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Short Communication

Serum Amyloid A Gene Transcription in Synovial Cells During Retroviral Arthritis

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In situ *hybridization was used to demonstrate serum amyloid A (SAA) gene expression in arthritic joint tissue from lentivirus-infected sheep and goats. SAA gene transcription occurs in isolated individual synovial cells and occasional giant cells but not in uninfected or uninflamed synovial tissue. These findings support the notion (derived from* in vitro *observations) that at least one member of the SAA gene/protein family may function as an autocrine collagenase inducer in inflammatory arthritis. Since we used heterologous DNA probes derived from human clones, our findings also support the growing evidence for interspecies conservation of SAA genes. (Am J Pathol 1992, 141:525–529)*

The small serum amyloid A (SAA) proteins are the most prominent serum proteins in the acute phase response. They are cleaved to give shorter polypeptides that aggregate in the amyloid deposits of secondary or reactive amyloidosis. The N-terminal sequences of SAA proteins isolated from serum of humans, mice, horse, mink, duck, and hamster are closely related to the consensus sequence R-S-F-F-S-F-L-G.[1,2] Despite this remarkable conservation and their relationship to the acute-phase response, no distinct function has been found for SAA proteins although they are small (104 aa in humans) to be enzymes. In serum, SAA proteins are apoproteins of high density lipoproteins[3,4] but this may not be their only function.

In both humans and mice, there are clustered families of genes encoding closely related but distinct members of a family of SAA-related proteins.[5,6] One member of the human SAA gene family, GSAA3 (also called SAAg9), encodes the known serum protein.[7,8] We recently reported the genomic sequence of GSAA1, another member of the human SAA gene family, and noted that the N-terminal sequence predicted for its product differed from the consensus despite its structural similarity to other SAA genes (exon-intron organization, length, etc.[9]). Interestingly, this unexpected N-terminal sequence was virtually identical to that of an "SAA-like" protein produced by rabbit synovial fibroblasts in tissue culture after treatment with phorbol myristate.[10] This rabbit protein was secreted along with collagenase from the cultured synovial cells and was independently capable of stimulating collagenase synthesis in the same cells in an autocrine manner *in vitro*. We proposed that the human GSAA1 protein, although closely related to the serum SAA protein, could be an autocrine mediator of inflammation which might be restricted to local effects, possibly limited to synovia, and not be present in the serum.

We have thus sought evidence for SAA transcription in actual arthritic synovia and report our study of the arthritis induced by caprine arthritis-encephalitis virus lentivirus (CAEV) and ovine-lenti virus (OVLV) infection in goats and sheep, respectively. This retrovirus-induced arthritis is characterized by invasion of the joint by macrophages (the host cell for virus replication) proliferation of macrophages and lymphocytes, prominent joint effusions, and the ultimate destruction of synovial surfaces.[11,12]

This study addressed two questions: 1) Does SAA transcription occur in synovial tissues of goats and/or sheep with lentivirus-induced arthritis and, if so 2) which cells in the inflamed joint transcribe SAA?

Materials and Methods

DNA Probes

We constructed pGS103, a truncated genomic clone, based on the human SAA clone GSAA1.[9] As shown in

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Figure 1, the new recombinant contains all four exons in a total insert length of 3.2 kb cloned into the vector pIBI30 (International Biotechnologies, Inc, New Haven, CT). The insert fragment was released from the recombinant by *Xba* I digestion. Other DNAs used included the pIBI30 vector alone and the human SAA cDNA pA1.[13]

In Situ *Hybridization*

Joint tissues from lentivirus-infected goats and sheep were fixed in 10% formalin, dehydrated, and paraffin-embedded. *In situ* hybridization was performed on 6 μm tissue sections as described.[14] Briefly, the 3.2 kb *Xba* I insert fragment from clone pGS103 (Figure 1) was radiolabeled by nick translation[15] using [^{35}S] dATP and [^{35}S] dCTP and 25 ng/ml DNase[16] to specific activities of greater than 7×10^8 cpm/μg. Tissue sections were pretreated with 0.2 N HCl for 20 minutes and proteinase K (25 μg/ml) for 15 minutes followed by acetylation and dehydration. Labeled DNA at a concentration of 0.2 μg/mL in a solution of 50% formamide, 100 mg/mL dextran sulfate, 0.63 mol/l NaCl, 10 mmol/l Tris-HCl, 0.5 mmol/l Na ethylenediamine tetraacetic acid (EDTA), 0.02% polyvinyl-pyrrolidone-40, 0.02% Ficoll-400, 1.5 mmol/l dithiothreitol (DTT) and 200 μg/ml sheared, denatured salmon sperm DNA was applied to the tissue sections. After incubation for 16 hours at 37°C, the slides underwent four 30-minute washes in 1 × SSC, 0.13% Triton-X 100, 1.25 mmol/l Na EDTA and 1.25 mmol/l DTT followed by a 20-minute rinse in 1 × standard saline citrate (SSC) in 50% formamide at 37°C and a rinse in 1 × SSC for 1 hour. After a further 2-hour rinse in 0.6 mol/l NaCl, 10 mmol/l Tris-HCl, 1.0 mmol/l Na EDTA, 1.25 mmol/l DTT, the sections were dehydrated in graded alcohol solutions containing 0.3 mol/l ammonium acetate, air-dried, dipped in NTB3 autoradiographic emulsion (Eastman Kodak Co., Rochester, NY), and allowed to expose for 2 to 5 days.

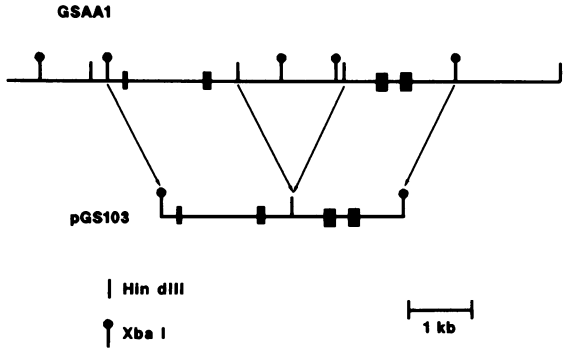

Figure 1. *Structure of clone pGS103, a truncated version of the genomic clone GSAA1[9] (top line). Cloning vector pIBI30. The heavy bars represent the four exons. Restriction enzyme cleavage sites shown by vertical lines.*

Developed slides were examined by light microscopy; the presence of target RNA was indicated by silver grains over cells.

Lentivirus-infected and uninfected cultured goat synovial membrane cells and sections of uninfected and lentivirus-infected goat synovium hybridized with an irrelevant probe (pIBI30) were used as positive and negative controls for every hybridization reaction.

RNA Detection

Joint tissue from normal and infected, inflamed animals was used to prepare RNA using guanidinium thiocyanate and cesium chloride centrifugation.[17] The RNA was used for dot-blot analysis[18] by hybridization with SAA probes labeled with [^{32}P] dCTP to greater than 10^9 cpm/μg by random primers.[19]

Results and Discussion

Sections of arthritic joints from two goats and two sheep with lentivirus-induced arthritis consisted of large areas of bland connective tissue with multifocal areas of lymphocyte- and macrophage-rich inflammation. One joint from a goat had multiple areas of dystrophic calcification surrounded by reactive (foreign body) multinucleated giant cells, probably of macrophage origin. The labeled SAA probes hybridized to fibroblast-like cells in the connective tissue (Figure 2A) which appeared to be of mesenchymal origin. The probes also detected RNA in the cytoplasm of multinucleate giant cells (Figure 2B). By contrast, the other macrophages and inflammatory cells were conspicuously unreactive with the probe (Figure 2B).

The same pattern of hybridization was seen in affected joint tissue from another CAEV-infected goat and 2 OVLV-infected sheep (not shown). No hybridization was seen in synovial tissue from uninfected animals. There was no hybridization to the pIBI30 plasmid itself. The hybridization patterns produced with a human SAA cDNA probe (pA1[13]) and the truncated genomic probe pGS103 were indistinguishable despite the presence of introns (containing reiterated $[CA]_n$ regions) in the latter.[9] We conclude that, under conditions of appropriately low stringency, both probes hybridize with the sheep and goat counterparts of human SAA gene transcripts. Hybridizing these probes to dot-blots of as much as 200 ng of RNA from infected and control joints showed no signals, compatible with the small numbers of positive cells seen with *in situ* hybridization (data not shown). The results are consistent with the report that exposing cultured rabbit synovial fibroblasts to phorbol esters causes re-

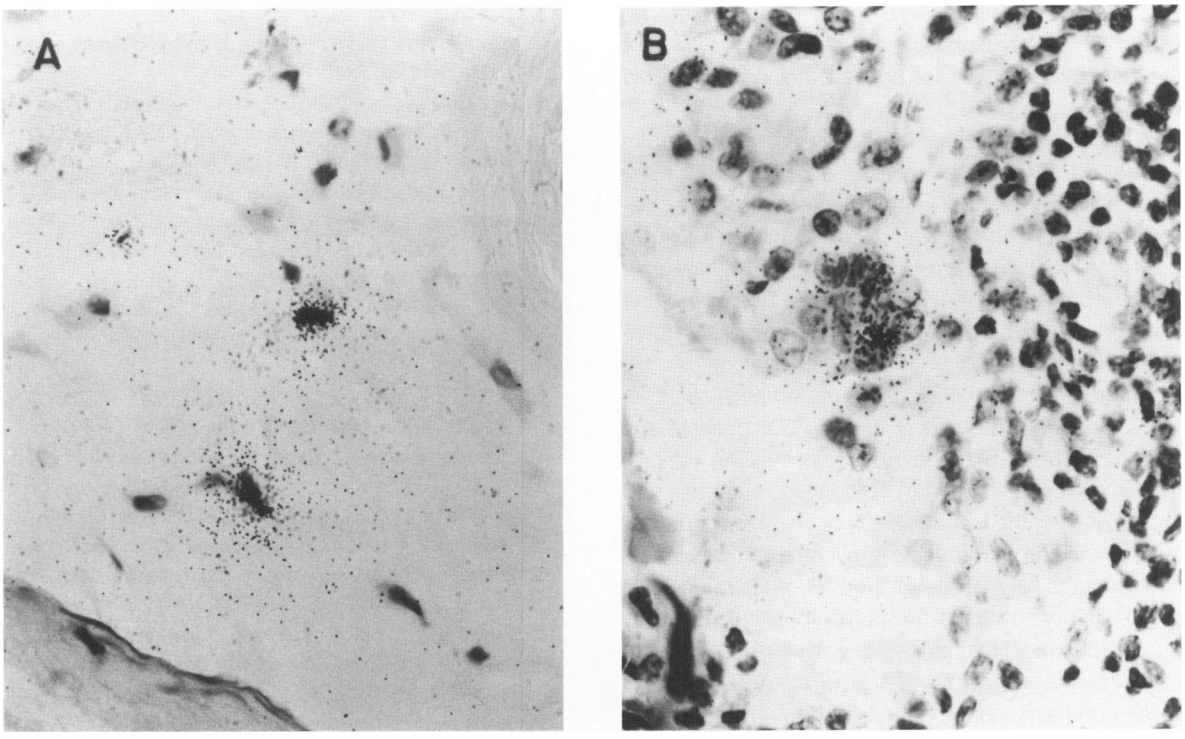

Figure 2. *Photomicrographs showing* in situ *hybridization with labeled pGS103 on inflamed synovium of carpal joints of animals infected 6 months previously with CAEV as described.*[11] *Control hybridizations using pIBI30 vector as well as uninfected control joints showed no signal under identical hybridization conditions (not shown).* **A**: *Isolated bipolar cells with cigar-shaped nuclei, consistent with fibroblasts with prominent hybridization to two cells while others are unreactive [from sheep, magnification ×520].* **B**: *Prominent hybridization to a multinucleated giant cell adjacent to an area of mineralization consistent with macrophage origin of this foreign body giant cell [from goat, magnification ×600].*

lease of "SAA-like" protein.[10] They also localize SAA transcription to the resident synovial fibroblasts of the joint and the occasional multinucleated foreign body giant cell.

We purposely selected probes representing two different members of the human SAA gene family. The cDNA clone, pA1, represents the coding sequence for the SAA protein found in serum (which we have referred to as the product of gene GSAA3[9,13]). The other clone pGS103 was derived from the GSAA1 gene and may be expressed only in synovium.[9,10] The differences in the proteins encoded by these DNAs are largely confined to the N-termini[9] reflecting conservation of the C-terminal sequences of most SAA proteins. We used hybridization stringencies that did not distinguish between our two probes because the nucleotide sequences of sheep and goat SAA genes are unknown, although some SAA protein sequence data are available for the sheep and closely resemble human SAA.[20]

All fibroblast-like cells in the synovia did not react equally well with the hybridization probes. While there can be local variations in access to the probe, there also may be differences in the synovial cells themselves, which cannot be distinguished by morphology. Three types of synovial cells have been distinguished by culture techniques[21] and these appear to differ in function. For example, only one synovial cell type, the dendritic cell, appears to produce collagenase.[22]

In contrast to SAA expression in joint fibroblasts, earlier studies of CAEV gene sequences demonstrated that viral gene expression is confined to the invading macrophages and is not present in the synovial cells. This finding is consistent with the recognized host range of CAEV which is tropic for cells of macrophage lineage.[11,12] It also may help explain that we did not find SAA transcription in the macrophages of the joint since they are the site of viral replication.

The predominant cells in the synovial fluid and inflamed joint tissue of ruminants with lentivirus-induced arthritis are macrophages and CD8+ lymphocytes.[23] Little free virus is found in the synovial fluid, possibly reflecting virus-induced interferon, which has been found to restrict viral replication as well as inhibit monocyte maturation and proliferation.[24] Our studies indicate that some factor(s) associated with arthritis induces SAA transcription in certain cells but the nature of the factor(s) has not been established. Since SAA isolated from human serum (the product of the GSAA3 gene) has been shown to inhibit

lymphocyte proliferation and E-stable rosette formation *in vitro*,[25] the SAA originating in the synovium may have a feedback inhibitory function on the invading inflammatory cells.

As an acute phase reactant, the SAA protein in serum is believed to be largely derived from hepatic synthesis.[26,27] However, earlier immunofluorescent analysis detected SAA in embryonal, dermal, and other fibroblasts.[28] Thus, the notion of extrahepatic SAA synthesis is not new but has not been emphasized because of the attention directed toward the prominent hepatic synthesis of the serum proteins.

We have shown that SAA gene transcription occurs in CAEV-induced inflammatory arthritis. Our observations are compatible with the possibility that the SAA-like protein produced is involved in collagenase synthesis and propagation of the joint destruction. This important finding strengthens our underlying hypothesis that at least one (perhaps all) member of the SAA-related gene and protein family functions as an inflammatory mediator. Such a role would be compatible with several features of the SAA proteins including their small size (few functional protein domains, hence unlikely to be an enzyme), dynamic synthesis control (prominent acute-phase synthesis[26] and rapid reduction in peak serum levels) and broad species conservation of their basic structure. At least one member of the SAA gene and protein family may be central to the pathogenesis of arthritis and its continuing joint destruction.

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