RHEUMATOID ARTHRITIS AND THE COMPLEMENT SYSTEM

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Abstract. The complement system represents a major part of the immune response. Its normal functioning is mandatory for the organism’s defence against pathogens and for the clearance of immune complexes, apoptotic cells and cellular debris. Its over-activation however can lead to generation of many pro-inflammatory and cytotoxic mediators, causing inflammation and tissue damage. The participation of the complement system in rheumatoid arthritis (RA) pathogenesis is indisputable but its precise mechanisms and the opportunities for influencing them are still an object of investigation. The aim of this review is to outline and systematize all the relevant scientific evidence.

Key words: complement system, rheumatoid arthritis

RHEUMATOID ARTHRITIS

RA is autoimmune, extravascular, immunocomplex disease affecting the synovial membrane and other structures in the joint. When there is certain genetic predisposition, under the influence of different extrinsic factors (e.g. smoking, infections) in a not fully understood way immune reaction is initiated, which escapes the control of the regulatory mechanisms. The result is production and accumulation of autoantibodies, reacting with their antigens to form immune complexes. Also other parallel pathogenetic mechanisms start operating including complement activation, production of various inflammatory cytokines, activation of aggressive cells leading to tissue inflammation and destruction and to the clinical presentation of the disease RA.

Many such pathogenetic steps are depicted in details and evidence is demonstrated in vitro and in animal models, as well as in real life patients. Other moments are still vague or ambiguous as is the case with the role of complement in initiating and developing joint inflammation and destruction. Some scientists put complement in the center of these processes, whereas others regard it as ancillary and/or secondary part. Scientific interest towards this still open question underlies the present review.

COMPLEMENT SYSTEM

Complement system is a part of the innate immunity. It is comprised of more than 30 proteins as well as membrane expressed receptors/regulators. It functions in plasma, tissues, on cell surface, and within the cells. It was first described more than a century ago as a bactericidal aid to antibodies [1]. For a long time it is considered to play mainly supportive role and receives little scientific attention. It became clear over the years that complement has versatile functions, extending beyond the simple bactericidal activity.

Immediately after the first encounter with the pathogen complement is triggered in a cascade fashion, as every factor activates the next through its enzymatic action. So, its major responsibilities are: pathogen opsonization and tagging for recognition by antigen presenting cells; driving the inflammatory process; modulation of the activity of T- and B-lymphocytes. Together with the specific antibodies it functions to eliminate pathogens and clear immune complexes. Under normal circumstances it directs the immunologically silent clearance of own apoptotic cells.

Complement can be activated via three pathways – classical, lectin and alternative (Fig. 1).

Activation of the classical pathway is dependent on immunoglobulins (Ig), IgM or IgG, present in immune complexes, leading to binding of the C1 complex via the C1q subunit [2]. Activation of the lectin pathway starts after the recognition of different carbohydrate ligands on the surface of the microorganisms by mannan-binding lectin or ficolins [3]. The alternative pathway is started by auto-activation of unstable complement factor C3 and its subsequent deposition on activating pathogen surfaces. Activa-
Activation of the complement system through any of the three pathways leads to activation of C3. The classical and lectin pathways generate the same C3 convertase, whereas the alternative pathway generates a different C3 convertase. Similarly, the classical and lectin pathways generate the same C5 convertase (C3bC4bC2a), whereas the alternative pathway generates a different C5 convertase (C3bBbC3b). The three pathways converge at the activation of C5 to form C5a (a potent chemoattractant) and the membrane attack complex (MAC) C5b-9, which can lead to cell lysis [3].

The regulatory mechanisms of complement are finely balanced so that, on one hand, the activation of complement is focused on the surface of invading microorganisms and, on the other, the deposition of complement on normal cells and tissues is limited [4]. When these mechanisms malfunction, complement system can cause injury while inducing and potentiating inflammation.

**Complement Synthesis**

The plasma levels of most complement components are maintained by hepatic synthesis. Studies on the acute phase response have shown that complement proteins may act as acute phase proteins, with increased hepatocyte synthesis occurring in response to cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and interferon-γ [5-9]. Moreover, complement is synthesized and activated also in extrahepatic sites. Currently there is evidence that all normal tissues are capable of synthesizing at least one complement component [10]. Such synthesis is demonstrated also in chronically inflamed tissues, such as rheumatoid joints [12, 17, 28]. A large number of different cell types have been shown to synthesize complement components. These include mononuclear phagocytes, fibroblasts, endothelial cells, epithelial cells, alveolar type 2 epithelial cells and adipocytes [13-16]. In particular, the cells of synovial membrane which are responsible for the synthesis of complement components are the lining cells (type A-mononuclear phagocyte, type B-fibroblast like), fibroblasts, mononuclear phagocytes and endothelial cells [17].

Almost all of complement components are synthesized in joint structures. Human articular chondrocytes produce clusterin, C1s, C1q, Ctr, C4, C2, C3, factor B and C1-inh [18-20]. C5 and C9 are made in the hypertrophic zone of cartilage [21]. All the complement components and regulators, excluding C8 and properdin, are produced from human fibroblasts [22]. C5a receptor (C5aR; CD88) are expressed on human articular chondrocytes [23];
C3a receptor (C3aR) and C5aR are also present in synovial tissue [24,25]. Monocytes in synovial fluid and synovial membrane macrophages also produce most complement components [26] and are thought to supply complement components in the synovial space. From all the aforementioned, it is clear that every component from C1 to C9 can be produced locally and supplied into the synovial space.

In RA patients, it is presumed that synthesis and activation of complement factors and receptors are intra-articular events. First reports from Brodeur, 1991 [27] are supported by many others applying various techniques. Complement factors and receptors have been thought to play an important role in RA since and synthesis and activation of complement take place at distinct sites within rheumatoid synovium. In 1974 Ruddy and Colten demonstrated synthesis of complement components C2, C3, C4 and C5 by rheumatoid synovial tissue by three techniques: in vivo metabolic studies with Radio-labeled C3; in vitro biosynthesis of C2, C3, C4 and C5; staining of cell in rheumatoid synovium with fluorescent antiserums to C3 and C4 [10]. Neumann et al. demonstrated that complement components and receptors are readily produced in situ in the inflamed RA synovium [25]. Using in situ hybridization, immunohistochemistry, and Western blot techniques, they localized the sites of complement messenger RNA (mRNA) expression in rheumatoid synovium. They also found increased expression of mRNA for C3 and FB, as previously reported [28] and for the first time they reported increased expression of mRNA for C3a and C5a receptors in synovial tissue [25]. These findings are reproduced in more recent studies [29] and support the idea that C3a and C5a are produced locally in RA synovium and are not plasma derived.

To some point this evidence answers the question which is leading in RA – systemic or local complement production.

The synovial tissues have the potential to act as a selective molecular filter [30]; as a consequence, the joint might be a relatively enclosed and protected environment. Systemic complement consumption by intravenous injection of cobra venom factor (CVF) did not suppress the development of inflammatory mono-arthritis in rat [31], whereas local complement suppression by administration of soluble complement receptor 1 (sCR1) into the joint space was effective [32], suggesting that complement activation in plasma and the joint are regulated independently. This underlines the importance of local complement synthesis and activation for the development of arthritis. On the other hand, systemic complement suppression seems effective in collagen-induced arthritis [33-35]. So, we can logically presume that in different stages of arthritis development systemic or local complement synthesis prevail.

**COMPLEMENT ACTIVATION IN RA**

There are a number of studies, identifying complement activation as a main event in the inflammatory cascade in RA [36-39] (fig. 2).
Apart from bacteria, complement is usually activated by immune complexes. RA patients have increased levels of circulating immune complexes [40, 41]. Part of them contain rheumatoid factors (RFs) and they are capable of activating complement via the classical pathway [42-46], with IgM-RF being more effective in complement activation than IgG-RF [47].

All the changes in the chemical structure or property of a protein occurring after or at the same time of its translation are called post-translational modifications. Impairments in their regulation have been linked to different inflammatory and autoimmune conditions [48]. These modifications may cause formation of neoepitopes with resulting generation of autoantibodies. [49, 50]. Usually, RA is associated with three types of post-translational modifications — citrullination, glycosylation and carbamylation.

Citrullination is the deimination of the amino acid arginine by a family of enzymes called peptidylarginine deiminase (PAD or PADI). Citrullination of the proteins renders them immunogenic and various anti-citrullinated peptide antibodies are found in RA. Trouw et al. suggest that anti-cyclic citrullinated peptide/protein antibodies (anti-CCP) from RA patients may activate complement via both the classical and alternative pathways [52]. Also, Garred et al. [53] suggested MBL, interacting with multiple IgG0 may be a potential cause of extra inflammation in late onset and rapidly progressing RA.

Carbamylation is called the addition of a cyanate group to a protein. Carbamylation of self-proteins causes a loss of the native structure and can lead to a break of tolerance and synthesis of anti-carbamylated proteins (anti-CarP) autoantibodies. Moreover, carbamylation of IgG is demonstrated in vivo in the synovium and the synovial fluid of RA patients and it is found to make IgG completely unable to bind C1q and to activate the classical complement pathway [55, 56]. It is thought to inevitably reduce the complement-mediated clearance of immune complexes from the joint, leading to their probable deposition.

It is not clear to what extent circulating immune complexes contribute to complement activation in RA. If it can’t be postulated that immune complexes are the only complement activators in RA, we should consider other potential triggers. Such a trigger for example could be C-reactive protein (CRP), since it can activate complement both in vitro and in vivo [57-60]. There is a study demonstrating that the plasma levels of activated complement and CRP—complement complexes are increased in most of the patients with RA and that these levels correlate with parameters of disease activity [61]. It means that CRP participates in complement activation and CRP-mediated complement activation may play a role in the pathogenesis of RA [61].

**Clinical evidence for complement involvement in RA**

In the synovial fluid of RA patients increased levels of certain complement cleavage products, such as C3a, C3c, C5a, sC5b-9, Bb, C1-CqINH complexes can be found [62-65]; as well as evidence of consumption of C3 and C4 complement components [27, 66-70] and activated complement components and complement activator molecules [71]. Therefore complement activation by IC in the arthritic joint is likely to be a mediator, which attracts effector cells (Fig. 2). Activation of infiltrated macrophages, mast cells, fibroblasts, and granulocytes by proinflammatory cytokines leads to their degranulation and subsequent detrimental release of proteolytic enzymes with ensuing joint erosion [72-74].

Also increase of soluble MAC (sMAC) was demonstrated in synovial fluid of RA patients [27, 66]. Non-lethal amounts of the MAC can be an inflammatory mediator in synovial cells in RA [75]. MAC binding to synovial fibroblast cells abundantly increased expression of collagenase-specific mRNA [76]. These results suggest that MAC triggers inflammation in arthritis and contributes to joint destruction.

In RA complement activation seems to cause local inflammation via the release of the small anaphylatoxins C3a and C5a. The increased serum or synovial levels of C5a are found to correlate with more severe inflammation [65, 69]. In inflammatory and proliferative synovial tissues, upregulation of C5aR was reported [23-25], possibly priming these cells to respond to C5a and/or scavenging excess C5a. C3a, another complement-derived anaphylatoxin, has also been found at elevated levels in synovial fluid in RA [70]. In contrast, C5a and C3a were not increased in synovium of OA patients.
Several studies failed to demonstrate a correlation between local and systemic activation of complement system in RA and reported higher levels of complement cleavage products in synovial fluid than in plasma [62, 77, 78], and these findings are consistent with a prevalent local activation of the complement cascade. Association between complement activation and disease activity is another aspect of great interest. In the study from Wouters and coworkers, a correlation between plasma levels of C1q-C4 complexes and disease activity expressed as DAS28 was found [79], likewise Doherty et al. reported raised synovial C3d levels in active compared with inactive RA joints [62].

The leading pathway of complement activation in RA is still disputable. Probably complement activation is not confined only to the classical pathway as there are data that the concentration of Bb fragments generated during the assembly of the C3-convertase of the alternative pathway, is higher in the synovial fluid of RA patients [27]. Some studies suggest that in juvenile arthritis the alternative pathway is the main player [80] and factor Bb concentrations were found to correlate better with the level of circulating immune complexes [81]. One possible explanation depends on activation of the alternative pathway by IC bearing IgA RF. Indeed, direct activation of the alternative pathway by IgA has been described [82], and high-molecular weight IgA RF-containing IC have been reported in children with polyarticular disease [83] as well as adults with RA [84]. Another explanation relates to the fact that the alternative pathway can be activated directly by collagen type II [85], which is specific for cartilage and becomes exposed as a result of proteolysis during the course of disease. Furthermore, one has to remember that the alternative pathway always acts as an efficient amplification loop for the classical one at the stage of generation of C3b. Therefore, the alternative pathway is crucial also for processes that were initiated via activation of the classical pathway.

**Cartilage molecules involved in complement regulation in RA**

It is well known that joint replacement surgery ameliorates inflammation. It appears that molecules present in or released from cartilage may have a role in propagating this inflammation by activating complement. One candidate is fibromodulin (FM) [86].

The cartilage contains relatively small number of cells, located in predominating extracellular matrix abundant in supramolecular assemblies. These include the family of leucin-rich repeat proteins such as decorin, biglican, osteoadherin, FM, lumican, chondroadherin [87]. Some of them are known to react with complement factors. For example C1q binds to decorin [88] and biglycan [88] as well as non-connection fibronectin [89]. In none of these examples do the interactions lead to activation of complement cascade.

In inflammatory joint disease proteases, particularly metalloproteinases family members, degrade the cartilage molecules. The initial event in this process is the liberation of N-terminal part of FM by the action of MMP-13.

FM binds to globular heads of C1q. As a result, similarly to immune complexes ligands, complement activation follows. FM activates complement predominantly via the classical pathway [86]. This activation leads to pronounced deposition of C3b and C4b and less pronounced deposition of MAC.

Interestingly, decorin [88,90] and biglycan [88] bind to C1q but instead of activating complement they inhibit the ability of C1q to trigger the classical pathway. These interactions take place at physiological ionic strength and may be relevant for regulation of complement activation and C1-mediated effects on the cells in the inflamed joints [88].

To sum up, there are several molecules in the cartilage capable of binding C1q. While some of them (FM) activates complement the others probably act as local complement inhibitors. Thus, the result depends on many local parameters.

Undoubtedly, complement plays an important role in the macroorganism’s immune reactions directed either against foreign or own structures. In health the system is finely balanced and performs its physiological functions without causing harm. Its dysregulation however, no matter congenital or acquired during disease, is a double-edged sword. On one hand, the insufficient activation of complement may lead to defective clearance of cell debris and/or immune complexes. On the other, its excessive activation may cause release of cytotoxic and inflammatory mediators resulting in tissue damage. Keeping in mind all the evidence for the importance of this complex enzymatic cascade we can only underline the necessity of its deepening research in order to find new pathogenetic insights in disease, including RA, better understanding of disease processes and their better treatment.

**Authors’ statement:**

We state that the presented figures are original as was required.

Authors declare that the article is published for the first time.


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Rheumatoid arthritis and the complement system


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