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Fibrin Deimination in Synovial Tissue Is Not Specific for Rheumatoid Arthritis but Commonly Occurs during Synovitides

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Autoantibodies to deiminated (citrullinated) proteins are the most specific serological markers of rheumatoid arthritis (RA). Deimination is critical in generating the peptidic epitopes they recognize. In the synovial tissue (ST), deiminated forms of the α- and β-chains of fibrin are their major autoantigenic targets (anti-human fibrin(ogen) autoantibodies (AhFibA)). We investigated whether the presence of deiminated fibrin in the ST was specific for RA, because this could explain why AhFibA are RA specific. In 13 patients with RA and 19 patients with various other rheumatological disorders, knee ST biopsies were collected in macroscopically inflamed areas identified under arthroscopy. Synovitis was histopathologically confirmed in all of the biopsies. By immunoblotting, using antisera to fibrin, Abs to citrullyl residues, and AhFibA purified from RA sera, deiminated fibrin was evidenced in ST extracts from all of the patients. Moreover, variations in the degree of fibrin deimination were observed that were not related to the disease. Immunohistochemical analysis, using Abs to citrullyl residues and an antisera to fibrin on adjacent serial sections of ST, confirmed the results because deiminated proteins colocalized with fibrin in RA as well as in control patients. Therefore, fibrin deimination in the ST is a general phenomenon associated to any synovitis, which does not necessarily induce a B autoimmune response with production of AhFibA. The Journal of Immunology, 2005, 174: 5057–5064.

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3 Abbreviations used in this paper: RA, rheumatoid arthritis; AFA, antifilaggrin autoantibody; AhFibA, anti-human fibrin(ogen) autoantibody; AMC, Ab to modified citrullyl residues; AS, ankylosing spondylitis; MM, multiple myeloma; PAD, peptidyldiamarginine deiminase; PAA, psoriatic arthritis; ST, synovial tissue.
than 50 years ago (for review, see Ref. 30). Moreover, PADs are expressed in a wide variety of vertebrate tissues. Nonetheless, only very few physiological PAD substrates have been characterized to date, and the probably very numerous and important biological consequences of this posttranslational modification have not yet all been elucidated. In the rheumatoid synovium, deimination of fibrin could make it immunogenic, the deiminated fibrin eliciting or at least sustaining production of the RA-specific autoantibodies. To fully consider this hypothesis, it was essential to know whether the presence of deiminated fibrin was, or was not, specific for the rheumatoid ST. Indeed, a close association to RA would explain why AhFibA are so highly specific for the disease.

To answer this question, we analyzed synovial biopsy samples from a series of 32 patients with RA or with non-RA rheumatological disorders (control patients). The presence of deiminated fibrin in the ST was analyzed by immunoblotting and immunohistology, using Abs directed to citrullyl residues (targeting any deiminated proteins) and to fibrin, and also AhFibA, immunopurified from a pool of RA sera.

Materials and Methods

Patients

Among consecutive patients undergoing needle arthroscopy for knee pain and/or arthritis in the Department of Rheumatology of Ghent University Hospital, 32 were selected who exhibited synovitis as asserted by macroscopical examination under arthroscopy. Thirteen patients were diagnosed as having RA, according to the American College of Rheumatology classification criteria (31). The other 19 patients (control patients) included 13 patients with spondyloarthropathies (classified in 4 ankylosing spondylitis (AS), 4 psoriatic arthritis (PsA), 1 reactive arthritis, 4 undifferentiated spondyloarthropathy, according to the criteria of the European Spondylarthropathy Study Group (32)); 1 patient with systemic lupus erythematosus (33); 4 patients with osteoarthritis of the knee, classified according to the American College of Rheumatology criteria (34); and 1 patient with a multiple myeloma (MM) presenting a synovial extension. All patients gave their informed consent as approved by the Ethics Committee of the Ghent University Hospital.

Tissues

ST biopsies were obtained during arthroscopy, as previously described (35). Briefly, after careful inspection of the joint cavity, the biopsies (14 per patient) were collected from the macroscopically inflamed areas using 2-7 mm biopsy forceps (Karl Storz). For histological and immunohistological analyses, 8 biopsies were fixed in 3% formaldehyde and then embedded in paraffin. For immunochemical analyses, the remaining 6 biopsies were snap frozen and stored at −80°C until ST extraction was performed.

Histology and immunohistology

Histological analyses were performed after staining with H&E. To evaluate ST inflammation, histological parameters were scored by two independent observers unaware of the diagnosis and clinical data, as previously described (36) (see Table I). To assess the presence of fibrin, sections were probed with a rabbit antiserum directed to the B-chain of human fibrinogen (1:4000; Cambio), followed by Ab to rabbit IgG coupled to a peroxidase-enzyme immunoperoxidase reaction (1:2000; DakoCytomation). On adjacent sections, the presence of deiminated proteins was probed using an Ab to modified citrullyl residues (AMC; purified rabbit IgG, a generous gift of Dr. T. Senshu, Graduate School of Integrated Science, Yokohama City University, Japan) (37). AMC was used at 0.66 μg/ml after Ag retrieval by a 40-min incubation in 10 mM citrate buffer (pH 6.0) in a boiling water bath and in situ modification of citrullyl residues, as previously described (38). This procedure was also applied to sections of formaldehyde-fixed and paraffin-embedded skin, which served as positive control. AMC binding was visualized using protein-coupled goat IgG to rabbit IgG (1:100; Southern Biotechnology Associates), followed by biotinyl tyramide- and peroxidase-based amplification using the Tyramide Signal Amplification Biotin System (PerkinElmer Life and Analytical Sciences), as suggested by the manufacturer. Finally, whatever the Ag detected, the peroxidase activity was visualized using 3-amino-9-ethylcarbazole, and all of the sections were counterstained with hematoxylin. In both staining procedures, a normal rabbit antiserum was used as negative control.

Sequential protein extraction from synovial tissues

ST biopsies (median wet weight 28.5 mg; range 8–119 mg) were sequentially extracted with an Ultra-Turrax homogenizer (T25 basic; IKA Labortechnik) in 500 μl of ice-cold 40 mM Tris-HCl-based buffers (pH 7.4; three times in each buffer), containing first 150 mM NaCl (TBS extract), then 8 M urea, deionized by incubation with a mixed bed resin (AG 501-X8; Bio-Rad), then 8 M urea, deionized by incubation against 50 mM EDTA were performed using disposable ultrafiltration devices with a 3-kDa molecular mass cutoff (Centricon YM-3; Millipore). After concentration so that the total suspension volume equaled 2.5 μl/mg ST sample, the protein solution was placed in SDS-PAGE sample buffer.

Immunoblotting

Whenever possible, equal amounts of total proteins from all of the 32 urea-DTT ST extracts (as adjusted by visual examination of Coomassie blue-stained gels) were separated by SDS-PAGE on 7.5% polyacrylamide gels and then electrotransferred onto reinforced nitrocellulose membranes (Hybond-C extra; Amersham). Each membrane was successively detected
by several primary probes diluted in 40 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl, 0.05% Tween 20, and 2.5% powdered skimmed milk (blotting buffer). To make sure that the signals observed were actually due to binding of the probing primary Ab used, each time that the membrane was probed, the process was preceded by an Ab removal procedure that included a 5-min wash in 0.2 M glycine-HCl (pH 2.7) and then a 30-min incubation at 50°C in blotting buffer containing 14 mg/ml SDS and 0.2 M 2-ME. The primary probes used were: AhFibA purified from a pool of RA sera (10 μg/ml), then the antiserum to the β-chain of human fibrin(ogen) (1:800,000), then a sheep antiserum to the α-chain of human fibrin(ogen) (1:4,000; Cambio), and, finally, after chemical modification of citrullyl-residues (37), AMC (0.8 μg/ml). Peroxidase-conjugated secondary probes were used for detection of all the primary Abs: protein A (Sigma-Aldrich), goat Abs to rabbit IgG (H + L) (Zymed Laboratories), and rabbit F(ab'2) to sheep IgG (Southern Biotechnology Associates) for the detection of human, rabbit, and sheep IgG, respectively. Peroxidase activity was visualized using ECL reagents (Amersham), as suggested by the manufacturer. Negative controls included probing with the secondary probe only.

The specificity of the staining obtained with AhFibA was further checked using human IgG purified from healthy individuals (Sigma-Aldrich). Fibrinogen, purified from human plasma, and a urea-DTT extract from a rheumatoid ST sample previously demonstrated to contain deiminated fibrin (8) were used as controls and systemically included in the SDS-PAGE gels for simultaneous immunoblotting.

Fibrinogen deimination

Plasminogen-depleted human fibrinogen (95% pure; Calbiochem) was further purified, as suggested by the manufacturer, by affinity chromatography on a protein G column (Hitrap protein G, 1 ml; Amersham) to eliminate residual contamination by IgG. Deimination was then performed at 0.86 mg of fibrinogen/ml with rabbit skeletal muscle PAD (Sigma-Aldrich; 7 U/mg fibrinogen) in 0.1 M Tris-HCl (pH 7.4), 10 mM CaCl2, and 5 mM DTT for 2 h at 37°C.

Autoantibody purification

AhFibA were purified, as previously described, from the IgG fraction of a pool of 38 high-titered RA sera (8). Briefly, the IgG were loaded onto a 5-ml N-hydroxysuccinimide Hitrap column (Amersham) coupled with 14.4 mg of deiminated human fibrinogen. After a 2-h incubation at 4°C, the column was washed with 20 mM phosphate buffer (pH 7.0) containing 2 M NaCl and then bound Abs were eluted with 0.2 M glycine-HCl (pH 2.7) and immediately neutralized by addition of 2 M Tris. This purification procedure was repeated several times, and the fractions corresponding to the eluted AhFibA were pooled and concentrated using affinity chromatography on protein G.

AhFibA assay

The titer of AhFibA in the sera was evaluated with a recently developed ELISA using in vitro deiminated human fibrinogen as immunosorbent (11). Sera were classified as positive for the presence of their own target, deiminated fibrin.

Statistical analyses

Median differences were tested with the Mann-Whitney U test. The p value adjustment for multiple comparisons was done by the Holm (sequential Bonferroni) correction method (39). Values of p less than or equal to 0.05 were considered significant.

Results

Histopathological examination confirms the existence of synovitis in all RA and control patients

Clinical data of the patients are shown in Table II. Knee synovitis was arthroscopically asserted in all of the patients. All but one (RA13) had an associated synovial fluid effusion. As expected, upon histological examination of the ST (Table I), the individual scores obtained for mean lining thickness, vascularity, and infiltration by cells of hematological origin clearly indicated that all of the synovial membranes were actively inflamed, even though, on comparison, the groups of RA and control patients tended to show differences reflecting disease-related features (36).
amount of deiminated fibrin assessed by immunoblotting in the ST biopsies (data not shown).

**Deiminated proteins and fibrin are histologically colocalized in the synovial tissue of both RA and control patients**

The presence of deiminated fibrin in the synovial membrane was also indirectly assessed in 19 of the 32 patients by immunoperoxidase staining of adjacent serial sections of synovial biopsies with the antiserum to the \( \beta \)-chain of fibrinogen (with and without AMC) (illustrated in Fig. 2). In 12 patients (corresponding to 6 RA and 6 control patients), the anti-fibrin Ab stained interstitial amorphous deposits of varying abundance located in the lining layer and/or in the deep synovium. In 2 of these 12 patients (RA 9 and RA 11), AMC did not stain the fibrin deposits, whereas in the other 10 patients, fibrin deposits were labeled. The labeling intensity of the fibrin deposits was variable from one patient to another, but also, in each ST sample, within a given fibrinous zone. As illustrated in Fig. 2, high, medium, or low degrees of deimination of the fibrin deposits were observed in both RA and control patients.

Therefore, unlike with immunochemical analysis, the less sensitive immunohistological methods did not allow detection of deiminated fibrin in all of the ST samples. Nevertheless, the results of both analyses are in perfect agreement to confirm that the presence of deiminated fibrin in the ST is not specifically associated to RA. Moreover, the deiminated fibrin deposits did not appear more prominent in RA than in other synovitides.

**Discussion**

Using an Ab directed to citrullyl residues, produced by immunization of rabbit with l-citrullin coupled to keyhole limpet hemocyanin (in this work referred to as anti-l-Cit), we previously explored, by immunohistology, the association between RA and the presence of deiminated proteins in the ST (40). With this Ab, deiminated proteins were evidenced in the ST of RA patients, located in the cytoplasm of rare cells (2–5/biopsy section) of the lining and sublining layers, these labelings being totally absent from the ST of patients with other rheumatologic disorders, including spondylarthropathy and osteoarthritis (40). However, the presence of deiminated fibrin could not be reliably investigated because anti-l-Cit did not allow reproducible staining of deiminated extracellular fibrin deposits to be obtained. Moreover, anti-l-Cit is unable to recognize deiminated proteins in immunohistology, because, by ELISA as well as immunoblotting, it fails to detect many deiminated proteins such as the deiminated \( \alpha \)- and \( \beta \)-chains of human fibrinogen, and deiminated filaggrin purified from human epidermis (our unpublished results). Therefore, in the present study, AMC, an Ab to citrullyl residues developed by Senshu et al. (37) and known to be reactive with deiminated fibrin both by immunohistology and immunoblotting (8), was used to assess whether the presence of deiminated fibrin in the ST was specific for RA or not. The results clearly demonstrate that the presence of deiminated fibrin in the synovial membrane is not specific for RA. Moreover, as already observed (8), AMC decorated the cytoplasm and/or the nucleus of often quite numerous cells that morphologically evoked macrophages and fibroblasts (data not shown). This cellular labeling was not confined to RA patients, but was also seen in two cases of ankylosing spondylitis, one case of psoriatic arthritis, one case of undifferentiated spondylarthropathy, and one case of osteoarthritis. In contrast, when the ST samples of the present study were probed with anti-l-Cit according to our protocol (40), the results obtained were very consistent with those of our previous study: rare citrulline-positive cells were detected in 7 of 13 RA patients and in none of the 19 control patients (data not shown). Therefore, the two Abs to citrullyl residues, AMC and anti-l-Cit, obviously do not recognize the same sets of deiminated proteins. Anti-l-Cit defines a subset of intracellular deiminated protein(s) that is specific for RA. The molecular characterization of these proteins is therefore of great interest for RA pathogenesis.
Nevertheless, to date, anti-i-Cit is the only Ab showing such specificity, but it does not work in immunochemical techniques. Therefore, it cannot help the characterization. This exciting future challenge is beyond the scope of the present study.

Because the presence of fibrin deposits is linked to tissue inflammation, to check the association between RA and deiminated fibrin, we had to compare the ST of RA patients with other inflamed ST. The controls were therefore chosen among patients with non-RA inflammatory rheumatological disorders. Patients with osteoarthritis and one with knee localization of a multiple myeloma were also included in our study because even though they are not classified as inflammatory disorders, these diseases can also lead to intense local synovitis. Accordingly, all of the control ST samples in our study, including those from osteoarthritic and multiple myeloma patients, as well as the rheumatoid ST samples, exhibited local synovitis, as indicated by arthroscopy and histology. Therefore, the presence of deiminated fibrin that we observed in all the ST is probably a consequence of their inflammatory state. In agreement with this hypothesis, and with our results, is the fact that the presence of deiminated fibrin in the ST has recently been demonstrated in two mouse arthritis models, namely acute streptococcal cell wall-induced arthritis and chronic destructive collagen-induced arthritis, whereas the synovial membrane of naive control mice was devoid of fibrin and of deiminated proteins (41). Although it remains to be established, it is also highly conceivable that deimination of fibrin may not be restricted to ST inflammation, but also may be observable during every tissue inflammation in which fibrin deposition is prominent, such as, for example, glomerulonephritis or acute lung injury. More generally, deimination of fibrin during inflammation could occur in numerous organs and play a major role in the physiology of inflammation. Confirmation of this hypothesis would have a wide impact in human pathology.

As mentioned earlier, PADs are expressed in a wide variety of tissues, but in most of them, the biochemical nature of their substrate(s) and hence their function still remain to be unraveled. Myelin basic protein (42), type I and type II cytokeratins (38), filaggrin (43), and trichohyalin (44) are among the few proteins that have been demonstrated to be in vivo PAD substrates. A common consequence of deimination of these proteins is a decrease of their net charge that, in vitro, has been shown to modify intermolecular interactions (45–47). These modifications probably play a role in physiological processes such as terminal differentiation of epidermis (48), and skin and brain development (49, 50). Concerning pathophysiological processes, in multiple sclerosis, the associated increase in the relative amount of deiminated forms of myelin basic protein was proposed to be involved in the disease-characteristic instability of myelin sheaths (51). Deimination of fibrin could hamper its cleavage by plasmin, arginyl residues being included in the cleavage sites of this enzyme. The subsequent defect in fibrinolysis could have a role in perpetuating inflammation because fibrin accumulation in the ST has been demonstrated to play a pathogenic role in various animal models of arthritis (52–54). As, in this work, we demonstrate the presence of deiminated fibrin in other rheumatic diseases associated with synovitis, a deimination-related defect in fibrinolysis could constitute a general phenomenon contributing to the maintenance of ST inflammation.

FIGURE 2. Immunohistological analysis shows that deiminated fibrin is present in the ST of RA as well as control patients. Deiminated fibrin was searched in the ST biopsies by immunoperoxidase staining of adjacent serial sections (panel pairs) with the Ab to the β-chain of human fibrin(ogen) (Fibrin, left panels) and with AMC (Deiminated proteins, right panels). In the synovial membranes of RA (RA 6, RA 13, RA 7) and control patients (PsA 4, AS 2, MM 1), the presence of fibrin deposits can be evidenced by staining of interstitial amorphous zones of varying abundance. These zones are stained to a varying extent and intensity by AMC, in both RA and control patients. The degree of staining indicates the degree of deimination of the fibrin deposits, a high (RA 6, PsA 4), medium (RA 13, AS 2), or low (RA 7, MM 1) degree of deimination being observable in both patient groups. Bars = 50 μm.
When the presence of deiminated fibrin in the ST was investigated by immunoblotting, in both RA and control samples, we noted that the intensities of AMC staining were generally higher for the α- than for the β-chain of fibrin. This suggests that the α-chain is more accessible to PAD than the β-chain. The staining intensities of the β-chain obtained with AMC and with AhFibA were well correlated with each other, whereas a high degree of deimination of the α-chain evidenced with AMC did not always correlate with strong staining by AhFibA. This suggests that while the in vivo deimination of the α-chain seems more effective than that of the β-chain, this does not necessarily lead to the generation of a higher number of AhFibA epitopes on the α- than on the β-chain. Moreover, the fact that the staining intensities of the α-chain obtained with the antisemur to the α-chain of human fibrin(ogen) and with AhFibA appeared inversely correlated in all samples tested is also noteworthy. One possible explanation for this observation is that while generating citrulline-containing epitopes on the α-chain of fibrin targeted by AhFibA, deimination at the same time disrupts arginine-containing epitopes targeted by the antisemur to the α-chain of human fibrin(ogen). This hypothesis is in agreement with the decreased intensity that we observe with this antisemur when probing in vitro deiminated human fibrinogen in comparison with an equal amount of its undeiminated form.

Even though the presence of deiminated fibrin is not specific for RA, the autoantibody response to it is tightly associated to the disease. The reason for this association therefore remains to be elucidated.

In this study, whatever the disease we analyzed, deiminated fibrin of the ST was recognized by AhFibA. This shows that the epitopes produced in the nonrheumatoid ST are either the same or largely overlap or are at least cross-reactive with those produced in rheumatoid ST. However, one cannot exclude that one or more immunogenic epitopes could be specifically generated during RA. Such epitope(s) could initiate an autoimmune response that would therefore be disease specific. Secondarily, epitope spreading could lead to the production of Abs directed to epitopes common to rheumatoid and nonrheumatoid ST. Generation of such RA-specific epitopes could be due to the activity of one or several RA-specific PAD isof orm(s) exhibiting variations in their fine substrate specificity. Interestingly, in a Japanese population, it was recently found that a haplotype of the gene encoding isof orm 4 of PAD (PAD4) was associated with an increased susceptibility to RA, and that individuals homozygous for this haplotype were more likely to be AFA positive (55). This haplotype was found to be associated with the production of a PAD4 mRNA with increased in vitro stability, suggesting that the amount of PAD4 and, consequently, the amount of deiminated proteins and particularly of deiminated fibrin could be higher in rheumatoid than in nonrheumatoid ST. However, in our study, we could not evidence quantitative differences in the amount of deiminated fibrin between RA and control patients. This could be related to differences in the populations analyzed because the association between susceptibility to RA and the described haplotype of the PAD4 gene has not been confirmed in a Caucasian population from United Kingdom (56). Alternatively to an increased total amount of PAD enzyme, the described RA-associated haplotype could rather encode a PAD4 whose fine substrate specificity leads to the generation of the above mentioned RA-specific immunogenic epitopes on deiminated fibrin. Finally, another possible source of RA-specific immunogenic epitopes corresponds to the still uncharacterized RA-associated intracellular deiminated protein(s) defined by the anti-L-Cit Ab. Indeed, some of these proteins seem to correspond to targets of the RA-associated autoantibodies to deiminated proteins because in some cells, anti-L-Cit and AFA reactivities were histologically colocalized (40).

An alternative to the above hypotheses would be that reactivity to deiminated proteins bearing epitopes that mimic epitopes targeted in deiminated fibrin is not subsequent to the appearance of RA-specific epitopes, but genetically determined. The Ab response could indeed be dependent on a T cell response restricted by the MHC class II molecules bearing the so-called shared epitope that characterizes the HLA-DR haplotypes associated with an increased susceptibility to RA. Supporting this hypothesis is the fact that we found that the titers of AFA are significantly higher in patients with susceptibility alleles (57) and that an association between the presences of autoantibodies to deiminated proteins and of shared epitope-bearing alleles was also described by other groups (13, 20, 21). The deiminated proteins eliciting the Ab response could either be expressed by infectious agents or be endogenous. Such an endogenous immunogen could be the RA-associated deiminated protein(s) recognized by the anti-L-Cit Ab or deiminated vimentin, which moreover could correspond to the same single Ag. Indeed, this Ag was recently demonstrated to be the target of the anti-Sa autoantibodies (58), another family of RA-associated autoantibodies that therefore belongs to the family of autoantibodies to deiminated proteins and probably overlaps with the other members of the family. Interestingly, although T lymphocytes specific for deiminated fibrin have not been described yet, a recent study showed that, in a given vimentin-derived peptide, the conversion from arginine to citrulline at the peptide side-chain position interacting with the shared epitope increases the affinity of the interaction with the MHC molecule (59). Although these results remain to be confirmed with a larger set of peptides, they open a very interesting road toward the understanding of how shared epitope-bearing DRB1 alleles could contribute to the autoimmune response to deiminated self Ags in RA patients.

Be that as it may, in our study, the presence of susceptibility alleles was investigated in 10 of the 13 RA patients and 8 of the 19
control patients (data not shown). The results were consistent with the largely reported association between RA and these alleles, because 10 of 10 RA patients had a genotype containing at least one susceptible allele. They also confirmed the association between these alleles and the Ab response to deiminated proteins because, among the 7 of the 10 shared epitope-positive RA patients in whom the presence of AhFibA was investigated, 6 were AhFibA positive. However, none of the control patients had detectable AhFibA, indicating that even if they possibly play an important role to generate the AhFibA response, deiminated fibrin, present in all control patients, and shared epitopes bearing HLA-DR alleles, present in 4 of the 8 analyzed control patients, are not the only factors involved.

Although our results exclude a direct relationship between autoantigen expression and specific autoimmune response, we cannot exclude that a large overproduction of deiminated fibrin contributes to drive the AhFibA response in RA. Interestingly, some target autoantigens of systemic sclerosis-associated autoantibodies were found to be selectively overexpressed in the dermal fibroblasts of diseased patients (60). It has been reported that fibrin deposits are particularly abundant in the rheumatoid synovium vs the ST from patients with osteoarthritis or joint trauma (61). However, in the present work, we could not detect that the amount of fibrin per gram of ST was increased in RA patients vs other rheumatological disorders. This apparent discrepancy may be related to differences in the inflamed character of the control ST analyzed, because, in our study, no bias relative to the inflammatory status of the synovial membranes was introduced when the RA and control patients were compared. Nonetheless, generally, in RA compared with other rheumatological disorders, the ST is particularly more hyperplastic, and the number of joints inflamed is higher; therefore, the global amount of fibrin in the body is higher. Because we did not find any particular decreases in the ratio of deiminated fibrin to fibrin in the rheumatoid compared with nonrheumatoid ST, that means that the global amount of deiminated fibrin is probably often greater in RA patients. Although, in our study, we could not evidence a relationship between the serum AhFibA titer of RA patients and the amount of deiminated fibrin in the ST of one inflamed joint, this does not exclude that a positive correlation may exist with the total body amount of deiminated fibrin.

In conclusion, even if to date the specific presence of AhFibA in RA remains unexplained, we think that the role of the autoimmune response to deiminated fibrin in the disease pathophysiology is undoubtedly important. We propose that fibrin deimination in the ST is a key element of the disease-characteristic perpetuation of synovitis because it feeds an immunological conflict that has proinflammatory effects that promote the formation of new fibrin deposits in the tissue, which are secondarily deiminated (Fig. 3). The precise biological pathways leading to fibrin deimination in the human ST are currently the object of investigations in our laboratory, in particular, determination of which isoforms of the PAD are expressed in the ST and what their cellular origin is.

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Disclosures

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