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Novel insights into the expression pattern of anaphylatoxin receptors in mice and men

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ABSTRACT

The anaphylatoxins (AT) C3a and C5a play important roles as mediators of inflammation. Further, they regulate and control multiple innate and adaptive immune responses through binding and activation of their cognate G protein-coupled receptors, i.e. C3a receptor (C3aR), C5a receptor 1 (C5aR1) and C5a receptor 2 (C5aR2), although the latter lacks important sequence motifs for G protein-coupling. Based on their pleiotropic functions, they contribute not only to tissue homeostasis but drive, perpetuate and resolve immune responses in many inflammatory diseases including infections, malignancies, autoimmune as well as allergic diseases. During the past few years, transcriptome expression data provided detailed insights into AT receptor tissue mRNA expression. In contrast, our understanding of cellular AT receptor expression in human and mouse tissues under steady and inflammatory conditions is still sketchy. Ligand binding studies, flow cytometric and immunohistochemical analyses convincingly demonstrated tissue-specific C5aR1 expression in various cells of myeloid origin. However, a detailed map for C3aR or C5aR2 expression in human or mouse tissue cells is still lacking. Also, reports about AT expression in lymphoid cells is still controversial. To understand the multiple roles of the ATs in the innate and adaptive immune networks, a detailed understanding of their receptor expression in health and disease is required. Recent findings obtained with novel GFP or tdTomato AT-receptor knock-in mice provide detailed insights into their expression pattern in tissue immune and stroma cells. Here, we will provide an update about our current knowledge of AT receptor expression pattern in humans and mice.

1. Introduction

Sensing of exogenous or endogenous threats by C1q, Mannan-binding lectin or ficolins induces a cascade of proteolytic events, eventually resulting in the cleavage of C3 and C5 through canonical complement activation pathways (Merle et al., 2015a). Further, any nucleophilic attack including spontaneous hydrolysis of the thioester domain in C3 initiates proteolytic C3 cleavage by canonical alternative pathway activation. C3 and C5 cleavage by such events results in the generation of the anaphylatoxins (AT) C3a, C5a and its primary degradation product C5a-desArg. In addition to canonical generation, ATs can be generated in, on or in the vicinity of cells in through cell-derived proteases (Kolev et al., 2014), plasma proteases of the contact system (Merle et al., 2015b) or pathogen-derived proteases (Potempa and Potempa, 2012).

C3a and C5a exert their multiple effector and regulatory functions through binding and activation of their cognate G-protein-coupled AT receptors. While C3a binds to C3a receptor (C3aR) (Crass et al., 1996; Ames et al., 1996), C5a and C5a-desArg specifically interact with C5a receptor 1 (C5aR1, CD88) (Gerard and Gerard, 1991; Boulay et al., 1991) and C5a receptor 2 (C5aR2, C5L2 or GPR77) (Cain and Monk, 2002a; Okinaga et al., 2003). Further, some studies suggest that C3a-desArg, which has also been named Acylation-Stimulating Protein (ASP), binds to C5aR2 (Cui et al., 2009; Kalant et al., 2003, 2005).

Initially, C5aR1 was considered to mediate mainly the many pro-inflammatory effector functions of C5a/C5a-desArg (Gerard and Gerard, 1994). During the past 25 years, it became clear that C5aR1 exerts pleiotropic regulatory roles and contributes to cell and tissue homeostasis (Verschoor et al., 2016), metabolism (Hess and Kemper, 2016) and differentiation (Arbore et al., 2016). The second receptor for C5a, C5aR2, is uncoupled from G-proteins, which suggested a function as a negative regulator of C5aR1. However, several reports demonstrated pro-inflammatory effector functions, either independent of or in concert with C5aR1 (Li et al., 2013). Like C5aR1, activation of C3aR was initially shown to drive mainly pro-inflammatory effector functions (Klos et al., 2009, 2013). However, several reports showed strong anti-inflammatory activity, often counteracting C5aR1-driven effects (Coulthard and Woodruff, 2015), suggesting a complex and

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multifaceted, context-dependent function.

Many of the multiple AT receptor functions have been uncovered using cells or tissues from C5aR1- (Höpken et al., 1996), C5aR2- (Gerard et al., 2005) or C3aR-deficient mice (Humbles et al., 2000), experimental models using such mice or inhibitors that selectively target C5aR1 (Köhl, 2006) or C3aR (Ames et al., 2001). However, although such approaches helped to delineate the roles of AT receptor activation in health and disease, they did not provide detailed insights into their cellular expressing pattern. Precise mapping of AT receptor expression and monitoring the dynamics of such expression under physiologic and pathophysiologic conditions will help to better understand the pleiotropic biologic functions of AT in health and disease.

Tissue and cell expression analyses of AT receptors at the mRNA level provide no information about intracellular or cell surface protein expression. AT receptor analyses by western blot, immunohistochemistry and flow cytometry using polyclonal or monoclonal antibodies have been used to assess such expression patterns in tissues, immune and stromal cells. However, controversial data have been obtained suggesting false positive results due to antibody cross-reactivity (Karsten et al., 2015; Tschernig et al., 2007). An elegant way to monitor the expression pattern and dynamics of AT receptor expression is to examine the expression of fluorescent proteins such as green fluorescent protein (GFP) or the tandem-dye Tomato fluorescent protein (tdTomato) under the control of AT receptor promoters. In the past, we and others have successfully used this technology to examine the expression of C5aR1 using a floxed GFP-C5aR1 knock-in mouse (Karsten et al., 2015; Dunkelberger et al., 2012). In addition to this mouse, we have now generated floxed tdTomato-C5aR2 and tdTomato-C3aR knock-in mice, which were used to determine C5aR2 and C3aR expression in various immune and stromal cells from different tissues. Here, we will summarize and update the current knowledge about the expression pattern of C3aR, C5aR1 and C5aR2 in mice, with a particular emphasis on recent results obtained with the novel AT receptor reporter mouse strains.

2. Anaphylatoxins and their receptors

The three AT receptors belong to the large family of G-protein coupled receptors (GPCRs). The GPCR-family is the largest group of transmembrane receptors in mammals, counting more than 800 members in humans (for review Munk et al., 2016). Structurally, all GPCR share a core segment of seven transmembrane (TM) helices. Ligands either bind to specific sites located within the 7-TM segment or to sites within the extracellular loops and/or the N-terminus. The intracellular loops and the Carboxy-terminal tail form the pockets for G-protein binding. Activation of the serine and threonine phosphorylation sites is important for the initiation of intracellular signaling pathways, internalization and the recycling of the GPCRs.

C5a is a pro-inflammatory effector molecule involved in many immune responses. It is a highly potent chemoattractant and activator for neutrophils, macrophages, and mast cells (MC). Activation of neutrophils and MCs results in degranulation, which fuels the pro-inflammatory environment (Klos et al., 2009; Kemper and Köhl, 2013; Schmudde et al., 2013). C5a exerts its functions via two different receptors: C5aR1 (CD88) and C5aR2 (C5L2/GPR77). Most of the pro-inflammatory and regulatory functions of C5a are mediated through activation of C5aR1. C5aR1 and 2 cluster with other chemoattractant receptors including the formyl peptide receptor family, bradykinin receptors, type II-angiotensin-II receptors, Chem23 and several orphan receptors. C5aR1 is a 45 kDa protein binding C5a with high affinity. In C5aR2, the DRY motif located at the boundary between transmembrane domain (TM) III and intracellular loop 2 and the NPXXY motif in helix TM VII are altered, thus preventing the binding of G proteins and the initiation of G protein dependent signaling (Okinaga et al., 2003). Because of this property, C5aR2 was initially considered as a decoy-receptor only regulating the functions of C5aR1 by capturing excessive C5a (Okinaga et al., 2003). However, additional studies have shown that binding of C5a to C5aR2 promotes either pro- or anti-inflammatory properties (Gao et al., 2005; Croker et al., 2014; Chen et al., 2007). Like C5aR1, C5aR2 can bind β-arrestins, and regulates C5aR1-dependent activation of MAPK-dependent signaling pathways (Bamberg et al., 2010). Indeed, binding of C5a to both receptors triggers their phosphorylation, facilitating their association with β-arrestin. C5aR1/β-arrestin association leads to ERK1/2 phosphorylation, whereas C5aR2/β-arrestin complexes result in the inhibition of ERK1/2 phosphorylation, suggesting that the ratio between the expression of C5aR1 and 2 is determining the outcome of C5a-mediated cellular responses (Bamberg et al., 2010).

C3a exerts a wide range of pro- and anti-inflammatory functions (Coulthard and Woodruff, 2015), through binding to its cognate receptor C3aR (Crass et al., 1996; Ames et al., 1996). The binding of C3a to C3aR leads to rapid C3aR internalization that depends on phosphorylation of serine and threonine residues at the receptor C-Terminus (Settmacher et al., 1991, 2003) and the activation of pertussis toxin-sensitive G-proteins (Klos et al., 2013). In the circulation C3a and C5a are quickly converted into C3a-desArg (ASP), and C5a-desArg through serum carboxypeptidase N. C3a, but not C3a-desArg (ASP) is binding to C3aR. In contrast, C5a and C5a-desArg bind to both C5aRs. C5a binds to C5aR1 with a higher affinity than C5a-desArg. Conversely, the affinity of C5a-desArg to C5aR2 is higher than to C5aR1 (Cain and Monk, 2002b). The findings regarding the of binding of ASP to C5aR2 are controversial (Cui et al., 2009; Kalant et al., 2003, 2005; Honczarenko et al., 2005; Johswich et al., 2006). Several studies from the Cianflone laboratory suggest that ASP interacts with C5aR2 in adipocytes and regulates immunometabolism (summarized in Poursharifi et al. (2014a)). However, findings from the Klos lab did not confirm ASP binding to C5aR2 (Johswich et al., 2006).

3. Designing knock-in gene constructs to generate reporter mice

3.1. Genomic structures of C3AR, C5AR1 and C5AR2 loci

The structural organization of the *C3ar1* locus is identical in humans and mice. It comprises a small exon 1 separated from exon 2 by an intron (human: 6 kb, mouse 4,7 kb) (Paral et al., 1998). In mice, the mRNA contains an open reading frame of 1431 bp, a short 5′ non-translated region and a long 3′ UTR (Hollmann et al., 1998). Similarly, the structural organization of the genes encoding C5aR1 are identical in mice and men and contain 2 exons (Gerard et al., 1992; Gerard and Gerard, 1991). Exon 2 comprises the entire coding sequence (CDS) except the ATG start codon, which is located in exon 1. In human, both exons are separated by a 9 kb intron (Gerard et al., 1993), and by 5.3 kb in mouse (Dunkelberger et al., 2012). The 5′ UTR harbors cell-specific promoter and suppressor regions. The importance of the 3′ UTR region for the C5aR1 expression and translocation to the cell surface has been identified in a C5aR1 knock-in mouse. In this reporter mouse, the gene encoding GFP has been cloned downstream of the *C5ar1* CDS resulting in a mutant 3′ UTR that abolishes C5aR1 surface expression (Dunkelberger et al., 2012). In addition, the 3′ UTR region contains regulatory sequences important for the expression of C5aR1 (Palmer et al., 2012). Also, the human and mouse *C5AR2/C5ar2* genes have the two-exon structure, which is characteristic for chemoattractant receptor family members comprising the 5′ UTR and initiating methionine encoded in the first and the CDS as well as the 3′ UTR in the second exon (Gerard et al., 1993). Several splicing variants exist in different organs suggesting a role for the UTRs in the control and specificity of C5aR2 expression (Okinaga et al., 2003).

3.2. Fluorescent dye-coupled anaphylatoxin receptor knock-in mice as tools to study AT receptor expression

GFP fusion proteins have been widely used to study the dynamics