INFLAMMATION

Igniting the flame in arthritis: C5aR2 controls endothelial transcytosis of C5a

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C5aR2 transports C5a generated in the arthritic joint to the blood vessel endothelium as the first step in C5aR1-driven neutrophil arrest and crawling.

Neutrophils are critical to driving tissue inflammation in several autoimmune diseases. They must first move from the circulation to target tissues to exert these undesirable and harmful effector functions. This process is complex and includes initial transient and weak interactions between neutrophils and endothelial cells (ECs), followed by firm adhesion, crawling, and the identification of sites that allow for neutrophil transmigration through EC barriers. In this issue, Miyabe *et al.* (*1*) have identified a mechanism by which the complement 5 cleavage fragment C5a initiates these inflammatory events in the K/BxN arthritogenic serum transfer (AST) model of inflammatory arthritis. In this model, antibodies against glucose-6-phosphate isomerase (G6PI) form immune complexes (ICs) in joints, driving complement activation by the alternative pathway and massive generation of C5a (*2*). This local complement activation occurs as early as 1 hour after AST, long before chemokine production. Intravital multiphoton imaging studies revealed that C5a, bound to heparan sulfate proteoglycans (HSPGs) on the luminal site of blood vessel ECs, activates C5a receptor 1 (C5aR1) on circulating neutrophils, resulting in their firm adhesion. Instrumental to this adhesion process is the up-regulation of the β2-integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) on neutrophils (*2*). Until now, the mechanisms that mediated the trafficking of tissue-generated C5a from the joint to the blood vessel lumen were not clear.

C5aR1 (CD88) is a G protein–coupled receptor (GPCR) that is widely expressed on cells of myeloid origin and exerts many effector functions of C5a and its primary degradation product, C5a-desArg, which lacks the C-terminal arginine residue. In addition to C5aR1, C5a can bind with high affinity to C5aR2 (formerly C5L2 or GRP77), another seven-transmembrane receptor. In contrast to C5aR1, C5aR2 is uncoupled from G proteins because of amino acid changes in the DRY and NPXXY motifs located in the second intracellular loop and transmembrane domain VII (*3*). Since the first description of C5aR2 almost 20 years ago, conflicting reports have described its ligands, cellular localization, signaling, and function (*3*).

This study by Miyabe *et al.* (*1*) provides experimental evidence that C5aR2 enables transcytosis of C5a from the joint to the luminal site of the joint endothelium, thereby bridging the gap between C5a generation in the joint and C5aR1-mediated adhesion of neutrophils on the surface of vascular ECs (Fig. 1). The authors show that, in addition to C5aR2, the atypical chemokine receptor 1 (ACKR1) contributes to neutrophil adhesion by transportation of CXC chemokines CXCL1 and CXCL2 from the joint endothelium to the lumen of the blood vessel in vivo (Fig. 1). Previously, specific chemokine uptake and transcytosis from the abluminal to the luminal site of ECs have only been shown in vitro (*4*). In contrast to canonical chemokine receptors and similar to C5aR2, ACKR1 lacks the DRYLAIV motif in the second intracellular loop, resulting in uncoupling from G proteins and canonical GPCR signaling. The new study shows that the shared structural properties of C5aR2 and ACKR1 are associated with similar functions. The authors found markedly reduced disease development in $C5ar2^{-/-}$ and $Ackr1^{-/-}$ mice that was associated with impaired neutrophil recruitment into the joint of C5aR2- and ACKR1-deficient mice. Using bone marrow (BM) chimera, they observed that C5aR2 and ACKR1 can control neutrophil recruitment through effects on nonhematopoietic cells. This is contrary to what has been reported for the corresponding GPCRs C5aR1 and CXCR2, whereby only the absence in hematopoietic cells resulted in markedly decreased neutrophil recruitment (*2*). The authors also showed that C5aR2 is expressed on 20% of naïve joint ECs, which is a prerequisite to shuttle C5a through the joint ECs, and is strongly up-regulated 7 days after AST (>80% of ECs). Future studies are needed to define mechanisms that drive the up-regulation of C5aR2 on the ECs to judge the relative contribution of C5aR2 to neutrophil recruitment during the course of the disease.

The authors also showed that C5aR2 expressed on the abluminal site of joint ECs controlled the initial neutrophil adhesion through transcytosis of C5a to the luminal site of ECs (Fig. 1). It is noteworthy that the current (*1*) and a previous study (*2*) by Miyabe *et al.* suggest exclusive expression of C5aR2 on the abluminal site of joint ECs. For many cell types, coexpression of C5aR1 and C5aR2 has been observed, suggesting that their functions are tightly linked (*3*). Similar to ACKR1, C5aR2 can function as a decoy receptor to reduce the amount of its cognate ligands. Through sequestering β-arrestins from C5aR1, C5aR2 can suppress C5aR1-mediated neutrophil effector functions induced by ERK1/2 phosphorylation (*5*). An important issue arising from the study is clarification of which signaling pathways trigger C5aR2-mediated C5a transcytosis. In C5aR2-transfected HEK cells, C5a induced C5aR2 phosphorylation in a Ser-Thr–rich C-terminal region associated with β-arrestin 2 binding, internalization, intracellular colocalization with Ras-like small G proteins (GAB) 5, 7, and 11, and partial recycling back to the plasma membrane (*6*). It remains to be determined whether C5aR2 uses this or other transcytotic pathways, as ACKR1-bound chemokines have been shown to be targeted to caveolae (*4*). To further elucidate the role of C5aR2 on the joint endothelium in vivo, floxed tdTomato-C5aR2 reporter mice are

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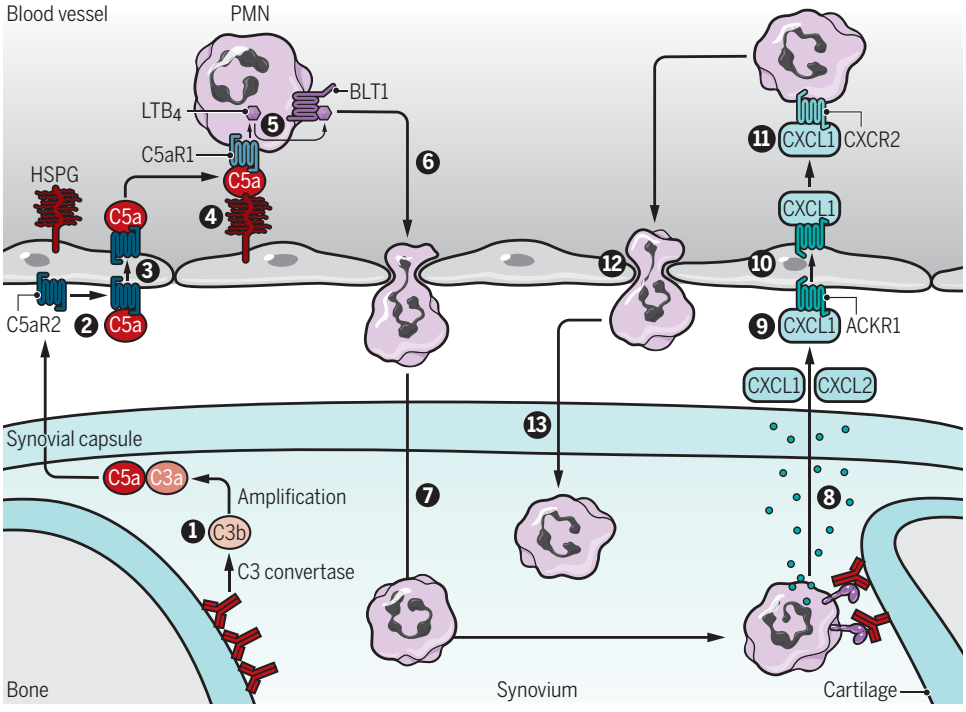

Fig. 1. A model detailing the sequence of events underlying C5a and CXC chemokine generation, transcytosis, and their impact on early and late neutrophil adhesion, activation, and extravasation into the joint. (1) G6PI ICs formed in the joint trigger alternative complement pathway activation resulting in the formation of the anaphylatoxins C3a and C5a. (2) C5a binds to C5aR2 expressed on joint ECs and (3) transports the anaphylatoxin to the blood vessel luminal site of the EC, where it (4) binds to HSPG. (5) Such C5a is recognized by C5aR1 expressed on circulating neutrophils, resulting in the up-regulation of LFA-1 and MAC-1 integrins critical for firm neutrophil arrest (not shown). Further, C5aR1 signaling induces autocrine LTB_4 release, which binds to BLT1 on neutrophils initiating (6) their extravasation into the joint space. (7) Within the joint, G6P1-ICs activate FcγRs on neutrophils, eventually resulting in (8) the production of CXCR2 ligands that bind to ACKR1 (9) expressed on the joint endothelium. (10) Analogous to C5aR2, ACKR1 enables transcytosis of its ligands CXCL1 and CXCL2 to the luminal site of the EC, where they can bind (11) to CXCR2 expressed on circulating neutrophils. (12) CXCL1- and CXCL2-driven activation of neutrophils results in their transendothelial migration into the joint, further fueling joint inflammation (13).

available (*7*). Such mice will allow the tracking of C5aR2 expression and will potentially verify the role of C5aR2 in endothelial transcytosis of C5a using endothelial-specific C5aR2 knockout mice.

In contrast to ACKR1, C5aR2 deficiency had no impact on neutrophil extravasation from the blood vessel into the joint. The available data suggest a sequel of events, in which locally generated C5a is transported by C5aR2 from the joint through the EC and is exposed on HPSGs in the blood vessel lumen to activate neutrophils via C5aR1 for firm arrest. However, C5aR1-activated neutrophils do not pass the EC barrier (*2*). In this context, it will be important to delineate why neutrophil activation through other chemoattractant receptors such as leukotriene B4 (LTB_4) receptor BLT1 and CXCR2 can drive neutrophil adhesion and extravasation (Fig. 1), whereas C5aR1 signaling exclusively controls the adhesion process.

Together, these new findings add to the growing number of studies showing important functional properties for C5aR2 independent of C5aR1, mediated by exclusive expression of C5aR2 on nonhematopoietic ECs (*1*) or hematopoietic cells such as B and NK cells (*7*). It is important to note that C5aR2 can exert anti-inflammatory functions, as demonstrated in several models of lung (*8*) or skin (*9*) inflammation. Future studies will define the conditions in which C5aR2-driven transcytosis of C5a is required to present and activate C5aR1-expressing inflammatory effector cells. With respect to rheumatoid arthritis, it will be important to delineate whether collaboration and division of labor between canonical C5aR1 and noncanonical C5aR2 occur in the asymptomatic preclinical phase of the disease that is already associated with complement activation (*10*). This could result in novel approaches for disease prevention.

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