

# Class A scavenger receptors mediate cell adhesion via activation of $G_{i/o}$ and formation of focal adhesion complexes

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**Abstract** Class A macrophage scavenger receptors (SR-A) are multifunctional receptors with roles in modified lipoprotein uptake, innate immunity, and macrophage adhesion. Our previous studies conducted in mouse peritoneal macrophages demonstrated that pertussis toxin (PTX) mediated inhibition of  $G_{i/o}$  attenuated SR-A-dependent uptake of modified lipoprotein. The finding that SR-A-mediated lipoprotein internalization was PTX-sensitive led us to hypothesize that SR-A-mediated cell adhesion might be similarly regulated by  $G_{i/o}$ -dependent signaling pathways. To test this hypothesis, SR-A was expressed in HEK cells under inducible control. Relative to HEK cells that lack SR-A, SR-A expressing cells displayed enhanced adhesion to tissue culture dishes. SR-A-mediated adhesion was significantly reduced following PTX treatment and was insensitive to chelating divalent cations with EDTA. SR-A-expressing cells exhibited a distinct cell morphology characterized by fine filopodia-like projections. Both polymerized actin and vinculin were codistributed with SR-A in the filopodia-like projections indicating the formation of focal adhesion complexes. Overall, our results indicate that the ability of SR-A to enhance cell adhesion involves  $G_{i/o}$  activation and formation of focal adhesion complexes.—Post, S. R., C. Gass, S. Rice, D. Nikolic, H. Crump, and G. R. Post. Class A scavenger receptors mediate cell adhesion via activation of  $G_{i/o}$  and formation of focal adhesion complexes. *J. Lipid Res.* 2002, 43: 1829–1836.

**Supplementary key words** atherosclerosis • endocytosis • lipoprotein • actin • vinculin • G protein

Class A macrophage scavenger receptors (SR-A) are trimeric membrane proteins that bind a diverse array of polyanionic ligands including modified LDL, polynucleotides, bacterial products, and extracellular matrix proteins (1–4). In isolated macrophages, SR-A mediated endocytosis of chemically modified forms of LDL [e.g.,

oxidized LDL (oxLDL) and acetylated LDL (AcLDL)] promotes the formation of lipid-laden foam cells and was therefore thought to contribute to the development of atherosclerotic lesions (5, 6). In addition to modified LDL, SR-A mediates uptake of multiple bacterial-derived products by macrophages suggesting that, in addition to contributing to atherosclerosis, SR-A might be an important component in host defense (1, 3, 7). Overall, these and other studies conducted in isolated cells support a role for SR-A-mediated ligand internalization in normal immune function and pathological settings such as atherosclerosis.

In addition to mediating ligand endocytosis, an important role for SR-A in cell attachment was supported by the observation that the monoclonal SR-A antibody 2F8 inhibited macrophage adhesion to serum-coated tissue culture dishes (8). Enhanced adhesion in the presence of serum was also noted following transfection of SR-A into cultured cells (9, 10). Although the components of serum that mediate enhanced SR-A adhesion have not been identified, additional studies have shown that SR-A mediates macrophage attachment to glycosylated collagen IV and denatured forms of type I collagen (11, 12). The ability of SR-A to bind modified extracellular matrix proteins suggests that SR-A is important for macrophage attachment and retention at sites of vascular injury and thus SR-A may contribute to atherosclerotic lesion development by two distinct activities; specifically by mediating lipoprotein uptake and macrophage adhesion.

The cellular mechanisms that regulate SR-A dependent adhesion are not as well studied as those involved in integrin-mediated adhesion. For example, integrin-mediated

Abbreviations: AcLDL, acetylated low density lipoproteins; DiI-AcLDL, 1,1'-dioctadecyl-3,3',3'-tetramethyl-indocarbocyanine perchlorate labeled acetylated low density lipoprotein; FACS, fluorescence-activated cell sorting; MPM, mouse peritoneal macrophage; PTX, pertussis toxin; SR-A, class A scavenger receptor.

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cell attachment to extracellular matrix proteins involves the recruitment of cytosolic scaffold proteins, actin polymerization within peripheral filopodia and lamellipodia, and ultimately the formation of focal adhesion complexes (13–15). Additionally, integrin-mediated cellular adhesion is regulated by the intracellular environment through a process known as “inside-out” signaling (16, 17). Whether SR-A mediated cell adhesion involves focal adhesion formation or is regulated by intracellular signals has not been addressed. However, our recent finding that inhibiting  $G_{i/o}$ -dependent signaling pathways with pertussis toxin (PTX) decreased SR-A-mediated lipoprotein uptake supports the possibility that SR-A-mediated cell adhesion might also be regulated by PTX-sensitive pathways (18).

Mechanistic analysis of SR-A function in cells is complicated by the expression of additional receptors that serve similar functions. To facilitate studies aimed at elucidating the role of intracellular signaling pathways in regulating SR-A-mediated cell adhesion, SR-A was expressed in HEK cells under control of the inducible ecdysone promoter. Using SR-A transfected HEK cells, we found that SR-A-mediated cell adhesion was inhibited by PTX treatment. Furthermore, we showed that SR-A codistributed in actin-containing filopodia-like projections together with vinculin, but not clathrin, suggesting that SR-A expression promotes formation of focal adhesion complexes. Inhibition of actin polymerization with cytochalasin D reduced SR-A-mediated adhesion to a similar extent as PTX treatment. Overall, our data indicate that SR-A promotes cellular adhesion through a process that involves SR-A activation of a  $G_{i/o}$ -dependent signaling pathway, actin polymerization and formation of focal adhesion complexes.

## EXPERIMENTAL PROCEDURES

### Chemicals

Dulbecco's modified Eagle's medium (DMEM) with L-glutamine and high glucose, bicarbonate-free DMEM buffered with 25 mM HEPES, and heat-inactivated fetal bovine serum (FBS) were purchased from GibcoBRL (Grand Island, NY). Pertussis toxin (*Bordetella pertussis*) was purchased from Calbiochem (La Jolla, CA). The rat monoclonal SR-A antibody 2F8 and its fluorescent derivative 2F8-RPE were purchased from Serotec (Raleigh, NC). Fluorescently labeled lipoproteins, phalloidin, and secondary antibodies were from Molecular Probes (Eugene, OR).

### Cell culture and transfection

Human embryonic kidney (HEK) cells expressing the ecdysone receptor (EcR-293; Invitrogen; Carlsbad, CA) were maintained in DMEM containing penicillin (10 U/ml), streptomycin (10  $\mu$ g/ml), 10% FBS (DMEM/FBS), and bleocin (20  $\mu$ g/ml). The cDNA encoding the type II murine scavenger receptor was subcloned into the pIND vector (Invitrogen) and transfected into HEK cells using GenePorter transfection reagent (Gene Therapy Systems, San Diego, CA) according to the manufacturer's protocol. Using this expression system, SR-A is only expressed when an inducing agent (e.g., muristerone) is added to the cell culture media. Transfected cells were selected with G418 (800  $\mu$ g/ml) and inducible expression confirmed by immunostaining adherent cells with 2F8 and Western blotting of cell lys-

ates with a guinea pig anti-SR-A antibody (provided by A. Daugherty, Univ. of Kentucky). Cells expressing the highest level of receptor were selected by fluorescence activated cell sorting (FACS) using a fluorescently-labeled SR-A ligand 1,1'-dioctadecyl-3,3',3',3'-tetramethyl-indocarbocyanine perchlorate labeled acetylated low density lipoprotein (DiI-AcLDL). Isolated cells were then expanded in culture and cell-surface SR-A expression determined by flow cytometry using a fluorescently labeled SR-A specific antibody (2F8-RPE).

Resident peritoneal macrophages were collected from 6–8 week old male outbred Cr:NIH (S) Swiss mice (National Cancer Institute, Charles River Laboratories, Frederick, MD) by peritoneal lavage with 5 ml ice-cold sterile saline. Isolated cells were resuspended in DMEM/FBS. After an overnight incubation at 37°C, non-adherent cells were removed by washing, and adherent cells cultured in DMEM/FBS.

### Detection of AcLDL association and SR-A expression

HEK cells were incubated for 24 h with increasing concentrations of muristerone to induce SR-A expression. In some experiments, cells were treated with both muristerone (4  $\mu$ M) and PTX (100 ng/ml, 16 h). To assess SR-A-mediated lipoprotein uptake, HEK cells were preincubated for 2 h in serum-free DMEM and then incubated for 2 h with DiI-AcLDL (10  $\mu$ g/ml). Where indicated, the SR-A antagonist polyinosine (10  $\mu$ g/ml) was added 5 min prior to addition of lipoprotein. Unbound lipoprotein was removed by washing cells with PBS, and cells were then suspended by trypsinization and associated DiI-AcLDL fluorescence determined by flow cytometry. A similar approach was used to detect SR-A protein. Trypsinized cells were resuspended in DMEM at  $10^7$  cells/ml and incubated with a fluorescently labeled anti-SR-A antibody (2F8-RPE) for 10 min at room temperature. Labeled cells were washed in PBS and cell-associated fluorescence assessed by flow cytometry.

For Western blotting, cell lysates were prepared by incubating cells in solubilization buffer (25 mM MES; 150 mM NaCl; 60 mM octylglucopyranoside; 1% Triton X-100; pH 6.4) for 60 min on ice. Insoluble material was pelleted by centrifugation and protein concentration of supernatant determined using the BioRad DC assay (Hercules, CA). For electrophoresis, 15  $\mu$ g of cell protein was resolved by 10% SDS-PAGE and transferred to PVDF membrane. To detect SR-A protein, membranes were incubated with a guinea pig SR-A specific antisera (provided by A. Daugherty, University of Kentucky) followed by a HRP-coupled secondary antibody. Bands were detected by chemiluminescence and quantified using a Kodak Image Station 440.

### Cell adhesion assay

HEK cells were cultured in 6-well plates in DMEM/FBS. To induce SR-A expression, muristerone (4  $\mu$ M) was added and cells cultured for an additional 24 h. In some experiments, cells were treated with both muristerone and PTX (100 ng/ml, 16 h). Subsequently, cells were dissociated with trypsin, washed with DMEM, and resuspended in DMEM/FBS at  $10^6$  cells/ml. To confirm the specific requirement for SR-A in cell adhesion, cells were incubated with 2F8 (25 ng/ml) for 30 min or polyinosine (10  $\mu$ g/ml) for the final 5 min. Treated cells were then replated into 6 well plates and allowed to adhere for 10 min at 37°C. To remove non-adhered cells, the media was removed and wells were washed once with DMEM. Adhered cells were suspended with trypsin and cell number determined using a hemacytometer.

### Fluorescent staining and confocal imaging

HEK cells were plated (50,000 cells/well) on two-chambered LAB-TEK® slides (Nalge Nunc International; Naperville, IL). To

facilitate adhesion of uninduced cells, the slides were treated with BD Cell-Tak™ (BD Biosciences; Bedford, MA) according to manufacturers' adsorption protocol. Cell morphology was not significantly affected by culturing on Cell-Tak. Where indicated, SR-A expression was induced with muristerone (4  $\mu$ M). After 16 h, cells were gently washed twice for 2 min with warm phenol red free-DMEM, fixed for 30 min at room temperature with 4% paraformaldehyde in PBS, and washed once for 5 min. Fixed cells were permeabilized with 0.1% Triton X-100 in PBS, washed, and blocked with 1% BSA for 30 min. To detect SR-A, cells were incubated with 2F8 (diluted 1:300; v/v) for 15 min at room temperature, fixed, permeabilized, and then incubated with AlexaFluor-488 Goat Anti-Rat IgG (1:500; v/v) for 60 min. To assess polymerized actin (F-actin), cells were fixed, permeabilized, and incubated with AlexaFluor-568-conjugated phalloidin (diluted 1:100; v/v) for 20 min. To detect vinculin or clathrin, fixed cells were incubated with either a vinculin specific antibody (1:100; v/v; 60 min; Upstate Biotechnology) or a clathrin heavy chain specific antibody (1:100; v/v; 60 min; BD Transduction Laboratories) followed by fluorescently-labeled secondary antibodies for 20 min. Cells were then mounted in the embedding medium Mowiol containing 1% n-propyl gallate and dried overnight at 4°C. Images (100 $\times$ ) were digitally captured using a Leica TCS SP confocal microscope and processed with the MetaMorph® software package.

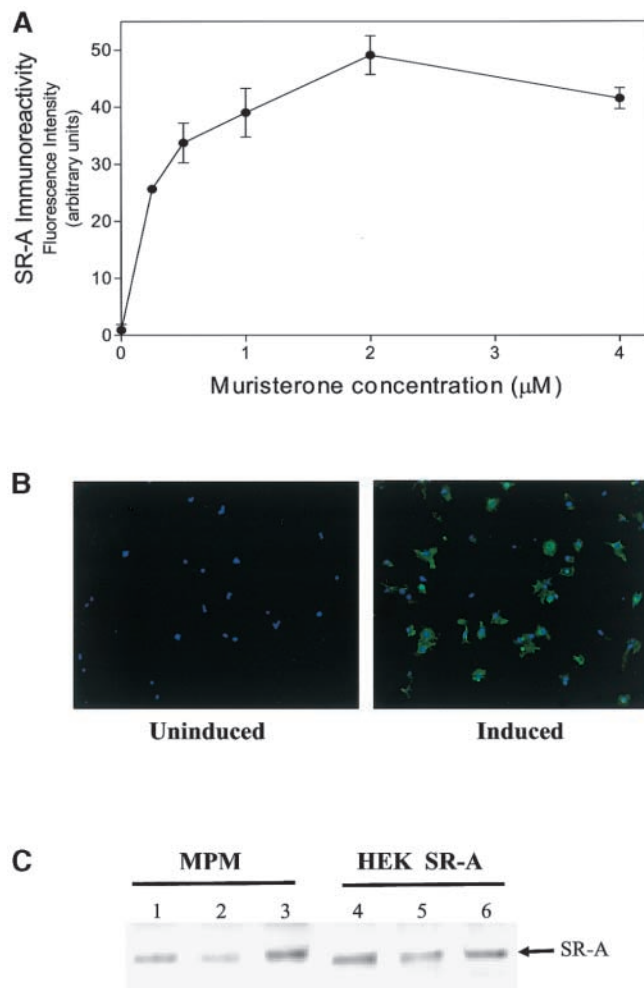
### Statistical analysis

For statistical analysis, data were analyzed using the GraphPad Prism program. Comparisons involving more than two groups were analyzed by one-way ANOVA. When a statistical difference was indicated, results were compared using a Bonferroni's post-test.

## RESULTS

### Inducible expression of SR-A in HEK cells

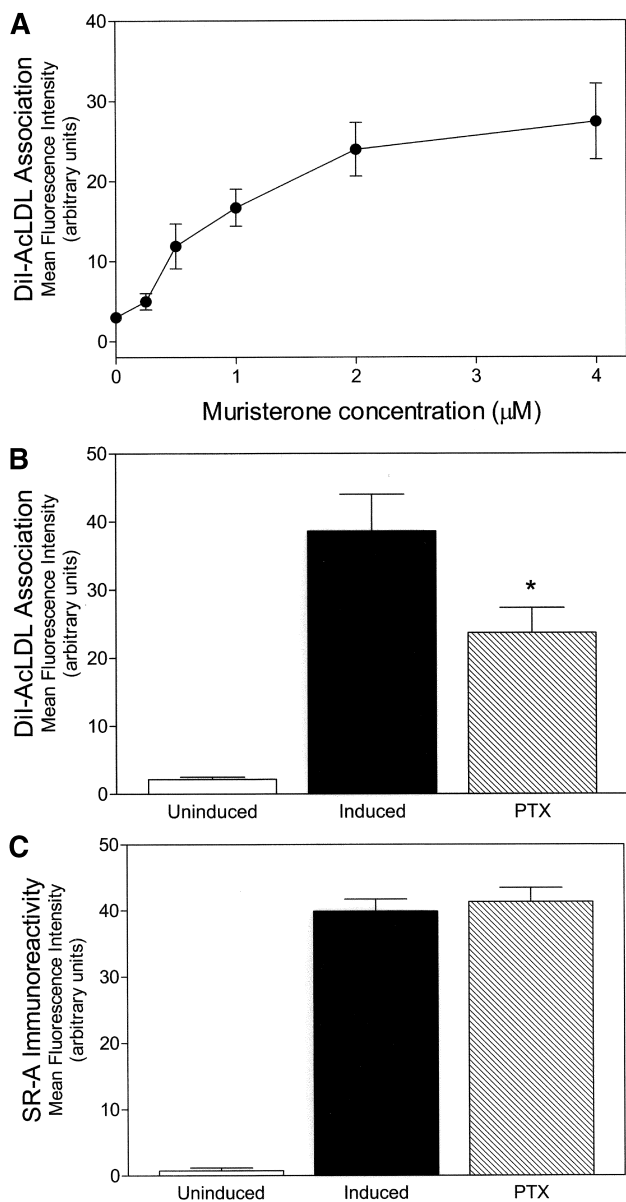
To facilitate studies related to the intracellular mechanisms that regulate SR-A-mediated endocytosis and adhesion, SR-A was expressed in HEK cells under the control of an ecdysone responsive promoter. The ability to control expression levels of SR-A minimizes potential problems associated with continuous high-level protein expression (e.g., upregulation of compensatory pathways). Muristerone-dependent SR-A expression in transfected HEK cells was assessed by flow cytometry and immunostaining with the SR-A-specific antibody 2F8 and by Western blotting using a guinea pig antisera against SR-A. As shown in **Fig. 1A**, muristerone induced SR-A expression in a concentration-dependent manner that was maximal by 2  $\mu$ M. Inducible SR-A expression in transfected cells was verified by immunostaining cells treated with muristerone (4  $\mu$ M) for 24 h. Consistent with results from flow cytometry, SR-A was not detected in uninduced cells (**Fig. 1B**) or in HEK cells transfected with  $\beta$ -galactosidase (data not shown) but was readily detected in cells treated with muristerone (**Fig. 1B**). Western blotting of equivalent amounts of cell lysates with a guinea pig SR-A antisera demonstrate that SR-A expression following muristerone treatment was comparable to endogenous expression levels observed in isolated mouse peritoneal macrophages (MPM) (**Fig. 1C**).



**Fig. 1.** Muristerone induces class A scavenger receptor (SR-A) expression in transfected HEK cells. **A:** HEK cells were incubated for 24 h with increasing concentrations of muristerone to induce SR-A expression. SR-A expression was assessed by determining the cell-association of a fluorescently labeled anti-SR-A antibody (2F8-RPE) as described in Experimental Procedures. Data represent the mean fluorescence intensities  $\pm$  SD from two separate experiments. **B:** HEK cells were cultured in the absence (left) or presence (right) of muristerone to induce SR-A expression. SR-A was detected using 2F8 and a fluorescent secondary antibody. Nuclei were stained with DAPI. Images (10 $\times$ ) were digitally captured using an Olympus IX70 inverted fluorescent microscope with the blue (DAPI) and green (SR-A) intensities kept constant for both the induced and uninduced cells. **C:** SR-A expression in cell lysates prepared from three separate mouse peritoneal macrophage (MPM) isolations (lanes 1–3) or three different HEK cell lysates induced with 4  $\mu$ M muristerone (lane 4–6) was determined by Western blotting of 15  $\mu$ g total cell lysates with a guinea pig SR-A-specific antisera.

### SR-A-mediated AcLDL uptake in transfected HEK cells is PTX sensitive

To assess SR-A-mediated endocytosis, DiI-AcLDL association was determined in uninduced or muristerone-treated (induced) HEK cells. Muristerone treatment increased DiI-AcLDL association in a concentration-dependent manner (**Fig. 2A**) that paralleled SR-A expression (**Fig. 1A**). Consistent with previously published results (9) and data



**Fig. 2.** Pertussis toxin (PTX) inhibits SR-A-mediated lipoprotein association but does not alter SR-A expression in transfected HEK cells. **A:** HEK cells were incubated with increasing concentrations of muristerone for 24 h to induce SR-A expression. SR-A-mediated lipoprotein uptake was assessed by determining the cell-association of a fluorescently labeled SR-A ligand (DiI-AcLDL) as described in Experimental Procedures. Nonspecific lipoprotein association was determined in the presence of the SR-A antagonist polyinosine (10 μg/ml) and was subtracted from each value. Data represent the mean fluorescence intensities ± SEM of three separate experiments. **B:** SR-A expression was induced by incubating cells with 4 μM muristerone for 24 h. As indicated, PTX (100 ng/ml) was added to the induced cells 16 h prior to assessing DiI-AcLDL association. Data are the mean fluorescence intensities ± SEM of sixteen separate experiments. **C:** HEK cells were incubated with muristerone (4 μM) to induce SR-A expression. As indicated, PTX (100 ng/ml) was added to the induced cells 16 h prior to assessing SR-A expression as described in Experimental Procedures. Data represent the mean fluorescence intensities ± SEM of four separate experiments.

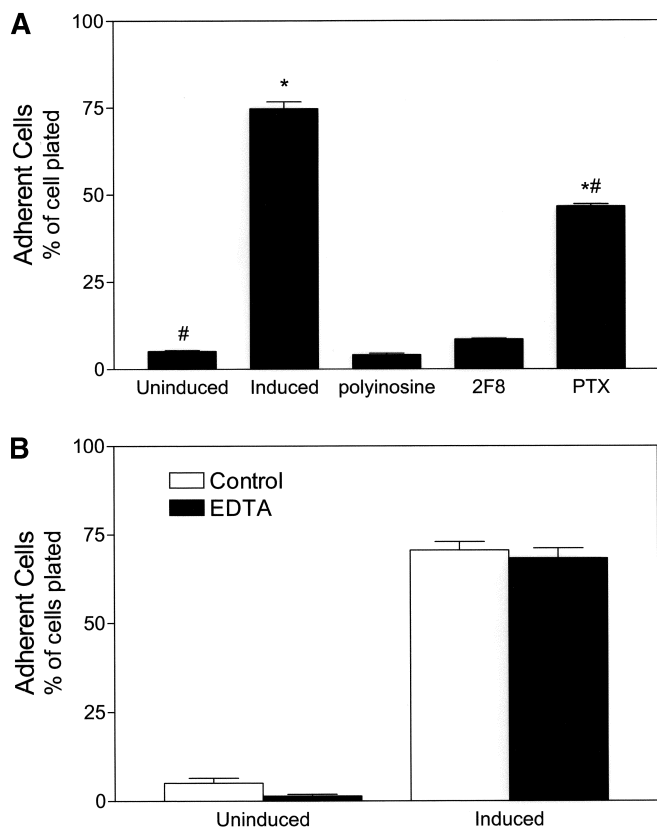
presented in Fig. 1, DiI-AcLDL did not associate with uninduced HEK cells, confirming the lack of endogenous SR-A expression in HEK cells. The role of  $G_{i/o}$  proteins in SR-A-mediated AcLDL uptake was assessed by treating HEK cells that express SR-A with PTX (100 ng/ml). Similar to our previous findings in MPM, PTX-treatment significantly reduced (by  $45.8 \pm 5.5\%$ ,  $P < 0.05$ ; Fig. 2B) DiI-AcLDL uptake in transfected HEK cells, indicating that  $G_{i/o}$  activation is involved in SR-A-mediated ligand internalization. PTX treatment did not alter the abundance of SR-A detected (Fig. 2C) by flow cytometry following incubation of intact cells with a fluorescently-labeled SR-A specific antibody (2F8-RPE). Similar association of SR-A antibody with untreated and PTX-treated cells indicates that decreased DiI-AcLDL uptake observed in PTX treated cells did not result from decreased SR-A expression on the cell surface. In contrast to its effect on DiI-AcLDL association, PTX treatment did not inhibit the association of DiI-LDL, an LDL receptor ligand, with HEK cells (data not shown), indicating that PTX treatment did not cause a generalized inhibition of receptor-mediated endocytosis. Together, these results indicate that SR-A-mediated endocytosis in transfected HEK cells is regulated by  $G_{i/o}$ -dependent signaling pathways in a manner similar to that described for SR-A endogenously expressed in MPM (18).

#### SR-A-mediated cell adhesion is PTX sensitive

To examine the role of SR-A in cell adhesion, we assessed the ability of HEK cells to adhere to tissue culture plates. As shown in Fig. 3, only  $5.2 \pm 0.5\%$  of uninduced cells adhered to tissue culture plates following a 10 min incubation compared to  $74.7 \pm 4.5\%$  of the cells treated with muristerone. Longer incubation times were required for adhesion of uninduced cells such that by adhesion of induced and uninduced cells was not significantly different after 60 min. Enhanced adhesion of induced cells was abolished by polyinosine and 2F8 (Fig. 3A), demonstrating that the observed increase in cellular adhesion was indeed mediated by SR-A. To examine the role of  $G_{i/o}$  activation in SR-A-mediated adhesion, we assessed adhesion of induced HEK cells pretreated with PTX (100 ng/ml; 16 h). Similar to results obtained for DiI-AcLDL uptake (Fig. 2B), PTX treatment reduced SR-A-mediated cell adhesion by  $37.3 \pm 4.6\%$ ,  $P = 0.04$ , with no further inhibition observed at higher PTX concentrations. PTX treatment did not alter cell viability as assessed by trypan blue exclusion in either adhered or non-adhered cells (data not shown). Furthermore, as shown in Fig. 3B, adhesion of induced cells was insensitive to chelation of divalent cations with EDTA (5 mM), indicating that enhanced cell attachment was independent of integrin binding.

#### SR-A expression promotes actin polymerization and formation of focal adhesion complexes

To address the role of the actin cytoskeleton in SR-A-mediated cell adhesion, we assessed the effect of SR-A expression on actin polymerization using phalloidin staining. As shown in Fig. 4A and B, SR-A staining was



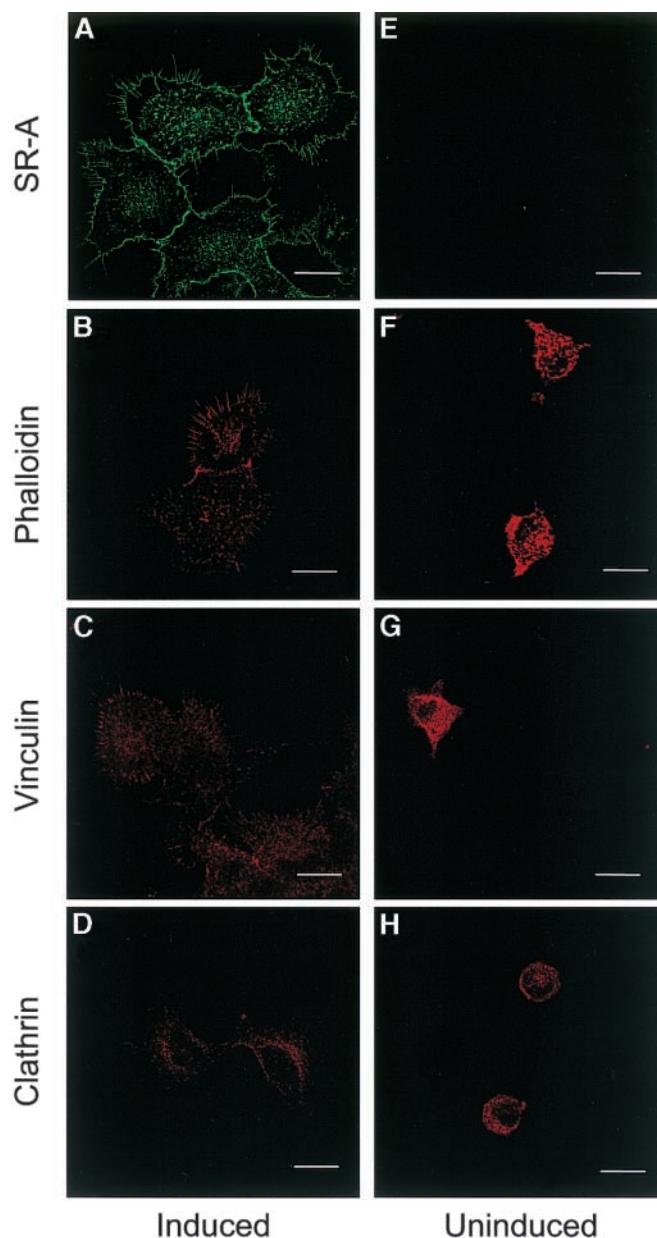
**Fig. 3.** SR-A expression enhances HEK adhesion by a PTX-sensitive mechanism. Except for uninduced cells, cells were incubated in the presence of muristerone for 24 h. As indicated, cells were also treated with PTX or polyinosine as described in Experimental Procedures. Treated cells were suspended with trypsin/EDTA, washed, and replated. The number of cells adhering after 10 min was determined and is represented as a percentage of the total number of cells plated. Data are means  $\pm$  SEM of at least three experiments. \*Denotes significantly different from uninduced values. #Denotes significantly different ( $P < 0.05$ ) from induced values. B: Cell adhesion was determined as described above with the following modifications. Prior to plating cells for 10 min, 5 mM EDTA was added to the incubation media to chelate divalent cations. Data are means  $\pm$  SEM of four experiments.

prominent in actin-containing processes that resemble filipodia. To determine whether SR-A-mediated adhesion was associated with formation of focal adhesion complexes, transfected HEK cells were immunostained with an antibody to vinculin. Vinculin is an actin binding protein that is considered a marker of focal adhesion complexes (13, 14, 19, 20). As shown in Fig. 4C, punctate vinculin immunostaining was observed in the cellular processes of induced cells, indicating that SR-A expression promotes formation of focal adhesion complexes. Because SR-A is endocytosed via clathrin-coated pits (21–23), we determined whether clathrin codistributed with SR-A. In contrast to phalloidin and vinculin, clathrin immunoreactivity was not observed in the filipodia-like projections (Fig. 4D). In the absence of SR-A expression (Fig. 4E), HEK cells did not exhibit filipodia-like projections when stained with phalloidin (Fig. 4F) or vinculin (Fig. 4G). The cellular distribution of clathrin immu-

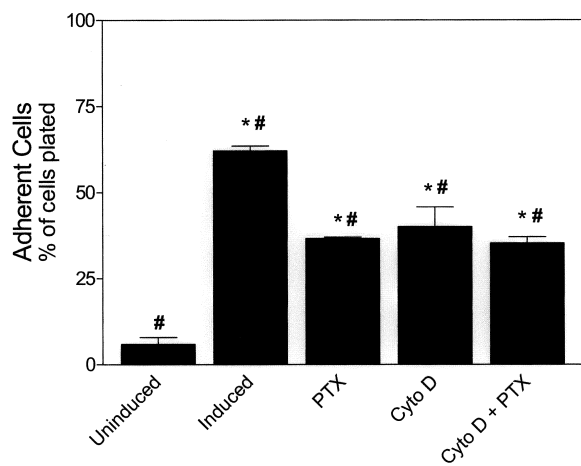
noreactivity in uninduced cells (Fig. 4H) was similar to that observed in induced cells (Fig. 4D).

### Inhibiting actin polymerization reduces SR-A mediated adhesion

To determine if actin polymerization was required for SR-A-mediated cell adhesion, we assessed the effect of inhibiting actin polymerization with cytochalasin D on adhesion of induced HEK cells. For these experiments, SR-A-expressing HEK cells were treated with cytochalasin D (2



**Fig. 4.** Expression of SR-A induces filipodial formations that contain SR-A and polymerized actin. HEK cells were plated on slides, fixed, and permeabilized as described in Experimental Procedures. Cells shown in A–D were induced to express SR-A, whereas cells shown in E–H lack SR-A expression. Cells were immunostained for SR-A expression (A and E); polymerized actin (B and F); vinculin (C and G); and clathrin (D and H). The scale bar represents 16  $\mu$ m.



**Fig. 5.** Inhibition of actin polymerization reduces SR-A-mediated adhesion. Except for uninduced cells, cells were incubated in the presence of muristerone for 24 h. As indicated, cells were also treated with PTX for 16 h. Cells were suspended with trypsin/EDTA, washed and treated with cytochalasin D (2  $\mu$ M) for 30 min prior to plating. The number of cells adhering after 10 min was determined and is represented as a percentage of the total number of cells plated. Data are means  $\pm$  SD of at least three experiments. \*Denotes significantly different from uninduced values. #Denotes significantly different ( $P < 0.05$ ) from induced values.

$\mu$ M) for 30 min prior to plating. As shown in **Fig. 5**,  $40.1 \pm 5.8\%$  of cytochalasin D treated cells adhered to the plate compared to  $62.1 \pm 1.3\%$  of untreated cells. No further inhibition of cellular adhesion was observed using cytochalasin D concentrations up to 10  $\mu$ M (data not shown). Cytochalasin D-mediated inhibition of cell attachment was similar in magnitude to that observed following PTX treatment ( $36.6 \pm 0.4\%$ ). Moreover, combined PTX and cytochalasin D treatment had no additional inhibitory effect on cell attachment ( $35.3 \pm 1.8\%$ ) relative to PTX or cytochalasin D treatment alone. The lack of additive effects suggests that PTX and cytochalasin D reduce SR-A-mediated adhesion via inhibition of a common pathway.

## DISCUSSION

The ability of SR-A to internalize a diverse array of ligands and mediate cell adhesion suggests that this receptor may serve multiple physiologic functions. At the cellular level, SR-A-mediated lipoprotein uptake is the best studied function of SR-A. SR-A binds modified lipoprotein and mediates ligand endocytosis via clathrin-coated pits (21–23). SR-A internalization is regulated by alterations in receptor expression and localization (24–28). In addition, ligand binding to SR-A promotes receptor phosphorylation and localization in clathrin-coated vesicles suggesting that SR-A-mediated uptake of modified lipoprotein is regulated by intracellular signaling pathways (21, 22, 29, 30). We recently reported that PTX treatment reduced AcLDL association with MPM, indicating the involvement of  $G_{i/o}$  signaling pathways in regulating SR-A-mediated lipopro-

tein internalization (18). The current studies demonstrate that SR-A-mediated cell adhesion is also regulated by activation of  $G_{i/o}$  signaling cascades and involves both actin polymerization and formation of focal adhesion complexes.


SR-A promotes excessive cellular uptake of modified lipoprotein to promote foam cell formation, suggesting a proatherogenic role for SR-A. Several studies demonstrate that changing SR-A expression alters atherosclerotic lesion development and immune function. For example, SR-A deficient mice exhibited a 58% reduction in atherosclerotic lesion formation (7). However, mice that overexpress SR-A also showed decreased lesion formation (31). Together, studies conducted in SR-A-deficient or overexpressing mice indicate both pro- and anti-atherogenic roles for SR-A (4, 32). In addition to mediating the endocytosis of modified LDL, SR-A binds to certain bacterial products including endotoxin and lipoteichoic acid, and SR-A mediates phagocytosis of apoptotic thymocytes by macrophages (33–35). A physiological role for SR-A in host defense is further supported by the observation that SR-A deficient mice exhibited impaired clearance of bacteria from sites of infection and a greater susceptibility to infection with *Listeria monocytogenes*, *Staphylococcus aureus*, and herpes simplex virus type 1 (33, 36). Together, results obtained with SR-A deficient mice clearly demonstrate the importance of SR-A in normal and pathophysiologic processes.

Several studies suggest that SR-A may also be important for macrophage recruitment, adhesion, and retention at sites of tissue damage. For example, relative to macrophages isolated from wild-type mice, SR-A-deficient macrophages exhibit reduced spreading on tissue culture plates (7). In contrast, peritoneal macrophages isolated from transgenic mice with macrophage-specific overexpression of SR-A showed enhanced spreading relative to cells isolated from nontransgenic mice (37). Moreover, SR-A overexpressing transgenic mice displayed enhanced granuloma formation resulting from enhanced macrophage accumulation at the site of a subcutaneous injection of the SR-A ligand carrageenan (37). Additional studies indicate that the contribution of SR-A to cell adhesion may depend on the activation state of the macrophage (38). Relative to resident peritoneal macrophages, thioglycollate-elicited peritoneal macrophages displayed a significant increase in SR-A-mediated adhesion. Consistent with such results obtained in macrophages, HEK cells that express SR-A display enhanced adhesion and spreading relative to control HEK cells (Fig. 5).

To date, the best studied adhesion pathways are those associated with integrins. Integrin binding to extracellular matrix proteins initiates a series of intracellular events including assembly of cytosolic protein complexes, reorganization of cytoskeletal components, filopodia or lamellipodia formation, and ultimately the organization of focal adhesion complexes (13–15, 20). The codistribution of vinculin and polymerized actin in the filopodia-like projections of SR-A expressing cells suggests that SR-A promotes formation of focal adhesion complexes and en-

hances cell attachment by a process similar to that utilized by integrin proteins (19, 39–41).

In addition to cytosolic proteins, integrin function can be regulated by interaction with other membrane proteins (16, 42). For example, growth factor receptors, integrin associated protein/CD47, and the GPI-anchored uPA receptor form functional complexes with integrin proteins (43–48). Although our data do not exclude the possibility of an interaction between SR-A and an integrin, several observations indicate that the enhanced adhesion of induced HEK cells is mediated by SR-A and is independent of integrin binding. First, in the absence of SR-A, HEK cells adhere poorly to tissue culture plates. Second, the SR-A ligand polyinosine and the SR-A specific antibody, 2F8 completely attenuated enhanced adhesion of SR-A expressing cells. Lastly, in contrast to integrin-mediated adhesion, adhesion of SR-A expressing cells does not require divalent cations.

We have previously reported that in MPM,  $G_{i/o}$  activation increases SR-A-dependent AcLDL uptake. Our current results indicate that  $G_{i/o}$  activation similarly promotes SR-A-mediated cell adhesion, indicating that enhanced lipoprotein uptake and cell adhesion may be initiated by a common mechanism. Taken further, our results suggest that the cellular consequence of SR-A binding may depend on the ligand. For example, interaction of SR-A with AcLDL or other soluble ligands may promote receptor endocytosis by enhancing an interaction of SR-A with endocytic proteins (e.g., clathrin). On the other hand, receptor clustering on the cell surface, as may occur when SR-A binds to modified extracellular matrix proteins or other immobilized ligands, would induce actin polymerization, formation of focal adhesion complexes, and enhanced cell adhesion. Future studies will examine the signaling pathways that preferentially regulate SR-A-mediated ligand uptake or cell adhesion. 

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