Published in final edited form as:
Oral Microbiol Immunol. 2009 December ; 24(6): 456–463. doi:10.1111/j.1399-302X.2009.00538.x.

Scavenger receptor A is expressed by macrophages in response to *Porphyromonas gingivalis*, and participates in TNF-α expression

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Abstract

Introduction—*Porphyromonas gingivalis* is a periodontopathic bacterium closely associated with generalized aggressive periodontal disease. Pattern recognition receptors (PRRs) participate in host response to this organism. It is likely that PRRs not previously recognized as part of the host response to *P. gingivalis* also participate in host response to this organism.

Methods and Results—Employing qRT-PCR, we observed increased *msr1* gene expression at 2, 6, and 24h of culture with *P. gingivalis* strain 381. Flow cytometry revealed increased surface expression of SR-A protein by the 24h time point. Macrophages cultured with an attachment impaired *P. gingivalis fimA*- mutant (DPG3) expressed intermediate levels of SR-A expression. Heat-killed *P. gingivalis* stimulated SR-A expression similar to live bacteria, and purified *P. gingivalis* capsular polysaccharide stimulated macrophage SR-A expression, indicating that live whole organisms are not necessary for SR-A protein expression in macrophage response. As SR-A is known to play a role in lipid uptake by macrophages, we tested the ability of low-density lipoprotein (LDL) to influence the SR-A response of macrophages to *P. gingivalis*, and observed no effect of LDL on *P. gingivalis*-elicited SR-A expression. Lastly, we observed that SR-A knockout (SR-$A^{-/-}$) mouse macrophages produced significantly more tumor necrosis factor (TNF)-α than wild type mouse macrophages cultured with *P. gingivalis*.

Conclusion—These data identify that SR-A is expressed by macrophages in response to *P. gingivalis*, and support that this molecule plays a role in TNF-α production by macrophages to this organism.

Introduction

Periodontal disease is one of the most common chronic inflammatory diseases of humans. Indeed, an estimated 100 million individuals in the United States possess clinically measurable oral bone loss (62). *Porphyromonas gingivalis* is a Gram-negative anaerobe bacterium, comprising part of the sub-gingival plaque, and is considered an organism closely associated with generalized forms of periodontal disease (63). *P. gingivalis* possess a broad armamentarium of identified virulence factors (36), including gingipains (50,61), fimbriae (31,72), lipopolysaccharide (LPS; (3,39)), and capsular polysaccharide (6,35,71). As this organism is present in both healthy individuals and patients with periodontal disease, it is unclear which aspects of this host – pathogen interaction allow for disease to ensue.

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Inflammation elicited by periodontopathic bacteria is thought to play a significant role in the development of periodontal disease, accounting for the soft and hard tissue destruction that accompanies this disease. Indeed, elevated levels of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 (15,55,74), chemokines including IL-8, MCP-1, RANTES (12,69), C-reactive protein (9,56), and mRNA for toll-like receptors (53) have been detected in samples obtained from patients with periodontitis. Yet not all studies are in agreement as to the particular mediators that are best associated with periodontal disease (14,54,74). Thus, a complex and likely incompletely defined picture has emerged regarding our understanding of the innate immune response during periodontal disease and to organisms associated with periodontal disease including *P. gingivalis*.

Pattern recognition is a principal strategy for host recognition of pathogens (30). Germ-line encoded receptors, termed pattern recognition receptors (PRR), provide immunological sensing and recognition of conserved microbial epitopes, thus providing sensitivity to a vast array of pathogens through recognition of a limited number of epitopes (40). Several families of PRRs have been identified and include toll-like receptors, NODs, CD14, CR3, lectins, and scavenger receptors (1). Phagocytic cells such as macrophages are known to express many of these PRRs (19). Ligation of cognate antigen by PRRs such as TLRs on macrophages induces a series of intracellular signaling events, NF-κB activation, and culminating in expression of inflammatory mediators (26). Clinical and experimental studies have identified that PRR-dependent recognition of *P. gingivalis* is an initial step in host response to this organism, and TLRs have emerged as a major group of PRRs involved in recognition and signaling in the context of *P. gingivalis* exposure. Indeed, experimental studies have identified that signaling through TLR2 leads to oral bone loss in mice (5,17), and that TLRs participate in epithelial cell, endothelial, and monocytic cell inflammatory response to *P. gingivalis* and several of its antigens including LPS, and FimA protein (2,10,13,24,27,28,32,52,57,68,75). Yet despite these reports, clinical studies employing a genetic polymorphism approach are not in agreement regarding major roles for TLRs in periodontal disease. Schroder *et al*. (59) reported that a TLR4 polymorphism is associated with periodontitis, while Folwaczny *et al*. (14) failed to observe associations for TLR2 or TLR4. Thus it is likely that in addition to TLRs, other unrecognized PRR may contribute to periodontal disease and host response to periodontal pathogens including *P. gingivalis*.

Scavenger receptors are a broad group of PRRs with at least 8 identified classes, possess little by way of sequence homology, but which recognize similar polyanionic ligands (42). Scavenger receptors were first identified for binding and uptake of modified low-density lipoproteins by macrophages (4), and subsequent studies have shown that several scavenger receptors may play a key role in atherosclerotic cardiovascular disease due to their ability to promote lipid uptake by cells (34,60). The best-characterized scavenger receptors are the class A scavenger receptor, SR-A, and class B scavenger receptor, CD36. SR-A is expressed primarily by macrophages (29) is encoded by the gene *msr1*, and alternative splicing leads to three isoforms. Two (SR-AI and SR-AII) are expressed on the surface of cells, and one (SR-AIII) is sequestered to endoplasmic reticulum and likely functions as a dominant negative isoform to regulate SR-A expression (33). SR-A has been implicated in atherosclerosis due to its localization in human vascular lesions (43), and its binding and uptake of low-density lipoprotein (34), and in Alzheimer's disease, in which it localizes to microglia in senile plaques (7). Emerging data point to a significant role for SR-A in host defense. SR-A is implicated as receptor for bacteria including *Escherichia coli* (46), *Enterococcus faecalis* (21), *Mycobacterium tuberculosis* (76), and *Staphylococcus aureus* (11) and binds enterobacterial LPS (23), LTA (11), and bacterial surface proteins (47). Studies using SR-A knockout (SR-A$^{-/-}$) mice have identified that SR-A deficiency renders increased susceptibility to *Listeria* and herpes simplex virus type 1 (65), *Staphylococcus aureus* (67), and *Neisseria meningitidis* (49) infections. Haworth *et al*. (25) reported that SR-A$^{-/-}$ mice

challenged with *Bacillus Calmette Guérin* (BCG) formed normal granuloma, but were more susceptible to subsequent LPS exposure and endotoxic shock mediated by increased levels of TNF-α. SR-A$^{-/-}$ mice have also been shown to produce a more robust pro-inflammatory IL-6 response than matched wild type animals challenged with *N. meningitides* (49). Little is known about scavenger receptors in periodontal disease or host response to periodontal pathogens such as *P. gingivalis*. Use of SR-A$^{-/-}$ mice has shown an important role for SR-A in normal osteoclast function (37). Recently, Triantafilou *et al*. (68) reported that the class B scavenger receptor, CD36, is localized to lipid rafts with TLR1, TLR2, CD11b, and CD18, and thus may participate in uptake of *P. gingivalis* by human vascular endothelial cells.

As scavenger receptors play a role in innate immunity and host response to infection, we hypothesized that SR-A is induced in macrophages in response to *P. gingivalis* and participates in host pro-inflammatory response to this organism. We report elevated expression of the previously unrecognized PRR, SR-A, in murine macrophages exposed to *P. gingivalis*. We investigated the contribution of *P. gingivalis* virulence factors on the macrophage SR-A response, and observed that the capsular polysaccharide of *P. gingivalis* elicits SR-A expression in macrophages. Lastly, employing SR-A$^{-/-}$ macrophages we observed a role for this scavenger receptor in TNF-α production following challenge with *P. gingivalis*. Collectively, our data suggest that SR-A contributes to the host response to *P. gingivalis*.

Materials and Methods

P. gingivalis strains and cultivation

Wild type *P. gingivalis* strains 381, as well as the *P. gingivalis* strain 381 *fimA*-deficient mutant DPG3 were grown 3–4 days anaerobically on BHI blood agar plates followed by 24 hour growth in BHI broth supplemented with hemin and menadione as described previously (8). An additional supplement for growth of strain DPG3 was 10μg/ml erythromycin (75). Following broth growth, the bacteria were collected, washed, suspended to an OD_{660} of 1 (equivalent to 1×10^9 CFU/ml) in RPMI-1640 + 10% heat-inactivated FBS without antibiotics, diluted to the indicated multiplicity of infection (MOI), prior to addition to macrophage cultures. Heat-killed *P. gingivalis* preparations were generated from broth growth by incubating an aliquot at 60°C for 30 minutes; plate counts were used to confirm kill. These heat-killed preparations were then added to macrophage cultures as indicated above for live *P. gingivalis*.

Purification of *P. gingivalis* capsular polysaccharide

Capsular polysaccharide of *P. gingivalis* was purified from strain W83 using a modified version of the procedure of Schifferle *et al*. (58), as we described previously (8). In brief, overnight broth cultures of *P. gingivalis* W83 were washed, subjected to hot phenol-water extraction, the aqueous phase collected, and was sequentially treated with RNAse, DNAse and proteinase K. The enzyme treated extract was then concentrated, applied to a S-400 resin column for separation of material by size, and fractions containing only the high molecular mass capsular polysaccharide were pooled, lyophilized, and stored frozen at −20°C. Purity was confirmed as described previously (18), and included spectrophotometric scans, silver staining of SDS-PAGE gels, and endotoxin assessments. *P. gingivalis* capsule was suspended in culture medium and added to cells as indicated.

Mouse strains and murine macrophages

The C57BL-6 mice used in these studies were purchased (Jackson Laboratories, Bar Harbor, ME). A colony of SR-A knockout mice was established from purchased breeding pairs (strain *msr1*$^{-/-}$; SR-A$^{-/-}$; Jackson Laboratories), and the genotype of the SR-A$^{-/-}$ mice was

confirmed by PCR using the vendor described protocol. All mice received food and water *ad libitum*. All studies were performed in accordance with Boston University Institutional Animal Care and Use Committee approvals. Peritoneal macrophages were harvested 3 days after interperitoneal injection of sterile 3% thioglycolate broth. These cells were washed, counted, diluted to 5×10^5 cells/ml in RPMI-1640 + 10% heat-inactivated fetal bovine serum and penicillin/streptomycin, and were added to wells of cell culture dishes. Media was replenished every other day with fresh antibiotic-free medium 24h prior to macrophage challenge.

Macrophage challenge

P. gingivalis organisms suspended in antibiotic-free RPMI-1640 complete medium were added to macrophage cultures at MOIs of 1, 10, and 100, as indicated. In similar assays, cells were incubated with heat-killed *P. gingivalis* strain 381, or purified *P. gingivalis* capsular polysaccharide. In some assays, human low-density lipoprotein (LDL; Intracell, Frederick, MD) was added to determine the effect of cholesterol on *P. gingivalis*-elicited macrophage SR-A expression. All cells were challenged for up to 48 h. RNA was harvested from macrophages challenged for 2, 6, and 24 h and analysis of *msr1* gene expression was accomplished by quantitative RT-PCR. Macrophage surface expression of SR-A protein was determined by flow cytometry at 6, 24, or 48 h of *P. gingivalis* exposure. For TNF-α measurements, thioglycolate-elicited macrophages from C57BL-6 and SR-$A^{-/-}$ mice were cultured with *P. gingivalis* strain 381 at MOI of 100 for 6h, culture supernatant fluids were collected, and were stored at −20°C for analysis by ELISA.

Extraction of RNA and real-time RT-PCR

After removal of culture supernatants, macrophages were washed with PBS, and total RNA was extracted using RNEasy kit (Qiagen, Valencia, CA) following manufacturers instructions. Quantitative RT-PCR (qRT-PCR) was performed on mRNA using QuantiTect SYBR Green PCR kit (Qiagen) with QunatiTect primers (Qiagen) based on murine *msr1* (NM001113326) in an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA). GAPDH was used as an endogenous control to normalize expression levels. Expression of *msr1* was determined using delta delta Ct method, and fold expression of *msr1* gene by *P. gingivalis* challenged macrophages was calculated in relation to *msr1* gene expression of unchallenged macrophages at each time point.

Flow Cytometry

Macrophages were harvested from culture dishes by scraping, washed with sterile PBS + 2% BSA, and immediately incubated with the FITC-labeled 2F8 monoclonal antibody (AbD Serotec, Raleigh, NC), or FITC-labeled isotype matched antibody for 30 minutes on ice. A portion of unstained cells from each population served as unlabeled controls. The cells were then placed into a FACScan microflourimeter, and 10,000 events were recorded for histograms generation and determination of mean fluorescence intensity (MFI) using CellQuest software (BD Bioscience, San Jose, CA).

ELISA

Frozen cell culture supernatant fluids were thawed and TNF-α was determined using a murine specific ELISA kit per manufacturers instructions (DuoSet, R&D Systems, Minneapolis, MN). To accommodate for experimental variation we calculated the percent change in TNF-α expression by the SR-$A^{-/-}$ macrophages as a function of the WT macrophage response. We first normalized the TNF-α pg/ml values for the *P. gingivalis* challenged wild type macrophages for each experiment. We next calculated the percentage of TNF-α present in each sample as compared to the wild type challenged macrophage mean

within each experiment (n = 3, total of 11 mice). We then calculated the TNF-α levels in SR-A$^{-/-}$ macrophage cultures within each experiment (n = 3, total of 11 mice) as a percentage of mean TNF-α level for WT macrophages cultured with *P. gingivalis*.

Statistical analyses

Experiments were performed at least 3 times. Data were imported into Prism V analysis package (Graphpad Inc, La Jolla, CA) and descriptive and comparative statistical analyses were performed as indicated. With exception of histograms, all data are presented as means ± standard error of the mean (SEM). A $P < 0.05$ was considered significant.

Results

P. gingivalis stimulates *msr1* gene expression in mouse macrophages

P. gingivalis is known to stimulate an array of inflammatory markers from macrophages including PRRs. As SR-A functions as a PRR we wanted to determine if SR-A was expressed in macrophages cultured with *P. gingivalis*. Employing thioglycolate-elicited C57BL-6 mouse macrophages we observed elevated levels of *msr1* expression as early as 2 hours post-challenge (1.9-fold ± 0.3 over unchallenged macrophages). Moreover, elevated expression was observed 6 and 24h following challenge, with 2.7-fold ± 0.3 and 2.6-fold ± 0.7 increases over unchallenged controls, respectively (Fig. 1). These data provide evidence for increased expression of the SR-A gene, *msr1*, by macrophages exposed to *P. gingivalis*.

P. gingivalis stimulates increase in SR-A surface protein expression in macrophages

In light of elevated *msr1* gene expression elicited by *P. gingivalis*, we were interested to determine if an increase in surface expressed SR-A protein could be detected. To accomplish this, murine macrophages were cultured with *P. gingivalis* and SR-A protein expression was measured on the surface of challenged cells by flow cytometry. First we assessed the temporal kinetics of SR-A expression in response to *P. gingivalis* strain 381 at a MOI of 100. We observed no detectable change in macrophage surface expressed SR-A following 6 hours of *P. gingivalis* exposure (data not shown); however, SR-A expression was significantly increased in macrophages cultured with *P. gingivalis* for 24h (Fig. 2A). Assessing SR-A expression at 48 h we found no significant differences in SR-A expression by macrophages exposed to *P. gingivalis* for 24h (data not shown). To determine the sensitivity of macrophages to numbers of *P. gingivalis*, we incubated macrophages with increasing MOIs of *P. gingivalis* from 1 to 100 and assessed SR-A expression following 24h exposure. Low-level expression of SR-A by macrophages was observed in response to a MOI of 1, while maximal expression occurred in response to a MOI of 100 (Fig. 2B). Taken collectively, these data show increased macrophage SR-A protein expression in response to *P. gingivalis* exposure.

P. gingivalis FimA plays a partial role in stimulating SR-A expression in macrophages

The major fimbriae of *P. gingivalis* are known to play an important role in oral bone loss elicited by this organism (38), and contribute to *P. gingivalis* attachment and invasion of cells (44,73). Thus we were interested to determine if *P. gingivalis* FimA was necessary for *P. gingivalis* to elicit macrophage SR-A expression. To test this, we challenged macrophages with wild type *P. gingivalis* strain 381 or the *fimA*- mutant DPG3 at MOI of 100 for 24h and measured SR-A expression by flow cytometry. Unchallenged macrophages produced low levels of SR-A, while wild type *P. gingivalis* elicited high levels of macrophage SR-A expression. We observed that *P. gingivalis fimA*- mutant challenged macrophages expressed lower levels of SR-A than macrophages cultured with wild type *P. gingivalis*; however, the level of SR-A was not significantly different between the two

groups (Fig. 3A). Further analysis revealed that expression of SR-A in unchallenged macrophages and macrophages cultured with the *fimA*- mutant were statistically similar despite the slight increase in SR-A elicited by *fimA*- challenged macrophages (Fig. 3A). These data suggest that FimA protein may play a partial role in the ability of *P. gingivalis* to stimulate macrophages to express SR-A.

Viability is not required for *P. gingivalis* stimulation of SR-A, and capsular polysaccharide from this organism will induce a SR-A response

As the *fimA*- mutant stimulated low-level SR-A expression from macrophages, we were interested to know if killed *P. gingivalis* stimulated SR-A expression. To address this, we challenged macrophages with live and heat-killed *P. gingivalis* and assessed SR-A expression by flow cytometry. We observed that macrophages cultured with live or heat-killed *P. gingivalis* produced similar levels of SR-A expression (Fig. 3B). Taken together with results in macrophages cultured with the attachment attenuated *fimA*-mutant, we reasoned that other *P. gingivalis* antigens might possess SR-A stimulating activity. To test this, we incubated macrophages with a surface expressed antigen of *P. gingivalis*, capsular polysaccharide. Indeed, we observed that macrophages incubated with 10 μg/ml of *P. gingivalis* capsular polysaccharide stimulated a SR-A response (Fig. 3C).

Exogenous LDL does not influence SR-A expression by macrophages cultured with *P. gingivalis*

As SR-A is best known for lipid uptake by cells leading to the macrophage foam cell phenotype characteristic of atherosclerotic lesions (34,64), we tested the ability of exogenous LDL to influence SR-A expression by macrophages cultured with *P. gingivalis*. In these studies, macrophages were pre-incubated with either medium alone or medium containing human LDL. Cells were then washed and replenished with fresh culture medium with or without LDL to which was added *P. gingivalis* 381 at a MOI of 100, and flow cytometry was performed 24h later. We observed that macrophages incubated in medium either in the presence or absence of LDL expressed similar low levels of SR-A (Fig. 4). As expected, *P. gingivalis* stimulated macrophage SR-A expression in the absence of LDL; however, addition of exogenous LDL did not influence SR-A expression in macrophages cultured with *P. gingivalis*. These data indicate that exogenous LDL does not affect SR-A expression by macrophages cultured with *P. gingivalis*.

SR-A participates in TNF-α expression in macrophages cultured with *P. gingivalis*

TNF-α is though to play a key role in host pro-inflammatory responses seen in periodontal disease as well as in host responses to *P. gingivalis*. TNF-α is the factor in culture supernatants collected from *P. gingivalis* challenged macrophages that drives *in vitro* osteoclastogenesis, and TNF-α antagonists reduce bone resorption elicited by *P. gingivalis* infection (20,22,66,70), supporting a key role for TNF-α in bone loss. Moreover, it has been reported by others that SR-A participates in the magnitude of pro-inflammatory cytokine IL-6 production in mice infected with *N. meningitidis* (49), and in TNF-α expression by LPS following BCG exposure (25). To determine if SR-A participates in the pro-inflammatory host response to *P. gingivalis*, we cultured macrophages from WT and SR-A$^{-/-}$ mice in medium alone or medium containing *P. gingivalis* strain 381 at a MOI of 100 for 6h, and measured culture supernatant fluid levels of TNF-α by ELISA. As anticipated, WT and SR-A$^{-/-}$ mice cultured in medium alone failed to produce detectable levels of TNF-α (data not shown); however, we observed that macrophages from SR-A$^{-/-}$ mice expressed significantly more TNF-α than WT macrophages in response to *P. gingivalis* (Fig. 5). These data indicate that SR-A is not merely expressed by macrophages exposed to *P. gingivalis*, but support a role for SR-A in the inflammatory response of macrophages to *P. gingivalis*, specifically through modulating expression of the pro-inflammatory molecule TNF-α.

Discussion

Here we report that a molecule not previously recognized in host responses to *P. gingivalis*, SR-A, is expressed by macrophages exposed to *P. gingivalis*. We provide kinetic data detailing transcriptional analysis of the *msr1* gene as well as surface expression of SR-A protein. Moreover, we report that major fimbriae of *P. gingivalis* may play a partial role in evoking this response from macrophages, as a *P. gingivalis fimA*- mutant elicited SR-A albeit less than wild type *P. gingivalis* strain 381 challenged macrophages. Heat-killed *P. gingivalis* stimulated SR-A expression similar to live organisms and purified capsular polysaccharide of *P. gingivalis* possesses the ability to evoke a SR-A response. These latter findings identify that viability of *P. gingivalis* in not required for SR-A stimulation, and support that at least one *P. gingivalis* antigen has the ability to elicit SR-A expression in macrophages independent from live bacteria. Moreover, use of SR-A$^{-/-}$ macrophages implicated SR-A in TNF-α expression by macrophages exposed to this organism.

It is widely accepted that the host inflammatory response to periodontopathic bacteria such as *P. gingivalis* plays a major role in the soft and hard tissue responses that lead to loss of dentition. To date SR-A has not been reported as part of the molecular repertoire of PRRs present in periodontal lesions, or to experimental *P. gingivalis* exposure, yet pattern recognition is a key instructive component to host innate immune response to pathogen (41). Indeed, SR-A and other scavenger receptors participate in host pathogen interaction, and roles for scavenger receptors in immunity have emerged (45,48). We observed that loss of SR-A led to a more robust TNF-α response then that observed with wild type macrophages exposed to *P. gingivalis*. Indeed, SR-A and other scavenger receptors have been reported to play roles in host pathogen interaction, and in regulation of pro-inflammatory cytokine expression during infection. Employing a *N. meningitidis* model, it was recently reported that SR-A$^{-/-}$ mice produced a more robust circulating IL-6 response to infection than wild type mice (49). Haworth *et al*. (25) reported that BCG primed SR-A$^{-/-}$ mice were more susceptible to LPS and endotoxic shock linked to elevated TNF-α expression. In the context of periodontal disease, a key role for TNF-α has been demonstrated in host response to *P. gingivalis*, including regulation of osteoclastogenesis *in vitro* (70), and *in vivo* (20). We observed elevated TNF-α production in the absence of SR-A to pathogen exposure as the previous studies, albeit in our study we employed macrophages while the prior studies employed mouse infection models (25,49). Regardless of model system, our studies are in agreement with these two studies, that in the absence of SR-A, a more substantial inflammatory response to infection can occur, thus implicating SR-A as a regulator of inflammation.

We do not know the specific consequence of our findings in the context of *P. gingivalis*-infection. Animal modeling and *in vitro* assays identify TNF-α as a factor driving osteoclast formation (20,70). Moreover, elevated levels of TNF-α have been detected in gingival crevicular fluids of individuals with periodontal disease (55). As we observed that absence of SR-A leads to a more robust TNF-α response by macrophages to *P. gingivalis*, we speculate that increased expression of SR-A as a result of cell exposure to this organism and its antigens could provide a measure of immunological control to fine-tune TNF-α production at the site of infection, thus limiting development of the inflammatory lesion, tissue destruction, and bone loss characteristic of periodontal lesions. Conversely, the absence of functional SR-A would permit for increased TNF-α expression resulting in enhancement of inflammation, tissue destruction, and bone loss. Precisely how SR-A functions to regulate inflammation in the context of *P. gingivalis* infection is not known, thus detailed studies are needed to define the precise role of SR-A in the context of cellular inflammatory lesion development and oral bone loss that accompanies *P. gingivalis* infection.

SR-A is known to play an important role in atherosclerosis. Interestingly, our observation that SR-A is induced in macrophages cultured with *P. gingivalis* may provide a mechanistic link to lipid accumulation resulting in foam cell formation in macrophage, a characteristic of insipient atherosclerotic lesions. Elevated SR-A is expressed in atherosclerotic lesions (43), and hyperlipidemic mice lacking SR-A present with reduced levels of atherosclerotic plaque (65). At the cellular level SR-A imparts uptake of LDL by macrophages (34). Previously several groups have reported that *P. gingivalis* stimulates macrophage foam cell formation (16,51). As we observed that macrophages exposed to *P. gingivalis* as well as its capsular polysaccharide have increased SR-A expression, it is conceivable that increased SR-A expression facilitates increase lipid accumulation in infected cells, resulting in elevated foam cell formation. Additional studies are needed to determine if SR-A plays a role in *P. gingivalis*-elicited macrophage foam cell formation and atherosclerosis.

Prior to our current study, the only report to date implicating a scavenger receptor in host response to *P. gingivalis* reported that the class B scavenger receptor, CD36 co-localized with additional molecules in lipid rafts to bind *P. gingivalis* by vascular endothelial cells (68). As the class B scavenger receptor, CD36, is associated with host recognition of *P. gingivalis*, and SR-A expressed by macrophages regulates TNF-α expression by these cells exposed to *P. gingivalis*, it is clear that additional studies are necessary to determine the importance of scavenger receptors in host response to *P. gingivalis* in the context of inflammation, bone loss, and atherosclerosis.

Acknowledgments

The authors do not possess any conflicts of interest related to these studies. PHS funding principally supported this work though NIH/NIDCR grants DE014774 and DE018318 (FCG), and also by a Department of Medicine Pilot Project grants (FCG). The authors wish to thank Dr. Ellen Weinberg for critical reading of this manuscript, Ms. Deepa Vyas for technical assistance, and the BUMC FACS core.

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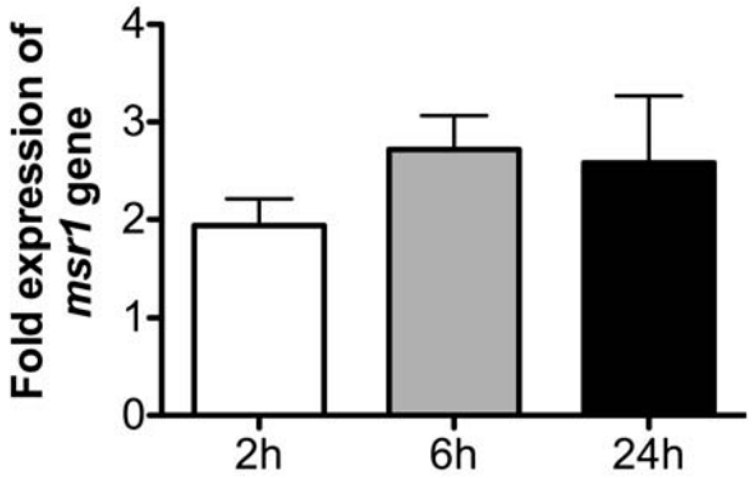

Fig. 1.

Temporal expression of the *msr1* gene by macrophages cultured with *P. gingivalis*. C57BL-6 mouse macrophages were cultured with *P. gingivalis* strain 381 at a MOI of 100, and RNA was harvested at 2, 6, and 24h of co-culture. Expression of the *msr1* gene was determined by qRT-PCR for both unchallenged and challenged macrophages and each sample was normalized to GAPDH. Values are presented as mean fold-expression of *msr1* gene for the challenged macrophages relative to unchallenged macrophage *msr1* gene expression from 3 separate experiments. Bar mean ± SEM.

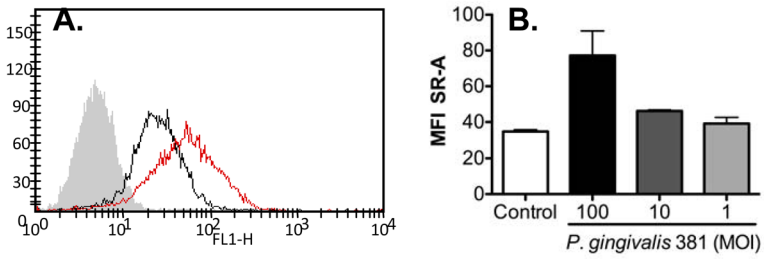

Fig. 2.

SR-A is expressed on the surface of macrophages cultured with *P. gingivalis*. C57BL-6 mouse macrophages were cultured in medium alone or medium with *P. gingivalis* strain 381 at a MOI of 100 for 24h, or *P. gingivalis* strain 381 at MOIs of 1, 10, or 100 for 24h and SR-A expression was determined by flow cytometry using FITC-2F8 monoclonal antibody. (A) Representative histogram of five separate experiments, unchallenged isotype labeled (grey shaded trace), unchallenged – 2F8 labeled (black trace), and *P. gingivalis* challenged – 2F8 labeled (red trace); (B) mean fluorescence intensity (MFI) of macrophage SR-A expression from cells cultured in medium alone (Control), or medium containing live *P. gingivalis* at the indicated MOIs. Bar mean ± SEM.

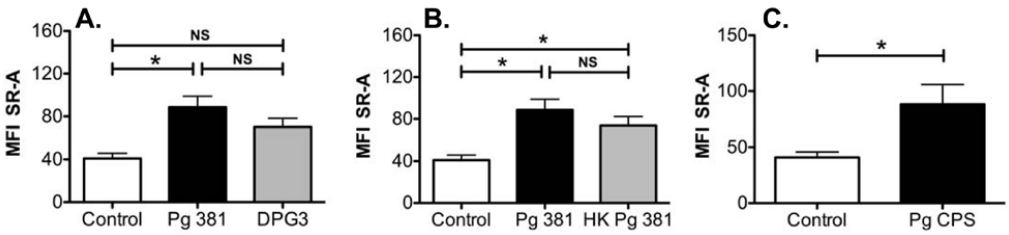

Fig. 3.

Attributes of *P. gingivalis* that stimulate SR-A expression by macrophages. Macrophages from individual mice were cultured in medium alone (Control, n=5), or medium containing *P. gingivalis* strain 381 (n=5), *P. gingivalis fimA*- mutant strain DPG3 (DPG3, n=5), heat-killed *P. gingivalis* 381 at an equivalent of MOI of 100 (HK Pg 381, n=5, or *P. gingivalis* capsular polysaccharide (Pg CPS, n=3) for 24h and mean fluorescence intensity (MFI) of SR-A was determined for each group of cells when stained with FITC-labeled 2F8 monoclonal antibody. (A) DPG3 stimulates a slight increase in SR-A expression by macrophages; (B) heat-killed *P. gingivalis* stimulate similar SR-A responses from macrophages; (C) *P. gingivalis* stimulates macrophages to produce SR-A. Bars represent mean fluorescence intensity (MFI) ± SEM. Fig. 3A and 3B were analyzed by ANOVA with Tukey post-tests, while Fig. 3C was analyzed using unpaired t test. * = $P < 0.05$, NS = not significant.

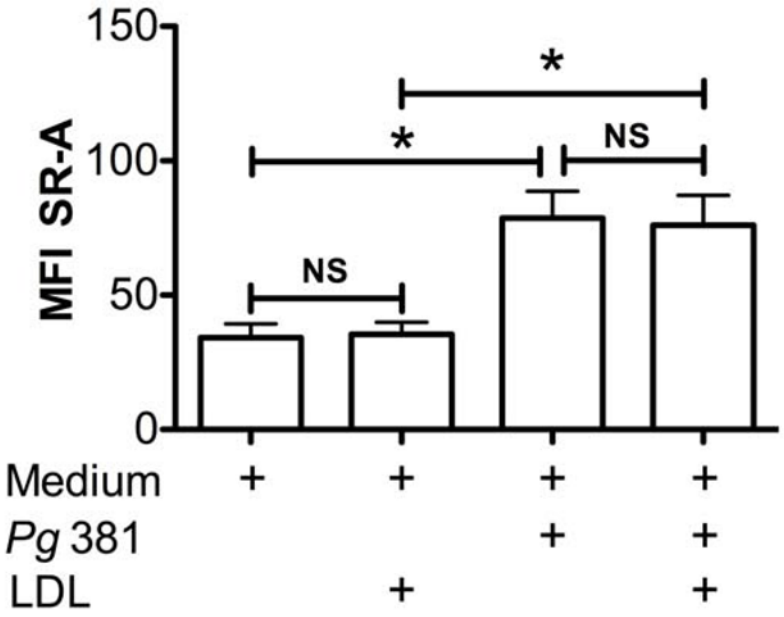

Fig. 4.

LDL does not affect SR-A expression by macrophages in response to *P. gingivalis*. Macrophages were cultured on medium alone (Medium), or with *P. gingivalis* strain 381 at MOI of 100 (*Pg* 381), either in the presence or absence of human LDL (200 μg/ml; LDL), and after 24h of culture, SR-A expression was determined by flow cytometry. Bars represent mean fluorescence intensities (MFI) ± SEM (n=3 mice per group). Data analyzed by ANOVA with Tukey post-tests. * = $P < 0.05$, NS = not significantly different.

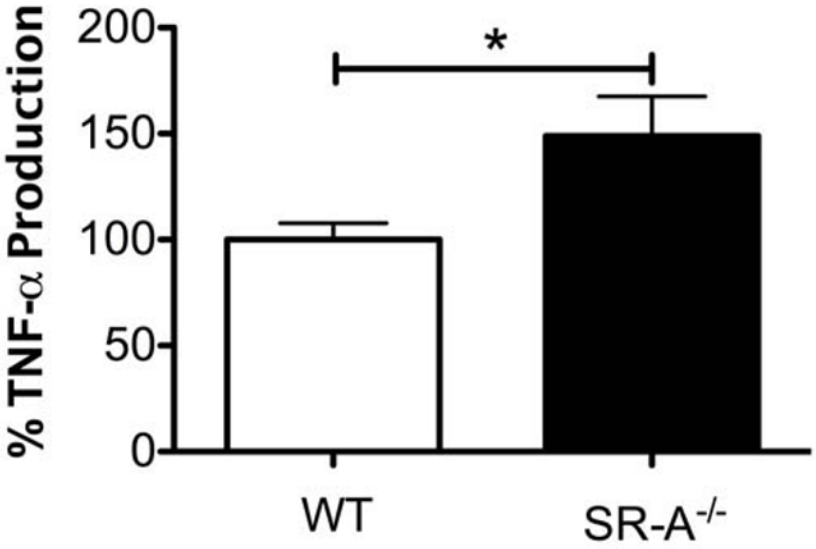

Fig. 5.

SR-A participates in the macrophage TNF-α response to *P. gingivalis*. WT and SR-A$^{-/-}$ mouse macrophages were cultured in medium alone or with *P. gingivalis* strain 381 at a MOI of 100 for 6h and culture supernatant levels of TNF-α were determined by ELISA. Due to experimental variability, the pg/ml value of TNF-α was transformed to % TNF-α produced and WT macrophage production to *P. gingivalis*, and the percentage change in TNF-α expression was then calculated for each mouse in relation to this mean. Bar represents mean ± SEM for 3 separate experiments using a total of 11 mice per group.