al., 1991; Matto et al., 1996). Because of the wide range of the ligand-binding capacity of these receptors, they have a broad spectrum of biological roles in not only atherogenesis but also host defense against bacteria or exogenously invading pathogens as well as the removal and clearance of various arrays of negatively charged macromolecules, including waste products. The scavenger receptors are classified into class A type I and type II macrophage scavenger receptors (MSR-A) (Kodama et al., 1990; Rohrer et al., 1990; Freeman et al., 1990; Matsusoto et al., 1990; Naito et al., 1991; Matto et al., 1996), and a macrophage receptor with collagenous structure (MARCO) (Elomaa et al., 1995; van der Laan et al., 1997), class B (CD36: Endermann et al., 1993 and SR-BI: Acton et al., 1994, 1996), and class C (dSR-CI: Pearson et al., 1995; Landshulz et al., 1995, Fc γ-RII-B2: Stanton et al., 1992, and C68/macrosialin: Ramaprasad et al., 1995; Holness et al., 1993). Among these scavenger receptors, MSR-A is a trimeric glycoprotein expressed in macrophages in various tissues which binds to a precursor of Gram-negative bacterial lipid A (Hampton et al., 1991; Ashkenas et al., 1993) and a cell wall component of Gram-positive bacteria (Dunne et al., 1994). It

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was recently shown that MSR-A-knockout mice were more susceptible to *Listeria monocytogenes* and herpes simplex virus infections, indicating important roles for MSR-A in host defense mechanisms (Suzuki et al., 1997). MARCO (Elomaa et al., 1995; Van Der Laan et al., 1997) is also a trimeric glycoprotein which is only expressed on macrophages in the marginal zone of the spleen and in the cells constituting marginal sinuses of lymph nodes in normal unstimulated conditions (Elomaa et al., 1995; Van Der Laan et al., 1997). MARCO expression is induced in macrophages in several tissues of mice infected with *Klebsiella pneumoniae*, and COS cells transfected with MARCO cDNA are specifically bound to *Escherichia coli* and *Staphylococcus aureus*, or acetylated LDL (Elomaa et al., 1995; Van Der Laan et al., 1997). These findings suggest that MARCO plays important roles in the recognition, binding, and internalization of bacterial antigens as well as chemically modified LDL.

The macrophage colony stimulating factor (M-CSF) is known to upregulate MSR-A expression *in vitro* (De Villiers et al., 1994). To elucidate the effects of M-CSF on the expression of MSR-A *in vivo*, osteopetrotic (op/op) mice serve as an interesting model, because the mice are defective in the production of M-CSF (Yoshida et al., 1990) and do not have marginal zone macrophages or marginal metallophilic macrophages (Takahashi et al., 1994). Therefore, op/op mice are a unique model for examining the expression of MARCO as well as that of MSR-A.

In the present study, we examined the expression of MARCO in various tissues after the administration of lipopolysaccharide (LPS), zymosan, BCG, and *L. monocytogenes*. The purpose of the present investigation is to demonstrate that the expression of MARCO is induced in macrophages by various bacterial antigens. MARCO expression in M-CSF-deficient op/op mice and the role of MARCO in the uptake of BCG were also examined.

**MATERIALS AND METHODS**

**Animals**

BALB/c mice were purchased from Charles River Inc. Japan (Tokyo, Japan). Osteopetrotic mice, (C57BL/6J X C3HeB/HeJ) F1-op/op, were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained under routine conditions at the Laboratory Animal Center, Niigata University School of Medicine. Heterozygotes (op/+ ) were used as littermate control mice. Eight-week-old male mice were intraperitoneally (i.p.) injected with 100 μg of LPS (Sigma, St Louis, MO). The liver was removed at various time points after LPS injection. Three mice were killed by ether anesthesia at each of these time points. Some mice were intravenously (i.v.) injected with zymosan (1 mg/mouse, Sigma), BCG (8 × 10^6 CFU/mouse, Japan BCG Inc., Tokyo, Japan), and *L. monocytogenes* (5 × 10^4 CFU/mouse, EGD strain, kindly provided by Dr. M. Mitsuya, Department of Microbiology, Graduate School of Medicine, Kyoto University, Kyoto).

**Antibodies**

The monoclonal antibodies, BM8, ER-TR9, and MOMA-1, were purchased from BMA Biomedicals (Augst, Switzerland) and used at a dilution of 1:100. These monoclonal antibodies recognize antigens on macrophages. Monoclonal antibody, mcnC5-3 (Matsunaga et al., 1994), was kindly provided by Prof. S. Yamamoto and F4/80 (Austyn and Gordon, 1981), and 2F8 (Fraser et al., 1993; Hughes et al., 1995) by Prof. S. Gordon and coworkers. Anti-MARCO antisera against domain I (N-terminal; residues 1-50) and domain IV plus domain V (C-terminal; residues 369-518) were raised in rabbits (Elomaa et al., 1995). The antisera were used at a dilution of 1:1,000. Table 1 shows the reactive cells and specificities of antibodies used in the present study.

**Histology**

Tissues were fixed in 10% phosphate-buffered formalin and processed routinely for paraffin sections. Sections, 5 μm thick, were prepared, deparaffinized, hydrated, and stained with hematoxyline and eosin. Neutrophils were stained by the ASD-chloroacetate esterase method (Maloney et al., 1960).

**Immunohistochemistry**

The liver, spleen, lung, heart, kidney, pancreas, lymph nodes, uterus, ovary, testis, and skin were fixed for 4 h at 4 °C in periodate-lysine-paraformaldehyde, washed for 4 h with phosphate buffer saline containing 10, 15, 20% sucrose, embedded in OCT compound (Miles, Elkhart, IN), frozen in dry ice-acetone, and cut with a cryostat (Bright, Huntington, UK) into 6 μ-thick sections. After the inhibition of endogenous peroxidase activity by the method of Isobe et al. (1977), we performed immunohistochemistry using the anti-mouse monoclonal antibodies described above. As a secondary antibody, we used anti-rat Ig-horseradish peroxidase-linked F(ab')2 fragment (Amersham, Poole, UK). As a secondary antibody for rabbit polyclonal antibodies, anti-sheep Ig-horseradish peroxidase-linked F(ab')2 fragment
Table 1. The reactive cells and specificities of antibodies used.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reactive cells</th>
<th>Specificity</th>
<th>Reference</th>
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<tr>
<td>F4/80</td>
<td>Tissue macrophages, monocytes</td>
<td>Single chain glycoprotein</td>
<td>Austyn et al., 1981</td>
</tr>
<tr>
<td>BM8</td>
<td>Tissue macrophages, monocytes</td>
<td>BM8 antigen</td>
<td>Malorny et al., 1986</td>
</tr>
<tr>
<td>MOMA-1</td>
<td>Marginal metallophilic macrophages</td>
<td></td>
<td>Kraal and Janse, 1986</td>
</tr>
<tr>
<td>ER-TR9</td>
<td>Marginal zone macrophages</td>
<td></td>
<td>Van Vliet and Van Ewijk, 1985</td>
</tr>
<tr>
<td>rmC5-3</td>
<td>Macrophages, neutrophils</td>
<td>CD14</td>
<td>Matsura et al., 1994</td>
</tr>
<tr>
<td>2F8</td>
<td>Macrophages, hepatic sinusoidal cells</td>
<td>Scavenger receptor type I and type II</td>
<td>Fraser et al., 1993</td>
</tr>
<tr>
<td>Anti-MARCO antisera</td>
<td>Marginal zone macrophages, lymphatic sinus</td>
<td>Domain I and IV + V</td>
<td>Eломаа et al., 1995</td>
</tr>
</tbody>
</table>

(Amersham) was used. Following visualization with 3, 3′-diaminobenzidine (DAB; Dojin Chemical, Kumamoto, Japan) and nuclear staining with methylene green, the sections were mounted with resin.

**Double immunohistochemical staining**

Double immunohistochemical staining with F4/80 and 2F8 was performed according to the method previously described (YAMAMOTO et al., 1996) with a minor modification. In brief, after inhibiting the endogenous peroxidase activity as described above, cryostat sections were incubated with the primary monoclonal antibody. After incubation with anti-rat Ig-horseradish peroxidase-linked F(ab)² fragment, the reaction was stained brown with 3,3′-diaminobenzidine. The sections were washed twice with glycine-HCl buffer for 1 h, and then incubated with the secondary monoclonal antibody. After incubation with anti-rat Ig-horseradish peroxidase-linked F(ab)² fragment, they were incubated with nickel chloride solution in 3,3′-diaminobenzidine substrate kit (Vector Lab, Burlingame, CA) and processed as above to stain positive cells blue black. Double stainings with F4/80, MOMA-1, or 2F8 and anti-MARCO antisera (domain V-VI) were performed by the same method with a minor modification. In brief, the sections were immunostained using the first antibody, washed with phosphate buffer saline, and incubated with the antiserum following the incubation with anti-rabbit Ig-horseradish peroxidase-linked F(ab)² fragment (Amersham).

**Immunelectron microscopy**

For immunelectron microscopy, the liver was perfused with PLP through the portal vein. After washing in 0.05 M cacodylate buffer, the tissues were sliced into 50 μm-thick sections using a vibratome (Lancer, St. Louis, MO), and then incubated with 2F8 and anti-MARCO antibodies as described in the section of Immunohistochemistry. After incubation, the sections were fixed with 1.5% glutaraldehyde, post-fixed with 1% OsO₄, dehydrated in a graded ethanol series, and embedded in Epok. Ultrathin sections were observed under an electron microscope (H-800: Hitachi, Tokyo) after staining with lead citrate.

**Detection of BCG in the spleen**

Mice were intravenously injected with 8 × 10⁵ CFU of BCG (Japan BCG Inc., Tokyo), and sacrificed at 1 h after injection. The spleens were fixed with PLP solution, and frozen sections were immunostained with MOMA-1, ER-TR9, or anti-MARCO antibodies. The sections were then stained by the method by Ziehl-Neelsen to localize the bacilli.

**RNA isolation and mRNA analysis by reverse transcriptase polymerase chain reaction (RT-PCR)**

Total cellular RNA was isolated by guanidinium thiocyanate-phenol-chloroform extraction from the liver and spleen of mice at various time intervals after LPS injection (YAMAMOTO et al., 1996). RNA was also isolated from cultured peritoneal macrophages. Two micrograms of total RNA were converted to cDNA by reverse transcription using a SuperScript Preamplification (Gibco BRL, Gaithersburg, MD) with the oligo (dt) primer. PCR amplification was performed by using a Program Temp Control System PC-700 (ASTEC, Tokyo). The reaction mixture consisted of 5 μl of sample cDNA, 5 μl of PCR amplification buffer, 2 μl of 25 mM MgCl₂, 4 μl of 2.5 mM dNTPs, 0.3 μl of Taq DNA polymerase (5 U/μl; Promega), 2 μl of 20 mM primer, and 29.7 μl of double-distilled water to bring the final volume to 50 μl. All the PCR primers were made to order by BEX.
(Tokyo, Japan). The oligonucleotides used are listed in Table 2. The mixture was first incubated at 95°C for 5 min, then cycled 30 times at 95°C for 1 min, 57°C for 2 min, 72°C for 2 min, and elongated at 72°C for 10 min. The samples were separated on a 2.0% SeaKem GTG agarose gel (TMC, Rockland, ME) containing 0.5 μ/ml of ethidium bromide, and bands were visualized and photographed using ultraviolet transillumination.

RESULTS

Histological changes after administration of LPS and bacteria

Following LPS injection of BALB/c and op/op mice as well as littermate mice, histological changes were similar. The infiltration of neutrophils and blood congestions were observed in several organs, being especially marked in the liver, spleen, and lungs. At 2 h after LPS injection, neutrophils started to infiltrate the liver; their number peaked at 8 h and returned to normal levels by day 3. Hepatocytes showed a cloudy swelling, and single cell necrosis and microabscess were scattered in the liver on day 1. In the spleen, neutrophils infiltrated the red pulp of the spleen after 4 h, but not so remarkably as in the liver. Expansion of the splenic red pulp occurred from day 1 to day 3. In the white pulp, numerous large macrophages containing a large number of cell fragments appeared on day 1 and disappeared on day 3. In the lung, neutrophils infiltrated the alveolar septa from 4 h, peaking at 8 h, but neutrophil infiltration and edema were not clearly observed in the alveolar spaces.

After i. v. injection of zymosan, BCG, and L. monocytogenes into BALB/c mice, granulomas were formed in the liver. Zymosan-, BCG- and Listeria-induced granulomas became largest on days 7, 21, and 3, respectively.

Table 2. The oligonucleotides used.

<table>
<thead>
<tr>
<th>Primers (5’-3’)</th>
<th>Products (bp)</th>
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<tr>
<td>CD14 Sense</td>
<td>ACG ATG TAA GGA AAG AAA GG</td>
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<tr>
<td>CD14 Antisense</td>
<td>CAA AAC AAA CAA AAC CAC AA</td>
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<tr>
<td>MSR-A Sense</td>
<td>CCA AGT CCT TGC AGA GTG TG</td>
</tr>
<tr>
<td>MSR-A Antisense</td>
<td>GTC TGA GGT GTG TGG TGA TG</td>
</tr>
<tr>
<td>MARCO Sense</td>
<td>GGG TCA AAA AGG CGA ATC T</td>
</tr>
<tr>
<td>MARCO Antisense</td>
<td>ATG TTC CCA GAG CCA CCT</td>
</tr>
<tr>
<td>C-fms Sense</td>
<td>GGA ATG ACA CCC ACC CTG AA</td>
</tr>
<tr>
<td>C-fms Antisense</td>
<td>CAG TAG CAC CAG CAG AGA CA</td>
</tr>
<tr>
<td>M-CSF Sense</td>
<td>ACT GTA GCC ACA TGA TTG G</td>
</tr>
<tr>
<td>M-CSF Antisense</td>
<td>GTC GTT GGT GCA GTT CCT G</td>
</tr>
<tr>
<td>IL-1β Sense</td>
<td>CTC TAG AGC ACC ATG CTA CAG AC</td>
</tr>
<tr>
<td>IL-1β Antisense</td>
<td>TGG AAT CCA GGG GAA ACA CTG</td>
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<tr>
<td>IFN-γ Sense</td>
<td>AGC GGC TGA CTG AAC TCA GAT TGT AG</td>
</tr>
<tr>
<td>IFN-γ Antisense</td>
<td>GTC ACA GTT TTC AGC TGT ATA GGG</td>
</tr>
<tr>
<td>MIP-1α Sense</td>
<td>GCC CTT GCT GTT CTT TCT CTC TG</td>
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<tr>
<td>MIP-1α Antisense</td>
<td>GGC AAT CAG TTC CAG GTC AGT</td>
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<td>MCP-1 Sense</td>
<td>CTC ACC TGC TGC TAC TCA TCT</td>
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<td>MCP-1 Antisense</td>
<td>GCA TGA GTG GTG TGT GAA AAA</td>
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<tr>
<td>iNOS Sense</td>
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</tr>
<tr>
<td>iNOS Antisense</td>
<td>GGC TGT CAG AGC CTC GTG GCT TTG G</td>
</tr>
<tr>
<td>TNF-α Sense</td>
<td>GGC AGG TCT ACT TGG GAG TCA TTG C</td>
</tr>
<tr>
<td>TNF-α Antisense</td>
<td>ACA TCC GAG GCT CCA GTG AAT TCG G</td>
</tr>
<tr>
<td>β-actin Sense</td>
<td>TGG AAT CCT GTG GCA TCC ATG AAA C</td>
</tr>
<tr>
<td>β-actin Antisense</td>
<td>TAA AAC GCA GCT CAG TAA CAG TCC G</td>
</tr>
</tbody>
</table>
Receptor expression in macrophages after LPS challenge and bacteria administration

In BALB/c mice, Kupffer cells were specifically stained with F4/80 and BM8 and distributed predominantly in the peripheral zone of the hepatic lobules. MSR-A was expressed both in Kupffer cells and sinusoidal endothelial cells in the liver, while MARCO and CD14 immunoreactivities were not observed in any cell types of the non-treated mouse liver (Fig. 1a). The number of Kupffer cells increased slightly after LPS injection. The number of CD14-positive round cells morphologically identified as neutrophils increased from 2 h after injection. MARCO-expressing Kupffer cells appeared on day 1, their number peaking on day 2 (Figs. 1b, 2). On double immunohistochemical staining, MARCO-expressing Kupffer cells expressed MSR-A (Fig. 1c), and more than half of the F4/80-positive Kupffer cells expressed MARCO on day 2 (Fig. 2).

F4/80- and BM8-positive macrophages were found in the red pulp of the spleen, while MOMA-1-positive marginal metallophilic macrophages and MARCO-expressing macrophages or ER-TR9-positive macro-
phages were present in the marginal zone of BALB/c mice and littermate (op/+) mice (Fig. 3a). Immunohistochemical double staining demonstrated that there were MARCO-positive, ER-TR9-positive, and MARCO/ER-TR9 doubly positive macrophages in the marginal zone (Fig. 3c). There were small numbers of MOMA-1-positive macrophages and MSR-A-positive macrophages in the red pulp. After LPS injection, the numbers of MSR-A-expressing cells increased in the splenic red pulp and marginal zone, with these MSR-A-expressing cells most numerous on day 2. MARCO-expressing cells appeared in the red pulp at 6 h following LPS injection, and their number peaked on day 2 (Fig. 3d). MOMA-1-positive marginal metallophilic macrophages increased, but the white pulp became irregular in outline. MOMA-1-positive tingible body macrophages were abundant in the germinal center of splenic white pulp on day 2 following LPS injection. MOMA-1-positive macrophages were also abundantly present in the splenic red pulp from 8 to 48 h after LPS injection, while CD14-positive macrophages were observed in the red pulp from 4 to 24 h after LPS administration.

In the lymph nodes of normal and LPS-stimulated mice, endothelial cells of the marginal sinus expressed both MSR-A and MARCO. Macrophages in the lymphatic sinuses also expressed MSR-A, but there were no MARCO-expressing macrophages. MARCO-expressing macrophages appeared in the lymphatic sinuses after LPS injection.

Macrophages in the heart, lungs, pancreas, kidneys, uterus, ovaries, testis, skin, and salivary glands expressed MSR-A but not MARCO in both normal and LPS-stimulated mice. After administration of BCG, L. monocytogenes, and zymosan, Kupffer cells in the liver expressed MARCO. These bacteria and fungus induced MARCO expression and enhanced MSR-A expression in macrophages in and outside the granulomas (Fig. 1d). Double immunohistochemical staining showed a simultaneous expression of both MSR-A and MARCO in almost all the macrophages. In the spleen, red pulp macrophages expressed MARCO by 1 or 2 days after the administration of these bacteria.

In op/op mice, MSR-A expression was observed in tissue macrophage populations, liver sinusoidal endothelial cells, and macrophages and endothelial cells in the lymphatic sinus. However, MSR-A-positive macrophages in op/op mice were far fewer than those in littermate mice as reported previously (Platt et al., 1996). MARCO expression was observed in marginal zone macrophages in op/op mice (Fig. 3b). There was no difference in the number of MARCO expressing marginal zone macrophages in op/op mice and op/+ mice. After LPS injection, Kupffer cells and splenic red pulp macrophages expressed MARCO. The number of MARCO-positive Kupffer cells in op/op mice was approximately one third of that in op/+ mice at days 2 and 3.

Ultrastructural expression of MSR-A and MARCO

Immunoreactivity against anti-MSR-A and anti-MARCO antibodies was localized on the cell surface membrane of macrophages in all mouse strains examined. The membrane of some endocytic vesicles was positive for MSR-A and MARCO (Fig. 4).

Expression of receptor and cytokine mRNAs

In the liver of BALB/c mice, MSR-A mRNA expression was enhanced after LPS treatment. C-fms mRNA was upregulated from day 1 to 5. Expressions of MARCO and CD14 were induced by LPS administration (Fig. 5a). TNF-α, IL-1β, and MIP-1 α mRNA expressions were detected at 30 min after LPS injection. M-CSF, MCP-1, iNOS, and IFN-γ mRNAs were enhanced markedly from 2-4 h after injection. The expression of TNF-α in the liver was less remarkable than that in the spleen (Fig. 5a). The expressions of MSR-A and MARCO mRNAs were augmented in BCG-, Listeria-, and zymosan-treated mice (Fig. 5b). In op/op mice, the levels of MSR-A, MARCO, and
CD14 expressions in the liver after LPS injection were lower than those of op/+ mice (Fig. 5c).

Uptake of BCG by splenic macrophages

At 1 h following BCG injection, bacilli were taken up by the splenic macrophages. BCG-containing macrophages were abundant in the marginal zone, whereas macrophages in the red pulp contained small amounts of bacilli. Combined immunohistochemical staining using MOMA-1, ER-TR9 or anti-MARCO antibody and Ziehl-Neelsen staining showed that MARCO- and ER-TR9-expressing marginal zone macrophages in the littermate mice possessed larger amounts of BCG than MOMA-1-positive marginal metallophilic macrophages (Fig. 6). In op/op mice lacking MOMA-1- or ER-TR9-positive macrophages, MARCO-positive macrophages in the marginal zone contained a large amount of BCG (Fig. 6).
DISCUSSION

The present study demonstrated the constitutive expression of MSR-A in Kupffer cells and other tissue macrophages, whereas MARCO expression was restricted to certain splenic macrophage populations and to macrophages and endothelial cells in the lymph nodes. The marginal zone macrophages included MARCO-positive, ER-TR9-positive, and doubly positive populations in BALB/c mice as well as /op+ littermate control mice. However, /op/op mice possessed only MARCO-positive marginal zone macrophages. LPS administration induced the expression of MARCO in macrophages of various tissues, especially in Kupffer cells and splenic red pulp macrophages. The administration of BCG, L. monocytogenes, or zymosan also induced MARCO expression. Intravenous injection of BCG resulted in a heavy accumulation of the bacilli in marginal zone macrophages and marginal metallophilic macro-

Fig. 4. Immunelectron micrograph of a MARCO-expressing Kupffer cell. Reaction products for MARCO are localized on the cell membrane of Kupffer cells at 2 days after LPS administration. No counterstaining. × 6,000.

Fig. 5. a. The expressions of receptor and cytokine mRNAs in the liver and spleen of LPS-treated mice. b. The expressions of MSR-A and MARCO mRNAs in the liver of BCG-, Listeria-, and zymosan-treated mice. c. The expressions of CD14, MSR-A, and MARCO mRNAs in the liver of LPS-treated /op/op and /op+/+ mice. RT-PCR.
Fig. 6  a, c and e. The uptake of BCG in the spleen of littermate (op/+) mice at 1 h after the intravenous injection of BCG. ×200. b, d and f. The uptake of BCG in the spleen of op/op mice at 1 h after intravenous injection of BCG. ×200. a. Macrophages in the marginal zone incorporate a large amount of BCG (red). MOMA-1-positive marginal metallophilic macrophages (brown) also take up a moderate amount of BCG. b. Macrophages in the marginal zone incorporate a large amount of BCG (red). MOMA-1-positive marginal metallophilic macrophages are absent. a and b: Combined immunostaining using MOMA-1 and Ziehl-Neelsen staining. c. ER-TR9-positive macrophages in the marginal zone incorporate a large amount of BCG. d. There are no ER-TR9-positive macrophages in the marginal zone. However, macrophages in the marginal zone incorporate a large amount of BCG. e and d: Combined immunostaining using MOMA-1 and Ziehl-Neelsen staining. e. MARCO-positive marginal zone macrophages phagocytize a large amount of BCG (red). f. MARCO-positive marginal zone macrophages are present in op/op mice and phagocytized a large amount of BCG (red). Inset: ×800. e and f: Combined immunostaining using anti-MARCO antibody and Ziehl-Neelsen staining.
phages in op/+ mice, whereas bacilli were abundant in MARCO-positive marginal zone macrophages in op/op mice. These results indicate that MARCO is inducible by bacterial antigens and plays an important role in the recognition and uptake of bacteria.

Scavenger receptors have been shown to be involved in host defense mechanisms against various bacteria. MSR-A recognizes and internalizes lipid A in LPS (Hampton et al., 1991; Ashkenas et al., 1993) and Gram-positive bacteria (Dunne et al., 1994). MSR-A knockout mice showed an increased susceptibility to infection with L. monocytogenes, BCG, and herpes simplex virus (Suzuki et al., 1997; Haorth et al., 1997), indicating important roles for MSR-A in host defense against pathogens. MARCO also plays a role in host-defense mechanisms against bacteria (Elomaa et al., 1995; Van Der Laan et al., 1997). MARCO was constitutively expressed in marginal zone macrophages in spleen and endothelial cells in the lymphatic sinuses of lymph nodes situated in a strategically advantageous position to trap blood- or lymph-borne microorganisms and other pathogens (Humphrey, 1981; Dijkstra et al., 1985; Groeneveld et al., 1986; Kraal et al., 1986, 1988, 1989). In the spleen, there are several specific macrophage subpopulations, such as marginal zone macrophages and marginal metallicophilic macrophages around the white pulp, macrophages in the red pulp, and tingible body macrophages in the white pulp. Marginal zone macrophages are localized in the outer side of the white pulp and possess a higher phagocytic activity than marginal metallicophilic macrophages which are located in the inner side of the white pulp (Humphrey, 1981; Kraal et al., 1986, 1988, 1989; Dijkstra et al., 1985; Groeneveld et al., 1986; Wijffels et al., 1994; Van Rooijen and Kors, 1989; Van Rooijen et al., 1989; Han et al., 1994). Both macrophage populations are thought to play pivotal roles in certain immune responses. The antigen recognized by the MOMA-1 antibody is expressed by the marginal metallicophilic macrophages and capsular and medullar sinus macrophages in peripheral lymph nodes (Wijffels et al., 1994; Van Rooijen and Kors, 1989; Van Rooijen et al., 1989). These populations are characterized by a high level of nonspecific esterase activity, suggesting a degrading and detoxifying function because of their location. Injection of the MOMA-1 monoclonal antibody into mice led to a significant reduction in responses against thymus-dependent and thymus-independent type 2 antigens. ER-TR9 antigen expression is characteristic of splenic marginal zone macrophages and macrophages in the medullary and trabecular sinuses of lymph nodes. Highly phagocytic populations, they retain and ingest neutral polysaccharides, such as Ficoll and dextran (Humphrey, 1981; Kraal et al., 1989). In the present study, we confirmed that ER-TR9- and MARCO-positive marginal zone macrophages as well as MOMA-1-positive marginal metallicophilic macrophages were involved in the selective uptake of BCG, and both marginal zone macrophage populations showed a higher phagocytic activity than the latter. Furthermore, in op/op mice which lack MOMA-1-positive marginal metallicophilic macrophages and ER-TR9-positive marginal zone macrophages, MARCO-expressing marginal zone macrophages exhibited the active binding and removal of BCG from blood. These findings and the previously reported data on the binding of MARCO cDNA-transfected COS cells and bacteria (Elomaa et al., 1995; Van Der Laan et al., 1997) support the notion that MARCO plays an important role in the binding and incorporation of bacterial pathogens.

The observation that marginal zone macrophages in op/op mice express MARCO further indicates that marginal zone macrophages comprise a heterogeneous population. Immunohistochemical double staining demonstrated that marginal zone macrophages include ER-TR9-positive, MARCO-positive, and double-positive cells. It has been shown in vitro that the antigen recognized by MOMA-1 antibody is differentially induced in bone marrow-derived macrophages depending on the colony-stimulating factor (i.e., IL-3, M-CSF, and GM-CSF) used. However, a limited expression of ER-TR9 antigen is induced in cultured macrophages by IL-3, but not GM-CSF or M-CSF (Wijffels et al., 1993). These in vitro results are consistent with the finding that MOMA-1-positive macrophages are absent in op/op mice. The mechanism of failure of ER-TR9-positive macrophage differentiation in the mutant mice is unknown, however. It has been demonstrated that a daily injection of M-CSF results in the repopulation of MOMA-1-positive marginal zone metallicophilic macrophages in op/op mice, but the repopulation of ER-TR9-positive marginal zone macrophages in these mice was incomplete (Takahashi et al., 1994). These in vivo and in vitro results suggest that MOMA-1-positive marginal metallicophilic macrophages are an M-CSF-dependent macrophage population, and ER-TR9-positive marginal zone macrophages are partially M-CSF-dependent cell population. MARCO-positive marginal zone macrophages are apparently an M-CSF-independent population similar to dendritic cells which are present in normal numbers in op/op mice (Takahashi et al., 1993). The differentiation mechanism of MARCO-positive macrophages and their relationship to ER-TR9-positive macrophages deserves investigation in
further studies.

The present study demonstrated that MARCO is not only expressed constitutively in specific macrophage subpopulations, but is also induced in Kupffer cells and red pulp macrophages in the spleen following the administration of LPS, bacteria, and fungi. The biological significance of LPS-induced MARCO expression in macrophages is unknown. It might be postulated that marginal zone macrophages function as a major filter for a physiological concentration of blood-borne LPS and that splenic red pulp macrophages and Kupffer cells participate in scavenging excess LPS. The spleen and liver form an effective biodefense system against gut-derived endotoxins. We observed differences in the expression of TNF-α between the spleen and liver after LPS stimulation, indicating that Kupffer cells and splenic macrophages have different sensitivities to LPS. Since MSR-A is expressed both in the liver and spleen (KODAMA et al., 1988; NAITO et al., 1991) the constitutive expression of MARCO in the spleen may be one of the mechanisms for the difference in reactivity against LPS between the spleen and liver.

In conclusion, MSR-A is constitutively expressed on macrophages distributed ubiquitously in many tissues, whereas MARCO is constitutively expressed in certain specific macrophage populations and is inducible in other macrophages by microbial stimuli. MARCO expressed in marginal zone macrophages may be an important molecule responsible for the specific binding and incorporation of microbes and contributors to the heterogeneity of splenic marginal zone macrophages.

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