Plasma Apolipoprotein(a) Co-Deposits with Fibrin in Inflammatory Arthritic Joints

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Extravascular coagulation and diminished fibrinolysis are processes that contribute to the pathology of both inflammatory arthritis and atherosclerosis. We hypothesized that, given its homology with plasminogen, apolipoprotein (apo) (a), the distinctive glycoprotein of the atherogenic lipoprotein (Lp) (a), may be equally implicated in inflammatory arthritis. We detected the presence of apo(a) as part of Lp(a) in human arthritic synovial fluid. The abundance of apo(a) in synovial fluid rose in proportion to plasma apo(a) levels and was higher in inflammatory arthritides than in osteoarthritis. In addition, apo(a) immunoreactive material, but not apo(a) transcripts, was detected in inflammatory arthritic synovial tissues. These data indicated that synovial fluid apo(a) originates from circulating Lp(a) and that diffusion of Lp(a) through synovial tissue is facilitated in inflammatory types of arthritis. In synovial tissues, apo(a) co-localized with fibrin. These observations could be reproduced in a model of antigen-induced arthritis, using transgenic mice expressing human Lp(a). Although in this mouse model the presence of apo(a) did not change the severity of arthritis, the co-localization of apo(a) with fibrin in synovial tissue suggests that, in humans, apo(a) may modulate locally the fibrinolytic activity and may thus contribute to the persistence of intra-articular fibrin in inflammatory arthritis. (*Am J Pathol* 2001, 159:1445–1453)

Patients suffering from rheumatic diseases are at increased risk for cardiovascular morbidity and mortality,^{1,2} suggesting that common factors may promote the development of both atherosclerosis and arthritis.³ Among the many etiological pathways, the role of lipoproteins and diminished fibrinolysis need to be considered.^{4–6} Lipoproteins differ in their size, composition,

and atherogenic properties. One of the most atherogenic lipoproteins is lipoprotein (a) [Lp(a)]. Lp(a) is made of a low-density lipoprotein (LDL) particle and a highly polymorphic glycoprotein called apo(a), which is covalently attached to apoB-100 of LDL.⁷ Apo(a), which is only present in humans, great apes, and hedgehogs, is homologous to the fibrinolytic proenzyme plasminogen.⁸ Plasminogen contains five kringles (numbered from K1 to K5) and the protease domain, whereas apo(a) contains a variable number of tandemly repeated copies of a motif resembling K4, followed by a single copy of K5 and the protease domain, which is functionally inactive.

The exact mechanism whereby apo(a) is atherogenic remains to be elucidated. Apo(a) has a high affinity for lysine-binding sites on fibrin(ogen)^{9,10} and may therefore compete with plasminogen at sites of fibrin deposition and thus interfere with the fibrinolytic system.¹¹ In the same line apo(a), or fragments of apo(a) that have been found in human plasma and urine,¹² may antagonize the effect of angiostatin, a biologically active fragment of plasminogen that inhibits angiogenesis.^{13,14} Finally, Lp(a) may contribute to lipid delivery to atherosclerotic plaques.

In arthritis, pathological mechanisms that may be modulated by Lp(a) in the joint include the equilibrium between extravascular coagulation and fibrinolysis, plasmin-mediated cartilage and bone matrix degradation, and angiogenesis.^{15–22} Despite numerous reports on elevated concentrations of Lp(a) in plasma from patients with rheumatoid arthritis (RA)^{19,23–25} or other rheumatological diseases,^{23,26,27} little is known on the biology and the role of Lp(a) within the joint.

In the present study, we examined human arthritic synovial fluid for the presence of apo(a). We demonstrate that apo(a) is present in human synovial fluid as part of Lp(a) and we provide evidence that Lp(a) in synovial fluid originates from the liver. Next, because of difficulties in examining the function of apo(a) in humans, we tested whether apo(a) is beneficial or deleterious to the joints by using transgenic mice expressing human apo(a)^{28,29} in an experimental model of immunological arthritis, anti-

Supported by the Swiss National Foundation for Scientific Research (no. 44971.95 to V. M. and no. 56710.99 to N. B.), The Octave Botnar and Placide Nicod Foundation, and The Kamillo Eisner Foundation.

Accepted for publication July 10, 2001.

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gen-induced arthritis (AIA), which recapitulates some of the features of RA.³⁰

Materials and Methods

Collection of Human Samples

Patients were diagnosed with osteoarthritis (OA) based on clinical and radiological criteria ($n = 8$), with RA if they fulfilled at least four of the seven American Rheumatism Association revised criteria for RA ($n = 12$), or with other types of inflammatory arthritis ($n = 12$) (two patients had Still's disease, two had gout, three had spondylarthritis, one had Reiter's disease, one had chondrocalcinosis, and three had inflammatory monoarthritis of unknown origin). For all patients, synovial fluid, as well as venous blood, were collected into citrated tubes, subjected to centrifugation for 10 minutes at $1500 \times g$ and supernatants were stored at -70°C until use. In addition, specimens of synovial tissue from seven OA and seven RA patients undergoing joint surgery of the knee or the hip for advanced disease were obtained from the local Department of Orthopedics. All tissues were cut into small pieces, embedded in Tissue-Tek OCT, and immediately frozen in precooled hexane to be stored at -70°C until use.

Immunological Analysis of Apo(a) in Human Plasma and in Synovial Fluid and Tissue

Apo(a) was quantitated in plasma, synovial fluid, and tissue using a sandwich enzyme-linked immunosorbent assay using antibodies IgG-a6 as capture antibody and horseradish peroxidase-conjugated IgG-1-1 as detecting antibody, two mouse monoclonal antibodies of well-defined specificity against apo(a),³¹ as described.³² Immunoblot analysis of apo(a) was performed after size-fractionation of synovial fluid and plasma proteins using a 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis³³ and immunoblotting using IgG-a5, a mouse anti-human apo(a) monoclonal antibody directed against K4-type 2.³¹ This analysis was performed on untreated samples. In addition, we incubated plasma and synovial fluid samples with heparin-coated beads, and analyzed the heparin-bound fraction [corresponding to apo(a) bound to apoB-100 of LDL to form Lp(a)] and the heparin-unbound fraction [corresponding to apo(a) or fragments of apo(a) circulating free of Lp(a)], exactly as described.³³ Concentrations of total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, and albumin were measured in cell-free synovial fluid and plasma samples using the Unimate5-chol (Roche, Basel, Switzerland), the HDL-kit (Behring, Deerfield, IL), the Unimate7-trig kits (Roche), and by nephelometry (Behring), respectively, as described.³² LDL-cholesterol was calculated using the Friedewald formula. D-dimer concentration in mouse plasma was measured by a commercially available enzyme-linked immunosorbent assay kit designed for human D-dimer (Asserachrom D-Di, Diagnostica Stago, Asnières, France), which cross-reacts with

murine D-dimer. The content of murine D-dimer was calculated according to the human D-dimer standard curve.

Immunohistochemical Analysis of Apo(a) and Fibrin

Immunohistochemistry on human tissues was performed on air-dried 5- μm cryostat synovial tissue sections, fixed for 10 minutes in acetone at 4°C before use. Each slide was incubated for 30 minutes with 10% normal human serum, 10% normal goat serum, and 1% bovine serum albumin (BSA). For fibrin immunohistochemistry, slides were then overlaid for 30 minutes at room temperature with a murine monoclonal antibody against fibrin (Y22 monoclonal antibody, recognizing fibrin but not fibrinogen, TNO), used at 5 $\mu\text{g}/\text{ml}$ final concentration. For apo(a) immunohistochemistry, slides were overlaid for 2 hours at room temperature with IgG-a5, used at 5 $\mu\text{g}/\text{ml}$ final concentration. Immunohistochemical analyses on murine tissues were performed on paraffin-embedded sections of knee joints that were deparaffinized and rehydrated, then incubated for 30 minutes at room temperature with 5% BSA and 20% normal goat serum. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 minutes. Slides were then overlaid with rabbit anti-mouse fibrin(ogen) antiserum (diluted 1/1000) for 30 minutes at room temperature or with rabbit anti-human apo(a) polyclonal antibody (Europa, Cambridge, UK), at a concentration of 5 $\mu\text{g}/\text{ml}$, for 2 hours and 30 minutes at room temperature. Bound antibodies were visualized using an adequate biotinylated secondary antibody and the avidin-biotin-peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). The color was developed by 3,3'-diaminobenzidine (Sigma, St. Louis, MO) containing 0.01% hydrogen peroxide. After extensive washing in water, slides were counterstained with Papanicolaou and mounted in Merckoglass. Staining specificity for apo(a) and fibrin immunohistochemistry was confirmed using, as primary antibodies, preadsorbed antibodies onto clotted fibrin or Lp(a)-containing serum, respectively. Incubation in which the first antibody was omitted served as a negative control.

Apo(a) mRNA Expression in Synovial Tissues

Cryostat sections from synovial or liver tissues were homogenized in Trizol reagent (Gibco BRL, Basel, Switzerland) and total RNA was extracted according to the manufacturer's instructions. A total of $\sim 5 \mu\text{g}$ of total RNA was hybridized overnight at 52°C with 3×10^5 cpm of the $\alpha^{32}\text{P}$ -UTP labeled apo(a) probe, obtained by *in vitro* transcription of a linearized pGEM-T plasmid (Promega, Madison, WI) containing an insert corresponding to sequence 10,467 through 10,807 of the apo(a) cDNA.⁸ A labeled GAPDH riboprobe served to normalize the apo(a) gene expression. Samples were then treated with an RNase cocktail (Ambion, Austin, TX), RNase was removed by proteinase K treatment,

and samples were purified by phenol/chloroform extraction followed by ethanol precipitation using glycogen as carrier. Protected fragments were resolved through a 5% sequencing gel.

AIA in Genetically Modified Mice

LDL-receptor-deficient (LDL-R^{-/-}) mice expressing a human apoB-100 transgene (hApoB ^{+/+}) and a human apo(a) transgene containing 17 K4 repeats under the control of the mouse transferrin promoter [hApo(a) ^{+/-}]²⁹ were used in these experiments. [LDL-R^{-/-}, hApoB ^{+/+}, hApo(a) ^{-/-}] littermates served as controls. Mice were of the haplotype H-2b, and were 10 to 12 weeks of age at the start of the experiment. AIA was elicited as previously described.³⁰ Briefly, mice were immunized at day 0 and day 7 by intradermal injection of 100 μ g of methylated BSA (mBSA, Sigma) emulsified in 100 μ l of complete Freund's adjuvant containing 200 μ g of mycobacterial strain H37RA (GD Diagnostics, Sparks, MD), and by intraperitoneal injection of 2×10^9 heat-killed *Bordetella* Pertussis organisms (Berna, Bern, Switzerland). Arthritis of the right knee was induced at day 21 by intra-articular injection of 100 μ g of mBSA in 10 μ l of sterile phosphate-buffered saline (PBS), whereas the left knee was injected with sterile PBS alone. Grading of arthritis was performed after knees had been dissected and fixed in 10% buffered formalin for 4 days. Fixed tissues were decalcified for 5 days in a solution of 25% formic acid and 7.5% w/v sodium formate, dehydrated, and embedded in paraffin. Sagittal sections (6 μ m) of the whole knee joint were stained with Safranin-O and counterstained with fast green/iron hematoxylin. Histological sections were graded by two observers unaware of the animal genotype. Infiltration of cells was scored on a scale from 0 to 3, depending on the amount of inflammatory cells in the synovial cavity and synovial tissues (0 = no inflammatory cells to 3 = massive exudate or infiltrate). Cartilage proteoglycan depletion, reflected by loss of Safranin-O-staining intensity, was scored on a scale from 0 (fully stained cartilage) to 3 (totally unstained cartilage) in proportion to severity. The intensity of fibrin(ogen) deposition in the joints was scored on a 0 to 6 scale where 0 corresponded to complete absence and 6 to massive deposition of fibrin(ogen).

Statistical Analyses

Nonparametric Mann-Whitney two-sample test was used to compare groups. Analysis of statistical correlation was performed with Spearman's test of rank correlation. For animal studies, the Wilcoxon rank-sum test for unpaired variable (two-tailed) was used to compare differences in histological scoring between groups. *P* values < 0.05 were considered significant.

Figure 1. Relationship between concentrations of apo(a) in paired plasma and synovial fluid samples collected from 32 individuals with OA (filled circles, *n* = 8), RA (open squares, *n* = 12), or other inflammatory types of arthritis (open triangles, *n* = 12). Note the different log scales. Apo(a) was quantitated by a sandwich enzyme-linked immunosorbent assay using mouse anti-apo(a) monoclonal antibodies of well-defined specificity,³¹ as described.³²

Results

Apo(a) Is Present in Synovial Fluid from Human Arthritic Joints

Apo(a) was quantitated in paired plasma and synovial fluid samples collected from 32 arthritic patients (8 patients diagnosed with OA, 12 with RA, and 12 with miscellaneous inflammatory arthritides). Concentrations of apo(a) in plasma varied widely between individuals (from 0.7 to 145.1 nmol/L) and the distribution was markedly skewed toward lower values (median, 11.5 nmol/L), as in other Caucasian populations.⁷ In synovial fluid, the level of apo(a) ranged from 0.5 to 43.1 nmol/L (median, 7.8 nmol/L) and rose in proportion to plasma apo(a) levels (Figure 1, *r* = 0.88). In addition to apo(a), we detected the presence in synovial fluid of cholesterol [from 0.5 to 4.4 mmol/L, 2.4 ± 0.2 mmol/L (mean $\pm$ SEM)], triglycerides (0.1 to 1.6 mmol/L, 0.5 ± 0.1 mmol/L), HDL-cholesterol (0.1 to 1.2 mmol/L, 0.7 ± 0.1 mmol/L), and albumin (11 to 44 g/L, 23 ± 1 g/L).

Apo(a) in Human Synovial Fluid Corresponds to Full-Length Apo(a) as Part of Lp(a)

Immunoblot analysis of apo(a) was performed on paired plasma and synovial fluid samples, either untreated or after incubation with heparin-coated beads [that separate free apo(a) and apo(a) fragments from Lp(a)].³³ A representative blot performed on samples from two different individuals with moderately elevated apo(a) levels in plasma (28.4 and 18.1 nmol/L) and synovial fluid (10.6 and 8.2 nmol/L) is depicted in Figure 2. In both plasma samples, apo(a) was present as full-length apo(a) (lanes 1 and 7), and attached heparin as part of Lp(a) (lanes 3 and 9), whereas apo(a) fragments, which correspond to a

Figure 2. Immunoblot analysis of apo(a) in plasma and synovial fluid. A representative blot from two unrelated individuals with moderately elevated levels of apo(a) in plasma (28.4 and 18.1 nmol/L, respectively) and synovial fluid (10.6 and 8.2 nmol/L, respectively) is depicted. Plasma and synovial fluid samples were incubated with heparin-coated beads, which bind apoB-containing lipoproteins but not free apo(a) or fragments of apo(a), as described.³³ Untreated (U) samples [0.25 μ l of plasma (lanes 1 and 7) and 0.5 μ l of synovial fluid (lanes 4 and 10)], the heparin-unbound (UB) fraction in plasma (lanes 2 and 8) and synovial fluid (lanes 5 and 11), and the heparin-bound (B) fraction in plasma (lanes 3 and 9) and synovial fluid (lanes 6 and 12) were size-fractionated on a 5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted using horseradish peroxidase-conjugated mouse anti-apo(a) monoclonal antibody IgG-a5³¹ and the enhanced chemiluminescence technique, as described.¹²

small fraction (~2%) of apo(a) immunoreactive material, were hardly visible on this exposure (lanes 2 and 8). The size of apo(a) in untreated synovial fluid (lanes 4 and 10) was similar to the one of full-length apo(a) in plasma. Moreover, as was the case in plasma, full-length apo(a) in synovial fluid equally bound heparin (lanes 6 and 12), whereas apo(a) fragments were hardly visible on this exposure (lanes 5 and 11). This data demonstrated that apo(a) immunoreactive material in synovial fluid corresponds to full-length apo(a) as part of Lp(a) particles.

The Relative Abundance of Lipoprotein Particles in Human Synovial Fluid Depends on Their Size and the Type of Arthritis

Concentrations of apo(a) and the other particles examined were compared in arthritic synovial fluid and in plasma. The synovial fluid-to-plasma (SF/P) ratio varied widely between individuals and between the particles examined. However, patients who had a high ratio for one particle tended to have equally high ratios for the other particles, and a close correlation was observed between the SF/P ratios for apo(a) and LDL-cholesterol ($r = 0.63$, Figure 3A). A similar correlation was observed between the SF/P ratio for apo(a) and triglycerides ($r = 0.61$), total cholesterol ($r = 0.67$), HDL-cholesterol ($r = 0.51$), and albumin ($r = 0.62$). In Figure 3A, a lower SF/P ratio for apo(a) was apparent for patients with OA ($16 \pm 4\%$), as compared with patients suffering from RA or other inflammatory types of arthritis ($37 \pm 4\%$, $P < 0.001$). Moreover, the SF/P ratio for the particles examined rose in inverse proportion with their size (Figure 3B), ranging from $27 \pm 3\%$ (range, 3 to 61%) for triglycerides (mainly transported by large very low-density lipoprotein particles) to $32 \pm$

Figure 3. **A:** Relationship between the SF/P ratio for apo(a) and LDL-cholesterol in 32 patients with arthritis. Apo(a) was quantitated as described in Figure 1, whereas LDL-cholesterol levels were determined using the Friedewald formula. **B:** SF/P ratio for triglycerides (TG), apo(a), LDL-cholesterol, HDL-cholesterol, and albumin in 32 individuals, according to disease condition. *, $P < 0.05$; **, $P < 0.01$ versus OA.

4% (2 to 100%) for apo(a) [transported as Lp(a) particles], $42 \pm 5\%$ (1 to 100%) for LDL particles, $52 \pm 3\%$ (3 to 80%) for HDL particles, and $62 \pm 3\%$ (33 to 97%) for albumin. This data indicated that the SF/P ratio was mainly dependent on the type of disease (inflammatory versus noninflammatory) and the size of the particles examined.

Apo(a) Is Present in Human Synovial Tissues But Is Not Synthesized Locally

To determine whether apo(a) is also present in human synovial tissues, we quantitated apo(a) in homogenized synovial tissue collected from seven patients with OA and seven patients with RA (Figure 4A). In this limited sample, the amount of apo(a) in synovial tissues ranged from 52 to 1690 pmol/mg of protein, and seemed to be higher in synovial tissues of patients with RA compared to OA

Figure 4. **A:** Quantitation of apo(a) in synovial tissues. Synovial tissues collected from OA ($n = 7$) and RA ($n = 7$) joints were homogenized and apo(a) was quantitated as described in Figure 1. **B:** Apo(a) gene expression in synovial tissues. RNase protection assay was performed to detect apo(a) transcripts in human liver, in liver from transgenic mice expressing human apo(a), and in OA and RA synovial tissue, as described in Materials and Methods. No apo(a) transcripts were identified in synovial tissues. Three out of six representative samples from each disease group are shown.

(897 ± 194 pmol/mg protein versus 182 ± 26 pmol/mg protein, $P = 0.002$).

RNase protection assay was next performed to examine whether apo(a) present in synovial tissue was synthesized locally (Figure 4B). As positive controls, human and apo(a)-transgenic mouse liver mRNA was used. In these two samples, apo(a) transcripts were found; in contrast, apo(a) transcripts were undetectable in the human synovial tissues examined, even after prolonged exposure of the gel. This data provided strong evidence that the vast majority, if not all, apo(a) detected in synovial fluid and tissue was derived from circulating Lp(a).

Apo(a) Co-Localizes with Fibrin in Human Inflammatory Arthritic Joints and in a Murine Model of Experimental Arthritis

Immunohistochemical analysis was then performed to examine the localization of apo(a) in arthritic synovial tissue. A representative result of this analysis performed on human RA synovial tissues is illustrated in Figure 5.

Abundant amounts of apo(a) were identified in vascular and perivascular areas, in association with the extracellular matrix and in scattered cells, possibly macrophages and foam cells (Figure 5A). The specificity of the apo(a) immunoreactivity was confirmed by a pronounced attenuation of the signal when adjacent sections were incubated with the anti-apo(a) antibody preadsorbed with an Lp(a)-containing serum (Figure 5B). Fibrin staining performed on an adjacent section revealed a distribution of immunoreactivity similar to that of apo(a) (compare Figure 5, C and D), indicating that apo(a) co-localized with fibrin.

The same analysis was performed on LDL-R-deficient mice that expressed both human apo(a) and human apoB-100 [ie, these mice are able to synthesize human Lp(a) particles³⁴] using an experimental model of murine arthritis, the AIA. In apo(a)-expressing transgenic animals, large amounts of apo(a) were immunodetected in synovial tissue (Figure 6A) and in synovial fluid (Figure 6C), and the localization of apo(a) in synovial tissue closely resembled the pattern observed in human specimen. Moreover, we observed that apo(a) in mouse synovial tissue equally co-localized with fibrin(ogen), as was observed in humans [compare Figure 6E, apo(a), and 6F, fibrin]. The reaction was specific to apo(a), as confirmed by the absence of signal when examining the arthritic knee from a mouse that did not express apo(a) (Figure 6, B and D).

Apo(a) Does Not Affect the Severity of AIA in the Mouse

To evaluate the functional impact of apo(a) on the development of experimental arthritis, we next compared the severity of AIA in mice that expressed ($n = 20$) or did not express ($n = 19$) human apo(a). A similar abundance of inflammatory cells in synovial tissue (infiltrate) and fluid (exudate) and a similar cartilage damage were observed in both groups (Figure 7). Moreover, equivalent fibrin(ogen) content was found in the two groups. Finally, the concentration of D-dimers in the circulation, which reflects ongoing fibrinolysis, as measured 10 days after induction of AIA, was similar between mice expressing or nonexpressing apo(a) (3.8 ± 1.0 ng/ml versus 4.7 ± 0.9 ng/ml, $P = 0.20$). Taken together, this data indicated that, in this particular mouse model, apo(a) did not impact significantly on the development of experimental arthritis.

Discussion

In this study, we demonstrate for the first time that full-length apo(a) is present in human synovial fluid as part of Lp(a), and we show that Lp(a) in human arthritic joints originates from the circulation. Diffusion of Lp(a) from the circulation to synovial fluid is facilitated in inflammatory types of arthritis, and is slower for Lp(a) than for smaller particles. In addition, apo(a) was shown to co-localize with fibrin(ogen) in arthritic synovial tissues. This data established apo(a) as a potential link between athero-

Figure 5. Immunohistochemical analysis of apo(a) and fibrin in human RA synovial tissue. Cryostat sections of RA synovial tissue were incubated with IgG-a5, a mouse anti-human apo(a) monoclonal antibody, either untreated (**A** and **C**) or preadsorbed with an Lp(a)-containing serum (**B**), and with an anti-fibrin (**D**) monoclonal antibody. Detection was performed as described in Materials and Methods. Apo(a) was abundantly present in perivascular areas, in scattered cells, and in association with extracellular matrix. Adjacent sections stained for apo(a) (**C**) and fibrin (**D**) showed a co-localization of these two antigens.

sclerosis and arthritis, and prompted us to investigate whether apo(a) promotes arthritis in an animal AIA model. In this particular model applied to mice expressing a human apo(a) transgene, apo(a) did not impact on the severity of experimental arthritis.

Apo(a) was originally identified in human plasma, where its function remains elusive. Identification of fragments of apo(a) in urine³³ raised the hypothesis that apo(a) function may reside outside the circulation, ie, in the kidney or in the urinary tract. More recently, we have reported a strong association between apo(a) and apoE in the development of Alzheimer's disease.³⁵ The present demonstration that apo(a) is present in arthritic synovial fluid and tissue, a compartment where plasminogen has an important role and where the contribution of lipids has recently been highlighted, adds further support for a tissular role for apo(a).

Apo(a) in human synovial fluid was detected as a full-length glycoprotein. This is a somewhat unexpected finding, given the elastolytic activity present in synovial fluid,³⁶ and the fact that apo(a) is sensitive to digestion by elastase *in vitro*.³⁷ This suggests that, at least in the

samples examined, the elastolytic activity in synovial fluid was sufficiently inhibited by anti-proteases and/or that apo(a) is rather insensitive, *in vivo*, to digestion. As such, the present data are in line with our previous observation, in which we showed that no apo(a) fragments were generated in the circulation during cardiopulmonary bypass³⁸ or sepsis,³² two conditions characterized by marked release of elastase by polymorphonuclear cells.

Apo(a) is not the first apolipoprotein to be detected in synovial fluid, as the presence of apoA1, apoE, and apoB in this fluid has already been reported.³⁹ However, the mechanism responsible for the presence of these apolipoproteins in synovial fluid remained poorly understood. Here, we show that the concentration of lipoprotein particles in synovial fluid relative to plasma was smaller in OA than in inflammatory types of arthritis, and that the SF/P ratio inversely correlates with the size of the particles. This observation strongly suggests that lipoproteins diffuse from the circulation into synovial fluid and that the permeability of this barrier is increased in inflammatory types of arthritis. Whether the increased amount of apolipoproteins and their ac-

Figure 6. Immunohistochemical analysis of apo(a) and fibrin in arthritic knee joints from transgenic mice expressing human apo(a). AIA was elicited as described in Materials and Methods and knee joint sections were examined at day 10 of AIA by immunohistochemical analysis using a rabbit polyclonal anti-apo(a) (**A–E**) or anti-fibrin(ogen) (**F**) antibody. Apo(a) was abundantly present in extracellular matrix and in scattered cells (**A**), in synovial fluid (**C**) and co-localized with fibrin(ogen) [compare the distribution of apo(a) in **E** and fibrin in **F**]. Specificity of the apo(a) antibody was assessed using sections from a knee of a non-apo(a)-expressing mouse (**B** and **D**).

companying lipids contribute to the development of inflammatory types of arthritis, whether quantitation of these parameters in synovial fluid will allow, in clinical practice, to better evaluate the severity of the inflam-

matory reaction associated with arthritis, and whether diffusion of Lp(a) particles and binding to fibrin depends on the size of the apo(a) isoforms, remain to be established.

Figure 7. Histological grading of arthritic knee joints from non-apo(a)- and apo(a)-transgenic mice. Histology analysis and fibrin(ogen) immunohistochemistry were performed on knee joint sections after 10 days of AIA. Cell infiltrate (a), cell exudate (b), and cartilage damage (c) were scored using an arbitrary scale from 0 to 3. Intra-articular fibrin(ogen) deposition (d) was scored in the synovial joints using an arbitrary scale from 0 to 6. Human apo(a) transgenic mice [apo(a)+/−, *n* = 20] are noted apo(a) whereas apo(a)−/− mice (*n* = 19) are designated WT.

In the particular mouse model used here, the presence of apo(a) was not shown to impact on the severity of experimental arthritis or on the relative amount of fibrin(ogen) within the joint. This does not formally rule out, however, a role for apo(a) in the pathophysiology of arthritis in humans. First, mice do not express apo(a), and may not have the accessory machinery to allow the development of apo(a) function. Next, the concentration of apo(a) in the joint (which could not be assayed for technical reasons because of the very limited amount of material available) may not be sufficient in the mice examined here to inhibit locally the rate of plasmin generation. The similar plasma D-dimer levels in apo(a)-expressing or nonexpressing mice are in favor of this possibility. Moreover, it is conceivable that apo(a) in the joint may have contrasting effects, and that the net balance between the pro- and anti-arthritic effects may vary between species, or according to age, type of arthritis, or at different stages of the disease. Finally, mice expressing this human apo(a) transgene may not be an adequate model to study the function of apo(a). Indeed, despite ample demonstration that apo(a) is atherogenic in humans, no accentuation by apo(a) of the development of atherosclerosis has been observed in transgenic mice expressing apo(a).^{28,29}

To conclude, the presence of Lp(a) in human synovial fluid and tissue, the larger abundance of Lp(a) in synovial fluid from inflammatory arthritides, and the co-localization of apo(a) with fibrin in inflammatory synovial tissues suggest that, in humans, apo(a) may modulate locally the fibrinolytic activity and may thus contribute to the persistence of intra-articular fibrin and bone matrix degradation in inflammatory arthritis.

Acknowledgments

We thank Helen Hobbs (University of Texas Southwestern Medical Center, Dallas TX) for the generous gift of the plasmid containing part of the apo(a) cDNA and for the transgenic mice; Gilda Crespell and her colleagues for their excellent technical assistance; and Eric Kolodziec for his continuous support in microscopy analysis.

References

1. Wallberg-Jonsson S, Ohman ML, Dahlquist SR: Cardiovascular morbidity and mortality in patients with seropositive rheumatoid arthritis in Northern Sweden. *J Rheumatol* 1997, 24:445–451
2. Symmons DP, Jones MA, Scott DL, Prior P: Longterm mortality outcome in patients with rheumatoid arthritis: early presenter continue to do well. *J Rheumatol* 1998, 25:1072–1077
3. Pasceri V, Yeh ETH: A tale of two diseases—atherosclerosis and rheumatoid arthritis. *Circulation* 1999, 100:2124–2126
4. Hart DJ, Doyle DV, Spector TD: Association between metabolic factors and knee osteoarthritis in women: the Chingford study. *J Rheumatol* 1995, 22:1118–1123
5. Heliovaara M, Aho K, Knekt P, Reunanen A, Aromaa A: Serum cholesterol and risk of rheumatoid arthritis in a cohort of 52 800 men and women. *Br J Rheumatol* 1996, 35:255–257
6. James MJ, van Reyk D, Rye KA, Dean RT, Cleland LG, Barter PJ, Jessup W: Low density lipoprotein of synovial fluid in inflammatory joint disease is mildly oxidized. *Lipids* 1998, 33:1115–1121
7. Utermann G: The mysteries of lipoprotein(a). *Science* 1989, 246:904–910
8. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM: cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* 1987, 330:132–137
9. Sangrar W, Koschinsky ML: Characterization of the interaction of recombinant apolipoprotein(a) with modified fibrinogen surfaces and fibrin clots. *Biochem Cell Biol* 2000, 78:519–525
10. Klose R, Fresser F, Köchl S, Parson W, Kapetanopoulos A, Fruchart Najib J, Baier G, Utermann G: Mapping of a minimal apolipoprotein(a) interaction motif conserved in fibrin(ogen) β - and γ -chains. *J Biol Chem* 2000, 275:38206–38212
11. Soulat T, Loyau S, Baudouin V, Maisonneuve L, Hurtaud-Roux MF, Schlegel N, Loirat C, Anglès-Cano E: Effect of individual plasma lipoprotein(a) variations in vivo on its competition with plasminogen for fibrin and cell binding. An in vitro study using plasma from children with idiopathic nephrotic syndrome. *Arterioscler Thromb Vasc Biol* 2000, 20:575–584
12. Mooser V, Seabra MC, Abedin M, Landschulz KT, Marcovina SM, Hobbs HH: Apo(a) kringle-4 containing fragments in human urine—relationship to plasma levels of lipoprotein(a). *J Clin Invest* 1996, 97:858–864
13. O'Reilly MS, Holmgren L, Shing Y, Chen C, Rosenthal RA, Moses M, Lane WS, Cao Y, Sage EH, Folkman J: Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* 1994, 79:315–328
14. Schuster V, Koolwijk P, Peters E, Frank S, Hrenjak A, Graier WF, van Hinsbergh VWM, Kostner GM: Impact of apolipoprotein(a) on in vitro angiogenesis. *Arterioscler Thromb Vasc Biol* 2001, 21:433–438
15. Busso N, Péclat V, So A, Sappino AP: Plasminogen activation in

- synovial tissues: differences between normal, osteoarthritis and rheumatoid arthritis joints. *Ann Rheum Dis* 1997, 56:550–557
16. Ronday HK, Smits HH, Quax PH, van der Pluijm G, Lowik CW, Breedveld FC, Verheijen JH: Bone matrix degradation by the plasminogen activation system. Possible mechanism of bone destruction in arthritis. *Br J Rheumatol* 1997, 36:9–15
17. Del Rosso M, Fibbi G, Matucci Cerinic M: The urokinase-type plasminogen activator system and inflammatory joint diseases. *Clin Exp Rheumatol* 1999, 17:485–498
18. Walsh DA: Angiogenesis and arthritis. *Rheumatology* 1999, 38:103–112
19. Seriole B, Accardo S, Fasciolo D, Bertolini S, Cutolo M: Lipoproteins, antidiolipin antibodies and thrombotic events in rheumatoid arthritis. *Clin Exp Rheumatol* 1996, 14:593–599
20. Cheras PA, Whitaker AN, Blackwell EA, Sinton TJ, Chapman MD, Peacock KA: Hypercoagulability and hypofibrinolysis in primary osteoarthritis. *Clin Orthop* 1997, 334:57–67
21. Marty I, Péclat V, Kirdaite G, Salvi R, So A, Busso N: Amelioration of collagen-induced arthritis by thrombin inhibition. *J Clin Invest* 2001, 107:631–640
22. Prete PE, Gurakar-Osborne A: The contribution of synovial fluid lipoproteins to the chronic synovitis of rheumatoid arthritis. *Prostaglandins* 1997, 54:689–698
23. Wallberg-Jonsson S, Uddhammar A, Dahlen G, Rantapaa-Dahlqvist S: Lipoprotein(a) in relation to acute phase reaction in patients with rheumatoid arthritis and polymyalgia rheumatica. *Scand J Rheumatol* 1995, 55:309–315
24. Asanuma Y, Kawai S, Aoshima H, Kaburaki J, Mizushima Y: Serum lipoprotein(a) and apolipoprotein(a) phenotypes in patients with rheumatoid arthritis. *Arthritis Rheum* 1999, 42:443–447
25. Park YB, Lee SK, Lee WK, Suh CH, Lee CW, Lee CH, Song CH, Lee J: Lipid profiles in untreated patients with rheumatoid arthritis. *J Rheumatol* 1999, 26:1701–1704
26. Orem A, Deger O, Cimsit G, Karahan SC, Akyol N, Yildirmis S: Plasma lipoprotein(a) and its relationship with disease activity in patients with Behcet's disease. *Eur J Clin Chem Clin Biochem* 1995, 33:473–478
27. Takahashi S, Yamamoto T, Moriwaki Y, Tsutsumi Z, Higashino K: Increased concentrations of serum Lp(a) lipoprotein in patients with primary gout. *Ann Rheum Dis* 1995, 54:90–93
28. Mancini FP, Newland DL, Mooser V, Murata J, Marcovina SM, Young SG, Hammer RE, Sanan DS, Hobbs HH: Relative contributions of apolipoprotein(a) and apolipoprotein-B to the development of fatty lesions in the proximal aorta in mice. *Arterioscler Thromb Vasc Biol* 1995, 15:1911–1916
29. Sanan DA, Newland DL, Tao R, Marcovina S, Wang J, Mooser V, Hammer RE, Hobbs HH: Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). *Proc Natl Acad Sci USA* 1998, 95:4544–4549
30. Busso N, Péclat V, Van Ness K, Kolodziejczyk E, Degen J, Bugge T, So A: Exacerbation of antigen-induced arthritis in urokinase-deficient mice. *J Clin Invest* 1998, 102:41–50
31. Marcovina SM, Albers JJ, Gabel B, Koschinsky ML, Gaur VP: Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). *Clin Chem* 1995, 41:246–255
32. Mooser V, Berger MM, Tappy L, Cayeux C, Marcovina SM, Darioli R, Nicod P, Chioléro R: Major reduction in plasma Lp(a) levels during sepsis and burns. *Arterioscler Thromb Vasc Biol* 2000, 20:1137–1142
33. Mooser V, Marcovina SM, White AL, Hobbs HH: Kringle-containing fragments of apolipoprotein(a) circulate in human plasma and are excreted into the urine. *J Clin Invest* 1996, 98:2414–2424
34. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD, Hammer RE: Reconstitution of lipoprotein(a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein(a). *J Biol Chem* 1992, 267:24369–24374
35. Mooser V, Helbecque N, Miklossy J, Marcovina SM, Nicod P, Amouyel P: Interactions between apolipoprotein E and apolipoprotein(a) in patients with late-onset Alzheimer disease. *Ann Intern Med* 2000, 132:533–537
36. Moore AR, Appelboom A, Kawabata K, Da Silva JA, D'Cruz DG, Gowland G, Willoughby DA: Destruction of articular cartilage by alpha 2 macroglobulin elastase complexes: role in rheumatoid arthritis. *Ann Rheum Dis* 1999, 58:109–113
37. Edelstein C, Italia JI, Scanu AM: Polymorphonuclear cells isolated from human peripheral blood cleave Lp(a) and apo(a) at multiple interkringle sites via the enzyme elastase: generation of mini-Lp(a) particles and apo(a) fragments. *J Biol Chem* 1997, 272:11079–11087
38. Mooser V, Tinguely F, Fontana P, Lenain V, Vaglio M, Ruchat P, von Segesser LK, Marcovina SM, Markert M, Darioli R, Nicod P: Effect of cardiopulmonary bypass and heparin on plasma levels of Lp(a) and apo(a) fragments. *Arterioscler Thromb Vasc Biol* 1999, 19:1060–1065
39. Ananth L, Prete PE, Kashyap ML: Apolipoproteins A-1 and B and cholesterol in synovial fluid in patients with rheumatoid arthritis. *Metabolism* 1993, 42:803–806