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Targets Hidden by Fibrin Networks Still Need to Find Their Place in the Armamentarium for Rheumatoid Arthritis

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Introduction

The treatment of rheumatoid arthritis has undergone a huge revolution in the past two decades with the incoming of biological therapies, which have been designed out of a better understanding of pathogenic mechanisms. However, while these compounds successfully prevent -or at least slow down- joint damage in most of the patients, they all show an efficacy ceiling effect and their survival is limited. This reflects the complex and heterogeneous pathogenesis that characterises the disease, and is the reason why a continuous search for new strategies is necessary. In this context, the purpose of this commentary is to revisit intra-articular fibrin deposition as a yet little exploited approachable process in the treatment of rheumatoid arthritis. In the light of previous research, we underline the early place of fibrin in the pathogenic cascade as well as its involvement in the development of synovitis. In addition, we go through potentially targetable checkpoints and briefly discuss limitations to the development of fibrin-based therapies.

Fibrin Deposition and the Sequence from Early to Established Disease

As we understand it today, rheumatoid arthritis is the result of a succession of events targeting susceptible hosts [1-3]. The disease is thought to start as a non-specific inflammatory arthritis following the incidence of a variety of inducing triggers [4-8]. Next, the unbalance between resolving and perpetuating mechanisms results in the amplification of the inflammatory process. With spreading of pro-inflammatory mechanisms, it evolves to a more specific, usually non-subsiding process, characterised by the generation of immune memory, or established rheumatoid arthritis [3]. Pro-inflammatory cytokines are considered principal mediators of this transition, and are currently the cornerstone in the design of biological therapies. However, cytokines are interconnected in networks, characterised by their pleiotropic actions and multiplying circuits. These intricate interactions make it unlikely for cytokine-blocking drugs to completely stop the disease in the long run. It appears that therapeutic strategies should better be aimed at events upstream the cytokine induction in the pathogenic sequence.

Returning to early stages of the disease, joint swelling is associated to the effusion of plasma fibrinogen and zymogens to the joint cavity. Therefore, the struggle between resolving and perpetuating factors also involves clotting and fibrinolysis inside joints, and this battle is completely skewed towards fibrin deposition in rheumatoid hosts [8-13]. We can positively regard rheumatoid arthritis as an extravascular thrombophilia. The remarkable presence of fibrin in rheumatoid joints has been a matter of discussion for many years. A number of studies have addressed the specific alterations of extravascular hemostasis associated to the disease, the principal of which are summarised in Figure 1. On one hand there is an abnormally high procoagulant activity, with an increase in tissue factor (TF) and in plasminogen activator inhibitor (PAI) I [10]. On the other, fibrinolysis is not proportional, as reflects

the down-regulation of urokinase-type plasminogen activator (u PA) [11]. In addition, citrullination taking place within inflamed joints might facilitate the procoagulant deregulation. This post-translational modification usually leads to functional alterations of the substrates, and has been found to hamper antithrombin enzymatic activity, as well as the digestion of fibrin [12]. Also the degrading rate of carboxypeptidase B (CPB) has been found decreased in rheumatoid joints [13].

Fibrin Involvement in the Development of Synovitis

Synovitis, which is the defining feature of the disease, is thought to be a consequence of disease perpetuation. Synovitis can be defined as an invasive transformation of resident synovial cells leading to the destruction of cartilage and bone [14-16]. Studies based on histopathological findings have suggested that fibrin deposition could exert a central role in the development of rheumatoid synovitis [17,18]. Typically, the synovial tissue depicts in rheumatoid arthritis a mosaic-like architecture, in which areas of hypertrophic growth and invasion alternate with regions looking spared. One of the distinguishing features between these regions is precisely the presence of fibrin. Figure 2 shows a typical fibrin-rich region in a rheumatoid synovial tissue. In the photomicrograph, a high cell density, infiltration by macrophages, and fibrotic scarring can be observed. Positively, fibrin plays a physiological role in wound healing, promoting cell scatter through the matrix and its remodelling. It also contributes to transmigration of leukocytes through the vessels wall [19]. Using joint explants from patients undergoing prosthetic surgery, deposition of fibrin was shown at fronts of bone and cartilage invasion. In addition, fibrin co-localised with metalloproteinases 1 and 3, particularly at these sites [20]. All these facts led to propose a fibrin-based model for the development of synovitis based on the direct interaction between the fibrin networks and the resident fibroblasts [21].

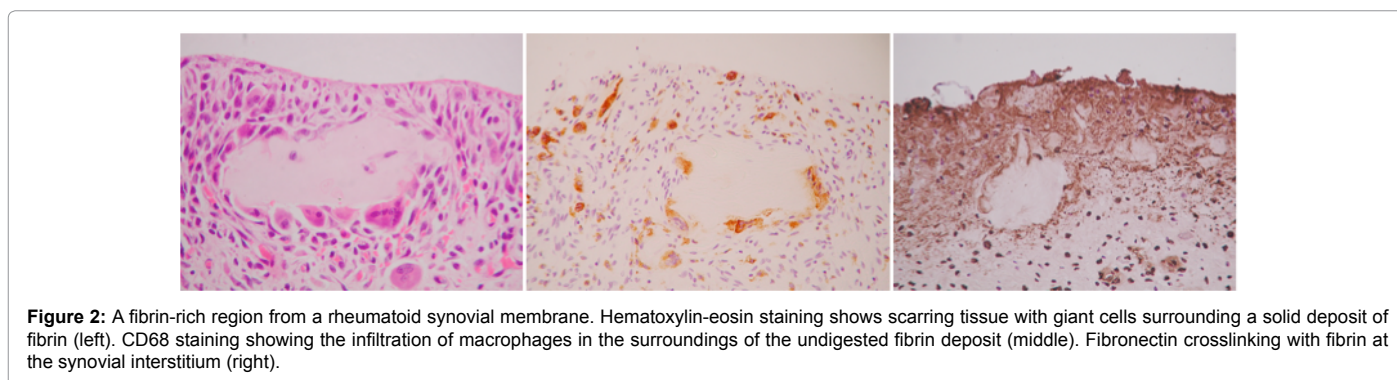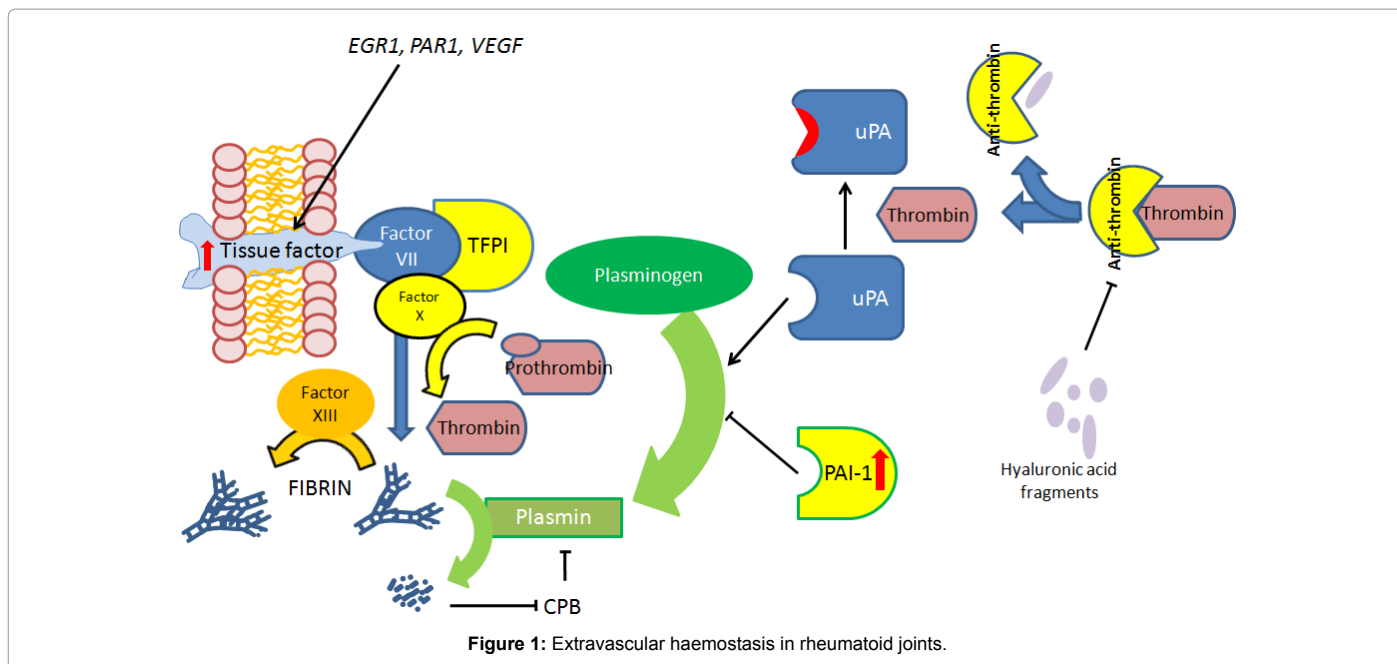## Fibrin Operates Ahead of the Cytokine Network

This synovitis model was thereafter challenged using an in vitro system in which fibrinogen was immobilised with the addition of thrombin directly on top of cell monolayers, to form fresh fibrin clots. This system had been formerly introduced to study cell transmigration [19]. This approach showed a powerful up-regulation of inflammatory mediators, growth factors and transcriptional activators by fibrin

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Figure 1: Extravascular haemostasis in rheumatoid joints.

Figure 2: A fibrin-rich region from a rheumatoid synovial membrane. Hematoxylin-eosin staining shows scarring tissue with giant cells surrounding a solid deposit of fibrin (left). CD68 staining showing the infiltration of macrophages in the surroundings of the undigested fibrin deposit (middle). Fibronectin crosslinking with fibrin at the synovial interstitium (right).

in rheumatoid synovial fibroblasts in the absence of collaboration of immune cells [22]. The fibrin transcriptome in these cells included some of the major pathogenic mediators of rheumatoid arthritis, such as interleukin 6, a variety of chemokines, vascular cell adhesion protein 1, intracellular adhesion molecule 1, tumor necrosis factor-related peptides, cyclooxygenase 2, and histone deacetylases. As represented in Figure 3 (left panel) the toll-like receptor (TLR) 4 - nuclear factor kappa B axis was found to mediate fibrin-dependent gene induction in synovial fibroblasts, while a similar pathway had been drawn in macrophages [22-24]. Globally, these findings support that synovial cells might, through their interaction with fibrin, fuel the principal pathogenic processes of rheumatoid arthritis, acting upstream cytokine networks and lymphocyte activation (illustrated in Figure 3, main panel).

Fibrin Networks are Scaffolds for Multi-Target Approaches

Interestingly enough, the stimulatory action of fibrin in the rheumatoid synovial membrane involves multiple therapeutic targets yet to be explored. As the principal receptor of fibrin inside the inflamed joint, TLR4 stands as an ideal molecular target of the fibrin-dependent pathogenic pathway (illustrated in Figure 3); moreover considering that

levels of expression of TLR have been found abnormally enhanced in rheumatoid synovial fibroblasts [25,26]. We will find out in the years to come if this approach can succeed in treating rheumatoid arthritis, since inhibitors of TLR4 are already undergoing development for other diseases. However, because of its widespread expression and its relevant role in the defence to gram-negative germs, TLR4 is a sensitive target, and the risk-benefit ratio of its inhibition would need to be individually considered.

In addition, a characteristic of fibrin is its multiple cell and matrix interaction domains. Fibrin networks provide sites for crosslinking of additional matrix proteins, such as fibronectin (Figures 2 and 3) [18,20]. The resulting multimolecular scaffold greatly enhances opportunities for cell attachment. It also offers binding sites for growth factors. Notably, vascular endothelium growth factor and fibroblastic growth factor 2 prolong their respective actions when anchored to fibrin networks [27,28]. The number of potential targets within this route is therefore high, and probably differing between subgroups of patients. Interestingly, fibrin and other components of the extracellular matrix undergo citrullination in rheumatoid joints, and this post-translational modification can further enhance mechanisms of cell activation, as has been demonstrated (Figure 3) [22,29-31]. In spite of not being specifically associated to the disease, this post-translational
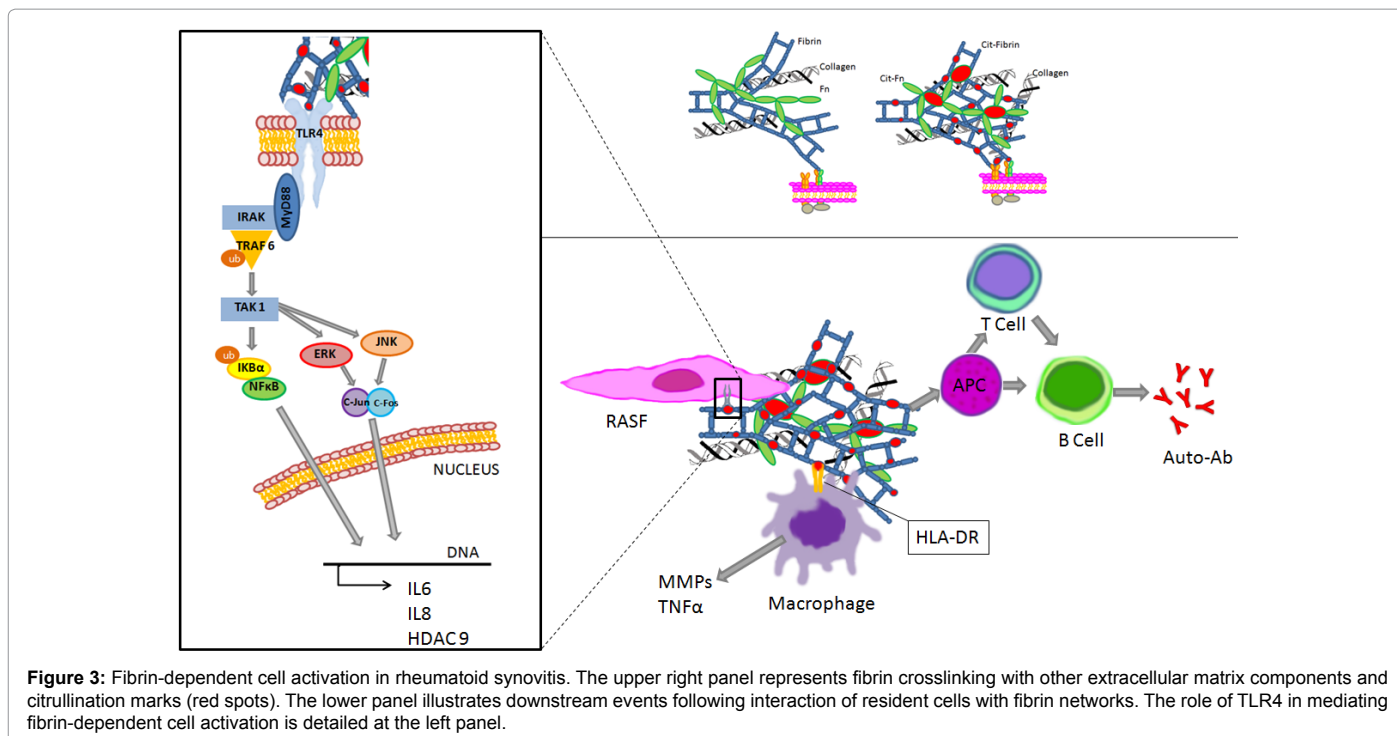
Figure 3: Fibrin-dependent cell activation in rheumatoid synovitis. The upper right panel represents fibrin crosslinking with other extracellular matrix components and citrullination marks (red spots). The lower panel illustrates downstream events following interaction of resident cells with fibrin networks. The role of TLR4 in mediating fibrin-dependent cell activation is detailed at the left panel.

modification of peptides facilitates their recognition as auto antigens [32]. Moreover, the development of antibodies to citrullinated peptides is relatively restricted to the disease. Citrullination is carried out by the family of peptidyl deiminases (PAD), out of which PAD2 emerges as an attractive therapeutic target. Of all isoforms, PAD2 shows the higher affinity for fibrin domains in vitro, and it has been found to be enriched in the synovial fluid of patients with rheumatoid arthritis and spondyloarthritis, as compared to osteoarthritis [33,34]. An increase in PAD has been put in relationship with erosive disease, and the presence of anti citrullinated antibodies is currently considered a marker of severe disease [35]. Altogether, this suggests that particular subgroups of patients would benefit from the inhibition of PAD2 activity.

Limitations to the Development of Fibrin-Based Therapies

Two major criticisms have hindered the progression of fibrin research in rheumatoid arthritis. The first one derived from the extrapolation of histopathological findings in synovial tissues from end-stage disease. Fibrin was for a long time considered a bystander product of inflammation. This hurdle was overcome with the studies performed in experimental models, and more recently, when fibrin was shown to be a major auto antigen of the disease. The second point raised was the lack of specificity of intra-articular fibrin deposition. This drawback is shared by most studies approaching the invasive phenotype of rheumatoid synovial fibroblasts, which have failed to show intrinsic alterations. In concordance, our group has found a similar profile of gene induction in fibroblasts from osteoarthritic joints to the one depicted by rheumatoid fibroblasts upon exposure to fibrin (unpublished data). This rather supports that fibrin networks provide a micro environmental imprinting, as responsible for the aggressive features of these cells. Besides, we did observe differences in the magnitude of the cell response between both conditions, probably due to the higher expression level of TLR4 in rheumatoid as compared to osteoarthritic synovial fibroblasts, as has been observed [25,26].

In conclusion, complexity of rheumatoid arthritis warrants a multi-target approach in order to shut down mechanisms involved in disease progression. In the next future, a better characterization of each phenotype will facilitate the use of tailored strategies, in which a place for fibrin-based therapies can be forecasted.

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