The primary structure of human macrophage receptor with collagenous structure (MARCO) was determined from cDNA clones and shown to be highly similar to that of mouse (Elomaa, O., Kangas, M., Sahlberg, C., Tuukkanen, J., Sormunen, R., Liakka, A., Thesleff, I., Kraal, G., and Tryggvason, K. (1995) Cell 80, 603–609). Features such as potential carbohydrate attachment sites in the extracellular spacer domain III and the interruption of Gly-Xaa-Yaa repeats in the collagenous domain IV were conserved between the two species. However, the human MARCO polypeptide chain lacked the intracellular cysteine present in mouse, as well as two extracellular cysteines that form interchain disulfide bonds in the murine protein. In situ hybridization showed MARCO to be strongly expressed in macrophages of several tissues of human individuals with sepsis. No expression was observed in other cell types. The bacteria-binding region of MARCO was determined in binding studies with full-length and truncated variants of MARCO, and localized to a domain proximal to the cysteine-rich part of the COOH-terminal domain V. The intrachain disulfide bond pattern of domain V was established showing that these bonds are between cysteine pairs C1-C5, C2-C6, and C3-C4.

Macrophages are bone marrow-derived cells that play a central role in host defense as well as in normal physiological processes such as the maintenance of tissues. Macrophages are present in most tissues, particularly in tissues that function in the filtration of blood or lymph fluid, such as liver, spleen, lung, and lymph nodes. A major characteristic of macrophages is their ability to recognize, internalize, and destroy harmful endogenous and foreign substances, thus functioning as scavengers. Macrophages have been shown to be able to bind pathogens either directly, or they recognize them as foreign after their coating by antibodies or complement (1, 2).

Macrophages contain scavenger receptors with a broad binding specificity that may be used to discriminate between self and non-self in the nonspecific antibody-independent recognition of foreign substances (2–4). The type I and II class A scavenger receptors (SR-AI and SR-AII) are trimeric membrane glycoproteins with a small NH2-terminal intracellular domain, and an extracellular portion containing a short spacer domain, an α-helical coiled-coil domain, and a triple-helical collagenous domain. The type I receptor additionally contains a cysteine-rich COOH-terminal (SRCR) domain (5, 6). These receptors are present in macrophages in diverse tissues throughout the body and exhibit an unusually broad ligand binding specificity. They bind a wide variety of polyanions, including chemically modified proteins, such as modified LDL, and they have been implicated in cholesterol deposition during atherosclerosis. They may also play a role in cell adhesion processes in macrophage-associated host defense and inflammatory conditions (3, 4).

We have recently characterized a novel murine macrophage receptor, MARCO, which has an approximately 80-nm-long extracellular collagenous domain and a COOH-terminal cysteine-rich domain (7). Although the overall structure resembles that of the type I scavenger receptor, MARCO differs in that the extracellular domain is longer and completely lacks an α-helical coiled coil domain. Furthermore, MARCO is only expressed in macrophages of the marginal zone of the spleen and in lymph nodes and thus differs substantially from the scavenger receptor, which is expressed in macrophages of most tissues. In our previous study MARCO was shown to bind Gram-positive and negative bacteria, and acetylated LDL, but not yeast. Based on its binding activity and tissue distribution, MARCO is likely to play a role in host defense.

The cysteine-rich motif (SRCR) present in SR-AI and MARCO has also been found in a number of other proteins. This motif is about 100 amino acid residues long and contains six or eight cysteine residues that were recently shown to form intrachain disulfide bonds in the globular SRCR domain of SR-AI (8). The biological function of SRCR domains has not yet been established, but their presence in proteins with binding functions suggests a role in ligand binding or adhesion. Recently, one of the SRCR domains in CD6 was reported to participate in ligand binding interactions (9).

In the present study, we have determined the primary structure of the human MARCO receptor and studied its spatial expression using in situ hybridization. The nature of the intrachain disulfide bonds of the COOH-terminal cysteine-rich domain V was determined, and using full-length and truncated variants of MARCO, the bacteria-binding region was localized immediately proximal to the cysteine-rich region of domain V.
Hybridization was carried out for 16 h at 52 °C, and high stringency
ter which cells were washed with PBS containing 0.1M glycine and
with sulfo-

EXPERIMENTAL PROCEDURES

Isolation and Characterization of cDNA Clones—Human liver and spleen cDNA libraries cloned in agt11 (CLONTECH) were screened with a 32P-labeled murine MARCO cDNA probe or by a PCR amplifi-
cation method using standard protocols (10, 11). The clones were iso-
lated, subcloned into pUC19 vector, and sequenced from both strands. The human MARCO clones obtained from the cDNA libraries did not extend to the region encoding the translation initiation site when com-
pared with the murine MARCO cDNA. To obtain the missing 5’ end sequence, human genomic libraries (CLONTECH) were screened and positive clones were subcloned into pUC19 vector for sequencing.

In Situ Hybridization—Human tissues obtained from a newborn child who died from meningitis were snap-frozen in liquid nitrogen at autopsies. Sections of 7 μm were placed on silane-coated slides, allowed to dry, and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and dehydrated in graded ethanol series. In situ hybridization was performed according to Wilkinson and Green (12) with some modifications. The sections were treated with 1 μg/ml proteinase K (Sigma) in 10 mM Tris-HCl, 2 mM CaCl2 at room temperature for 10 min. The samples were post-fixed by 4% paraformaldehyde in PBS and acetylated with acetic anhydride in 0.1 M triethanolamine (pH 8.0). The sections were washed with 50% deionized formamide and SSC both before and after acetylation. The slides were picked from the last washing solution one by one. Hybridization buffer (60% deionized formamide, 300 mM NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM EDTA (pH 8.0), 10% dextran sulfate, and 1 × Denhardt’s solution) containing 500 ng of yeast tRNA/ml without probe was then added and prehybridized for 2 h at 52 °C.

For the preparation of RNA probes, restriction fragments EcoRI-AccI (nucleotides 27–401) and KpnI-EcoRI (nucleotides 1327–1720) from human MARCO cDNA were cloned into pGEM-3Z plasmid vector (Promega). Probes were labeled with 32P-UTP using T3 and T7 polymerases. The probes were diluted to a final concentration of 40,000 cpm/ml. Hybridization was carried out for 16 h at 52 °C, and high stringency washes were carried out with 50% deionized formamide and 30 mM dithiothreitol at 42 °C for 60 min. The sections were treated with RNase (Boehringer Mannheim) for 30 min at 37 °C, washed, dehy-

Preparation of Antibodies—For the production of polyclonal antibod-
ies in rabbits, domains III (residues 71–147) and V (residues 421–520) of the human MARCO polypeptide were expressed in Escherichia coli as glutathione S-transferase fusion proteins using the pGEX-1AT vector (Pharmacia Biotech Inc.). DNA fragments encoding domains III and V were generated by PCR, and all sequences were confirmed by DNA sequencing. The fusion proteins produced in bacteria were purified using glutathione-Sepharose 4B (Pharmacia). Antiserum were raised in rabbits using standard procedures, and IgGs were first purified by protein A-agarose affinity (Pharmacia) and then by negative immunoadsorp-
tion of unspecific antibodies against glutathione S-transferase and E. coli proteins.

Plasmid Constructs and Cell Transfections—For cell transfections, cDNAs encoding the entire 520-residue sequence of MARCO (M-520) or truncated constructs encoding residues 1–420 (M-420), residues 1–431 (M-431), or residues 1–442 (M-442) were generated by site-directed insertion of a stop codon using PCR. The sequences of the PCR products were confirmed by DNA sequencing, and the products were cloned into the pcDNA3 expression vector (Invitrogen) using restriction sites en-
gineered into the primers. COS-7 cells were grown in high glucose (4.5 g/liter) Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml ampicillin. One day prior to transfection, the cells were plated at 0.5 × 105 cells/90-mm tissue culture dish and then transfected overnight by the calcium phosphate method using 20 μg of DNA isolated by CsCl gradient centrifugation. After 18 h the calcium phosphate precipitate was removed, and the cells were used after 24–48 h.

Analysis of Surface-bound MARCO—To examine whether the trans-
fected cells are able to transport the MARCO receptor to the cell surface, biotinylation of surface proteins was carried out, followed by their precipitation with streptavidin-agarose. For biotinylation, transfected COS-7 cells were grown in 90-mm Petri dishes, rinsed with ice-cold PBS (pH 8.3), containing 1 mM Ca2+ and 1 mM Mg2+, and cooled in this buffer on ice. Cell surface proteins were labeled for 30 min on ice with sulfo-N-hydroxysuccinimide-biotin (0.5 mg/ml in PBS; Pierce), after which cells were washed with PBS containing 0.1 M glycine and incubated in same buffer for 15 min on ice. For streptavidin precipita-
tion, cells were lysed into a triple detergent buffer (50 mM Tris-HCl (pH 8), 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxy-
cholate, 100 μg/ml phenylmethylsulfonyl fluoride, 0.02% sodium azide, 1 μg/ml aprotonin) followed by centrifugation to remove insoluble ma-
terial. The cell lystate was incubated with streptavidin-agarose (Sigma) overnight at 4 °C, after which the streptavidin-agarose was pelleted by centrifugation and washed five times with the triple detergent buffer. Precipitated proteins were eluted by heating the streptavidin-agarose in SDS sample buffer at 95 °C for 10 min.

Cell lyses and avidin-precipitated proteins were analyzed by SDS-
PAGE followed by immunoblotting using 5 μg/ml IgG against the mouse III of MARCO and peroxidase-conjugated rabbit anti-rabbit IgG (DAKO) as a secondary antibody. The immunoreaction was visualized using the chemiluminescence method (Renaissance; DuPont). Mouse IgG against paxillin (Transduction Laboratories), which is a cytoskeletal protein, was used as a negative control for cell surface biotinylation.

Bacteria and Ligand Binding Studies—Transfected cells cultured on coverslips were incubated with FITC-labeled E. coli or Staphylococcus aureus (Molecular Probes) for 1 h in 50:1–100:1 ratios. Following incu-
bation cells were washed three times with PBS buffer, fixed in 4% paraformaldehyde solution, and stained using anti-MARCO IgG and FITC-labeled secondary antibody and rhodamine-conjugated phalloidin (Molecular Probes). The fluorescence staining was localized with a fluorescence microscope.

Production of Recombinant Domain V Using Baculovirus—A cDNA fragment encoding domain V (starting from serine residue 421) was cloned into a baculoviral transfer vector pVL1393 (13) containing the coding sequence for the amino-terminal half of human matrix metallo-
protease MMP-2 (residues 1–448). The resulting transfer vector, termed pVL1393–72FX, contained the coding sequence for the signal peptide, the three fibronectin type II-like repeats and the catalytic site of MMP-2 (14), followed by an artificial factor X cleavage site (Ile-Glu-

Determination of Disulfide Bond Pattern in Domain V—The recom-
binant protein was purified by gelatin-affinity chromatography and ion exchange HPLC as described earlier (15). The purified fusion protein, in 20 mM Tris (pH 8.0), 1 mM CaCl2, 0.1 mM NaCl, was concentrated to 1.5–2 mg/ml and digested with factor Xa at room temperature for two hours using an enzyme/substrate mass ratio of 1:50. To exclude rearrange-
ment of disulfide bonds during the manipulation, control experiments with 5 μm iodosacamide added prior to the factor Xa digestion was carried out. Digestion was terminated by adding 1.5 volumes of 0.1% trifiuoroacetic acid, and 200-μl aliquots were purified by HPLC on a Delta Pak RP-4 300-A 3.5 × 150-mm column, using a CH3CN gradient from 30% to 50% in 0.1% triffuoacetic acid over 20 min (flow 0.8 ml/min). Domain V of MARCO was identified by mass spectrophotom-
etry MALDI-TOF and NH2-terminal sequencing, dried in a Speed Vac, and dissolved in 8 μl urea, 0.4 mM NH3HCO3 (pH 7.8), to a concentration of 0.5–1 mg/ml. Three volumes of 0.1% trifiuoroacetic acid and 200-μl aliquots were digested with trypsin (ratio 1:20–1:50) at 37 °C overnight. Reduced (10% v/v mercaptoethanol) and unreduced aliquots were analyzed by reverse phase HPLC, using the same eluents as above, but the gradient was from 25% to 55% CH3CN in 60 min (flow 6.0 ml/min). Peaks likely to contain disulfides were analyzed by MALDI-TOF and NH2-terminal sequencing. Tryptic peptides containing disulfide bonds were evac-
urated and dissolved in 25 mM NH3HCO3 (pH 7.8), at 0.1 mg/ml and digested with V8 protease (enzyme substrate ratio 1:20) for several days at room temperature. Alternatively, 50 mM sodium phosphate (pH 7.8) or 130 mM NH4Ac (pH 4) was used as a buffer. Digestion products were purified by reverse phase HPLC with a CH3CN gradient from 10% to 35% in 50 min (flow 0.6 ml/min), and characterized by MALDI-TOF and NH2-terminal sequencing.

RESULTS

Primary Structure of Human MARCO and Similarity with the Murine Protein—Analysis of human MARCO cDNA clones revealed a 1533-base pair open reading frame and a 152-base pair 3’-end untranslatable sequence, starting with the TGA translation stop codon and containing a putative ATAAA polyadenylation signal. On the basis of Northern analyses and comparison with the murine sequence, the human MARCO mRNA has a size of about 1.8 kilo-base pairs, indicating that the cDNA clones lacked the 5’-most end sequence. To obtain the
missing 5' sequence, 5'-rapid amplification of cDNA ends was carried out, but this did not yield further sequence. The missing sequence encoding residues 1–11 (Fig. 1) was therefore obtained by analysis of the coding sequence in a genomic clone. Evidence for residue 1 in Fig. 1 being the actual initiator methionine came from the fact that there is an in-frame stop codon three codons upstream of the codon for this methionine residue (data not shown). Together, the results showed that the open reading frame encodes a polypeptide chain of 520 amino acid residues (Fig. 1). The identity of the amino acid sequence between the full-length human and murine polypeptide chains was only 68%, the similarity being 77%. The sequence identity of the chains between the two species is highest (74%) in the carboxyl-terminal cysteine-rich domain V and, as expected, in the collagenous domain IV (75%), which has glycine as every third amino acid (Table I). Between the intracellular domains I, the sequence similarity is surprisingly low (only 51%). Interestingly, the human MARCO subunit chain differs from the mouse chain in that it does not have any cysteine residues outside the cysteine-rich domain V, whereas the mouse chain has one cysteine residue within the intracellular domain I and two in the extracellular spacer domain III. Both potential carbohydrate attachment sites in the spacer domain III, as well as the interruption of Gly-Xaa-Yaa repeats in collagenous domain IV, are conserved between the two species. The transmembrane domain II has 69% sequence identity between mouse and man.

In Situ Hybridization Analysis—In situ hybridization, carried out on several human tissues obtained from autopsies of two newborn infants who died from sepsis, revealed expression in macrophages in several tissues. In thymus, the expression

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Fig. 2. In situ hybridization analysis of MARCO expression in a newborn child who died from septic meningitis. Distinct signals could be observed with the antisense probe in individual tissue macrophages (histiocytes) and their derivates. Light (left) and dark (right) fields are shown. A and B, strong signals are observed in the stromal compartment of thymus. C and D, positive cells are primarily seen beneath the mucosal membrane of the intestine. E and F, scattered positive cells can be observed in the spleen, but the morphology is not good due to poor quality of the sample. G and H, positive cells are apparent in the interstitium of kidney. I and J, intensively positive cells identified as Kupffer cells can be seen in liver.
Characterization of MARCO Variants Expressed by Transfected COS Cells—To determine the bacteria-binding region of MARCO, COS cells were transiently transfected with the full-length or truncated forms of MARCO cDNAs for binding studies. The truncated variants either completely lacked domain V (M-420) or contained up to 11 (M-431) or 22 residues (M-442) out of the 99-residue domain V. All the variants lacked that portion of domain V which contains the six cysteine residues. To verify production of the different MARCO variants, proteins produced by the various transfectants were studied by Western blotting (Fig. 3). The anti-MARCO antibodies reacted weakly with two sharp bands of about 60 and 43 kDa in reduced samples from mock-transfected cells (Fig. 3, lane 1). In the lysate prepared from cells transfected with the full-length construct, the antibodies specifically revealed a major diffuse band of about 60 kDa and a weaker, but a sharp doublet band of about 50 kDa. Under nonreducing conditions, the bands had similar sizes, of about 60 kDa and a doublet of weaker bands of about 50 kDa (Fig. 3, lane 2). Slightly smaller bands of about 45 kDa were observed in cell lysates of COS cells transfected with the truncated cDNAs M-431, M-442, and M-420 (Fig. 3, lanes 3–5). Under nonreducing conditions, the bands had similar sizes, indicating the absence of interchain disulfide bonds (data not shown).

Cell surface biotinylation was used to examine whether the truncated forms of MARCO were transported to the cell surface. In this experiment, COS cells transfected with the full-length and the shortest MARCO construct (M-420) lacking the entire domain V were biotinylated on ice, followed by precipitation of biotinylated proteins with streptavidin and Western blotting, essentially as described under “Experimental Procedures.” The results unequivocally showed that the 420-residue truncated form was present on the cell membrane (Fig. 4A, lane 3), similarly to full-length MARCO (Fig. 4A, lane 2). As a control, a parallel experiment with mock-transfected cells was carried out. In this case, streptavidin-agarose was not found to precipitate any biotinylated polypeptides that could be detected by the anti-MARCO antibodies (Fig. 4A, lane 1). As an additional control, we examined whether an intracellular protein, paxillin, is present in the protein fraction precipitated by streptavidin-agarose. However, paxillin was not detected in this fraction (Fig. 4B, lanes 1–3), even though it was easily detectable in the total cell lysate prepared from COS cells (Fig. 4B, lane 4).

Bacteria Bind to Proximal Region of Domain V—In our previous study, mouse MARCO was shown to bind both Gram-positive and Gram-negative bacteria, but not yeast (7). To study whether the human MARCO has binding properties similar to those of mouse MARCO, COS cells were transfected with full-length human MARCO cDNA, followed by incubation with FITC-labeled *E. coli*, *S. aureus*, or zymosan A (*Saccharomyces cerevisiae*), after which the binding was detected by fluorescence microscopy. Specific binding of bacteria was observed to COS cells expressing full-length human MARCO (Fig. 5A). In contrast, labeled zymosan A did not bind to MARCO expressing cells, as was shown previously for murine MARCO (data not shown).

To more specifically characterize the bacteria-binding region of MARCO, we carried out transfections with the truncated variants (Fig. 5F). The transfected cells were analyzed for their binding of FITC-conjugated bacteria. The results showed that cells expressing MARCO variants M-420 and M-431 did not bind bacteria (Fig. 5, C and D). However, cells expressing MARCO construct M-442 were found to bind bacteria, indicating that the cysteine-rich part of domain V is not needed for the bacteria binding activity of MARCO.

Determination of the Disulfide Bond Pattern of Domain V—The carboxyl-terminal domain V of MARCO contains six cysteines that probably form intrachain, but not interchain, disulfide bonds. Here, the nature of the intrachain disulfide bonds was studied in human domain V expressed as a recombinant protein in eukaryotic H5 cells using the baculovirus system. Domain V was expressed as a fusion protein with MMP-2 matrix metalloproteinase, where it replaced the COOH-terminal hemopexin domain of the enzyme. The expression construct was designed so that the MMP-2 fusion partner could be cleaved away with factor Xa. The final yield of purified protein was only 0.1 mg of protein/liter of cell culture medium, which is considerably lower than our normal yields of wild-type or point mutated MMP-2 (15). The fusion protein was first purified with a single-step affinity column containing gelatin immobilized on Sepharose. This purification method is based on the high gelatin binding activity of the fibronectin type II-like repeats of MMP-2 (15). MARCO domain V was then released from the MMP-2 fusion partner by cleavage with factor Xa, and the cleavage products were separated by HPLC, essentially as described under “Experimental Procedures.” Fractionation and amino acid sequence analysis of the cleavage products revealed that domain V itself contained two internal cleavage sites for the factor Xa, as illustrated in Fig. 6A.

The factor Xa fragment Xa2,3, which lacked 14 NH₂-terminal residues of MARCO domain V and was additionally cleaved after arginine 469 (Fig. 6A), was digested with trypsin. This yielded two trypsin fragments T1 and T2, which both contained
internal disulfide bond(s) (Fig. 6B). Amino-terminal sequencing and the molecular weight of T2 directly proved the presence of bonds between cysteine residues C4 and C3. Amino-terminal sequencing and mass spectrometric analyses of T1 showed that cysteine residues C1, C2, C5, and C6 were present in this fragment. In Fig. 6B, we refer to this fragment also as fragment ψT1, since 5 residues were sometimes cleaved away from the NH₂ terminus due to chymotrypsin contamination in the trypsin preparation used. Finally, cleavage of T1 with V8 proteinase generated three cleavage products for which sequence analyses and molecular weight calculations allowed the unambiguous assignment of cysteine bonds, as illustrated in Fig. 6C.

**DISCUSSION**

The present study provided the primary structure of the subunit chain of the trimeric human macrophage MARCO receptor. Comparison with the mouse polypeptide revealed a sequence identity of 68% and similarity of 77%. An interesting difference between the two species is that the human chain lacks cysteine residues outside domain V, while the mouse chain has one in the intracellular domain I and two in the spacer domain III. The role of the single cysteine in the intracellular domain in mouse is not known, but we have shown previously that the two cysteine residues in the spacer domain participate in interchain disulfide bonds in the MARCO receptor (7). Therefore, the three subunit chains of human MARCO are bound to each other only through the collagenous triple helix of domains IV, as the cysteine residues in the COOH-terminal domain V all form intrachain bonds. In the structurally related scavenger receptor SR-AI chain, a single cysteine residue in the spacer domain and another one in domain I are conserved in rabbit, mouse, bovine, and man (3, 16). However, the mouse class A scavenger receptor does contain a second cysteine in domain I, so the cysteine pattern is not completely conserved in this type of receptors. The present results further showed that two putative carbohydrate attachment sites in the

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**FIG. 4. Analyses of proteins expressed in MARCO-transfected COS cells.** A, streptavidin-precipitated biotinylated cell surface proteins. Mock-transfected COS cells (lane 1) or COS cells transfected with the full-length MARCO cDNA (lane 2) or cDNA M-420 lacking the entire COOH-terminal domain V of MARCO (lane 3) were biotinylated for cell surface proteins on ice followed by the precipitation with streptavidin-agarose. The precipitated proteins, separated by 8% SDS-PAGE, revealed diffuse bands of about 60 and 50 kDa when analyzed by immunoblotting with anti-MARCO antibodies. B, the filter used in A was probed with an anti-paxillin monoclonal antibody. Lanes 1–3 show that paxillin was not precipitated by streptavidin-agarose, even though it is easily recognizable in the total lysate of COS cells (lane 4). This result demonstrates that intracellular proteins were not biotinylated during the course of the experiment.

**FIG. 5. Determination of the bacteria-binding region in MARCO using COS cells transiently transfected with full-length and truncated variants of MARCO and FITC-labeled E. coli.** A, a MARCO positive COS cell expressing full-length MARCO binds FITC-labeled E. coli bacteria, while an adjacent cell not expressing MARCO fails to bind. B, cells expressing the M-442 variant also bind bacteria. Cells expressing the truncated constructs M-431 (C) and M-420 (D) fail to bind bacteria. E, mock-transfected cells did not show bacteria binding. F, scheme of the domain structure of constructs used in the study.
spac**er domain** III of MARCO, as well as an interruption in the collagenous Gly-Xaa-Yaa repeat sequence, are conserved, indicating a functional importance for these regions.

*In situ* hybridization analyses of tissues from two newborn infants revealed general expression of MARCO in macrophages of several organs such as thymus, intestine, and kidney and in Kupffer cells of the liver. In the spleen, expression was mainly seen in the white pulp region. The general macrophage expression observed here sharply contrasts what we previously found in the mouse, where expression was normally only present in macrophages of the marginal zone of the spleen and lymph nodes (7). However, the results are not completely comparable, as it turned out that the human autopsy samples analyzed in the present study were obtained from infants who both died from sepsis. Therefore, it is possible that the expression of MARCO is induced in macrophages outside spleen and lymph nodes as a result of the bacterial infection. In fact, this appears to be the case, as we have recently shown that expression of MARCO is up-regulated in lung and liver macrophages in mice developing endotox shock following infection with bacteria, such as *Klebsiella pneumoniae* (17). Together, these observations strongly associate the expression of MARCO with the response of the organism against bacterial infection. This, together with our data showing that cultured cells transfected with MARCO bind bacteria (Ref. 7 and this study), suggests that MARCO is an important component of the organism’s innate immunity.

The biological roles and functional relationship of MARCO and class A scavenger receptors SR-Ai and SR-AII are interesting aspects. MARCO has certain structural similarities with the class A scavenger receptors SR-AI and SR-AII, which are normally generated through alternative splicing of primary transcript (21), differ from each other in that SR-AII lacks the cysteine-rich domain SRAI. SR-AI and SR-AII have been associated with the binding of oxidized LDL and the process of atherogenesis (3, 4). Although MARCO has also been shown to bind acetylated LDL (7), it is not expressed in macrophages of atherosclerotic plaques or at least the expression level is extremely low; therefore, MARCO is not likely to be involved in the atherogenesis process. Similarly to MARCO, scavenger receptors SR-AI and SR-AII may also be involved in the removal of bacterial pathogens. Thus, SR-AI has been shown to bind Gram-positive bacteria (22) and lipid A as a surface component of Gram-negative bacteria (23). Furthermore, Suzuki et al. (24) recently reported that mice homozygous for a disrupted SR-A gene are more susceptible to bacterial and viral infections than wild-type mice. Therefore, it appears that both MARCO and SR-AI/SR-AII have roles in the host defense through their binding and removal of pathogens. However, the two classes of structurally related receptors also seem to have differences, as SR-AI and SR-AII exhibit constitutive macrophage expression, while the expression of MARCO is more regulated.

The present study clearly demonstrated that the cysteine-rich part of domain V is not needed for the binding of bacteria. Thus, we found that a truncated MARCO receptor containing only 22 residues of this domain bound bacteria. However, truncated variants either completely lacking domain V or containing 11 residues of it were not able to bind bacteria. Consequently, we may have identified the region of MARCO that is directly involved in the bacteria binding. If this amino acid sequence, BGrAEVYYSGT (residues 432–442), is indeed the interactive region, the two arginines might play a role in the binding activity, as it can be inhibited by poly(G), a known polyanionic inhibitor of scavenger receptors. It is also possible that the binding region extends to the NH2-terminal side of this sequence, even into the collagenous domain, but that the 22

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**Fig. 6.** **Identification of disulfide bonds in proteolytic fragments of domain V of human MARCO.** Amino acid sequences of the fragments were determined by amino-terminal sequencing of HPLC-purified proteolytic fragments, and the molecular weights (mw) were calculated based on amino acid composition and by mass spectrophotometry ([M + H]+). A, illustration of the fusion protein, which consists of a truncated matrix metalloproteinase 2 (MMP-2) connected to domain V of the human MARCO polypeptide chain. The amino acid sequence of MARCO domain V is shown with the single-letter code with the six cysteine residues in boxes. The locations of factor Xa cleavage sites between the fusion partners and within domain V, as shown are the trypsin (T) and pseudochymotrypsin (VT) cleavage sites. Xa1 refers to the expected factor Xa fragment, *i.e.* the full-length domain V, and Xo2,3 refers to the obtained factor Xa fragment, which lacks 14 NH2-terminal residues and is cleaved after arginine 469. B, sequences of two disulfide-bonded trypsin fragments T1 and T2. C, sequences and disulfide bonds of three V8 protease fragments obtained from T1.

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2 G. Hansson, personal communication.

residues of domain V are required for correct folding of this region. The MARCO receptor contains a cluster of basic residues at the end of the collagenous domain, which in SR-A has been shown to mediate the binding of acetylated LDL (25).

Further sequence comparisons (Fig. 7) revealed that the sequence GRAEVYY is, with the exception of one residue, found in three other proteins, i.e. pema (26), neurotrypsin (27), and lysyl oxidase-related protein (28), which belong to a family of proteins containing SRCR domains. The SRCR motif contains about 100 residues and belongs to a family of well conserved cysteine-rich protein domains present in diverse proteins (29, 30). At least 16 different proteins, each having 1–11 SRCR motifs, have been described (8, 27, 28). Pema, which is a membrane glycoprotein of the sea lamprey, contains two SRCR domains and five epidermal growth factor-like repeats. Pema has been suggested to play a role in highly selective functions of the nervous system, where it may play a role in the binding of ligands, as well as studies with mice homozygous for an inactivated MARCO gene, may provide important information on the significance of this receptor in host defense.

Acknowledgments—We are grateful to Eila Cederlund, Irene Byman, and Tomas Bergman for amino acid sequencing and mass spectrometry analyses of the intrachain disulfide pattern of the SRCR domain. The disulfide bond pattern of the compacted MARCO SRCR domain was determined and shown to be identical to that of the corresponding domain in SR-A. The present and previous work imply some specific roles for the MARCO receptor in pathogen removal in vivo. Further studies on the MARCO receptor, such as the determination of the bacteria-binding sequence and ligands, as well as studies with mice homozygous for an inactivated MARCO gene, may provide important information on the significance of this receptor in host defense.

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Structure of the Human Macrophage MARCO Receptor and Characterization of Its Bacteria-binding Region
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