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A Role for the High-Density Lipoprotein Receptor SR-B1 in Synovial Inflammation via Serum Amyloid-A

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Acute phase apoprotein Serum Amyloid A (A-SAA), which is strongly expressed in rheumatoid arthritis synovial membrane (RA SM), induces angiogenesis, adhesion molecule expression, and matrix metalloproteinase production through the G-coupled receptor FPRL-1. Here we report alternative signaling through the high-density lipoprotein receptor scavenger receptor-class B type 1 (SR-B1). Quantitative expression/localization of SR-B1 in RA SM, RA fibroblast-like cells (FLCs), and microvascular endothelial cells (ECs) was assessed by Western blotting and immunohistology/fluorescence. A-SAA–mediated effects were examined using a specific antibody against SR-B1 or amphipathic α-Helical Peptides (the SR-B1 antagonists L-37pA and D-37pA), in RA FLCs and ECs. Adhesion molecule expression and cytokine production were quantified using flow cytometry and ELISA. SR-B1 was strongly expressed in the RA SM lining layer and endothelial/perivascular regions compared with osteoarthritis SM or normal control synovium. Differential SR-B1 expression in RA FLC lines ($n = 5$) and ECs correlated closely with A-SAA, but not tumor necrosis factor α–induced intercellular adhesion molecule-1 upregulation. A-SAA–induced interleukin-6 and -8 production was inhibited in the presence of anti–SR-B1 in human microvascular endothelial cells and RA FLCs. Moreover, D-37pA and L-37pA inhibited A-SAA–induced vascular cell adhesion molecule-1 and intercellular adhesion molecule expression from ECs in a dose-dependent manner. As SR-B1 is expressed in RA synovial tissue and mediates A-SAA–induced pro-inflammatory pathways, a better understanding of A-SAA–mediated inflammatory pathways may lead to novel treatment strategies for RA. ***(Am J Pathol 2010, 176:1999–2008; DOI: 10.2353/ajpath.2010.090014)***

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease characterized by proliferation of the synovial membrane (SM), which leads to degradation of articular cartilage and subchondral bone. Normal SM consists of a monolayer of synoviocytes including fibroblast-like cells (FLCs) and macrophage-like synoviocytes, which produce a proteoglycan-rich synovial fluid to lubricate the joint and provide nutrition to the avascular cartilage. A critical early event in synovial inflammation is angiogenesis, where new blood vessels develop from existing blood vessels and act as a conduit for the delivery of nutrition and invading immune cells into the joint. Recruitment of immune cells into the joint is mediated by tissue expression of chemokines and by expression of cell surface adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which selectively recruit mononuclear cells through their integrin receptors ligands.[1–3] In RA, activation of the SM transforms the lining layer into a hyperplastic tumor-like 'pannus' composed primarily of activated FLCs, macrophage-like synoviocytes, and lymphocytes, which through self perpetuating and persistent pro-inflammatory activation, are capable of destroying adjacent articular cartilage and bone.[4–6]

Acute phase serum amyloid A (A-SAA) is a highly-conserved acute phase apoprotein whose serum levels increase up to a 1000 fold within hours of an inflammatory stimulus.[7] Unlike other acute phase proteins, which are synthesized primarily in the liver as part of the systemic acute phase response, A-SAA is also markedly expressed at local sites of tissue inflammation. A-SAA is also known to be present at high levels in wound repair and in cancer tissues.[8] A-SAA, at normal serum levels,

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associates with high-density lipoprotein (HDL) forming a heterogenous HDL population containing both A-SAA and apolipoprotein A-1 (ApoA-1).[9] During the inflammatory response, however, A-SAA is dramatically elevated in serum (1 to 1000 μg/ml), at which levels A-SAA displaces ApoA-1 and saturates HDL resulting in high levels of free circulating A-SAA.[10,11] Our group has demonstrated a strong correlation between serum A-SAA and disease activity in RA.[12] Furthermore, we and others have demonstrated that A-SAA is produced by synovial FLCs and articular chondrocytes, where it is a powerful inducer of matrix metalloproteinases in these cells *in vitro*.[13–15] A-SAA induces the secretion of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-8 (IL-8) and acts as a chemoattractant for human monocytes, neutrophils and T cells.[16,17] A-SAA has high affinity for the extracellular matrix components laminin and fibronectin and has been shown to enhance leukocyte binding to extracellular matrix through a β1 integrin dependent mechanism.[18,19] We have demonstrated increased ICAM-1/VCAM-1 expression, increased endothelial cell (EC) migration, EC tubule formation, and matrix metalloproteinase 1 production in response to A-SAA in RA FLCs and ECs with an effect equipotent to TNF-α and IL-1β.[17] These studies together indicate that A-SAA may play a primary pathogenic role locally in joint destruction and mediating cartilage destruction.

A-SAA is known to bind to a formyl peptide receptor-like 1 (FPRL-1), a seven-transmembrane G protein–coupled receptor. Through FPRL-1, A-SAA interacts with a diverse array of exogenous and endogenous ligands in the inflammatory process including leukocyte migration, IL-8 induction, and matrix metalloproteinase production.[16,20–22] We, and others, have shown overexpression of A-SAA and its receptor FPRL-I in RA SM.[13,14,16] Lipoxin (LXA4) A4, an ecosanoid metabolite with anti-inflammatory actions, has recently been shown to inhibit the pro-inflammatory actions of A-SAA by competitive binding with FPRL-1 and differential regulation of the transcription factor nuclear factor-kappa B (NF-κB).[16,22] Recent evidence has demonstrated alternative A-SAA binding to and signaling through the HDL receptor human scavenger receptor class B type 1 (SR-B1).[23] SR-B1, also known CD36 and LIMPII analogous-1 (CLA-1), is the human orthologue of rodent SR-B1, which functions as a membrane bound receptor for HDL.[24] A-SAA binding to SR-B1 expressed on a monocyte cell line, induces chemokine production, activates extracellular signal-regulated kinases 1/2 (ERK1/2) and p38 mitogen activated protein kinases, a process that may involve cholesterol efflux.[23,25] A-SAA binding and signaling through SR-B1 have been shown to be inhibited by co-incubation with ApoA-1 or a synthetic mimetic peptide of ApoA-1 known as L-37PA in a dose-dependent manner.[23] Ligand binding of L-37PA to SR-B1 has also been shown to block the pro-inflammatory cytokine response to LPS in THP-1 cells, although to date this effect has not been demonstrated for intact ApoA-1.[26]

In this study we provide further evidence of a pathophysiological role of A-SAA in driving the pro-inflammatory response in RA through SR-B1. We demonstrate strong expression of SR-B1 in RA SM and differential expression of SR-B1 on RA FLC lines that correlate with A-SAA responsiveness. Furthermore we demonstrate that the pro-inflammatory actions of A-SAA in RA FLCs and human microvascular endothelial cells (HMVECs) can be blocked both by specific antibodies directed against SR-B1 and by synthetic mimetic peptides of ApoA-1. These findings support a novel role for A-SAA as an important mediator of synovial inflammation and suggest a novel strategy for A-SAA as a potential future therapeutic target in the treatment of inflammation through modulation of SR-B1.

Materials and Methods

Patient Recruitment and Arthroscopy

Patients with RA ($n = 8$), according to the criteria of the American College of Rheumatology,[27] or osteoarthritis (OA; $n = 5$) were recruited from rheumatology outpatient clinics at St. Vincent's University Hospital along with one normal healthy control subject. RA patients had clinically active disease including at least one inflamed knee joint. When compared with OA patients, RA patients had statistically higher serum levels of systemic inflammation as measured by C-reactive protein (18 $\pm$ 16 RA vs 5 $\pm$ 2 OA, mmol/L $P < 0.05$) and erythrocyte sedimentation rate (29 $\pm$ 7 RA vs 18 $\pm$ 7 OA, mm/hr, $P < 0.05$, mean $\pm$ SEM). There was no difference in the age range of patients when RA (59 $\pm$ 4 yrs) was compared with OA (55 $\pm$ 24 yrs). After approval by the institutional ethics committee, all patients gave written informed consent, before a day case arthroscopy procedure under local anesthetic. Arthroscopy of an inflamed knee joint using a 2.7-mm-diameter needle telescope (Stortz, Tuttlingen, Germany) was performed and biopsies of inflamed SM were obtained under direct visualization as previously described.[3] Synovial biopsies were obtained from seven RA patients. SM Biopsies were snap frozen in OCT, foil wrapped, and stored at −70°C. Cryostat sections 7 μm thick were cut, dried overnight at 37°C, and stored at −70°C and used for immunohistochemistry. Further biopsies were placed in medium and used to isolate RA primary synovial fibroblasts as outlined below.

Isolation and Culture of RA FLC Cell Lines

Synovial biopsies were digested using 1 mg/ml collagenase (Worthington Biochemical, Freehold, NJ) in RPMI (Gibco-BRL, Paisley, UK) for 4 hours at 37°C in humidified air with 5% CO2. Dissociated cells were grown to confluence in RPMI, 10% fetal calf serum (FCS; Gibco-BRL), 10 ml of 1mmol/L HEPES (Gibco-BRL), penicillin (100 units/ml), streptomycin (100 units/ml), and fungizone (0.25 μg/ml) before passaging. Cells were morphologically homogeneous RA FLCs and were used for experiments between passages 4 and 8.

Isolation and Culture of HMVECs

Dermal-derived HMVECs (Clonetics, San Diego, CA) grown in endothelial basal medium supplemented with 5% FCS, 0.5 ml human epidermal growth factor, 0.5 ml