



Review

The immunoglobulin, IgG Fc receptor and complement triangle in autoimmune diseases

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ABSTRACT

Immunoglobulin G (IgG)-mediated activation of complement and IgG Fc receptors (FcγRs) are important defense mechanisms of the innate immune system to ward off infections. However, the same mechanisms can drive severe and harmful inflammation, when IgG antibodies react with self-antigens in solution or tissues, as described for several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, and immune vasculitis. More specifically, IgG immune complexes (ICs) can activate all three pathways of the complement system resulting in the generation of C3 and C5 cleavage products that can activate a panel of different complement receptors on innate and adaptive immune cells. Importantly, complement and FcγRs are often co-expressed on inflammatory immune cells such as neutrophils, monocytes, macrophages or dendritic cells and act in concert to mediate the inflammatory response in autoimmune diseases. In this context, the cross-talk between the receptor for the anaphylatoxin C5a, i.e. C5aR1 (CD88) and FcγRs is of major importance. Recent data suggest a model of bidirectional regulation, in which CD88 acts upstream of FcγRs and sets the threshold for FcγR-dependent effector responses by regulating the ratio between activating and inhibitory FcγRs. Vice versa, FcγR ligation can either amplify or block C5aR-mediated effector functions, depending on whether IgG IC aggregate activating or inhibitory FcγRs. Further, complement and FcγRs cooperate on B cells and on follicular dendritic cells to regulate the development of autoreactive B cells, their differentiation into plasma cells and, eventually, the production of autoantibodies. Here, we will give an update on recent findings regarding this complex regulatory network between complement and FcγRs, which may also regulate the inflammatory response in allergy, cancer and infection.

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Introduction

Complement is an important danger sensing system, recognizing and flagging pathogens and eventually eliminating the intruders. Further, complement senses and eliminates endogenous danger such as apoptotic cells or cell debris. In addition to these well-appreciated properties, complement plays an integral role in the regulation of innate and adaptive immune responses and is involved in tissue regeneration, angiogenesis, lipid metabolism, solubilization and clearance of immune complexes (ICs), mobilization of hematopoietic progenitor cells and neurologic synapse maturation among other functions (Köhl 2006; Ricklin et al. 2010).

During evolution, the complement system has been built around factors B and C3 (Janssen et al. 2005) stressing the critical

importance of C3 and complement in general for the survival of complex organisms. The central C3 molecule can be activated by different mechanisms. The evolutionary oldest pathway is the alternative pathway engaging C3, factors B and D and properdin. The initial step of alternative complement pathway activation is the thioester activation within C3 by polysaccharide hydroxyl groups (Sahu et al. 1994; Tack et al. 1980), nucleating the formation of the alternative pathway C3 convertase, a protease complex comprising C3bBb. As we will outline below, immunoglobulin G (IgG) IC can also activate the alternative pathway, which is critical for the solubilization of precipitated ICs. The lectin pathway and the classical pathway have evolved before the establishment of adaptive immunity and the appearance of immunoglobulins. The classical complement pathway is activated at the level of C1q when natural and induced IgG and IgM antibodies form ICs with their cognate antigens. Mannan-binding lectin (MBL), a C-type lectin, recognizes conserved carbohydrate patterns on pathogens and binds to MBL-associated serine proteases MASP-1 and MASP-2 (Fujita et al. 2004). Once the immune sensors of the complement system have recognized their targets, a cascade of proteolytic events is triggered in

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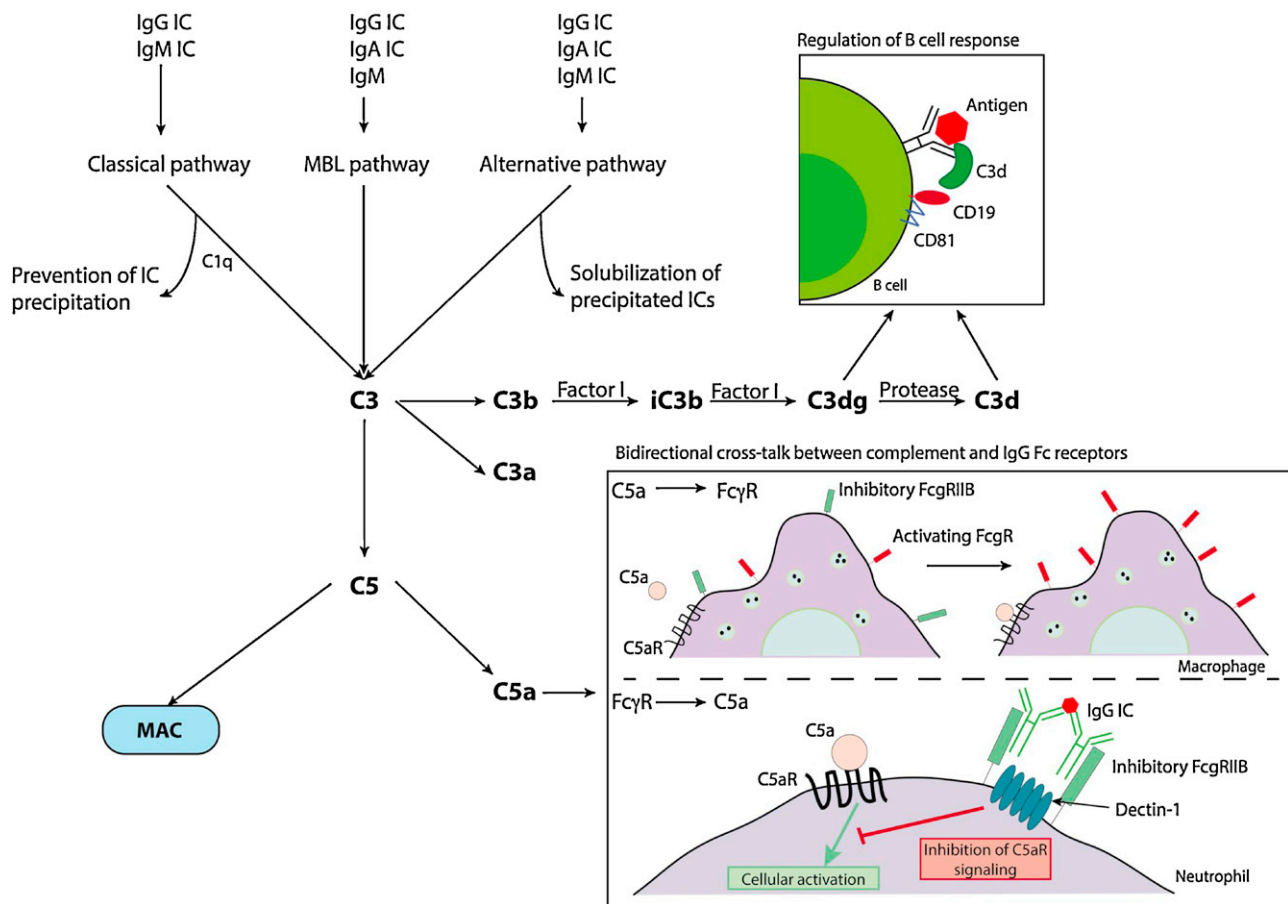


Fig. 1. Overview of the interactions between immunoglobulins, complement and FcγRs. Immune complexes comprising IgM, IgG or IgA and antigen activate the different pathways of complement. Complement activation is critical for appropriate handling of ICs, i.e. it prevents the formation of immune precipitates, can solubilize such precipitates and promotes IC clearance through activation of either the classical or the alternative pathway. Further tagging of antigens with C3 fragments and activation of complement receptors on B cells and follicular dendritic cells is an important mechanism of B cell regulation. More downstream in the complement cascade, C5a cross-talks with IgG Fc receptors on neutrophils and macrophages to lower the threshold for FcγR-mediated activation of such cells. Vice versa, an anti-inflammatory feedback loop exists, by which IC inhibit proinflammatory properties of C5a through engagement of the inhibitory FcγRIIB.

which complement components and factors in the fluid phase are cleaved to release smaller fragments which in turn nucleate the assembly of new protease complexes required for the cascade to proceed to the next step. Proteases of the lectin and the classical pathway promote the cleavage of C4 and C3, resulting in the formation of a C3 convertase composed of C4b2a (Fig. 1). Cleavage of C3 by either pathway drives the generation of the two fragments C3b and C3a. Depending on the environment, C3b can bind to different partner molecules which may either result in the amplification of the complement response, formation of the C5 convertase and activation of the terminal pathway leading to the generation of C5a and the membrane attack complex (MAC), or the irreversible degradation of C3b (reviewed in Gros et al. 2008). The MAC has an important function in destroying bacterial invaders. This is of particular importance with regard to infections with *Neisseria meningitidis*, as evidenced by a high frequency of early life meningococcal infections in people with deficiencies in one of the components of the terminal pathway (Skattum et al. 2011). On the other hand, MAC-mediated cell destruction can result in life threatening disease in case of paroxysmal nocturnal hemoglobinuria (PNH), which is due to an acquired defect of glycosylphosphatidylinositol-linked membrane proteins, resulting in the absence of complement control proteins CD55 and CD59 (Parker 2012). Regarding the cross-talk with immunoglobulins, the interaction of IC-bound C3b and its degradation products iC3b, C3dg and C3d with distinct complement

receptors expressed on phagocytes, B cells and follicular dendritic cells (FDCs) is of major importance. Here, we will discuss the activation of complement and of FcγRs by IC and the role of complement and FcγRs in the development of B cell immunity. Further, we will focus on the bidirectional cross-talk between C5a receptor (C5aR), activating FcγRs and the inhibitory FcγRIIB and its implications for autoimmune diseases (Fig. 1).

Activation of the complement system by immunoglobulins

Among the different classes of immunoglobulins, IgM and IgG can activate complement either via the classical, the alternative or the lectin pathway. IgA activates complement through the alternative or the lectin pathway. Activation of the classical pathway takes place when C1q recognizes IgG or IgM molecules that form IC with their cognate antigens. Similarly, unspecific aggregation of such antibodies (abs) and activation of C1q have been described in cryoglobulinemia (Adu and Williams 1984). IgM is more potent than IgG to activate complement. Even a single IgM pentamer can trigger the classical pathway of complement activation, whereas at least two IgG molecules are required for the same effect (Borsos and Rapp 1965). The IgM molecule also exists in hexameric forms, which is a 10–20 times more potent complement activator than its pentameric counterpart (Collins et al. 2002; Davis et al. 1988). Hexameric IgMs have been found in patients suffering

from Waldenström's macroglobulinemia, cold agglutinin disease or recurrent urinary tract infections. The specific role of hexameric IgM in these diseases remains elusive (Petrusic et al. 2011). The IgG subclasses differ in their potencies to activate the complement system. In humans, four different IgG subclasses have been described, i.e. IgG1–4. Human IgG activate C1q in the order IgG3 > IgG1 > IgG2, whereas IgG4 shows no to minor reactivity toward C1q (Ishizaka et al. 1967). Mice express the four subclasses IgG1, IgG2a/c, IgG2b and IgG3. Of those, the IgG2a/c, IgG2b and IgG3 subclasses activate complement, whereas IgG1 is not fixing complement via the classical pathway (Klaus et al. 1979).

The binding site for C1q has been located within the C_H2 domain of the Fc portion of IgG (Duncan et al. 1988). C1q is part of the C1 complex comprising the hexameric C1q molecule and the C1r2:C1s2 proenzyme complex. The tails of the C1q hexamer bind to the C1r2:C1s2 complex, whereas the C-terminal globular heads of the protein bind to the Fc part of the Ab. Because of its hexameric structure, the binding affinity of C1q to immunoglobulins increases markedly with the complexity of the IgG. Monomeric IgG bind to C1q with a 10,000-fold lower affinity than IgG that form IC (Burton and Woof 1992). The binding of C1q to IC results in a conformational change in the C1r2:C1s2 complex driving the autolytic activation of C1r, which in turn activates the C1s serine protease. The activated C1s enzyme then cleaves C4 resulting in two fragments, the large C4b and the small C4a molecule. C4b can covalently bind to cell surfaces via its labile thioester, driving the cleavage of C2 into the large C2a and the small C2b fragment. Unbound C4b is quickly inactivated. Within the C4bC2a complex, C2a is the active protease that can cleave C3 into the small C3a and the large C3b fragment, which can also covalently attach to cell surfaces. Importantly, bypass pathways exist, in which the C1 complex activation by IC results in direct cleavage of C3 by the alternative pathway without C2 (Matsushita and Okada 1986) or C4 and C2 cleavage (May and Frank 1973a,b,c). The *in vivo* importance of these bypass pathways has been demonstrated in a Forssman shock model in guinea pigs (Wagner et al. 1999) and in humans suffering from chronic urticaria (Ballow et al. 1975) hemolytic uremic syndrome (Nolin et al. 1979) or systemic lupus (Traustadottir et al. 1998).

In line with its evolutionary appearance before the development of the adaptive immune system and immunoglobulins (Nonaka and Kimura 2006), C1q not only binds to Abs but also to cell wall components and membrane proteins of all kinds of microorganisms, the prion infectious agent (Klein et al. 2001) as well as fragments of cellular and subcellular membranes (e.g. mitochondrial membranes) and other modified host proteins and phospholipids (Ghebrehwet et al. 1983; Kovacsics et al. 1985; Peitsch et al. 1988; Storrs et al. 1983). Further, it can bind C-reactive protein (CRP) and serum amyloid P (SAP) protein of the pentraxin family (Bottazzi et al. 2010).

Properties of IgG and IgM molecules that regulate classical pathway activation

It is surprising that the distinct IgG subclasses activate complement with different potencies as the C1q binding site within the C_H2 domain at positions Glu 318, Lys 320 and Lys 322 is highly conserved in IgG molecules of different species (Duncan et al. 1988). The difference might be explained by the variability in the structure of the hinge region, which separates the antibody binding moiety (Fab) from the Fragment crystallizable (Fc) part. More specifically, the length of the hinge regions between different IgG subclasses varies substantially, which results in different Fab arm segmental flexibility. The most effective complement activators among human IgGs, the IgG3 and IgG1 isotypes, have the longest hinge regions of the four IgG subclasses. The hinge region of the non

complement-activating human IgG4 isotype and its murine IgG1 counterpart display only limited flexibility as compared with other IgG subclasses, which results in limited movement of their Fab arms and less accessibility of this region for the C1q heads. The importance of the hinge region was strengthened by the observation that naturally occurring human IgG1 molecules with short and rigid hinges showed reduced ability to fix complement, as is the case for Dob and Mcg human IgG1 myeloma proteins (Guddat et al. 1993; Klein et al. 1981). However, antibody-engineering studies carried out by several groups (Norderhaug et al. 1991; Tan et al. 1990; Tao et al. 1993) suggest that the difference in the hinge region is only one important factor enabling IgG molecules to activate complement. In fact, differences within the hinge region do not fully explain the different potencies of IgG subclasses to activate C1q, as the available data show no strict correlation between segmental flexibility and the ability to activate complement. A modified IgG3 with the rigid hinge of IgG4 exhibits significant complement activation ability and an IgG4 with the hinge of IgG3, although as flexible as IgG3, displays no detectable complement activity (Norderhaug et al. 1991; Tan et al. 1990). In addition to the hinge region, the inter-H-chain disulfide seems to be important for complement activation. Schur and Christian (1964) showed that reduction of the inter-H-chain disulfide bridges in rabbit and human antibodies destroys their ability to interact with complement. More specifically, the amino acid composition of the C_H2 domain seems to be responsible for the isotype variation. Changing the C_H2 part from a human IgG1 to an IgG4 molecule created an IgG4 mutant that is almost as potent in activating complement as IgG1 (Greenwood et al. 1993; Tao et al. 1991). A Ser331Pro replacement improves complement activation of IgG4, suggesting that amino acids outside the C1q binding domain at positions 318, 320 and 322 contribute to classical pathway activation (Tao et al. 1993; Xu et al. 1994). Also, a fully functional and paired C_H3 domain is necessary for optimal complement activation (Utsumi et al. 1985). At this point, it is still unclear whether these structural properties are required for the appropriate orientation of the C_H2 domains or the functional integrity of the C1q-binding site (Brekke et al. 1995).

IgM mutant studies (Shulman et al. 1987) and X-ray analyses (Perkins et al. 1991) provided evidence that the C_H3 domain, which corresponds to the C_H2 domain in IgG, is critical for binding to C1q. Here, two clusters at the opposite ends of the C_H3 domain at positions Asp432, Pro434, Pro436, Asp356 and Asp417 may form a binding site for C1q (Arya et al. 1994). These data suggest that the C1q binding site for IgG and IgM are not identical. Taken together, several structural features within the IgG or IgM Ab promote the successful interaction of C1q with the antibody C_H2 and C_H3 regions.

IgG molecules activate the complement system by the alternative pathway

In addition to the classical pathway, IgG molecules have been shown to activate complement via the alternative pathway, e.g. in the mouse model of K/BxN serum-transferred arthritis, which is mimicking human rheumatoid arthritis (Ji et al. 2002) or in patients with juvenile arthritis (Aggarwal et al. 2000). In the K/BxN model, FcγRIII- and C5aR-deficient mice are equally protected from disease development whereas C1q-deficient mice are not. Surprisingly, also factor B-deficient mice are protected, suggesting a crucial role for alternative pathway activation in this model. Notably, IgG1, which does not activate the classical pathway, is the dominant isotype in the serum of K/BxN mice (Kouskoff et al. 1996) and transfer of such K/BxN IgG1 antibodies induces arthritis (Ji et al. 2002). Usually, the alternative pathway is associated with the detection of microbial surfaces, but what triggers this pathway in the arthritis model? It is well appreciated that spontaneous

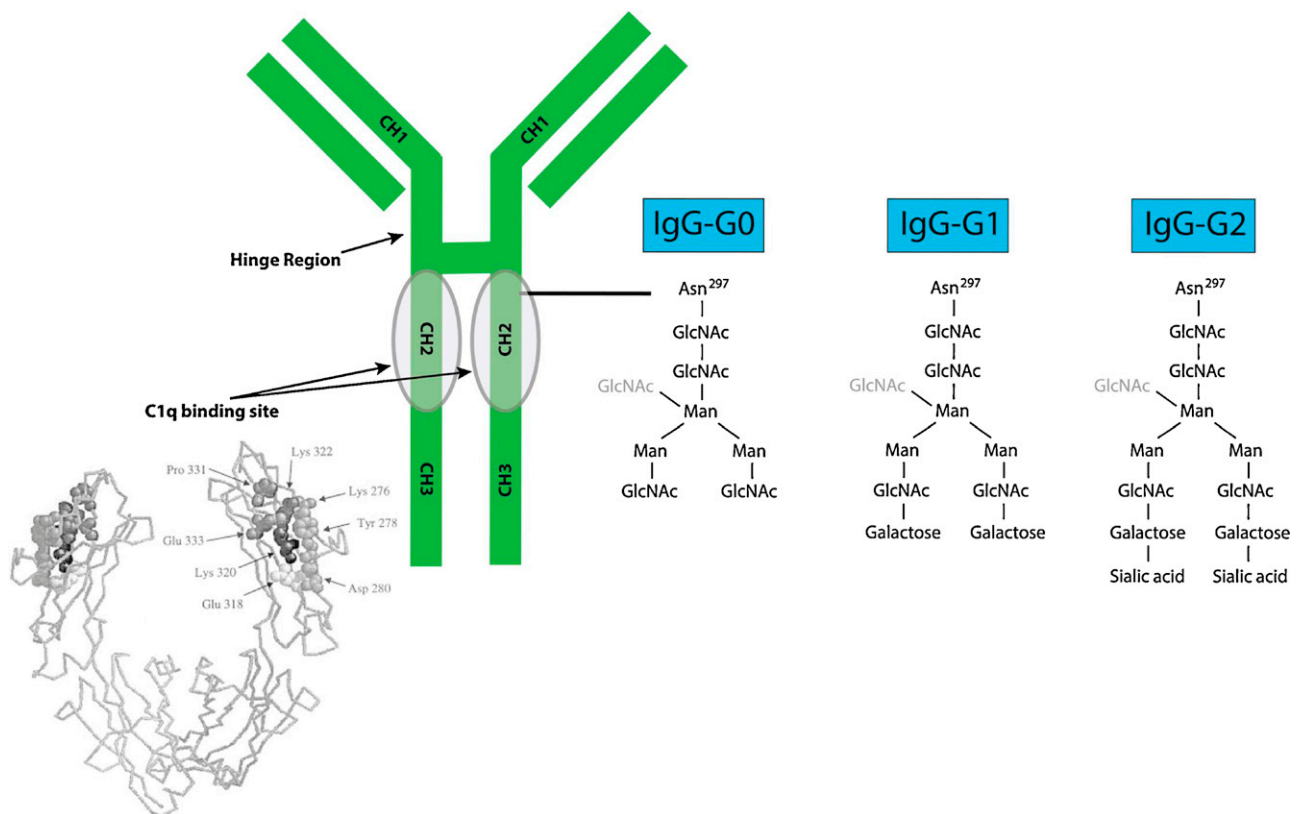


Fig. 2. N-glycan composition of IgG antibodies. A biantennary oligosaccharide core is attached to Asn297 within the IgG C_H2 domain comprising two N-acetyl-glucosamine (GlcNAc) residues and three mannose residues, two of which are linked to two terminal GlcNAc residues (IgG-G0). In addition to this core structure, IgG antibodies may contain additional galactose (IgG-G1) or galactose and sialic acid residues (IgG-G2). C1q binding site is highlighted; the detailed structure is based on crystallography works by Deisenhofer (1981) and Thommesen et al. (2000).

initiation of the alternative complement pathway takes place in the fluid phase, resulting in continuous cleavage of C3 into C3a and C3b (Pangburn and Müller-Eberhard 1980; Pangburn et al. 1981). Such C3b has affinity for IgG molecules (Kulics et al. 1983) and can form C3b₂-IgG complexes that are partially protected from inactivation by factors H and I resulting in an increased half-life of C3b₂-IgG complexes. Further, they can bind properdin bivalently, resulting in preferred sites for convertase formation (reviewed in Lutz and Jelesarova 2006). In contrast to C1q, which binds to the C_H2 domain, the docking site of nascent C3b is within the C_H1 domain of the IgG molecule (Shohet et al. 1993). Thus, certain IgG molecules can promote alternative pathway activation, which appears to be amplified by C5a-dependent neutrophil activation, resulting in C3, factor B and properdin production (Schwaebler and Reid 1999). Of note, naturally occurring antibodies against yeast or bacterial polysaccharide structures preferentially form C3b₂-IgG complexes, suggesting that this pathway has primarily evolved as a defense mechanism that efficiently attacks microbial invaders in the circulation (Lutz and Jelesarova 2006).

The impact of IgG glycosylation on complement activation

Another important parameter for effective complement activation by IgG is the glycosylation of the Fc portion. IgG molecules are N-glycosylated in their C_H2 domain at Asn297 (Fig. 2). The N-glycans within the Fc portion of the IgG molecule are highly heterogeneous with regard to the composition of their terminal sugar residues, which can be mannose, N-acetyl-glucosamine (GlcNAc), bisecting GlcNAc, galactose or sialic acid (Arnold et al. 2007).

Agalactosylated IgG molecules have been shown to either exert a reduced potency to activate complement or to lack it completely (Koide et al. 1977; Nose and Wigzell 1983; Tsuchiya et al. 1989; Williams et al. 1973). Further, when the terminal sugar is mannose, C1q binding and classical pathway activation is abrogated (Wright and Morrison 1994). This loss or decrease of C1q binding may result from allosteric changes of the C_H2 domain within the IgG molecule as the carbohydrate chains cover a hydrophobic patch in the C_H2 domain (Huber et al. 1976) that also involves regions between C_H1 and C_H2 (Silverton et al. 1977). As outlined above, the length and the accessibility of the hinge region is one important factor determining C1q binding. Notably, the terminal sugars in the biantennary glycan structure also define whether MBL can recognize the IgG molecule through its carbohydrate-recognition domains. Typically, MBL recognizes polysaccharide structures on bacteria, fungi, viruses and parasites as well as on apoptotic host cells (Thiel and Gadjeva 2009). When the Fc glycan terminates with a GlcNAc residue, MBL can bind to IgG and activate the lectin pathway (Malhotra et al. 1995). Human IgM comprises five N-linked glycosylation sites on each μ chain and one glycosylation site on the J chain. Some IgM glycoforms that express GlcNAc-terminating structures can bind to MBL. However, antigen-bound IgM ICs do not bind to MBL as such target glycan structures become inaccessible upon antigen binding. Nevertheless, MBL-mediated activation of the lectin-pathway may play a role in the handling of aggregated IgM molecules, i.e. in keeping such ICs soluble and remove them from the circulation. Rheumatoid arthritis patients express high levels of the agalactosyl-IgG-G0 glycoform, terminating in GlcNAc (Parekh et al. 1985) suggesting that the inflammatory response in the joint may result from undesired

activation of complement by the lectin pathway (Garred et al. 2000; Malhotra et al. 1995). Increased levels of the IgG-G0 glycoform have also been described in individuals suffering from systemic lupus erythematosus (SLE), Crohn's disease or tuberculosis (Bond et al. 1997; Parekh et al. 1985, 1989). In addition to MBL, some reports demonstrated a positive correlation between the terminal Fc glycan galactose content and C1q binding *in vitro* (Boyd et al. 1995; Hodoniczky et al. 2005; Kobata 2008; Raju 2008), suggesting that the adaptive immune system can fine-tune the activation of complement by either the classical or the lectin pathway at the level of IgG Fc glycan composition.

Other diseases where lectin-dependent activation of complement may play an important role are IgA-associated diseases such as primary IgA nephropathy and Henoch Schönlein purpura (reviewed in Floege 2011; Oortwijn et al. 2008). IgA can occur in monomeric and polymeric forms. The polymeric form contains a joining chain and may be associated with a secretory component. It can activate the alternative (Hiemstra et al. 1987) and the lectin (Roos et al. 2001) pathway. In patients with IgA nephropathy, mesangial deposition of IgA together with MBL, MASP-2 and C4 have been found, suggesting glomerular complement activation by the lectin pathway (Roos et al. 2006). At this point the glycan composition on polymeric IgA that triggers MBL binding and complement activation is unclear. Polymeric IgA heavy chains can be glycosylated via N-linked sugars or O-linked sugars (IgA1 heavy chain). Further the joining chain and the secretory component may be glycosylated (Royle et al. 2003). Of note, IgA nephropathy is frequently associated with an elevation of circulating poorly galactosylated IgA1 O-glycoforms, in particular in patients with active nephropathy and proliferative mesangial changes (Floege 2011). It is tempting to speculate that such IgA molecules may be targets for MBL recognition, driving the activation of the lectin pathway and disease progression.

The interaction of IgG with IgG Fc receptors

FcγRs belong to the large immunoglobulin superfamily (Nimmerjahn and Ravetch 2008b). Functionally, they serve as a bridge between the innate and the adaptive immune system as they can bind immunoglobulins by their Fc part thereby activating several types of innate immune cells. The prerequisite for efficient cell activation is the specific binding of immunoglobulins to their target antigen resulting in the formation of immune complexes (ICs) that aggregate the FcRs on the cell surface. FcγRs are expressed on the surface of monocytes, macrophages, dendritic cells (DC), neutrophils, natural killer (NK) and NKT cells and on B cells (Boruchov et al. 2005; Dhodapkar et al. 2005; Kim et al. 2006; Parren et al. 1992; van de Winkel and Capel 1993) but not on T cells (Takai 2002). Upon receptor aggregation, FcγRs initiate phagocytosis, antibody-dependent cellular cytotoxicity (ADCC), the transcription of cytokine genes and the release of inflammatory mediators (Dijstelbloem et al. 2001). In general, FcγRs can be divided into activating or inhibitory FcγRs (Kiener et al. 1993; Odín et al. 1991; Rankin et al. 1993). Further, they can be differentiated into high or low affinity FcγRs (Nimmerjahn and Ravetch 2008b). Except for FcγRIIIa, all activating FcγRs are composed of an IgG-binding α-chain and a signal-transducing adaptor molecule that contains an ITAM in its cytoplasmatic domain, i.e. the common γ-chain. In FcγRIIIa, the immunoreceptor tyrosine-based activation motif (ITAM) is part of the IgG-binding α-chain. In addition to its signaling function, the γ-chain promotes the assembly and the cell-surface transport of the different activating FcγR α-chains. Thus, mice that are lacking the γ-chain are functional FcγRs knock-outs (Takai et al. 1994). Crosslinking of activating FcγRs by IC leads to the initiation of signaling events, which are similar for all

activating FcγRs that couple to the accessory γ-chain. The first step is the tyrosine phosphorylation of the ITAM by a kinase of the SRC family (Ghazizadeh et al. 1994; Wang et al. 1994), followed by the recruitment of SYK-family kinases, which is then followed by the activation of different downstream targets including the activation of phosphatidylinositol 3-kinase (PI3K) and phospholipase Cγ (PLCγ) which are critical for increase in intracellular Ca²⁺, stimulation of mitogen-activated protein kinase and reorganization of the cytoskeleton (Kiener et al. 1993; Odín et al. 1991; Rankin et al. 1993). In contrast, the inhibitory FcγR, FcγRIIB, contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmatic domain. Co-aggregation of FcγRIIB with an ITAM-containing activating FcγR results in dominant negative regulation of the activating FcγR. This suppressive effect results from the phosphorylation of the ITIM by an SRC kinase, which promotes the recruitment of SH2-domain-containing phosphatases SHP1, SHP2 and the inositol polyphosphate 5'-phosphatase (SHIP) (D'Ambrosio et al. 1995; Ono et al. 1996, 1997). Importantly, SHIP can degrade phosphatidylinositol-3,4,5-triphosphate (PIP3) to PIP2 thereby blocking many of the signaling pathways downstream of activating FcγRs.

In the mouse, four different subclasses of FcγRs have been identified: FcγRI, FcγRIIB, FcγRIII and FcγRIV (Nimmerjahn and Ravetch 2006). Three of them belong to the group of activating FcγRs that is FcγRI, FcγRIII and FcγRIV. FcγRIIB is the only inhibitory FcγR. The activating and inhibitory FcγRs are co-expressed on most immune cells, except NK cells, which exclusively express FcγRIII and B cells, which solely express FcγRIIB. The FcγR genes cluster in close proximity on chromosome 1 in humans, chimpanzees and mice species (Nimmerjahn and Ravetch 2006). In humans, six different FcγRs have been described, i.e. FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA, FcγRIIB and FcγRIIB and several allelic variants that differ in receptor expression and IgG affinity (Bruhns 2012). The functional properties of the different human FcγRs in autoimmunity have been described elsewhere (Bruhns 2012; Dijstelbloem et al. 2001). As indicated by the nomenclature, some mouse FcγRs are similar to human FcγRs regarding their IgG binding and function whereas others show distinct IgG binding behaviors and functional differences. Thus it is difficult to transfer results obtained in the mouse system 1:1 to humans. For example, mouse FcγRIV is closely related to human FcγRIIIA and mouse FcγRIII to human FcγRIIA (Nimmerjahn and Ravetch 2006). However, there are also differences concerning the intracellular domains or the expression pattern of the FcγRs. For example, FcγRIIA, which is lacking in mice, is the only activating FcγR that expresses an ITAM in the cytoplasmatic tail of its α-chain and does not associate with the common γ-chain in order to signal.

As already outlined before, IgGs can be divided into four different subclasses in humans (IgG1–IgG4) and mice (IgG1, IgG2a, IgG2b, IgG3). The different IgG subclasses bind with varying affinities and specificities to the different FcγRs (Bruhns 2012; Bruhns et al. 2009; Dijstelbloem et al. 2001; Gavin et al. 1998; Nimmerjahn and Ravetch 2005; van der Poel et al. 2011) (Table 1). In humans and mice, only FcγRI binds monomeric IgG with high affinity. Consequently, the high-affinity FcγRI is constantly saturated with IgG ligands. However, it has also been demonstrated that it can bind IgG ICs with high affinity (10^{-8} – 10^{-9} M⁻¹). Mouse FcγRI preferentially binds IgG2a whereas human FcγRI can bind IgG1, IgG3 and IgG4 (Table 1). The other FcγRs bind IgG IC with markedly lower affinity than FcγRI (100–1000 fold lower) and are more promiscuous in IgG subclass binding. Further, the very low affinity of monomeric IgGs to activating FcγRs other than FcγRI prevents undesired cell activation by circulating IgG molecules. It is noteworthy that the binding IgG binding affinities are usually determined in *in vitro* assays and that *in vivo*, a restricted set of activating FcγRs is involved in mediating the activity of the individual subclasses (Hamaguchi et al.

Table 1
IgG subclass affinities of FcγRs.

Receptor class	Human ^a	Mouse ^b
FcγRI	3 = 1 > 4	2a ≫ 2b > 3
FcγRIIA ^{H131}	1 ≫ 3 > 2 > 4	–
FcγRIIA ^{R131}	1 ≫ 3 > 4 > 2	–
FcγRIIB	3 > 1 > 4 ≫ 2	1 = 2b > 2a
FcγRIII	–	2a = 2b > 1
FcγRIIIA ^{Val158}	3 > 1 > 4 > 2	–
FcγRIIIA ^{Phe158}	3 ≫ 1 > 4 ≫ 2	–
FcγRIIIB NA1	1 = 3 ≫ 2, 4	–
FcγRIIIB NA2	1 = 3 ≫ 2, 4	–
FcγRIV	–	2a = 2b

^a Dijkstra et al. (2001), Bruhns et al. (2009) and van der Poel et al. (2011).

^b Gavin et al. (1998), Ioan-Facsinay et al. (2002), Nimmerjahn and Ravetch (2005) and Bruhns (2012).

2006; Nimmerjahn and Ravetch 2005). For example, in several models of IC diseases, the activity of IgG2b antibodies is exclusively dependent on FcγRIV, despite its capacity to bind also to FcγRIII *in vitro* (Hamaguchi et al. 2006; Nimmerjahn and Ravetch 2005, 2006, 2007). The same is true for IgG2a, as demonstrated by specific pharmacological targeting of FcγRIV with neutralizing antibodies and FcγRIV-deficient mice (Hamaguchi et al. 2006; Nimmerjahn et al. 2010; Nimmerjahn and Ravetch 2005). In addition, it has also been described that FcγRI and FcγRIII contribute to IgG2a-mediated inflammation, suggesting that other factors like location, cytokine milieu and effector-cell type have to be considered for *in vivo* effects of IgGs (Biburger et al. 2011; Hazenbos et al. 1996; Ioan-Facsinay et al. 2002).

When an antibody is binding to its FcγR, it acquires a horse-shoe-like structure, which seems to be crucial for efficient binding. This conformational change is mediated by sugar moieties attached to asparagine 297 (Asp297) residues within the C_H2 domain of the Fc fragment (Nimmerjahn and Ravetch 2008b). Thus beyond subclass variations, the composition of the sugar moiety can influence the binding affinity of IgG molecules to different FcγRs. Importantly, the Fc N-glycans are highly dynamic and accessible to glycan-modifying enzymes or glycan recognition domains (Barb and Prestegard 2011) suggesting that modification of the Fc glycan composition may affect the biologic properties of the IgG molecules. As we will point out later in this review, terminal galactosylation of Fc-glycan can confers IgG1 binding to the C-type lectin receptor Dectin-1 (Karsten et al. in press).

The role of FcγRIIB in autoimmunity

The balance of activating and inhibitory signaling pathways regulates the immunologic response and its amplitude. If the negative signaling is abrogated, i.e. through a gene defect, the result is usually an overwhelming inflammation, which can lead to destruction of healthy tissues and development of chronic disease. FcγRIIB is an important negative regulator of inflammation in autoimmune diseases and infections (Brownlie et al. 2008). It is co-expressed with activating FcγRs on several immune cells including mast cells, neutrophils and macrophages, thereby regulating their cell activation and effector responses (Nimmerjahn and Ravetch 2008b). On B cells, where FcγRIIB is the only FcγR expressed, it regulates activating signals mediated by the B cell receptor. In B cells, FcγRIIB is important for peripheral tolerance, as FcγRIIB-deficient mice suffer from a higher frequency of autoreactive B cells than wt mice (Fukuyama et al. 2005; Paul et al. 2007; Takai et al. 1996; Xiang et al. 2007). Further, it has been demonstrated that mice with a polymorphism in the FcγRIIB promoter region, resulting in lower expression of FcγRIIB on B cells, are prone to develop autoimmunity (Nimmerjahn and Ravetch 2008b; Tsuchiya and Kyogoku 2005).

The ratio between activating and inhibitory FcγRs (A/I ratio) is one factor that regulates the magnitude of the inflammatory response mediated by of IC (Nimmerjahn and Ravetch 2006). In line with this view, the deletion of FcγRIIB results in a lower threshold for the activation of innate immune effector cells, which in turn modulates the severity of autoimmune disease (Takai et al. 1996). Specific overexpression of FcγRIIB in B cells is associated with an increased threshold for B cell activation resulting in a suppression of autoimmune disease (Brownlie et al. 2008). This effect is limited to T cell dependent B cell responses and affects mainly the late IgG but not the early IgM response, which is consistent with an earlier report (Fukuyama et al. 2005). Importantly, FcγRIIB overexpression leads to a shift toward high affinity B cells underscoring the role of this negative regulator in controlling B cell activation and shaping the antibody repertoire. Brownlie et al. showed that relatively minor changes in the expression pattern of FcγRIIB on B cells might have already substantial effects on the immune response and maintenance of tolerance (Brownlie et al. 2008; McGaha et al. 2005). In contrast, FcγRIIB overexpression in macrophages caused no significant suppression of autoimmune disease in models of arthritis and systemic lupus erythematosus (SLE). This minor effect of FcγRIIB on inflammatory effector cells can be explained by the IgG2a isotype, which is the main driver of the inflammation in these models. In fact, IgG2a IC bind with a 70-fold higher affinity to FcγRIV than to FcγRIIB (Nimmerjahn and Ravetch 2005).

A body of evidence has accumulated during the past decade that blocking of activating FcγRs or changing the A/I-ratio can suppress autoantibody-induced inflammation in mice and man (Bussell 2000; Nimmerjahn and Ravetch 2008b). Indeed, one of the many postulated mechanisms by which intravenous IgG (IVIg) exerts its anti-inflammatory properties is upregulation of FcγRIIB and simultaneous downregulation of activating FcγRs on macrophages to clear IC without detrimental cell activation (Kaneko et al. 2006; Nimmerjahn and Ravetch 2008a). Importantly, this effect is most effective in case of IgG1-mediated inflammation as FcγRIIB binds IgG1 with much higher affinity than IgG2a. In fact, the A/I ratios for IgG2a and IgG2b are 69 and 7 as compared with 0.1 for IgG1 (Nimmerjahn and Ravetch 2005). Taken together, the inhibitory FcγRIIB regulates several early and late events responsible for the initiation, the progress and the maintenance of autoimmune diseases.

Complement and FcγRs regulate the B cell response

The complement system has evolved before the development of the adaptive immune system. Thus, it may not be surprising that complement instructs the development of appropriate B cell responses (Fearon and Locksley 1996). This has already been noted in the 1970s when C3 was found to bind to circulating B lymphocytes (Nussenzweig et al., 1971) and FDCs within lymphoid follicles (Papamichail et al. 1975). C3 harbors a reactive thioester moiety that allows for covalent attachment to molecules or cellular surfaces. This is a crucial step within the complement activation, as it marks molecules or surfaces with a complement signature. Moreover, the tagged molecules or cells become targets for several complement receptors. With regard to B cell activation, CR1 and CR2 are of major importance. CR1 and CR2 are both type I transmembrane glycoproteins composed of similar repeating structural subunits (Ahearn and Fearon 1989). The cleavage fragments of iC3b, C3dg and C3d bind to CR2. In addition to these ligands, CR1 also binds C3b and C4b (Molina et al. 1994). In mice, both receptors are splice products of the same locus (Cr2) that is co-expressed on chromosome 1 (Kurtz et al. 1990; Molina et al. 1990). In humans they are encoded at separated loci (Ahearn and Fearon 1989; Carroll et al. 1988). Mature B cells, which are located in the B cell follicle,

express CR1/CR2 as part of the B cell receptor complex along with CD19 and CD81 (Bradbury et al. 1992; Carter and Fearon 1992). The activation of the B cell receptor complex in the presence of CR1/CR2 ligands enhances B cell differentiation at several stages. Interaction of C3d-bound antigens with CR2 results in dramatic (up to 10,000-fold) reduction of the B cell activation threshold (Dempsey et al. 1996) and promotes the migration of the activated B cells to the T cell:B cell boundary. Here, B cell differentiation occurs including somatic cell hypermutation and class switch recombination. In addition to the lowered threshold for B cell activation, CR1/CR2 co-receptor activation also rescues low affinity B cells from caspase-dependent apoptosis (Barrington et al. 2005). Further, when B cells lack CR1/CR2, they fail to survive in germinal centers (GC) as has been shown in an experimental system with lysozyme as a model antigen (Fischer et al. 1998). A prerequisite for the effective formation of GCs is an efficient antigen encounter of B cells. FDCs play a critical role in this regard as FDC retain antigen on their surface via CR1 and CR2. Upon activation by FDCs, cognate B cells present their antigen to follicular T helper cells and differentiate into effector and memory B cells. In the absence of efficient retention of antigen on FDCs through CR1/CR2, long-term antibody production as well as memory cells are markedly reduced (Barrington et al. 2002; Brockman et al. 2006). Complement is also important for trafficking of lymph-borne antigen into the B cell follicle. Recent data suggest that subcapsular sinus macrophages (SSMs) capture C3b-coated antigens via CR3 and FcγRIIB, which are widely expressed on SSMs (Phan et al. 2007). In a next step, noncognate naïve B cells take up the C3b (or C3d)-ICs from the SSMs and transfer such complexes to FDCs by a CR2-dependent process (Phan et al. 2009). At this point, it is still unclear what drives the transfer of complement-tagged ICs from naïve B cells to FDCs as both cells express CR1/CR2 on their surface. Further, it remains elusive, how large protein antigens are captured by SSMs prior to a specific immune response. Most likely, natural IgM or other innate sensors of the fluid phase such as C1q, MBL, ficolins, pentraxins and C-reactive protein will bind to the antigen and activate complement. It has also been shown that marginal zone B cells can capture ICs by a CR1/CR2-dependent mechanism and transport them to FDCs within the B cell follicle (Ferguson et al. 2004; Pozdnyakova et al. 2003; Youd et al. 2002). Of course, multiple pathways exist to transport antigens into the follicles that are not dependent on the activation of complement. For example, particulate antigens such as vesicular stomatitis virus (Junt et al. 2007) or protein-coated beads (Carrasco and Batista 2007) are rapidly phagocytosed by SSMs independent of C3. Of note, capture of influenza virus is mediated by a distinct pathway that involves MBL-dependent uptake by SSMs, transfer to medullary macrophages and recognition by resident dendritic cells through the C-type lectin receptor SIGN-R1 (Gonzalez et al. 2010). Taken together, signaling downstream of the complement receptors CR1, CR2 and CR3 on distinct and highly specialized immune cells within the peripheral lymphoid compartment builds a complex network that defines the activation and the outcome of the humoral immune response. The mechanisms and the pathways underlying such regulation have been outlined in detail in excellent recent reviews (Gonzalez et al. 2010, 2011).

Further, mice with disrupted *Cr2* locus are prone to develop autoimmunity as a result of defective elimination of self-reactive B cells. In addition to the regulatory effects on T cell-dependent antigens, an increasing body of data suggests a critical role for CR2 in shaping the natural antibody repertoire (reviewed in Holers 2005). Natural antibodies are mainly produced by a subset of long-lived, self-replenishing B cells, i.e. B-1 cells. As germline-encoded molecules, they serve as low affinity pattern recognition receptors that recognize pathogen as well as tissue-derived patterns. Importantly, *Cr2*^{−/−} mice lack a subset of the natural antibody repertoire that is critical to recognize hypoxia-related damage on endothelial

cells. On the one hand this can be beneficial, as *Cr2*^{−/−} mice are protected from intestinal ischemia/reperfusion injury. However, the impaired ability to recognize damaged cells may on the other hand lead to defective clearance and promote autoimmune responses. Collectively, these data demonstrate that the complement system is an integral part of the B cell compartment that shapes and regulates both the innate and the adaptive part of the B cell response.

Bidirectional cross talk between complement and IgG Fc receptors

Immune complexes drive the proinflammatory effector functions in autoimmune diseases including SLE, rheumatoid arthritis and Goodpasture's syndrome (Bolton 1996; Korganow et al. 1999; Madaio 1999) among others. The inflammatory properties of ICs have been extensively studied in the Arthus reaction, serving as a model of a type III hypersensitivity reaction in which soluble ICs are formed after repeated administration of foreign antigens. The Arthus reaction is characterized by infiltration of PMNs, hemorrhage and plasma exudation. Originally, horse serum has been repeatedly injected into the skin of rabbits, resulting in inflammation and neutrophil infiltration into the skin (Arthus 1903). For better standardization, the reverse passive model is frequently used, in which a specific antibody is injected at the desired site of inflammation, e.g. the peritoneum, the skin or the lung, followed by intravenous application of the corresponding antigen. The activation of complement has initially been considered as the main effector mechanism promoting the inflammatory response underlying the Arthus reaction (Colten 1994). Indeed, classical pathway activation results in the production of C5a, which leads to increased vascular permeability and promotes migration and activation of immune cells (Gerard and Gerard 1994). Several studies from the Ravetch lab published during the past 15 years have shown that the presence of activating FcγR is crucial, since *FcγR1*^{−/−} mice, deficient in the common γ-chain (i.e. lacking all activating FcγR) were protected from inflammation in different models of IC diseases (Clynes et al. 1998, 1999; Park et al. 1998). Based on such data, the authors came to the conclusion that the initiation of the inflammatory cascade depended entirely on FcγR-triggered activation of inflammatory cells such as mast cells (Sylvestre and Ravetch 1994). In support of this view, they found that complement-depletion by cobra venom factor (CVF) or genetic deficiency of C3 and C4 had at best minor effect on inflammation. This view was first challenged by data from cutaneous Arthus reaction model, pointing toward complement-dependent and FcγR-dependent pathways that were depending on the genetic background of the mice used for the study (Hazenbos et al. 1996). In support of the impact of the genetic background, two studies demonstrated that CVF-treatment had strong protective effects in peritoneal and cutaneous Arthus reaction models with C57BL/6 but not with a mixed C57BL/6/129 background (Baumann et al. 2000; Heller et al. 1999a). Also, the missing anti-inflammatory effect of C3 or C4 deficiency does not take into account the bypass pathways, in which ICs can drive direct cleavage of C3 by the alternative pathway without C2 (Knutzen Steuer et al. 1989) and/or C4 cleavage (May and Frank 1973a,b,c). Finally, several studies have shown that tissue-specific complement activation by resident and inflammatory cells promotes the production of several complement factors including C3a and C5a that are either activated locally by one of the three complement pathways or directly cleaved by proteases of cellular (Colten and Strunk 1993; Huber-Lang et al. 2002) or humoral origin (Huber-Lang et al. 2006, 2012; Amara et al. 2010). The importance of C5a for IC-mediated inflammation was clearly demonstrated in several models of pulmonary, peritoneal and cutaneous Arthus reactions, in which C5aR-deficient mice were protected from IC injury (Bozic

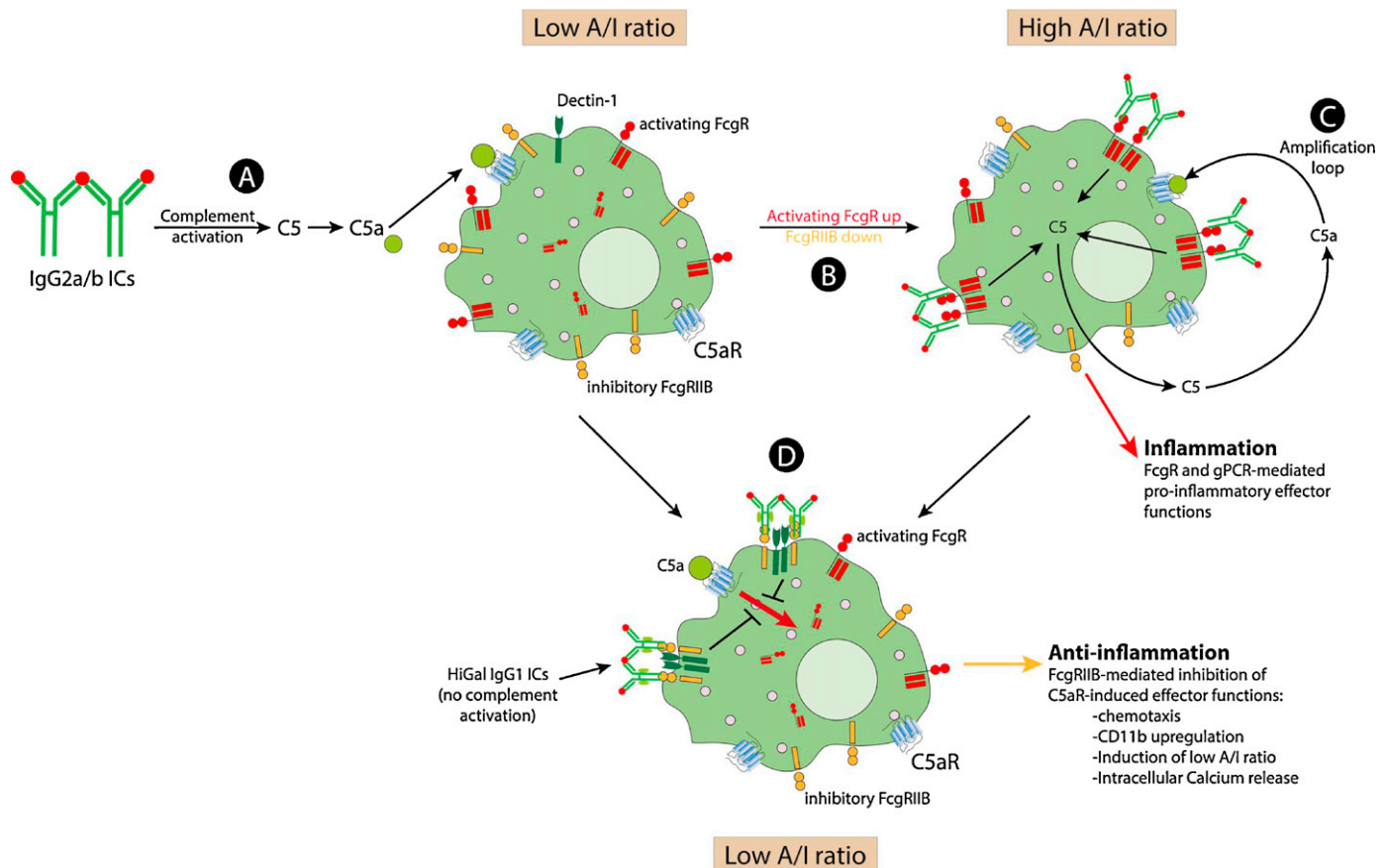


Fig. 3. Bidirectional cross-talk between C5a receptor and IgG Fc receptor signaling. (A) In the mouse system, IgG2a or IgG2b ICs activate complement, resulting in the generation of C5a. (B) Activation of the C5aR on macrophages results in the upregulation of activating FcγRs and downregulation of the inhibitory FcγRIIB, thereby reducing the threshold for FcγR-mediated stimulation of macrophages. (C) IC-triggered ligation of activating FcγRs promotes the production of C5 and activates proteases that cleave C5 into C5a, thereby amplifying the C5a-induced inflammation. (D) Highly galactosylated IgG1 ICs bind to FcγRIIB and associate FcγRIIB with Dectin-1. This heterodimer formation induces an anti-inflammatory pathway blocking C5aR-mediated effector functions.

et al. 1996; Heller et al. 1999b; Höpken et al. 1997). Further, in a model of collagen-induced arthritis, in which type II collagen-specific Ab were injected, C5aR-deficient mice were completely protected from disease development (Grant et al. 2002). Similarly, C5aR-deficient mice were protected in the K/BxN serum-induced arthritis model. Notably, FcγRIII-deficient mice were also protected in this model, pointing toward substantial cross-talk between the complement network and activating IgG Fc receptors (Ji et al. 2002).

First mechanistic insights into this cross-talk were provided using alveolar macrophages from C5aR-deficient mice. Treatment of such macrophages with C5a *in vitro* and *in vivo* resulted in strong transcriptional upregulation of FcγRIII (and the common γ-chain) and downregulation of the inhibitory FcγRIIB, resulting in a decreased A/I ratio (Shushakova et al. 2002). This threshold regulation of FcγRs has also been shown with peritoneal macrophages (Godau et al. 2004) and Kupffer cells (Kumar et al. 2006). Subsequent studies showed that G_{αi2}- and PI3Kγ-dependent signaling downstream of the G-protein-coupled C5aR is critical for the regulation of FcγR expression in macrophages and that cooperative signaling between activating FcγRs and C5aR drives the production of cytokines and chemokines that eventually recruit and activate inflammatory neutrophils (Konrad et al. 2008; Skokowa et al. 2005). Of note, a feedback loop has been described between the upregulation of activating FcγRIII on Kupffer cells by C5a and the local production of complement. In a mouse model of autoimmune hemolytic anemia, an Ab-dependent type II autoimmune disease, activation of the C5a–C5aR axis first upregulates activating FcγRs followed by binding of IgG-autoantibodies to such IgG Fc receptors

which promotes the production of C5 and its proteolytic cleavage into C5a. Eventually, these events further enhance the expression of activating FcγRs (Fig. 3) (Atkinson 2006; Kumar et al. 2006). In addition to FcγRIII, FcγRIV also seems to contribute to this vicious inflammatory circle between complement and IgG Fc receptors, at least in an acute IC pulmonary hypersensitivity model (Syed et al. 2009). Taken together, activation of C5 by systemic or local complement activation or direct proteolytic cleavage through humoral- or cellular-derived proteases sets the threshold for immunoglobulin-mediated activation of immune cells by FcγRs. This cross-talk, including the FcγR amplification loop that generates more C5a, is desirable in an infection to ward off invaders by a fully armed immune response. Complement receptors and FcγRs play critical roles in immune adherence of microorganisms to eliminate them from the blood stream and to promote their subsequent elimination by phagocytosis (Hess and Schifferli 2003; Nardin et al. 1999). The flip side is that in autoimmune disease or allergies, such full-blown inflammation may drive undesired tissue damage. Indeed, activating and inhibitory FcγRs reciprocally promote or suppress pulmonary allergy in experimental asthma (Bandukwala et al. 2007; Dharajiya et al. 2010) and autoimmune models (Nimmerjahn and Ravetch 2006). Several reports suggest that this regulation of the A/I ratio depends on sialylated IgG molecules that bind to the C-type lectin receptor SIGNR1 on macrophages in the splenic marginal zone (Anthony et al. 2008). This interaction is supposed to promote the production of an as yet unknown soluble factor that upregulates FcγRIIB on effector macrophages. Of note, the subset of highly sialylated IgGs has been suggested to be the active part

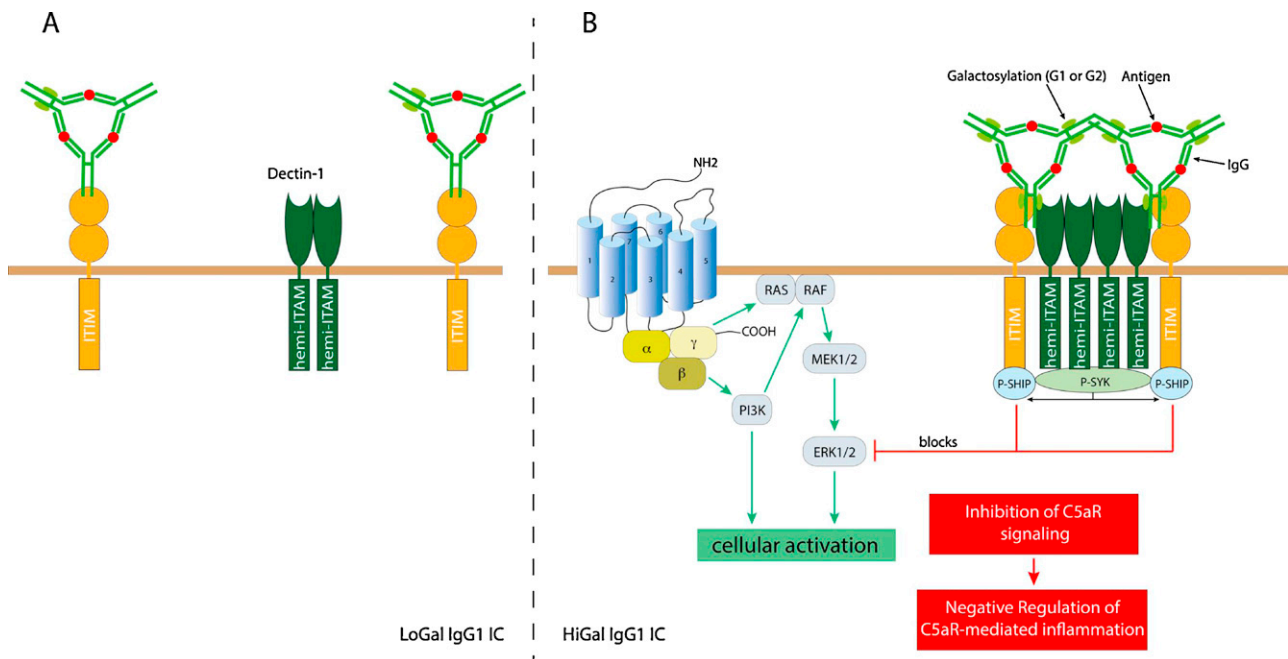


Fig. 4. Negative regulation of C5aR-mediated effector functions by highly galactosylated IgG1-IC which associate FcγRIIB with Dectin-1. (A) IgG1-IC binds to FcγRIIB independent of their N-glycan galactosylation. Low or agalactosylated IgG1-IC do not drive association of FcγRIIB with Dectin-1. (B) Highly galactosylated IgG1 IC links FcγRIIB to Dectin-1, resulting in tyrosine phosphorylation of the ITAM-like motif downstream of Dectin-1 and transient phosphorylation of Syk. Further, HiGal-IgG1-IC drives the association of SHIP with the ITIM within FcγRIIB and its phosphorylation. This pathway inhibits C5a-mediated ERK1/2 phosphorylation and several cellular effector functions mediated by C5aR signaling.

Modified from Karsten et al. (in press).

within intravenous IgG (IVIG), which is used at high concentrations to treat several autoimmune diseases including immune thrombocytopenia and the Kawasaki syndrome as well as rheumatoid arthritis, SLE and multiple sclerosis. An alternative mechanism for the effect of IVIG has been suggested by Basta et al., who found that IVIG is capturing C3a and C5a thereby neutralizing their pro-inflammatory effects (Basta et al. 2003). In light of the regulatory impact of C5a on the A/I ratio, it has been shown that IVIG treatment protects macrophages from the C5a-mediated upregulation of FcγRIII and suppression of FcγRIIB resulting in sustained high FcγRIIB:FcγRIII ratio (Konrad et al. 2006).

Recent evidence suggests that the cross-talk between C5aR and FcγR signaling is not uni- but bidirectional. In light of the strong direct and indirect pro-inflammatory properties of C5a through the ligation of its G-protein-coupled C5aR, it is surprising that our knowledge about the regulation of the C5a–C5aR axis is relatively scarce. In the fluid phase, the biologic activity of C5a is controlled by carboxypeptidases that rapidly cleave off a C-terminal arginine residue resulting in C5adesArg, which retains only 1–10% of the inflammatory activity of C5a (Bokisch and Müller-Eberhard 1970; Matthews et al. 2004). At the receptor level, TLR4 signaling has been shown to upregulate C5aR (Riedemann et al. 2002) and C5L2 expression (Raby et al. 2011). However, a detailed understanding of how the immune system regulates the effector responses of C5a has been lacking. A recent study provides evidence that IgG1-ICs can suppress C5aR-mediated effector functions in neutrophils by a novel anti-inflammatory pathway that involves the inhibitory FcγRIIB (Fig. 4) (Karsten et al. in press). More specifically, IgG1-ICs block C5a-mediated ERK1/2 phosphorylation and the phospholipase C/inositol phosphate pathway, both of which are critical for C5a-mediated pro-inflammatory effector functions. Notably, the effect was independent of activating FcγRs, suggesting that it is not initiated by the classical ITAM/ITIM pairing of activating FcγR

with FcγRIIB. In search for a potential partner that may promote the activation of FcγRIIB in the absence of the ITAM-bearing FcγR and that is expressed on neutrophils, we focused on the ITAM-like expressing C-type-lectin receptor Dectin-1. Our experiments revealed that IgG1-ICs engaged Dectin-1, resulting in Syk phosphorylation, which in turn promoted the phosphorylation of the ITIM within FcγRIIB and subsequent SHIP phosphorylation (Fig. 4). This pathway then blocked C5aR-mediated ERK1/2 phosphorylation and the phospholipase C/inositol phosphate pathway driven neutrophilic inflammation *in vitro* and *in vivo* in a model of IC peritonitis and of Epidermolysis bullosa acquisita, an autoimmune skin blistering disease. Surprisingly, this suppressive effect of IgG1 ICs was strictly dependent on high galactosylation of the Fc glycan portion. Agalactosylated IgG1 ICs exerted no inhibitory effect. Dectin-1 is well characterized as a receptor that recognizes β-1,3 glucans expressed by a broad range of fungal pathogens including *Candida albicans* and *Aspergillus* spp. (Geijtenbeek and Gringhuis 2009). However, no interaction with any Fc glycans has yet been reported. On the other hand, the N-glycan at Asn297 of the Fc moiety has been shown to promote IgG binding to FcγRs (Nimmerjahn and Ravetch 2005), the serum collectin mannose-binding lectin (Malhotra et al. 1995) and C1q (Raju 2008). Our findings demonstrate that high N-glycan galactosylation of IgG1 molecules promotes cooperative signaling of the FcγRIIB with Dectin-1 resulting in an inhibitory signaling pathway that blocks pro-inflammatory effector functions of the C5aR. This mechanism is distinct from the anti-inflammatory properties mediated by sialylation of Fc-linked N-glycans (Anthony et al. 2008). About 25–35% of Fc glycans from serum IgG are agalactosylated under steady state conditions. This frequency markedly increases in experimental and human autoimmune conditions including rheumatoid arthritis, SLE, inflammatory bowel disease as well as in infections (Arnold et al. 2007). Thus the inhibitory effect of highly galactosylated IgG1-ICs may serve as a feedback loop

to control complement and chemokine-mediated inflammation in autoimmunity and infection before IgGs can switch to the harmful agalactosyl glycoform. This effect may also contribute to the increased incidence of autoimmune phenomena in patients with hypogammaglobulinemia or common variable immunodeficiency (Agarwal and Cunningham-Rundles 2009).

Future perspectives

Cross-talk between Abs and complement protects the body from microbial invaders, apoptotic bodies and other potentially harmful cells. Further, complement prevents immune precipitation and subsequent deposition of ICs in small vessels or tissues through defined and elaborated pathways of immune adherence and IC clearance, thereby maintaining tissue hemostasis and avoiding tissue damage. Whereas the first mechanism depends on activation of the classical pathway, the solubilization of preformed precipitates is exclusively dependent on the alternative pathway. Although several pathways of IC clearance have been elucidated, how exactly complement and IgG Fc receptors communicate to safely remove ICs under steady state and inflammatory conditions is still incompletely understood and awaits further research. As outlined above, complement and FcγRs shape the B cell response in many ways and regulate the development of the natural and the induced antibody repertoire. The contribution of C3 cleavage fragments and the interaction with CR1 and CR2 on different types of B cells and FDCs has clearly been shown. At this point it is unclear whether other fragments of the complement system and their receptors may also contribute to B cell regulation. In particular, it will be of interest to delineate the molecular pathways underlying complement-mediated regulation of the innate B1 cell compartment, which is important for the control of infections with encapsulated bacteria. Finally, a bidirectional cross-talk between the C5a anaphylatoxin receptor with IgG Fc receptors exists that regulates IC-mediated inflammation in different ways. Under conditions where IgG IC strongly activates complement, such activation will reduce the threshold for IC-mediated activation of inflammatory effector cells including macrophages and neutrophils. This way, pathogens will be synergistically and most efficiently attacked by humoral and cellular effector systems. The flipside of such cross-talk are deleterious inflammatory responses in case of autoimmune diseases. Notably, an anti-inflammatory pathway has evolved that can control the vicious circle between C5aR and activating FcγR-mediated inflammation at the level of IgG glycosylation. More research is required to provide a comprehensive view of the pathways that control Fc-glycan composition. A detailed understanding of the mechanisms that control and regulate IgG, IgG Fc receptor as well as complement protein and complement receptor glycosylation may be key to uncovering novel pathways of complement and immunoglobulin interaction.

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