SREC-I, a Type F Scavenger Receptor, Is an Endocytic Receptor for Calreticulin*

Received for publication, June 3, 2004, and in revised form, August 5, 2004
Published, JBC Papers in Press, September 14, 2004, DOI 10.1074/jbc.M406202200

Brent Berwin‡§, Yves Delneste¶, Rachel V. Lovingood†, Steven R. Post**, and Salvatore V. Pizzo†§§

From the ‡Department of Pathology and ¶Cell Biology, Duke University Medical Center, Durham, North Carolina 27710, ¶¶Unité INSERM U564, University Hospital, Angers F49933, France, and **Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, Kentucky 40536

Calreticulin and gp96 (GRP94) traffic associated peptides into the major histocompatibility complex class-I cross-presentation pathway of antigen-presenting cells (APCs). Efficient accession of the cross-presentation pathway requires APC receptor-mediated endocytosis of the chaperone/peptide complexes. Previously, scavenger receptor class-A (SRA) was shown to play a substantial role in trafficking gp96 and calreticulin into macrophages, accounting for half of total receptor-mediated uptake. However, the scavenger receptor ligand fucoi din competed the chaperone uptake beyond that accounted for by SRA, indicating that another scavenger receptor(s) may also contribute. Consistent with this hypothesis, we showed that the residual calreticulin uptake into SRA−/− macrophages is competed by the scavenger receptor ligand acetylated-low density lipoprotein (LDL). We now report that an additional scavenger receptor, SREC-I (scavenger receptor expressed by endothelial cell-I), mediates the endocytosis of calreticulin and gp96. Ectopic expression of SREC-I in Chinese hamster ovary cells yielded chaperone recognition and uptake, and these processes were competed by the inhibitory ligands fucoi din and acetylated (Ac)LDL. Although AcLDL competes for the chaperone interactions with SRA and SREC, we showed that not all of the scavenger receptors, which bind AcLDL, bind calreticulin or gp96. The overexpression of SREC-I in macrophages increased chaperone endocytosis, indicating that SREC-I functions in APCs and that the cytosolic components necessary for the endocytosis of SREC-I and its cargo are present and not limiting in APCs. These data identify a novel class of ligands for SREC-I and provide insight into the mechanisms by which APCs and potentially endothelial cells traffic chaperone/antigen complexes.

Molecular chaperones are capable of stimulating a variety of immune responses. gp96 1 (also known as GRP94), hsp70, hsp90, and later, calreticulin (CRT) were isolated as tumor rejection antigens that prophylactically and therapeutically inhibit tumor metastasis (1–3). Chaperones, including gp96 and CRT, are also implicated in the pathophysiology of several autoimmune diseases including lupus, rheumatoid arthritis, atherosclerosis, and a model of autoimmune diabetes (4–8). Immunoregulation by chaperones derives from their ability to elicit a variety of effects on many cell types.

Chaperones are extremely efficient at raising peptide-specific immune responses. As a function of being chaperones, CRT and gp96 bind (poly)peptides in vitro and can bind synthetic peptides in vitro (1, 9–14). Although recent reports indicate proteasomal substrates, relative to chaperone-associated peptides, are predominant in raising endogenous CD8+ T cell-mediated immune responses (15, 16), chaperones are of increasing interest, especially therapeutically, because of their ability to be used as vehicles for escorting associated peptides into antigen-presenting cells (APCs) and eliciting subsequent peptide-specific CD8+ T cell responses (3, 9, 12). CD8+ T cell anti-tumor responses are reported to be generated from tumor-derived chaperone (17) as well as from chaperones complexed with synthetic tumor-specific peptides (18, 19). Upon endocytosis by APCs, chaperones deliver their associated peptides into the cellular antigen cross-presentation pathway(s), resulting in APC cell surface presentation of the peptides in the context of major histocompatibility class-I molecules and subsequent peptide-specific CD8+ T cell activation (1, 11, 12, 14, 20–23). Current evidence indicates that being complexed with a chaperone greatly increases the immunogenicity of a peptide and that receptor-mediated endocytosis is required for chaperone-associated peptides to efficiently access the peptide presentation pathway of APCs (11, 12, 23, 24).

Complementary to their ability to raise peptide-specific responses, chaperones also elicit peptide-independent responses both in vitro and in vivo. They stimulate APCs including the maturation of dendritic cells (DCs), induce cytokine responses, elicit peptide-independent anti-tumor responses, and can affect autoimmune disease (25–30). Additionally, perhaps linked to its ability to inhibit tumor metastasis, calreticulin has effects on endothelial cells. CRT can inhibit endothelial cell adhesion and proliferation and is reported to be anti-angiogenic (31, 32). Despite the pleiotropic immunological effects of chaperones, the cellular receptors that mediate their immunogenicity are not well understood. Here we identified a cell surface receptor...
for calreticulin that is present both on macrophages and endothelial cells.

Recent research has focused on the identification of cell surface chaperone receptors. Toll-like receptors and CD14 have been posited to transduce chaperone-mediated signaling (26, 29, 33–35). However, recent reports (36–39) indicate that some of the effects observed may have resulted from endotoxin contamination. Of particular interest has been the identification of endocytic receptors for chaperones, particularly in the context of chaperone/antigen complexes. CD91 (LDL receptor-related protein, an α2-macroglobulin receptor) was originally reported to be the unique endocytic receptor for a variety of chaperones including CRT, gp96, hsp70, and hsp90 (40, 41). However, other endocytic chaperone receptors have subsequently been identified, including a pair of scavenger receptors. LOX-1 was identified as an endocytic receptor for hsp70 but not gp96, whereas scavenger receptor class-A (SRA) was identified as an endocytic receptor for gp96 and CRT (24, 42). Both of these scavenger receptors trafficked chaperone-associated peptides into the APC antigen presentation pathway(s) (24, 42–44). The studies using SRA-/- peritoneal macrophages indicate that SRA accounts for approximately half of the endocytic capability for gp96 and CRT (24). Additionally intriguing, however, was the finding that a scavenger receptor ligand, fucoidin, competed >80% cellular gp96 and CRT binding and uptake more than could be accounted for by SRA (24). Here we identified a second scavenger receptor, SREC-I (scavenger receptor expressed by endothelial cell-1), as an endocytic receptor for the chaperones CRT and gp96.

SREC isoforms were originally cloned from endothelial cDNA libraries and subsequently identified in macrophages and phagocytic cells, including the CED-1 homologue in Caenorhabditis elegans (45–49). SREC-I, probably through epidermal growth factor-like repeats in its extracellular domain, binds and internalizes modified low density lipoproteins (LDL) and has an unusually long intracellular tail for a scavenger receptor (49). A second isoform, SREC-II, engages in heterophilic interactions with SREC-I but does not bind modified LDL well (47). As a follow up to our previous study identifying SRA as an endocytic chaperone receptor, we note that, in addition to fucoidin (24), AcLDL competes for chaperone binding to SRA and to macrophage cell surfaces. Fucoidin and AcLDL also partially compete for CRT uptake into SRA-/- cells, indicating the presence of another endocytic scavenger receptor. Screening genetic expression of scavenger receptors in CHO cells for augmented chaperone association, we have identified SREC-I as an endocytic receptor of CRT and gp96. The specificity of this binding was examined by competition with the SREC-I ligands fucoidin and AcLDL. Interestingly, the expression of other scavenger receptors, which bind modified LDL, did not confer endocytic activity for CRT, indicating a selectivity for CRT by SRA and SREC-I. Additionally, CRT and gp96 are structurally dissimilar and show no apparent similarity with AcLDL or fucoidin, potentially indicating that SREC-I binds these competing ligands on the basis of pattern recognition. Recombinant expression of SREC-I in macrophages increased the uptake of CRT, indicating that SREC-I functions as an endocytic receptor for chaperones in APCs and that receptor number is a limiting factor in chaperone uptake. These data identified CRT and gp96 as novel ligands for SREC-I and that SREC-I functions as an endocytic receptor in the trafficking of these chaperones.

MATERIALS AND METHODS

Antibodies and Proteins—Calreticulin was expressed and purified as described previously (50). The vector was a generous gift of Dr. David Williams (University of Toronto). gp96 was purified by the method of Wearsch et al. (51). AlexaFluor-succinimidyl esters, Alexa488-AcLDL, Alexa488-wheat germ agglutinin, and DiOC6 were obtained from Molecular Probes (Portland, OR). Protein labeling with the succinimidyl esters was performed according to the manufacturer’s protocols. Fucoidin and LPS were obtained from Sigma. Anti-SREC antibody was purchased from Santa Cruz Biochemicals.

Cell Culture and Transfection—Peritoneal macrophages were elicited in C57BL/6 mice (NIC, National Institutes of Health, Frederick, MD) as described previously (52, 53). SRA null mice (54, 55) were backcrossed into the C57BL/6J background and were a generous gift of T. Kodama (Tokyo University) and M. W. Freeman (NHLBI Program in Genomics Applications, Massachusetts General Hospital). Mice were killed 4 days post-injection, and macrophages were obtained by peritoneal lavage. Macrophages were enriched by adherence selection for 1 h in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum on 24-well plates (BD Biosciences) for flow cytometry experiments. RAW264.7 macrophages and CHO cells were cultured according to the published ATCC protocols. Human embryonic kidney (HEK 293) cells expressing SRA were used as described previously (24, 56, 57).

Bone Marrow-derived Dendritic Cell Culture—The DC culture protocol is described previously (10, 58) and is a modification of Inaba et al. (59). In summary, C57BL/6 mouse (Charles River Laboratories, Raleigh, NC) bone marrow-derived cells were resuspended at 10⁶ cells/ml in DC culture media (RPMI 1640 medium, 5% heat-inactivated fetal bovine serum) and cultured in 24-well plates (BD Biosciences) for flow cytometry experiments. RAW264.7 macrophages and CHO cells were cultured according to the published ATCC protocols. Human embryonic kidney (HEK 293) cells expressing SRA were used as described previously (24, 56, 57).

Detection of Fluorescent Ligand Association—Receptor-mediated ligand uptake studies were performed as described previously (42). These constructs were transfected into CHO cells using LipofectAMINE reagent (Invitrogen) according to the manufacturer’s protocol. Transfected cells were selected with hygromycin (500 μg/ml), and individual clones were selected and expanded. Scavenger receptor expression was confirmed by Western blotting of cell lysates with anti protein C-tag polyclonal antibody (Roche Applied Science) and, in some cases, by interaction with Alexa488-labeled acetylated LDL (Molecular Probes) (42).

RESULTS

Competition of CRT Endocytosis by AcLDL and Fucoidin Indicates the Activity of an Additional Scavenger Receptor(s)—Receptor-mediated endocytosis is required for efficient trafficking of molecular chaperones into APCs and representation of their associated antigens. SRA was previously identified as an endocytic receptor for CRT and gp96, and experiments using SRA-/- macrophages indicated that SRA accounts for ~50%
cell-surface CRT binding capacity on these cells (24). The interaction between SRA and CRT or gp96 was efficiently competed by the scavenger receptor ligand fucoidin (24, 60). However, fucoidin competed >80% CRT and gp96 binding to macrophages (24) and dendritic cells (Fig. 1E) more than could be accounted for by the SRA interaction. In the efforts to elucidate the identity of other receptors, we tested modified LDL for the ability to compete the uptake of CRT and gp96 to macrophages. Acetylated LDL competed the uptake of CRT into macrophages, blocking >80% of the uptake of fluorescently labeled CRT (Fig. 1, A–C). LDL oxidized with copper sulfate was similar in its ability to compete for CRT uptake (data not shown). AcLDL is an established ligand for SRA, so using a protocol similar to the one used to identify SRA as a chaperone receptor (24), we tested the ability of AcLDL to directly compete the chaperone/SRA interaction. HEK 293 cells transfected with SRA under an inducible promoter (HEK-SRAtet) (24, 57) were selected (as in Ref. 42). Receptor expression was verified by Western analysis (Fig. 3A). PSOX expression was not detectable by Western analysis (Fig. 3A) and by function (enhanced binding of fluorescent AcLDL, Fig. 3B). Untransfected CHO cells and those expressing scavenger receptors were then assayed for their relative ability to accumulate fluorescent CRT. CHO cells expressing the scavenger receptors LOX-1, Cla-1, or CD36 did not exhibit a reproducible increased CRT binding relative to untransfected CHO cells (Fig. 3C). PSOX expression was not as robust as that of the receptors observed in Fig. 3A (data not shown).}

**FIG. 1.** Acetylated LDL competes for SRA-mediated macrophage endocytosis of calreticulin. Elicited peritoneal macrophages were assayed for AcLDL-sensitive CRT uptake. A–C, macrophages were incubated for 30 min at 37 °C with 5 μg/ml Alexa647-labeled CRT in the absence (B) or presence (C) of 5 μg/ml Alexa488-labeled AcLDL. Unstained macrophages are shown in A. SRA binding of CRT is also competed by AcLDL (D). HEK cells ectopically expressing SRA (HEK-SRA<sup>wt</sup>) were incubated for 2 h with 20 μg/ml Alexa647-labeled CRT in the absence or presence of 10 μg/ml AcLDL or 75 μg/ml fucoidin. Receptor-mediated uptake of CRT was competed by AcLDL >80% in both macrophages and HEK-SRA<sup>wt</sup> cells. E, dendritic cells were incubated with 5 μg/ml Alexa488-CRT for 15 min at 37 °C in the presence (dots) or absence (dashes) of 10 μg/ml fucoidin. The solid line depicts autofluorescence.

**Fig. 2.** Acetylated LDL competes for the endocytosis of calreticulin into SRA<sup>−/−</sup> macrophages. Elicited peritoneal macrophages from SRA<sup>−/−</sup> (C57BL/6 background) mice were assayed for their ability to internalize CRT. A, macrophages were incubated for 30 min at 37 °C with 3 μg/ml Alexa647-labeled CRT in the absence (B) or presence (C) of 2 μg/ml Alexa488-labeled AcLDL. CRT uptake is shown on the y axis, AcLDL uptake is on the x axis. Autofluorescence is shown in A. D, bar graph showing mean Alexa647 fluorescence observed in A–C.
shown), but it also did not exhibit CRT binding ability (Fig. 3C). Multiple independently selected clones of each CHO-scavenger receptor transfectant were screened with similar results (data not shown). However, CHO cells expressing SREC-I (CHO-SREC) showed enhanced binding of chaperone relative to untransfected CHO cells (thin lines). Endocytosis of CRT was confirmed by confocal microscopy (C) with CRT (red) observed in punctate compartments within the cell surface (labeled with wheat germ agglutinin, seen as green). Fucoidin-sensitive uptake of Alexa647-labeled CRT into SREC-expressing CHO cells was assessed by FACS analysis with an EC_{50} of 17 ± 9 μg/ml (± S.D.), ~250 nM, observed for uptake of CRT (D).

**Fig. 3. Expression of scavenger receptors and analysis of their ability to endocytose calreticulin.** CHO cells were transfected with cDNAs for the scavenger receptors CD36, SREC, LOX-1, PSOX, and Cla. Expression was confirmed by Western analysis of whole cell lysates using anti-protein C-tag monoclonal antibody (A, lane 1, Bio-Rad molecular mass marker; lane 2, Cla-I; lane 3, SREC-I; lane 4, LOX-1) and by the enhanced ability of stable transfectants to accumulate Alexa488-labeled AcLDL as measured by FACS analysis (B). C. CHO cells stably transfected with LOX-1, PSOX, Cla, and CD36 (thin lines) did not exhibit a reproducible increased ability to bind 5 μg/ml Alexa647-labeled calreticulin compared with untransfected CHO cells (thick lines).

**Fig. 4. SREC-I expression is sufficient for the binding and uptake of calreticulin and gp96.** CHO cells were incubated with 5 μg/ml Alexa647-labeled CRT (A) or gp96 (B) for 30 min at 37 °C. Cells stably expressing recombinant SREC-I (thick lines; A and B) exhibited enhanced binding of chaperone relative to untransfected CHO cells (thin lines). Endocytosis of CRT was confirmed by confocal microscopy (C) with CRT (red) observed in punctate compartments within the cell surface (labeled with wheat germ agglutinin, seen as green). Fucoidin-sensitive uptake of Alexa647-labeled CRT into SREC-expressing CHO cells was assessed by FACS analysis with an EC_{50} of 17 ± 9 μg/ml (± S.D.), ~250 nM, observed for uptake of CRT (D).
effective in doing so and competed SREC-dependent CRT uptake (Fig. 6, A–C) with half-maximal inhibition observed at \( H_{1/2} \) concentration of fucoidin (Fig. 6D).

**SREC-I Functions in Macrophages to Endocytose CRT**—Having observed that ectopic SREC-I expression in CHO cells confers the ability to accumulate chaperones (Fig. 4), we then examined whether SREC-I functions in the endocytosis of chaperones into antigen-presenting cells. SREC was expressed on elicited macrophages (Fig. 7A) (46) and dendritic cells,\(^2\) and the expression levels were increased by exposing the macrophages to LPS (Fig. 7A) (46). To assist in studying SREC function, SREC-I was overexpressed in RAW264.7 macrophages with expression confirmed by Western analysis (Fig. 7B). SREC-I-transfected and -untransfected macrophages were then assayed for their relative ability to endocytose fluorescently labeled CRT. SREC-I-transfected macrophages bound fluorescent CRT at the cell periphery at 4 °C (Fig. 7C), and the ligand was internalized upon warming (37 °C) of the cells (Fig. 7D), confirming that SREC-I was internalizing its ligand. SREC-transfected and -untransfected macrophages accumulated CRT (Fig. 7, E and F) at least partly due to the presence of SRA (24), and consistent with this finding is the ability of fucoidin to inhibit CRT uptake in both SREC-untransfected (24) and -transfected macrophages (Fig. 7G). Recombinant SREC-I expression enhanced CRT accumulation >5-fold (Fig. 7H), indicating that SREC-I can endocytose chaperones into APCs and that the cellular machinery necessary for SREC-mediated endocytosis functions in macrophages. These data identified SREC-I as an endocytic receptor for molecular chaperones.

**DISCUSSION**

We report that SREC-I, a type F scavenger receptor present on endothelial cells and macrophages, is a cell surface receptor...
that binds the molecular chaperones CRT and gp96 and mediates their endocytosis. SREC-I expression in both CHO cells and RAW264.7 macrophages was sufficient to confer chaperone uptake and accumulation. This uptake was efficient (EC_{50} = 250 nM) and specific. Binding was competed by the scavenger receptor ligands fucoidin and acetylated LDL. The identification of SREC-I as a chaperone receptor extends the list of known chaperone receptors and augments the role of the scavenger receptor superfamily as endocytic chaperone receptors (24, 42). CD91, although not classified as a scavenger receptor, shares many traits of the scavenger receptor superfamily and was originally proposed as the sole APC endocytic receptor for a multitude of chaperones (40, 41). However, subsequent studies identified a CD91-independent pathway for chaperone uptake (53) followed by the identification of the canonical scavenger receptors LOX-1 and SRA (24, 42) as functioning in chaperone trafficking. LOX-1, a class-E scavenger receptor present on endothelial cells and APCs, binds and traffics the cytosol-derived chaperone hsp70 but not gp96 (42). SRA functions in the endocytosis of gp96 and CRT and is responsible for approximately half of the total receptor-mediated uptake of these chaperones into elicited peritoneal macrophages (24).

During the identification of SRA as a chaperone receptor, the scavenger receptor ligand fucoidin was found to be highly effective at competing gp96 and CRT uptake into macrophages as well as neutrophils (24, 60). Fucoidin abrogated SRA-mediated chaperone uptake and competed ~80% total chaperone uptake into primary and cultured macrophages (24) and into DCs (Fig. 1E). However, because subsequent experiments indicated that SRA accounts for ~50% total endocytic trafficking, roughly half of the residual SRA-independent trafficking was still competed by fucoidin. A second scavenger receptor ligand, AcLDL, is shown here to produce similar results, effectively competing the CRT/SRA interaction and partially competing the SRA-independent uptake. Using genetically SRA-deficient (SRA^{-/-}) macrophages, we bolstered the previous findings by showing competition of chaperone uptake by AcLDL, the levels of which correlate with the results obtained with fucoidin. Because the CD91 ligands α2-M and receptor-associated protein are not efficient in competing the interaction of chaperones with live unfixed macrophages (53, 61) and fucoidin inhibits chaperone uptake but not the α2-M/CD91 interaction (24), we hypothesized that additional scavenger receptor(s) may also function in the uptake of CRT and gp96.

Expression of SREC-I in multiple cell types bestowed the ability to bind and endocytose CRT and gp96. Scavenger receptors commonly function in clearing and mediating immune responses against ligands that are recognized as “foreign” either produced by exogenous organisms (e.g., LPS, bacteria) or endogenous ligands that have been modified (e.g., oxidized LDL, apoptotic cells). These ligands are often seemingly structurally unrelated, although the ligands tend to be “danger signals” associated with pathogenesis (reviewed in Ref. 62). Molecular chaperones were proposed to be danger signals, because they are normally retained within healthy cells but can be released during regulated and necrotic cell death (63–65). However,
SREC-I Is an Endocytic Receptor for Calreticulin

The mechanisms by which chaperones released from stressed or dying cell-elicited danger signals are not well understood. Chaperones released from necrotic tumor cells are reported to be immunostimulatory (27, 65–67); however, recent evidence indicates that the stimulatory activity may be independent of peptides with which the chaperones may be associated (15, 16). In common with other pathogenically released or derived molecules that are ligands of scavenger receptors, CRT and gp96 are polyanions (68, 69), although this characteristic is not sufficient. Scavenger receptors do not bind all polyanions. CRT and gp96 share no apparent sequence or domain similarity nor are they similar to the competitive ligands fucoidin and AcLDL. The ability of these ligands to compete for a common binding site(s) on SREC-I as well as SRA suggest that pattern recognition, a hallmark of the innate immune system, is being utilized. The basis of the pattern recognition, however, is not yet understood. Of note is that AcLDL and fucoidin were far more effective at competing CRT than CRT was at competing AcLDL (data not shown). This was consistent for the interaction of CRT with SRA, SREC, and macrophage cell surfaces. We speculate that AcLDL and fucoidin, as a function of being large polymers, may bind more tightly through avidity. Experiments are currently underway to discern the nature of the binding and competition. However, nonreciprocal competition has previously been reported for scavenger receptor ligands, although the underlying reasons are not yet clear (68, 70).

Of particular interest, as the molecular basis of pattern recognition continues to be explored, is the finding here that CRT and gp96 binding is restricted to a subset of the modified LDL-binding scavenger receptors. AcLDL effectively competed CRT from SRA and SREC-I, yet the modified LDL-binding scavenger receptors LOX-1, PSOX, Cla-1, and CD36 did not substantially bind CRT. This observation indicated selectivity for chaperone binding among scavenger receptors and, furthermore, that the ability to bind modified LDL did not correlate with chaperone binding. SRA and SREC-I both bind AcLDL, fucoidin, CRT, and gp96 yet also show no apparent similarity to each other in their binding domains. The extracellular portion of SREC is dominated by epidermal growth factor-like repeats, whereas SRA has collagen-like, cysteine-rich, and coiled-coil domains (49, 71). Additionally, a third scavenger receptor, LOX-1, which is distinct from SRA and SREC, binds modified LDL and hsp70 but not gp96 (42). The ability to compare and contrast the ligands (i.e., the ‘patterns’) and, just as importantly, the pertinent domains of the receptors will lend itself to better understanding the basis of pattern recognition and will allow for targeting of these receptors both selectively and generically.

Chaperones are noted both for their ability to access endocytic pathways and for their ability to induce signaling. Chaperones have been reported to elicit a variety of responses, primarily in and from immunoregulatory cells, including cytokine release, APC activation and DC maturation, up-regulation of cell surface co-stimulatory molecules, and stimulation of autoimmune responses (25–30). Of particular potential interest regarding the functions of SREC-I and LOX-1 are effects of CRT on endothelial cells on which both of these scavenger receptors are present. CRT inhibits endothelial cell proliferation and adhesion in vitro as well as having anti-angiogenic effects in vivo. The reported effects of CRT on endothelial cells are largely independent of antigenic peptides associated with CRT (31, 32). However, the signaling receptors for chaperones on both endothelial cells and APCs are possibly even more poorly defined than the endocytic receptors. Although both Toll-like receptors and CD14 have been reported to transduce a variety of these responses (25–30), confusion arose from reports that at least some of these responses are due to contaminating endotoxin (36–39). The use of endotoxin-free models is now helping to clarify this issue (5, 7, 72–74). SREC-I is an interesting candidate as not only an endocytic receptor but as a signaling receptor. Its intracellular domain comprises approximately half of the molecule, disproportionately long for a scavenger receptor (49). Additionally, there are several potential serine/threonine phosphorylation sites as well as a tyrosine within a consensus phosphorylation sequence (47, 49). The tyrosine was present in the endocytic SREC-I tail but not in the tail of SREC-II, which does not support internalization (47, 49). Further research is underway to elucidate roles for SREC-I in chaperone-mediated signaling.

In summary, we present data supporting the previous identification of fucoidin-sensitive SRA-independent chaperone trafficking and subsequently identify SREC-I as a novel endocytic receptor for the ER-derived chaperones CRT and gp96. Endocytic uptake of the chaperones by SREC-I is efficient with the specificity demonstrated by competition with the scavenger receptor ligands fucoidin and AcLDL. Ectopic expression of SREC-I in both CHO and RAW264.7 macrophages was sufficient to induce receptor-mediated endocytosis of chaperones. However, the ability to bind and internalize CRT appeared to be limited to a subset of scavenger receptors as the expression of several other receptors did not support chaperone trafficking. These findings expand the role and selectivity of scavenger receptors in chaperone trafficking. Additionally, as SREC-I binds multiple dissimilar chaperones for endocytosis, we present a system prime for studying the basis of pattern recognition and for studying the interface of adaptive and innate immunity.

Acknowledgments—We thank the Duke University Medical Center Comprehensive Cancer Center Shared Flow Cytometry Facility and the University of Kentucky Shared FACS Facility for FACS analyses and acknowledge the support of the DUMC Comprehensive Cancer Center Shared Confocal Microscopy Facility. We are grateful to Drs. Jo Rae Wright, Chris Nichitita, Cindy James, Justin Hart, and Eric Williams for advice and reagents.

REFERENCES

1. Basu, S., and Srivastava, P. K. (1999) J. Exp. Med. 189, 797–802
2. Srivastava, P. K., DeLeo, A. B., and Old, J. L. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3407–3411
3. Udono, H., and Srivastava, P. K. (1993) J. Exp. Med. 178, 1391–1396
4. Engleman, P., and Llewellyn, D. H. (1999) Science 286, 466–473
5. Liu, B., Dai, J., Zheng, H., Stoilova, D., Sun, S., and Li, Z. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 15824–15829
6. Martin, C. A., Carson, S. E., Kawakami, R., Bernstein, D., Valentino, M., and Santiago-Schwarz, F. (2003) J. Immunol. 171, 5736–5742
7. Millar, D. G., Garza, K. M., Odermatt, B., Ellford, A. R., Ono, N., Li, Z., and Ohashi, P. S. (2003) Nat. Med. 9, 1469–1476
8. Xu, Q. (2003) Arterioscler. Thromb. Vasc. Biol. 23, 1547–1559
9. Suto, R., and Srivastava, P. K. (1995) Science 269, 1565–1588
10. Berwin, B., Rossor, M. F., Brinker, K. G., and Nichitita, C. V. (2002) Traffic 3, 358–366
11. Singh-Jasuja, H., Toes, R. E., Spee, P., Munz, C., Hrif, N., Schonberger, S. P., Ricciardi-Castagnoli, P., Neefjes, J., Remmensee, H. G., Arnold-Schild, D., and Schild, H. (2000) J. Exp. Med. 191, 1965–1974
12. Blackshear, N. R., Li, L., Chaudhary, M. G., Sun, P., Xu, B., Sato, K. Y., U. K., Sato, K., Kajari, N. S., Basu, S., Udono, H., and Srivastava, P. K. (1997) J. Exp. Med. 186, 1315–1322
13. Argun, Y., and Simen, B. B. (1999) Semin. Cell Dev. Biol. 10, 495–505
14. Sabo, S., Weischaus, P. A., Rischel, D. A., Wassenberg, J. J., Gilboa, E., and Nichitita, C. V. (1999) J. Immunol. 162, 6426–6432
15. Norbury, C. C., Basta, S., Dohnoue, K. B., Teshar, D. C., Princicotta, M. F., Berglund, P., Gibbs, J., Bennink, J. R., and Yewdell, J. W. (2004) Science 304, 1318–1321
16. Shen, L., and Rock, K. L. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 3035–3040
17. Udono, H., Levey, D. L., and Srivastava, P. K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3077–3081
18. Manjili, M. H., Wang, Y. X., Chen, X., Martin, T., Repasky, E. A., Henderson, R., and Subjeck, J. R. (2003) J. Immunol. 171, 4054–4061
19. Manjili, M. H., Henderson, R., Wang, Y. X., Chen, X., Li, Y., Repasky, E., Kazim, L., and Subjeck, J. R. (2002) Cancer Res. 62, 1737–1742
20. Srivastava, P. K., and Udono, H. (1994) Curr. Opin. Immunol. 6, 728–732
21. Tamura, Y., Peng, P., Liu, K., Daou, M., and Srivastava, P. K. (1997) Science 278, 117–120
22. Castellino, F., Boucher, P. E., Eichelberg, K., Mayhew, M., Rothman, J. E., Houghton, A. N., and Germain, R. N. (2000) J. Exp. Med. 191, 1957–1964
23. Macary, P. A., Javid, B., Floto, A. R., Smith, K. G., Oehlmann, W., Singh, M.,
