VITRONECTIN EXPRESSION IN RHEUMATOID ARTHRITIC SYNOVIA—  
INHIBITION OF PLASMIN GENERATION BY VITRONECTIN PRODUCED IN VITRO

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SUMMARY

The plasmin-generating system controls, to a great extent, the degree of connective tissue destruction as well as fibrin deposition—two contributors to the pathogenesis generated in diseases such as rheumatoid arthritis. Vitronectin, an adhesive blood glycoprotein, has the potential to modulate this system by its known capacity to interact with plasminogen activator inhibitor-1, plasminogen activators, the urokinase plasminogen activator receptor, and plasminogen. The net effect of these interactions, in terms of plasmin generation, is not known as yet. In the present study, we have investigated the possible expression and role of vitronectin in rheumatoid arthritic synovia. Analysis of synovial frozen sections by immunofluorescence showed the presence of vitronectin in the 13 cases studied. In situ hybridization analysis demonstrated the presence of vitronectin mRNA in cells present in areas rich in infiltrating inflammatory cells. The adherent population of the rheumatoid arthritic synovial cells was isolated and found to synthesize and secrete vitronectin into the medium (seven of 10 isolates), as assessed by metabolic labelling and immunoprecipitation. Plasmin-generating activity was detected in the adherent synovial cells, and this activity was increased by antibodies to vitronectin. Our findings show, for the first time, that vitronectin can be endogenously produced in a pathophysiological system where it can inhibit the generation of plasmin.

KEY WORDS: Vitronectin, Plasmin, Fibrinolysis, Arthritis.

RHEUMATOID arthritis is a systemic inflammatory disease characterized by synovial tissue hyperplasia, connective tissue destruction, and increased leucocyte infiltration fostered, in part, by fibrin deposits [1]. The extensive connective tissue destruction observed in rheumatoid arthritis is largely due to the activity of latent metalloproteinases, some of which are activated by plasmin, the enzyme which is also responsible for the degradation of numerous connective tissue glycoproteins, as well as for the degradation of fibrin [2, 3].

Plasmin is derived from the zymogen plasminogen, through the actions of plasminogen activators, of which there are two types: tissue/blood vessel type plasminogen activator (tPA) and urokinase plasminogen activator (uPA). Plasminogen activators are, in turn, controlled primarily by plasminogen activator inhibitor-1 (PAI-1). PAI-1 is present in blood [4], and can be synthesized by a variety of cell types [5, 6]. Its activity is maintained by binding to vitronectin [7–10]. In addition to maintaining PAI-1 activity, the vitronectin–PAI-1 interaction can inhibit cell adhesion via the integrin αβ3 [11] or via the uPA receptor [12]. The net effect of the latter interactions, in terms of plasmin-generating activity, has not been studied as yet.

Vitronectin is an adhesion-promoting glycoprotein [13] which is found primarily in blood; it has also been immunohistochemically localized extravascularly, mostly deposited in diseased connective tissues [14–17]. Its primary site of synthesis is the liver [18, 19]; with a few exceptions, most cell types, other than hepatocytes/hepatoma cells, do not synthesize vitronectin in culture [20, 21].

The present study was undertaken to investigate whether vitronectin is expressed, and/or deposited, in inflammatory conditions, such as rheumatoid arthritis, and to determine its role in relation to plasminogen activation. Previous studies of the various processes involving the interaction of vitronectin with PAI-1 have been performed using exogenously added purified vitronectin, or vitronectin present in the fetal calf serum used in tissue culture. Our study shows, for the first time, that endogenously produced vitronectin inhibits plasmin generation in a pathophysiological system akin to that found in vivo.

METHODS

Antibodies

The monoclonal antibody to vitronectin, MaVN, previously referred to as MaSp (IgG1, purchased from Quidel, San Diego, CA, USA) and the rabbit anti-human vitronectin (Telios Pharmaceuticals, La Jolla, CA, USA) have been characterized previously [21]. The anti-vitronectin antiserum was produced in a pathophysiological system where it can inhibit the generation of plasmin.
Rheumatoid arthritic synovial tissues

Tissue samples were obtained from 13 patients undergoing elective surgery in connection with synovectomy of the knee joint due to rheumatoid arthritis. ‘Normal synovia’ were obtained from two patients with knee tissue showing no signs of local inflammation. Samples were frozen immediately to be cryosectioned and analysed by in situ hybridization, immunofluorescence, and/or for synovial cell isolation, as described below.

Immunofluorescence

Frozen sections were treated as described previously [17]. Sections were fixed in ice-cold acetone for 20 min, air dried, rehydrated, and blocked with 3% bovine serum albumin (BSA). Double immunofluorescence was carried out by incubation overnight at 10°C, with primary polyclonal and monoclonal antibodies, followed by incubation with biotin-conjugated secondary antibodies, and then with avidin–Texas Red (Vector, Burlingame, CA, USA) or by fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (Sigma). Fluorescence microscopy and photography were performed on a Nikon microscope with a microphotography attachment.

Rheumatoid arthritic synovial cell isolation and cell culture

Tissues were minced and treated with a solution of collagenase/DNAase as follows: 2 mg/ml collagenase type IV (Sigma), 140 μg/ml DNAase (Sigma) in RPMI (Gibco, Gaithersburg, MD, USA) containing 10% fetal calf serum (FCS) (National Veterinary Institute, Uppsala, Sweden), 25 mM HEPES (Gibco), 4 mM glutamine, 120 μg/ml penicillin and 100 μg/ml streptomycin, while shaking at 37°C for 2 h. The resulting cell suspensions were passed through a metal sieve to remove large aggregates; the cell suspensions were centrifuged at 1000 r.p.m. and the pellet resuspended in the above RPMI solution, without enzymes. Cells were then plated in the presence of 10% FCS for 1–2 h at 37°C. The unattached cells were discarded, and the attached cells were trypsinized and either replated for further study over a period of 24 h, or preserved frozen in liquid nitrogen. The adherent cell population was mostly composed of myeloid cells and fibroblasts. In three cases, rheumatoid arthritic synovial cells were cultured during a period of at least 2 weeks and passed at least three times in order to obtain synovial fibroblasts. The human foreskin fibroblasts AG1518 were obtained from ATCC (Rockville, MD, USA).

In situ hybridization

Biopsies from synovectomies were cryosectioned, fixed with 4% paraformaldehyde and stored in 70% ethanol at 4°C. In situ hybridization was carried out essentially as described previously [25]. The probes utilized were: a 1.5 kb polymerase chain reaction (PCR)-generated cDNA from HepG2 cells [21] encoding the full human vitronectin, sequence and a collagen z1(1) 372 bp human cDNA probe [25], kindly provided by Dr Eero Vuorio (University of Turku, Finland). Both probes were 32P labelled using a random priming kit (Megaprime, Buckinghamshire); ~106 d.p.m. (5 ng) were added to each slide in 50 μl hybridization solution [4 × standard saline citrate (SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 50% formamide, 1% dextran sulphate, Denhardt’s solution (0.02% Ficoll type 400, 0.02% polyvinylpyrrolidone, 0.02% BSA), 1% N-lauroylsarcosine and 20 mM Na3HPO4] containing 0.5 mg/ml salmon sperm DNA (Sigma). The slides were covered with siliconized coverslips and incubated for 18 h at 42°C in an air-tight box containing formamide buffer [50% formamide, 0.6 M NaCl, 10 mM Tris–HCl (pH 7.5) and 1 mM ethylenedinitrilo tetraacetic acid (EDTA)]. The slides were washed five times in 1 × SSC for a period of 15 min each at 55°C, allowing the last rinse to reach room temperature; they were then dehydrated in 70 and 99% ethanol, respectively, air dried, followed by immersion in film emulsion (NTB2; Eastman Kodak Company, Rochester, NY, USA) at 45°C and drying in a light-proof box containing silica gel. After 6 days exposure, the slides were developed by immersion in Dektol (Kodak) at 15°C for 2 min, followed by fixation for 5 min (Polymax, Kodak) and washing in water for 5 min; they were then counterstained with haematoxylin and eosin, and mounted. Control slides included: (1) the use of a collagen probe as (a) a negative control for hybridization of mRNA from myeloid cells and (b) as a positive control for hybridization to stromal cells; (2) frozen sections of liver known to express vitronectin, used as a positive control for the vitronectin probe; and (3) pre-treatment with RNase prior to hybridization in order to check that the probes were hybridized with cellular RNA in the tissue.

Metabolic labelling and immunoprecipitation

Rheumatoid arthritic synovial cells (1 to 3 × 106) were plated for 1 h in the presence of 10% FCS. The medium was removed and the cell monolayer was washed twice with phosphate-buffered saline (PBS). Methionine/cysteine-free MCDB medium (Swedish Veterinary Institute), containing 4 mM glutamine, 10 mM HEPES, 2% dialysed FCS, 120 μg/ml penicillin, 100 μg/ml streptomycin and 50 μCi/ml [35S]methionine/cysteine (Amersham, UK), was added and cells were incubated for 16 h at 37°C. The medium was removed, treated with 2 mM phenylmethylsulphonyl fluoride (PMSF) (Sigma), spun to remove cell debris, and
concentrated by the addition of 70% ammonium sulphate while mixing at 10°C for 1–3 h. Following centrifugation at 4000 r.p.m. for 40 min at 10°C, the pellet was resuspended in a minimum volume of Tris-buffered saline (TBS) and dialysed against TBS for 2–5 h at 10°C.

Attached cells were then incubated by addition of 1 ml of 0.5% deoxycholate (DOC; Merck, Darmstadt, Germany), 0.5% NP-40, 0.2% SDS (BDH Ltd, Poole), EDTA for 10 min on ice, followed by scraping and centrifugation at 12 000 r.p.m. for 10 min. The detergent-soluble extract and the conditioned medium were pre-cleared for immunoprecipitation by the addition of non-immune rabbit serum followed by Protein A– and/or Protein G–Sepharose or anti-mouse IgG1–Sepharose (Pharmacia, Uppsala, Sweden). Pre-cleared supernatants were incubated with the appropriate antibodies at 10°C, for ~16 h, followed by incubation with Protein A– or G–Sepharose for 1 h. Following centrifugation, the pellets were washed twice with 0.5% DOC, 0.5% Triton X-100 (Merck), 0.1% SDS in TBS, twice with 0.5% Triton X-100, 0.5 m NaCl in TBS, and once with 0.15% Triton X-100 in TBS. Electrophoresis sample buffer, with 10 mM β-mercaptoethanol, was added to the pellets and heated to 95°C for 10 min. The samples were then analysed on 7.5% gels by SDS–PAGE and followed by autoradiography.

Assay for plasmin-generating activity

Rheumatoid arthritic synovial cells or fibroblasts (3 × 10⁴ cells/well) were plated in duplicate in 96-well tissue culture-treated plates and incubated in either RPMI or MCDB containing 0.2% BSA and 10 mM HEPES for 1–2 h, before the addition of antibodies in the appropriate wells. Polyclonal and monoclonal antibodies were used at 80 and 50 μg/ml, respectively. The cells were then incubated overnight and plasminogen (isolated as described in [26]) was added to 10 μg/ml and incubated with the cells for 24 h. Duplicates of 80 μl were taken from each well and added to microtitre well plates to which 100 μl PBS per well were added, followed by 25 μl of 1 mg/ml plasmin substrate (S-2403) from Chromogenix AB (Molndal, Sweden). Absorbance was measured at 405 nm. A total of seven experiments were performed on five different synovial cell isolates in which polyclonal antibodies were tested. Monoclonal antibodies were tested in four of those experiments using three of the synovial isolates. Three different synovial fibroblast cultures, at passages between 3 and 11, were used to test the effects of polyclonal antibodies in six experiments. Monoclonal antibodies were tested in three of those experiments. Statistical analysis was performed using the t-test for the comparison of means of paired samples, in order to calculate the levels of significance (P values) of the effects obtained with the antibody treatments performed, as compared to treatment with no and/or control antibody.

Control experiments included: (1) the test of normal foreskin fibroblasts (AG1518 cells) for the generation of plasmin in the absence or presence of antibodies to vitronectin and PAI-1 (both are of isotype IgG1), (2) the test of conditioned medium without cells in the absence or presence of antibodies, prior to the addition of plasminogen, to test the requirement for cell surfaces for the generation of plasmin; and (3) the omission of plasminogen in wells containing antibodies to test for possible extraneous reactivities between the antibodies and the plasmin substrate.

RESULTS

Table I shows the distribution of vitronectin in rheumatoid synovia. Immunofluorescence analysis of synovia showing no signs of inflammation showed only weak and diffuse staining for vitronectin. In contrast, analysis of rheumatoid arthritic synovial tissues demonstrated the presence of vitronectin in all the cases studied (n = 13). Vitronectin was found either deposited in granulation tissue (Fig. 1) or cellulary associated (Fig. 2). In some cases, where there was no granulation tissue, vitronectin was present in fibrillar form in the stroma (not shown).

The staining for vitronectin shown in Figs 1 and 2 was performed with affinity-purified polyclonal antibodies to vitronectin. This staining was identical to that obtained with the monoclonal antibody MaVN. Figure 1 shows the double-immunofluorescence pattern of an area stained for vitronectin (Fig. 1a), and fibrin (Fig. 1b), demonstrating a staining representative of granulation tissue areas. PAI-1 was associated with cells present in granulation tissue (Fig. 1c), and was also found associated with blood vessels, but similar to vitronectin, PAI-1 was not present in undamaged areas of the connective tissue (not shown). Figure 1d shows the staining obtained with non-immune rabbit IgG.

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Table I. Localization of vitronectin in rheumatoid arthritic synovial tissue

gt, granulation tissue; representative staining is shown in Fig. 1. Cellular, representative staining is shown in Fig. 2. Stromal, denotes a fibrillar staining pattern in the stroma (not shown).

−, −/−, +, +/+, ++ + + + represent increasing degrees of staining intensity and extensiveness.
− indicates no positive staining.
*Denotes non-inflamed synovia.
Vitronectin was also found to be associated with both macrophage-like and fibroblastoid cells. Its association with macrophage-like cells is shown in Fig. 2. Figure 2a, c and e shows cells that are positive for vitronectin, most of which are positive for the macrophage markers, mAb M718 (Fig. 2b), recognizing the CD68 molecule mostly on monocytes and tissue macrophages [27], or RFD7 (Fig. 2d), recognizing mature tissue macrophages in rheumatoid arthritic synovia [23, 27]. The majority of the vitronectin-positive cells were also stained by antibodies against HLA-DR. However, some vitronectin-positive cells were negative for all of these markers and, in addition, negative for muscle cell and endothelial cell markers. These ‘undefined’ cells are likely to be fibroblasts, inasmuch as some of them stained with two fibroblast markers: the monoclonal antibody 5B5, which recognizes the sulphhydryl isomerase subunit of prolyl hydroxylase, and the monoclonal antibody to ‘fibroblast surface protein’ (not shown). In a few instances, there were vitronectin-positive cells in the vicinity of blood vessels, but as shown in Figs 2e and f the staining for vitronectin did not delineate blood vessels, in contrast to the staining observed for fibronectin. Figures 2g and h show the background staining obtained with non-immune rabbit IgG and mouse IgG, respectively.

In situ hybridization analysis of synovial tissues (Fig. 3) demonstrated the presence of vitronectin mRNA in some areas rich in inflammatory cells (Fig. 3a), but not in connective tissue areas devoid of an inflammatory reaction (Fig. 3b). Collagen mRNA was detected in stromal cells, but not in inflammatory cells (Fig. 3c). Figure 3d depicts some of the areas expressing vitronectin (V) or collagen (C). RNAase treatment abolished hybridization for both the vitronectin and collagen probes (not shown).

To study further the possible origin and role of the vitronectin found in rheumatoid arthritic synovia, we isolated the adherent population of cells and metabolically labelled them with [35S]methionine/cysteine. Immunoprecipitation from the conditioned media, with commercially available and previously characterized anti-vitronectin polyclonal antibodies [21], showed the presence of the 68 kDa polypeptide of vitronectin, as well as a faint 98 kDa band (Fig. 4) in seven of the 10 isolates studied.

Immunoprecipitation of vitronectin-producing isolates with monoclonal antibodies to vitronectin gave the same result as that obtained with the polyclonal antibodies (not shown). As controls for antibody specificity, the extracts were subjected to immunoprecipitation with anti-fibronectin antibodies and non-immune
rabbit serum. The anti-fibronectin antibody precipitated a 220 kDa band, corresponding to the expected size of fibronectin under reducing conditions, whereas the non-immune rabbit serum did not precipitate any detectable protein. Immunoprecipitation of conditioned media from synovial fibroblasts with anti-vitronectin antibodies showed the presence of a faint 68 kDa band, indicating a low level of vitronectin synthesis in these cells (not shown).

As shown in Fig. 5, plasmin activity can be generated by rheumatoid arthritic synovial cultures and synovial fibroblasts, upon addition of exogenous plasminogen in the absence of serum. Figure 5 shows a representative experiment in which the plasmin-generating activities of synovial cells and fibroblasts were determined in the absence and presence of various antibodies. Synovial cell cultures clearly displayed plasmin-generating activity. The monoclonal antibody to vitronectin, as well as both the polyclonal and monoclonal antibodies to PAI-1, enhanced the plasmin-generating activities in the synovial cultures. Synovial fibroblast cultures showed low levels of plasmin-generating activity, which could only be detected in the presence of antibodies to either vitronectin or PAI-1. In contrast, foreskin fibroblast cultures did not display any detectable plasmin-generating activity.
Fig. 3—Vitronectin is expressed by inflammatory cells. Frozen sections of rheumatoid articular synovia were processed for in situ hybridization analysis with cDNA probes for vitronectin (a and b) and collagen (c). (a) and (c) Areas rich in inflammatory cells; (b) an area devoid of an inflammatory reaction. The arrows indicate cells positive for hybridization. The areas in which vitronectin (V) or collagen (C) are expressed are depicted in (d).
regardless of the addition of antibodies to vitronectin or PAI-1, indicating that there are no artefactual effects of the antibodies themselves in plasmin generation. In the absence of added exogenous plasminogen, no plasmin was generated by any cell type, either in the absence or presence of antibodies to vitronectin or to PAI-1 (not shown). No plasmin-generating activity could be detected in conditioned media alone (not shown), indicating the requirement for cell surface components in the generation of plasmin. In accordance, uPA was detected in cell extracts of synovial cell isolates by immunoprecipitation (not shown).

The ratios of plasmin-generating activity obtained in the presence of the various anti-vitronectin or anti-PAI-1 antibodies, over the activities obtained with control antibodies are depicted in Fig. 6. In both rheumatoid arthritic synovial cells and fibroblast cul-

**Fig. 4.**—Vitronectin is expressed and secreted by isolated rheumatoid arthritic synovial cells. Synovial cells were isolated, metabolically labelled, and the medium immunoprecipitated and analysed by SDS-PAGE as described in Methods. Molecular weight markers are depicted at the left margin. Immunoprecipitation with polyclonal anti-vitronectin antibodies (lane 1) shows the typical 68 kDa vitronectin band and a faint 98 kDa band. Lanes 2 and 3 show immunoprecipitation with anti-fibronectin antibodies and normal serum, respectively, as controls for general protein synthesis and antibody specificity.

**Fig. 5.**—Plasmin-generating activity of synovial cells and fibroblasts. Plasmin was measured in rheumatoid arthritic synovial cells (RAS cells), rheumatoid synovial fibroblasts (RAS fibroblasts), or foreskin fibroblasts (1518 fibroblasts), in the absence and presence of the indicated antibodies, as described in Methods. The data originate from a representative experiment, showing the standard deviations obtained from quadruplicate samples per condition. PGN = 10 μg/ml plasminogen; VN = vitronectin; pAb and mAb represent the addition of 80 μg/ml polyclonal and 50 μg/ml monoclonal antibodies per well, respectively. Note that the plasmin-generating activity (1) is increased in the presence, particularly, of the monoclonal antibody to vitronectin in both rheumatoid arthritic synovial cells and synovial fibroblasts, (2) can be detected in synovial fibroblast cultures only in the presence of antibodies to vitronectin or PAI-1, and (3) is not detected in foreskin fibroblasts under any condition.

**Fig. 6.**—Summary of the effects of antibodies to vitronectin or PAI-1 on plasmin-generating activity in rheumatoid arthritic cells. The ratios of plasmin-generating activity obtained in the presence of the indicated antibodies (T) over that obtained with control antibody are shown for rheumatoid arthritic synovial cells (RAS cells; A) and rheumatoid arthritic fibroblasts (RAS fibroblasts; B). Standard deviations were calculated by comparison of ratios from the number of experiments and isolates indicated in Methods. An asterisk denotes effects with $P < 0.05$, compared to control antibody.

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tures, the monoclonal antibody to vitronectin had the most pronounced effect, inducing a marked increase in plasmin-generating activity. Figure 6A shows the ratios obtained from a total of seven experiments using five different synovial cell isolates. The monoclonal antibody to vitronectin and the polyclonal antibody to PAI-1 increased plasmin activity by 2.2 \( (P < 0.01) \) and 1.5 \( (P < 0.05) \) times, respectively. Neither the polyclonal antibody to vitronectin nor the monoclonal antibody to PAI-1 showed significant effects as compared to the control antibody, or to no antibody. A total of six experiments were performed with three different synovial fibroblast isolates (Fig. 6B), in which the monoclonal antibody to vitronectin had a dramatic effect, increasing the plasmin-generating activity 11 times over the control. In addition, the polyclonal antibodies to vitronectin and to PAI-1 increased the plasmin-generating activity in these fibroblast cultures by 3 \( (P < 0.01) \) and 1.5 \( (P < 0.02) \) times, respectively.

**DISCUSSION**

In this study we have shown, for the first time, that vitronectin present in rheumatoid synovia is associated with granulation tissue and that synovial cells express vitronectin, both at the mRNA and protein levels. The presence of vitronectin in rheumatoid synovia [28], and in synovial fluid [29], has previously been reported as part of studies investigating the presence of various matrix proteins, their receptors, and of inhibitors of complement-mediated cell lysis, respectively. However, neither the cellular origin nor the role of vitronectin were established in these reports. Two studies [28, 30] reporting the presence of vitronectin in synovia utilize a monoclonal antibody (mAb 8E6) that has previously been shown to be polyspecific, recognizing also the microfibrillar associated protein, MAGP [21]. Thus, stainings with this antibody cannot be attributed to vitronectin with certainty.

Vitronectin was found to be associated with diverse cell populations in the synovia studied. We concluded, based on immunostaining with various cellular markers, that most of the vitronectin-positive cells are macrophages and fibroblasts. In a few cases, cells lining or present in the vicinity of blood vessels were positive for vitronectin. Even though vitronectin did not outline blood vessels in the manner that fibronectin did—by marking the blood vessel basement membranes—this localization of vitronectin could be important with regard to the involvement of the vitronectin receptors, the \( \alpha_\text{v} \beta_3 \) and \( \alpha_\text{v} \beta_5 \) integrins, in angiogenesis [31, 32].

The cellular association of vitronectin in situ suggests that the vitronectin found in rheumatoid arthritic tissue arises, at least in part, locally rather than as a result of extravasation of blood components. This was confirmed by in situ hybridization analysis demonstrating the presence of vitronectin mRNA in infiltrating inflammatory cells. No vitronectin was detected in non-inflamed synovia, suggesting that cytokines or factors generated during the inflammatory response promote vitronectin synthesis. However, the specific inflammatory signals that direct vitronectin upregulation remain to be identified. In agreement, a previous study showed that no vitronectin mRNA was present in normal mouse blood cells or lymphoid organs [33]. The expression levels for vitronectin in rheumatoid synovia appeared to be similar to those previously demonstrated in advanced astrocytoma [34].

The major site for vitronectin synthesis, under normal physiological conditions, is the hepatocytes [19]. With a few exceptions, most primary cells [17], or cell lines [20, 21], do not synthesize vitronectin in culture. In the case of the astrocytomias, the upregulation of vitronectin synthesis appears to be dependent on the environment in situ, as cells derived from these tumours do not synthesize vitronectin in culture. In contrast, we show in this study that the rheumatoid synovial cells are capable of maintaining the signals leading to vitronectin upregulation when cultured in vitro for short periods.

Immunoprecipitation of metabolically labelled rheumatoid synovial cells demonstrated a 68 kDa band, expected for vitronectin, as well as a faint 98 kDa band whose identity remains to be clarified. The latter band has the expected size for SDS-stable, PAI-1–uPA complexes [35]. Thus, it is conceivable that its co-precipitation with anti-vitronectin antibodies arises from some of the vitronectin that remains associated with PAI-1–PA complexes. The co-precipitation occurred with both polyclonal and monoclonal antibodies to vitronectin, but not with non-immune sera or with antibodies to fibronectin, precluding the possibility of non-specific antibody interactions. Previous findings have indicated that either uPA or tPA can displace vitronectin from PAI-1 [7, 9]. However, this has only been tested in reconstituted systems with isolated proteins. It is possible that in complex systems, given that vitronectin can interact with tPA [36] and uPA [37], vitronectin remains associated with the inhibitor–protease complexes.

The ability of anti-vitronectin antibodies to enhance the plasmin-generating activities suggests that vitronectin may maintain PAI-1 activity in this system. If this were the case, it would indicate that the extravascular or ‘cellular’ vitronectin is similar to the liver-derived one. Thus, the inhibition of vitronectin function would result in the loss of PAI-1 activity, which would ultimately cause an increase in plasmin-generating capability. Antibodies to PAI-1 also cause an increase in plasmin-generating activity, but to a lesser extent than the vitronectin antibodies. In particular, the monoclonal antibody to native vitronectin, MaVN, had a dramatic effect on the plasmin-generating activities of the cells studied. An explanation for the strong effect of the anti-vitronectin monoclonal antibody, when compared to the PAI-1 antibodies, could be that: (1) the amount of vitronectin synthesized is lower than the amount of PAI-1 and, thus, the amount of antibodies applied might suffice to neutralize vitronectin, but not PAI-1 activity; (2) the monoclonal antibody used in this study might interfere with the precise site of vitronectin involved in the modulation of plasmin generation, i.e. the PAI-1-binding region, whereas the
monoclonal antibody to PAI-1 might recognize an irrelevant epitope; (3) there are inhibitors, other than PAI-1, for whose activity vitronectin might be important; (4) there are other operative mechanisms related to the binding of vitronectin to uPA [37], the uPA receptor [38], or to plasminogen [39], and/or tPA [36], which may affect plasmin generation in a manner not understood as yet. That the mAb to vitronectin might itself artefactually increase plasmin activity was negated by the fact that no plasmin could be detected in the presence of this or any other antibody in normal fibroblast cultures.

This is the first report showing the de novo synthesis of vitronectin by rheumatoid synovial cells. The system used should allow further elucidation of the mechanisms involved in vitronectin upregulation in inflamed tissue. This is also the first report demonstrating that endogenously produced vitronectin decreases plasmin-generating activity in a complex mixed system akin to that found pathophysiological. This system will allow us the possibility to study further the net effects and the relevance of the various interactions which are known to take place between vitronectin and components of the plasmin-generating system, as well as the coagulation system. In a preliminary study, we have found vitronectin strictly co-localized with fibrin in all the cases where fibrin was present (our unpublished observations). Because fibrin is an important contributing factor to persistent inflammation, these findings may prove to be of clinical significance. The importance of plasmin in the activation of metalloproteinases with the consequent destruction of connective tissue in arthritic joints is well recognized [40]. The present study suggests an important role for vitronectin as a modulator of plasmin generation in rheumatoid arthritis.

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Vitronectin expression in rheumatoid arthritic synovia—inhibition

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