Secretory Leukocyte Protease Inhibitor Suppresses the Inflammation and Joint Damage of Bacterial Cell Wall–induced Arthritis

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Summary

Disruption of the balance between proteases and protease inhibitors is often associated with pathologic tissue destruction. To explore the therapeutic potential of secretory leukocyte protease inhibitor (SLPI) in erosive joint diseases, we cloned, sequenced, and expressed active rat SLPI, which shares the protease-reactive site found in human SLPI. In a rat streptococcal cell wall (SCW)-induced model of inflammatory erosive polyarthritis, endogenous SLPI was unexpectedly upregulated at both mRNA and protein levels in inflamed joint tissues. Systemic delivery of purified recombinant rat SLPI inhibited joint inflammation and cartilage and bone destruction. Inflammatory pathways as reflected by circulating tumor necrosis factor α and nuclear factor κB activation and cartilage resorption detected by circulating levels of type II collagen collagenase-generated cleavage products were all diminished by SLPI treatment in acute and chronic arthritis, indicating that the action of SLPI may extend beyond inhibition of serine proteases.

Key words: inflammation • cartilage resorption • serine protease inhibitor

Injury and infection elicit a complex series of reactions in the host designed to isolate and/or eliminate the inciting agent(s) as well as to minimize and repair tissue damage. Precise regulation of these mechanisms is crucial for the maintenance of tissue integrity, and malfunction may result in detrimental tissue destruction characteristic of rheumatoid arthritis and other chronic inflammatory diseases (1, 2). In degenerative and inflammatory arthritis, degradation of the proteoglycans and collagen of articular cartilage is mediated by excess neutral serine proteases and metalloproteinases (3, 4). The serine proteases contribute to activation of matrix metalloproteinases, which are typically released in a latent form (3–7), and also to cleavage of fibronectin and other matrix molecules (8, 9). One of the most prominent cell types in effusions of inflamed joints is the neutrophil, the source of two serine proteases, elastase and cathepsin G. Since neither neutrophil elastase nor cathepsin G requires activation after release into the extracellular space, the activities of these enzymes must be modulated by appropriate inhibitors (10, 11). Serine protease inhibitors may thus play an important role in controlling matrix turnover in inflammatory joint diseases.

The secretory leukocyte protease inhibitor (SLPI)[1] is active against a variety of serine proteases including neutrophil elastase and cathepsin G (12). Human SLPI (hSLPI) is an 11.7-kD nonglycosylated protein originally identified from epithelial cells at mucosal surfaces (13–16). It is composed of two domains with the protease binding and inactivating site in the COOH-terminal domain (17). Moreover, recent evidence has revealed additional functions for this serine protease inhibitor including both antibacterial and antiretroviral activity, which may be associated with its NH$_2$-terminal domain (18–21). The production of SLPI by murine macrophages and its association with the host response to bacterial LPS suggested that SLPI might have an expanded involvement in innate host defense and inflammatory responses (21–24). In humans, SLPI is primarily associated with epithelia and has not been identified in macrophages (21).

To address these issues and define a role for SLPI in in-

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[1]Abbreviations used in this paper: AI, severity of arthritis; NF, nuclear factor; PM, peritoneal macrophage; SCW, streptococcal cell wall; SLPI, secretory leukocyte protease inhibitor.

flammatory joint diseases, we have cloned and sequenced rat SLPI (rSLPI) cDNA and overexpressed and purified the first biologically active recombinant rSLPI (rrSLPI) protein. Using the well-characterized streptococcal cell wall (SCW)-induced arthritis model (25), we initially monitored the endogenous expression of rSLPI during the course of the arthritis. Although it had not been observed previously, rSLPI is clearly upregulated during both the neutrophil-mediated acute phase and the T cell–dependent chronic destructive phase of arthritis. Subsequently, we studied the effects of administration of rrSLPI to the arthritic animals, and found that the overt tissue destruction and inflammation typically seen in this model can be suppressed by systemic injection of rrSLPI. Our results provide initial evidence that modulation of the level of this endogenous protease inhibitor may have therapeutic potential for treatment of destructive inflammatory diseases.

Materials and Methods

Cloning and Sequencing of rSLPI cDNA.   RNA extracted from peritoneal macrophages (PMs) was reverse-transcribed to cDNA using an oligo-dT primer (Promega). Two pairs of PCR primers were synthesized (22) to generate the complete open reading frame. The first pair: upstream primer 5′-GGAG-GCAAAAATGATGCTATC-3′, downstream primer 5′-CCGA-GCACGAGTCCAGAGCCG-3′; and the second pair: upstream primer 5′-CACCATGAAGTCCTGCGGCCT-3′, downstream primer 5′-GGCGCCAATGTCAGGGATCAG-3′. cDNA amplifications were performed using a Perkin-Elmer PCR kit. 10 μl of the PCR product using the first primer pair was reamplified with the second primer pair. PCR products were resolved on an agarose gel, transferred to a nitrocellulose membrane, and probed with murine SLPI (mSLPI) cDNA. The cDNA fragment that hybridized to mSLPI was subcloned and used to probe a rat macrophage cDNA library in Lambda ZAP II (Stratagene). Inserts of the positive clones were subcloned and sequenced with T3 and T7 primers at the National Institute of Dental and Craniofacial Research DNA core facility.

Sequence and Database Analysis.   The EMBL, Swissprot, and GenBank molecular biology databases were searched using the network service (National Center for Biotechnology Information, NLM, Bethesda, MD) and the FASTA program from the Genetics Computer Group (GCG) Wisconsin Sequence Analysis Software Package (University of Wisconsin). Multiple sequence alignments were performed using ClustalW alignment in MacVector software (Oxford Molecular Group, Oxford, UK).

RNA Isolation and Northern Blot Analysis.   PBMCs and polymorphonuclear neutrophils (PMNs) were isolated from female Lewis rats as previously described (25). Resident PMs were collected by PBS lavage of the peritoneal cavity. Total RNA was isolated by the RNeasy protocol (Qiagen). 8 μg of total RNA was then subjected to Northern blot analysis using [^{32}P]dCTP-labeled rSLPI and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA as the probes.

Expression of rSLPI cDNA and Purification of rSLPI Protein.   The rSLPI cDNA encoding the mature secreted protein was generated by PCR with an upstream primer 5′-GGCAAAAAT-GATGCTATC-3′ and a downstream primer 5′-TTACACT-GGGGGAAGGCAGA-3′ with BamHI and SalI adaptors, respectively. The BamHI–SalI cDNA fragment was subcloned into

a prokaryotic expression vector pQE30 (Qiagen Inc.) with an in-frame ATG and the hexahistidine tag to the NH$_2$-terminus. Bidirectional sequencing verified the correct sequence and reading frame. *Escherichia coli* strain M15 was transformed with the rSLPI expression construct and grown in a 10-liter fermentation system at 37°C with ampicillin (100 μg/ml) and kanamycin (25 μg/ml) until an OD of 10 was reached. rSLPI expression was induced by addition of 1 mM IPTG for 5 h at 37°C. The cells were then harvested and the pellet was resuspended in 50 mM Na-phosphate buffer (pH 8.0) containing 300 mM NaCl, and in 10 mM imidazole. After sonication and RNase A and DNase I treatment, sequential 15,000 and 50,000 *g* centrifugations were performed. The supernate was mixed with pre-equilibrated Ni-NTA resin and incubated at 4°C for 1 h before being packed into a chromatographic column. Step-elution with 50, 75, 100, 125, and 150 mM imidazole was then carried out. The eluate from 75 mM imidazole, which showed anti-elastase, anti-cathepsin G, and anti-chymotrypsin activities, was dialyzed against sodium phosphate buffer (PBS, 10 mM sodium phosphate, 0.9% saline, pH 7.4), concentrated, filter-sterilized, and stored in aliquots at −80°C. 25 mg of purified rrSLPI protein was isolated from 50 g of wet weight of *E. coli*, which constituted 0.6% of the total soluble protein. rrSLPI was sequenced by Edman degradation (CBER, FDA, Bethesda, MD) to confirm identity. The molecular weights of the purified proteins were determined by mass spectrometry (Dr. Lewis Pannel, NIDDK, NIH, Bethesda, MD). An 8.4-kD truncated HIS-rSLPI recombinant protein (the COOH-terminus ends at Arg[64] of the mature rSLPI protein) was obtained using the same purification scheme, which has no protease reactive site and lacked anti-elastase, anti-cathepsin G, and anti-chymotrypsin activities. Endotoxin levels of both the full-length and truncated rrSLPI protein preparations were found to be ≤25 pg/ml (detection limit).

Western Blot Analysis.   Rabbit polyclonal antibodies generated against the rSLPI peptide EGGKNDAIKIGAC (Quality Controlled Biochemicals, Inc.) were affinity purified and used for immunoblotting, ELISA, and immunohistochemical studies.

Protease Inhibition Assays.   Elastase activity was measured as the amidolytic effect of human neutrophil elastase (15 nM; Calbiochem) on pyroGlu-Pro-Val-pNA (0.5 mM; Chromogenix) after 10 min at 37°C (26). Trypsin activity was determined by measuring the amidolytic effect of 1-1-Tosylamide-2-phenyl-ethyl chloromethyl ketone (TPCK)-treated bovine pancreatic trypsin (Sigma Chemical Co.) on the chromogenic substrate *N*-α-benzoyl-1-Arg-pNA (Boehringer Mannheim, Inc.) (27). Chymotrypsin (12.5 nM) and cathepsin G (32 nM) (Calbiochem) activities were measured using the chromogenic substrate *N*-suc-Ala-Ala-Pro-Phe-pNA (Sigma Chemical Co.) at 0.1 and 0.4 mM concentrations for 15 and 20 min, respectively (28, 29). In all assays, the respective enzymes were incubated with or without different concentrations of purified rrSLPI at 37°C for 20 min. Then the substrate for each enzyme was added and the residual activity was measured as the change in absorbence at 405 nm after an appropriate incubation time at 37°C.

Induction and Monitoring of Arthritis.   Arthritis was initiated in female Lewis rats (Charles River Breeding Labs., Inc.) by an intraperitoneal injection of group A SCW (Lee Labs.) (25). The severity of arthritis (AI) was determined by blind scoring of each ankle and wrist joint, based on the degree of swelling, erythema, and disfigurement on a scale of 0–4 and adding the scores for all four limbs during the course of the study (26–28 d). Rats were randomly assigned to five groups for two separate

experiments (I and II) as follows: control animals, which received PBS on day 0 ($n = 6$ for both experiments); SCW-injected animals ($n = 6$ for both experiments); SCW-injected animals treated with rrSLPI on days 1 and 9 ($n = 6$ for each dosage group in experiment I, and $n = 12$ for experiment II); SCW-injected animals treated with rrSLPI on day 13 ($n = 3$ for each dosage group for experiment I, and $n = 12$ for experiment II); and SCW-injected animals treated with truncated rrSLPI on days 1 and 9 ($n = 3$ for each dosage group for experiment I, and $n = 9$ for experiment II). In experiment I, 100 μg or 1 or 5 mg of purified full-length or truncated rrSLPI was injected intraperitoneally at the times specified for each group of animals; in experiment II, 100 μg of full-length or truncated rrSLPI was used. Statistical significance was ascertained using the analysis of variance followed by Scheffe's post-hoc test. Radiographs were taken as previously described (25). Excised ankle joints were processed for histopathology (25), and sections were stained with anti-rSLPI antibody (5 μg/ml) using Vectastain-ABC kit with DAB substrate (Vector Labs.) and counterstained with methyl green (25).

ELISA Assays. Rat plasma samples (1:2) were diluted in the appropriate dilution reagent and the levels of TNF-α were measured by ELISA (Biosource International). The plasma levels of rrSLPI were measured by our ELISA assay. In brief, Immulon 4 HBX microtiter plates (DYNEX Technologies, Inc.) were coated with 200 μl of anti-tetra-HIS antibody (3 μg/ml; Qiagen), washed four times in PBS, and incubated in blocking buffer (2% sucrose, 0.1% BSA, and 0.9% sodium chloride) for 2 h. Standard ELISA procedure was then performed using rabbit anti-rSLPI (5 μg/ml) as the primary antibody, alkaline phosphatase–conjugated anti–rabbit antibody as secondary antibody (1:4,000; Boehringer Mannheim Biochemicals), and *p*-nitrophenyl phosphate as substrate (Sigma Chemical Co.).

Electromobility Shift Assay. Joint samples were obtained at the indicated times and processed in a freezer/mill (SPEX CertiPrep) in the presence of liquid nitrogen (25). Nuclear proteins were isolated from the powdered joint tissue by homogenization in lysis buffer (20 mM Tris, pH 7.6, 120 mM NaCl, 1% NP-40, 10% glycerol, 10 mM NaPPi, 100 mM NaF, 2 mM Na-orthovanadate, 1 mM AEBSF, and 5 μg/ml leupeptin). Electromobility shift assay (EMSA) for nuclear factor (NF)-κB was performed using a radiolabeled NF-κB consensus oligonucleotide probe (Promega) as previously described (30).

Collagen Cleavage ELISA. Rat plasma samples were collected by tail bleeding or intracardiac puncture. Plasma levels of type II collagen cleavage product Col2-3/4C long neoepitope were monitored by a solution phase ELISA assay based on a published method (31). The Col2-3/4C long neoepitope resides at the COOH terminus of the TC[A] piece produced by cleavage of the α1(II) chain by collagenase. The mouse mAb used in the assay is distinct from the rabbit antibody described previously (31) in that it recognizes only the cleaved α1(II) chain and thus is absolutely specific for type II collagen (Billinghurst, R.C., M. Ionescu, M.-A. Fitzcharles, E. Keystone, and A.R. Poole, manuscript in preparation).

Results

Cloning and Sequencing of rSLPI cDNA. rSLPI mRNA was identified by reverse transcriptase PCR using nested primers designed against the mSLPI open reading frame (22). A 400-bp cDNA fragment was isolated and used to probe a cDNA library from rat PMs. A 490-bp cDNA fragment was isolated, sequenced, and found to contain an open reading frame (bp 9–402, Fig. 1 A) encoding a predicted translation product of 131 amino acids with an estimated molecular mass of 14 kD. The putative rSLPI coding sequence shares 88% homology with murine SLPI (mSLPI) and 75% with hSLPI. At the amino acid level, rSLPI exhibits 83 and 58% identity to mSLPI and hSLPI, respectively (Fig. 1 B). 16 cysteine residues are conserved in the mature SLPI (residues 26–131, Fig. 1 B) and the serine protease binding site in hSLPI (Leu[72] of the mature protein) is identical in rSLPI (Fig. 1 B). However, by Northern blot analysis, distinct tissue distribution patterns compared with humans were observed (Song, X.y., L. Zeng, W. Jin, and S.M. Wahl, manuscript in preparation). Moreover, although no detectable hSLPI mRNA has yet been observed in human mononuclear cells and macrophages, rSLPI was constitutively present at very high levels in naive PMs, less abundant in PBMCs, and essentially absent in resting PMNs (Fig. 2 A).

Modulation of rSLPI Expression in SCW-induced Arthritic Joints. When rSLPI mRNA was monitored during the
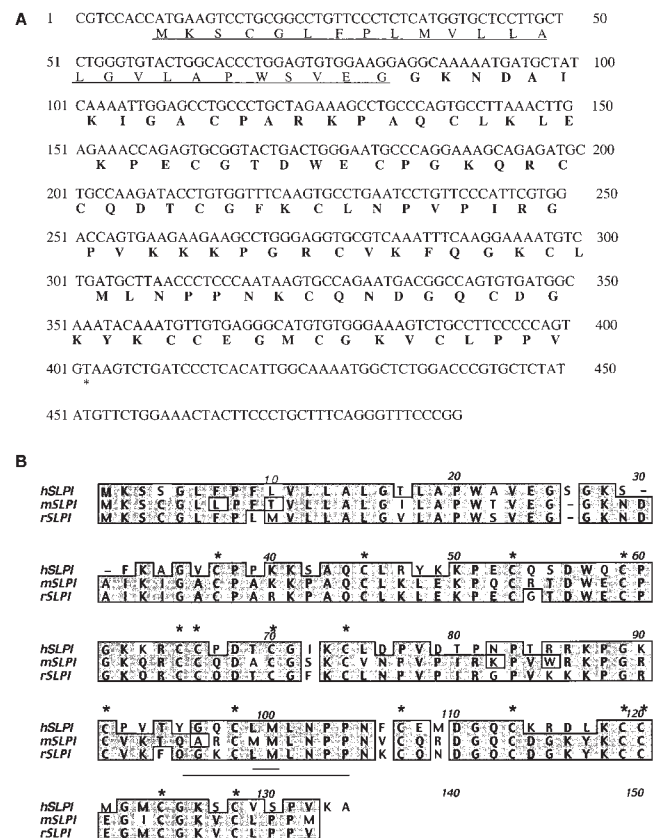
Figure 1. cDNA sequence of rSLPI and amino acid sequence comparison among human, murine, and rat SLPI. (A) Nucleotide sequence of rSLPI is shown in plainface, and the amino acid sequence of the mature protein in boldface below the nucleotide sequence. The amino acid sequence for the signal peptide is underlined. Asterisk, stop codon. (B) Comparison of amino acid sequences of human, murine, and rat SLPI using ClustalW alignment. Conserved residues are shaded in dark color, and conserved cysteine residues are indicated by asterisks. The active site for protease binding is underlined with the two key residues double underlined.
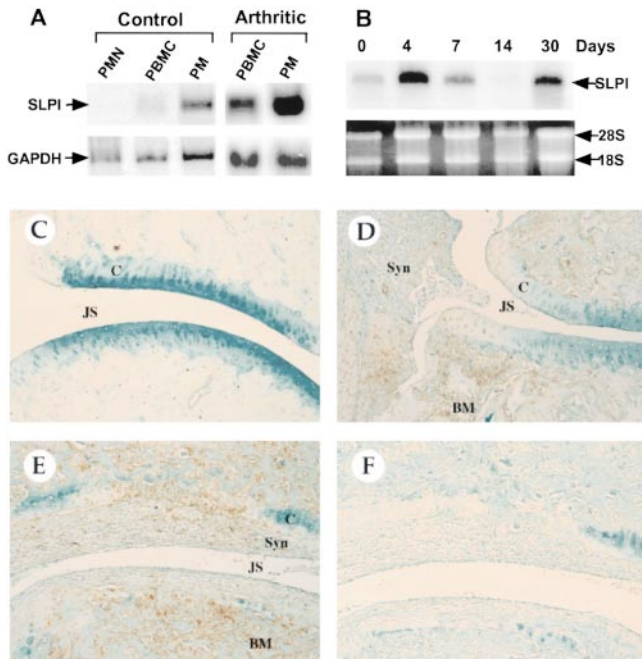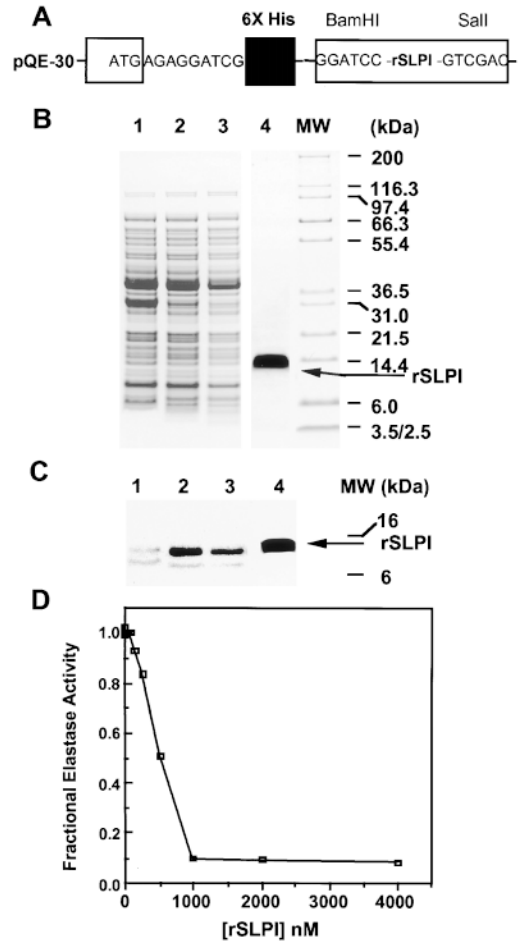
Figure 2. SLPI expression in SCW-induced arthritis. (A) RNAs from PMNs, PBMCs, and PMs of control and SCW arthritic (day 26) animals were probed with rSLPI and rat GAPDH cDNA probes. (B) Total RNA from ankle tissues at indicated times after SCW injection were blotted to a Nytran membrane and probed with rSLPI cDNA. The ethidium bromide–stained gel demonstrates the amount of 28S and 18S RNAs. Data are representative of three experiments. (C–F) Immunohistochemical detection of rSLPI protein (brown stain) in joint sections before (C), 4 d after (D), and 26 d after SCW injection (E, F). Rabbit IgG was used as control in F. Syn, synovium; JS, joint space; C, cartilage; BM, bone marrow.

Figure 3. Expression and purification of active rrSLPI. (A) Diagram of HIS–rSLPI fusion protein for step-elution from Ni-NTA column. (B) SDS-PAGE of purified proteins: 1, sonicated induced cells; 2, lysate from 15,000 g centrifugation; 3, dialyzed lysate from the sequential 50,000 g centrifugation; 4, 75 mM imidazole elute fractions; MW, markers. (C) Immunoblot of B using anti-rSLPI peptide antibody as described in Materials and Methods. (D) rrSLPI (lane 4) possesses antiprotease activity toward human neutrophil elastase (shown), cathepsin G, and chymotrypsin. Different concentrations of SLPI were shown on the x-axis, and the fractional elastase activity at each inhibitor concentration compared with uninhibited activity was shown on the y-axis.

course of synovial inflammation and compared with the low constitutive SLPI mRNA levels in control joints, a striking biphasic expression pattern was observed during the course of the disease. The increase in rSLPI mRNA coincided with the peak acute (day 4 after SCW injection) and chronic (day 30) inflammatory responses (Fig. 2 B), but decreased significantly at the onset of the remission phase (day 7), and was negligible at the onset of chronic disease (day 14). These findings suggest that failure to produce sufficient SLPI or to maintain the initial elevated levels of the protease inhibitor preceding chronic disease may facilitate joint destruction.

Assessment of the origins of SLPI in the inflamed synovium revealed that rSLPI protein was found in infiltrating inflammatory cells consistent with SCW-induced mRNA expression (Fig. 2 A), and was also associated with some chondrocytes and cells in the bone marrow (Fig. 2, D and E).

Recombinant rSLPI Inhibits SCW-induced Joint Inflammation. Because SLPI levels were minimal during the interval before joint destruction becomes most pronounced, we attempted to restore levels of the protease inhibitor by exogenous delivery of active rrSLPI. For this purpose, a mature rSLPI–HIS fusion protein was expressed, purified to homogeneity (Fig. 3, A–C), and found to contain a polypeptide recognized by both anti-rSLPI (Fig. 3 C, lane 4) and

anti-HIS tag antibodies (data not shown). This full-length fusion protein (molecular mass ∼12 kD; Fig. 3 C, lane 4) exhibited antiprotease activity toward human neutrophil elastase (Fig. 3 D), cathepsin G, and bovine chymotrypsin, but not trypsin (data not shown), and was used in the subsequent in vivo study. A COOH-terminally truncated form of the rrSLPI protein without anti-elastase, anti-cathepsin G, or anti-chymotrypsin activities was used as the control agent in the following animal experiments.

Based on the SLPI expression profile (Fig. 2, B–F), full-length or truncated rrSLPI (0.1, 1, and 5 mg) was administered intraperitoneally before the clinically evident acute response to SCW or preceding the chronic inflammatory response, when endogenous SLPI was at a low ebb. As early as 2 d after the first full-length SLPI-injection (0.1 mg),
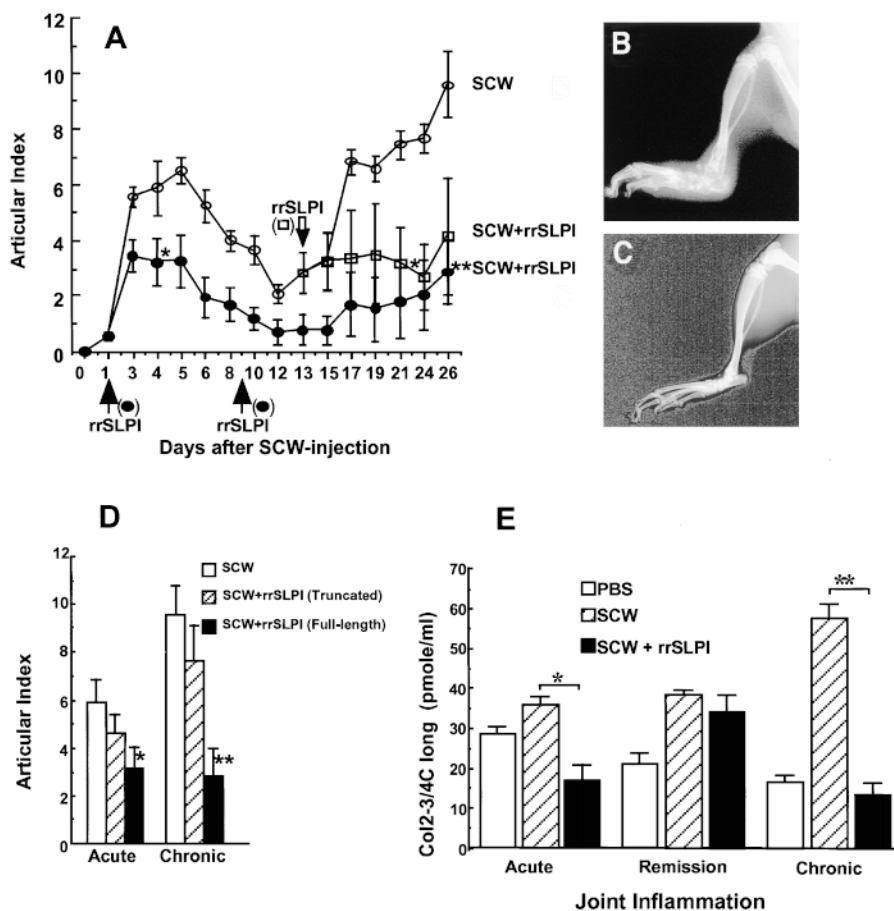
Figure 4. Suppression of arthritis by rrSLPI. (A) Arthritis was initiated by a single intraperitoneal injection of SCW on day 0 and the articular index (AI) measured as described in Materials and Methods for the course of the study (○). An intraperitoneal injection of 0.1 mg of the purified active rrSLPI protein was administered 24 h and again on day 9 after SCW (●) or only on day 13 (□). Each point represents the mean AI ± SEM for each group of animals ($n = 12$) and significant decreases in AI with treatment are indicated as *$P < 0.05$ and **$P < 0.01$. (B and C) Radiographs of representative animals 26 d after SCW injection (B) and rrSLPI-treatment on days 1 and 9 (0.1 mg) (C). (D) The effect of truncated rrSLPI (0.1 mg, hatched bars) administered on days 1 and 9 after SCW on acute and chronic arthritis was compared with that of the full-length rrSLPI (solid bars). The articular index of untreated SCW-injected animals was shown as open bars. Each point represents the mean AI ± SEM for each group of animals ($n = 12$) and significant differences are indicated by *$P < 0.05$ and **$P < 0.01$. (E) rrSLPI suppresses the increased cleavage of type II collagen. Plasma samples were collected during the acute (day 4), remission (day 10), and chronic phases (day 26) of arthritis and Col2-3/4C long neoepitope quantified by ELISA. Data shown are the means ± SEM for each group of animals ($n = 6$). SCW+rrSLPI represents the group of animals that have been injected with active rrSLPI on day 1 and 9 after SCW (solid bars) and PBS represents the control animals injected with phosphate buffered saline (open bars). *$P < 0.05$, **$P < 0.001$.

a reduction in AI was evident that was more significant by day 4 of the acute response (AI = 3.17 ± 0.86, $n = 12$; vs. 5.88 ± 0.98 in SCW animals, $n = 12$, $P = 0.035$, Fig. 4 A). The effects of 0.1, 1, and 5 mg of rrSLPI were similar based on AI (data not shown). However, the impact of a single injection of only 0.1 mg of full-length rrSLPI was transient, as the clinical and histopathological symptoms reappeared during the late chronic disease stage, albeit at reduced levels (data not shown). Importantly, joint swelling, erythema, and disfigurement, the hallmarks of chronic arthritis, a response typically considered T cell– and macrophage- rather than neutrophil-dependent, were drastically curtailed after a second rrSLPI injection 9 d after SCW (AI = 2.83 ± 1.17 vs. 9.58 ± 1.19, $n = 12$, day 26, $P = 0.0065$, Fig. 4 A) and remained suppressed for the duration of the experiment. Radiographic analysis revealed that compared with SCW-injected animals, which exhibit overt erosion of cartilage and bone (Fig. 4 B), full-length rrSLPI-treated animals had minimal pathological changes with only mild soft tissue swelling (Fig. 4 C). In contrast, treatment of SCW-injected animals with the antiproteolytically inactive truncated rrSLPI caused no significant changes in disease severity (Fig. 4 D). Moreover, a single

injection of full-length rrSLPI at the beginning of chronic arthritis (day 13) appeared to reduce the disease severity, achieving statistical significance within 10 d (Fig. 4 A). When plasma levels of rrSLPI were examined in rrSLPI-treated (1 mg) and untreated SCW animals, 2,652 pg/ml of rrSLPI was detected in rrSLPI-treated animals 2 h after injection, which declined over the next 16 h. SLPI levels were not detected in control or untreated arthritic animals. Finally, no apparent adverse effects were observed in rrSLPI-treated (1 mg) animals, which maintained their body weight at a level similar to the nonarthritic control animals (body wt = 175.7 ± 1.8 g in rrSLPI-treated arthritic animals vs. 158.6 ± 1.76 g in untreated SCW arthritic animals and 184.5 ± 2.4 in nonarthritic control animals, day 26).

Impact of rrSLPI on Extracellular Matrix Destruction. Circulating type II collagen collagenase–generated cleavage products reflect the increase in cleavage in degenerate articular cartilage in human osteoarthritis (31), but have not been analyzed in experimental animal models. A new assay to detect a hidden type II collagen epitope in the COOH-terminal three-quarter–length fragment (Col2-3/4C long neoepitope), which is exposed only when native triple he-

lical type II collagen is cleaved by collagenase, enabled us to quantify cartilage degradation in SCW arthritic rats. We demonstrate that Col2-3/4C long neoepitope increased in plasma with disease progression (Fig. 4 E), paralleling the evolution of cartilage destruction observed in these animals (Fig. 4 B) (25). Interestingly, during the remission phase, when inflammation and swelling typically decline, collagen cleavage appears to persist with the spill-over of cleavage products into the circulation. After a single full-length rrSLPI treatment on day 1 after an arthritogenic injection of SCW, a significant reduction (two- to threefold) in Col2-3/4C long neoepitope plasma levels was detected even during acute arthritis (Fig. 4 E). The lack of significant decrease in cleavage products in rrSLPI-treated arthritic animals during remission (day 10) is consistent with the clinical and pathological data in which a single treatment with full-length rrSLPI does not fully sustain suppression of the arthritis through the chronic phase. Although a second rrSLPI injection was administered on day 9, this probably represents an inadequate interval of time to effect a further reduction in enzymatic profile on day 10. However, the injection of SLPI on day 9 did have a profound impact on the subsequent evolution of cartilage and matrix destruction associated with chronicity. In fact, full-length rrSLPI appeared to nearly eliminate the joint destruction as quantified by multiple parameters, including AI, radiologic assessment of joint destruction, and the release of type II collagen cleavage products (Fig. 4).

Suppression of Inflammatory Mediators by rrSLPI. In addition to proteolytic blockade of matrix degradation, histopathological analysis revealed reduced evidence of synovial inflammation in rrSLPI-treated arthritic animals. It has been shown that TNF-α can induce SLPI production in epithelial cells (32), and SLPI can in turn inhibit TNF-α production in macrophages (21, 22). To determine whether exogenously administered active rrSLPI could reinforce the endogenously produced SLPI to antagonize SCW-induced TNF-α (25), we compared plasma levels of TNF-α in full-length rrSLPI-treated and untreated arthritic animals over the course of the disease. Plasma levels of TNF-α in arthritic animals were elevated during the acute phase, decreased in remission, and markedly increased in the chronic phases of the disease (reference 33; Fig. 5 A). However, after administration of full-length rrSLPI on days 1 and 9, there was a rapid and sustained decrease in detectable levels of circulating TNF-α (Fig. 5 A). Since SCW (30) and TNF-α both activate NF-κB, which is responsible for the transcription of multiple inflammatory mediators including TNF-α (34), the consequences of rrSLPI injection on NF-κB activity in active rrSLPI-treated and untreated animals during both acute (Fig. 5 B) and chronic (data not shown) arthritis were examined. By EMSA, NF-κB was strongly activated in SCW-induced arthritic joint tissue as compared with PBS-injected control animals, and this activation was suppressed by rrSLPI (Fig. 5 B). These data implicate a potential role for rrSLPI in breaking the positive feedback loop between TNF-α and NF-κB activation, thereby dampening SCW-induced joint inflammation.
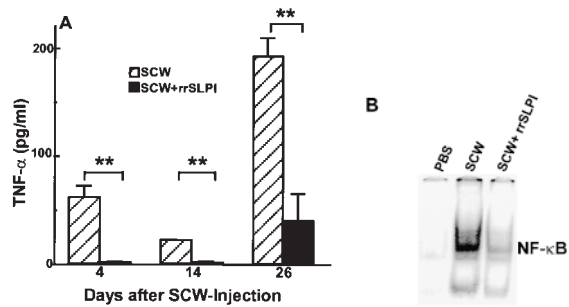
Figure 5. (A) rrSLPI inhibits TNF-α production. Plasma samples of SCW-injected (hatched bar) and SCW animals that have received 0.1 mg of active rrSLPI on days 1 and 9 (solid bar) were collected as indicated and analyzed by ELISA. Each point represents the mean $\pm$ SEM ($n = 6$). **$P < 0.01$. (B) rrSLPI suppresses NF-κB activity in SCW animals. Proteins were extracted from joint samples of PBS-injected nonarthritic and SCW-injected and active rrSLPI-treated SCW arthritic animals 3 d after SCW or PBS and 2 d after rrSLPI injection and subjected to EMSA.

Discussion

SLPI, a serine protease inhibitor with potent neutralizing activity for leukocyte enzymes such as neutrophil elastase, is upregulated in response to bacteria and bacterial products (21, 22, 24). Here we demonstrate that, in contrast to hSLPI, rSLPI, like its murine counterpart (24), is expressed in monocytes and PMs and, apparently, other cells exposed to bacterial cell wall components. Our identification of SLPI in articular chondrocytes is consistent with recent reports, although it is still controversial whether SLPI is produced by these cells in situ or is sequestered into the cartilage matrix (35–37). Moreover, we have expressed biologically active recombinant rSLPI, and show that when delivered to animals in which bacterial products induce arthritic lesions, SLPI can profoundly inhibit proteolytic tissue destruction and joint inflammation. This effect of rrSLPI on SCW-induced arthritis and the parameters monitored implies that SLPI inhibits multiple pathways either directly as a consequence of its antiprotease activity or via yet to be determined regulatory pathways.

SCW-induced arthritis presents a biphasic disease process with a neutrophil-dependent acute phase and a prolonged T cell–dependent, macrophage-mediated chronic phase that manifests ~2 wk after SCW injection (25). Although the acute phase is considered reversible, the chronic destructive disease presents irreversible changes with progressive pannus invasion of the cartilage and bone, and eventual loss of normal joint structure and function. The increasing differences between nonarthritic and SCW arthritic animals in type II collagen cleavage as disease progresses is, in part, due to a gradual decrease in normal type II collagen cleavage in young control animals as they age, associated with the closure of the growth plate (38). Parallel to the biphasic disease manifestation, the expression of endogenous SLPI is substantially upregulated at both mRNA and protein levels. The unexpected increases in endogenous SLPI observed during both acute and chronic arthritis most likely represent an attempted defense against the elevated serine pro-

teases triggered by SCW activation. However, expression of SLPI nearly vanishes in the interval preceding chronic destructive disease, suggesting that this loss of SLPI activity may contribute to the joint destruction typical of chronic arthritis. In this regard, the therapeutic effect of exogenously delivered rrSLPI might result from direct inhibition of the serine protease cascade, initiated not only by neutrophil elastase, but also by that derived from both lymphocytes and macrophages (39–41). Consistent with this hypothesis, denaturation of type II collagen in articular cartilage as reflected by quantifiable circulating levels of a neoepitope resulting from cleavage of collagen by collagenase, the Col2-3/4C long neoepitope, was significantly suppressed by exogenously delivered rrSLPI during both the acute and chronic phases of the disease. The persistent collagen cleavage during remission, when inflammatory activity is low, indicates that the devastating cartilage destruction associated with chronicity is the consequence of smoldering proteolysis. Consequently, the therapeutic effect of exogenous SLPI on chronic arthritis may also involve suppression of protease activities existing at the transition between acute and chronic arthritis.

By providing exogenous active rrSLPI, the severity of disease at the clinical, histological, and molecular levels can be reduced. By neutralizing elastase, which cleaves not only elastin, but also collagen, fibronectin, and proteoglycans leading to cartilage and matrix degradation, this protease inhibitor may have multiple beneficial actions (8, 9). Moreover, because elastase (3, 5) and the plasminogen–plasmin system (3) can activate latent matrix metalloproteinases, SLPI may also indirectly block matrix metalloproteinase–mediated matrix cleavage. Recent evidence indicates that SLPI inhibits synthesis of macrophage collagenase and impairs PGE synthesis (42), implicating SLPI in the interruption of a proteolytic cascade of inflammatory events that evolve to tissue destruction (43, 44).

In addition to inhibiting proteolytic tissue destruction, SLPI appears to have multiple antiinflammatory actions. SLPI inhibits TNF-α production, probably through its ability to block NF-κB activation (22, 45). Our demonstration of SLPI's effect on TNF-α production and NF-κB activity in vivo complements the in vitro findings that SLPI is upregulated by proinflammatory stimuli including LPS, TNF-α, IL-6, and IL-1β, and subsequently inhibits TNF-α and nitric oxide production. These pathways may underlie SLPI's antiinflammatory functions in vivo (22, 24, 32).

Based on the similarities between human rheumatoid arthritis and SCW-induced experimental arthritis, and the shared protease reactive site in hSLPI and rSLPI, our study provides initial preclinical evidence in support of the use of this serine protease inhibitor in alleviating arthritic pathology. SLPI may represent a prototype therapeutic approach for multiple inflammatory destructive diseases.

References

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