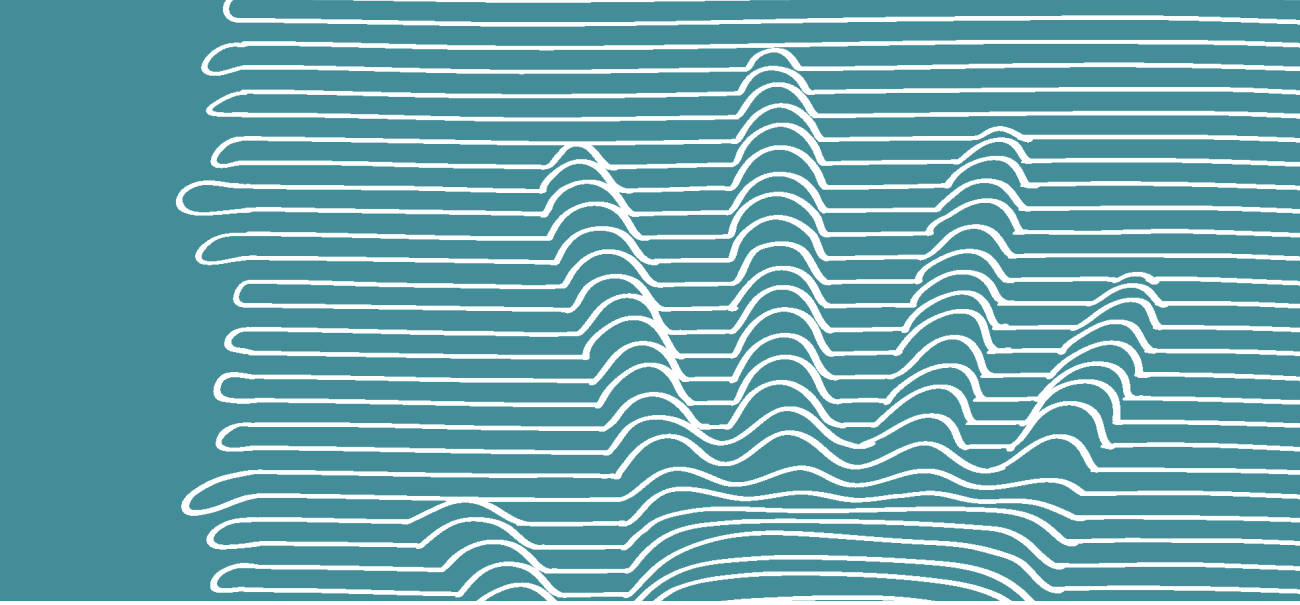

CHAPTER 8

Complement activation by (auto)antibodies

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ABSTRACT

The complement system is a key part of the innate immune system and plays an important role in the clearance of pathogens and apoptotic cells upon its activation. It is well known that both IgG and IgM can activate complement via the classical pathway by binding of C1q to the Fc regions of these immunoglobulins. Recent advances have shown that also IgA is capable of activating the complement system. Besides, more insight is gained into an additional role for antibodies in the activation of both the alternative and the lectin pathways. Mouse models have shown that autoantibodies can activate the alternative pathway and induce in cell lysis and tissue damage.

Besides the role of antibodies in complement activation, complement may also be a target for recognition by antibodies directed against autologous complement components. These autoantibodies play a role in several diseases, especially vascular diseases. Understanding how antibodies interact with the complement system will allow the manipulation of this interaction to diminish pathological consequences of autoantibodies and optimize the effect of therapeutic antibodies.

In the current review, we discuss complement activation by (auto)antibodies by the different pathways.

INTRODUCTION

As part of the innate immune system, complement is important in the clearance of pathogens and apoptotic cells. The activation of complement can occur by three different pathways, the classical pathway (CP), the lectin pathway (LP) and the alternative pathway (AP). All three pathways comprise different components and are activated in different fashions but use a common terminal pathway. The CP is activated when C1q interacts with its ligands such as immune-complexes (ICs), the LP is activated when MBL or ficolins binds to certain carbohydrates and the AP can be activated by spontaneous C3 hydrolysis or via properdin, thereby generating C3(H2O) which can bind factor B (fB). Activation of each of these pathways generates C3-convertases resulting in activation of the common terminal pathway and generating several effector processes, such as chemotaxis by C3a and C5a, opsonization by C3b, and lysis by the membrane attack complex (Ricklin et al., 2010). Additionally, the complement system provides a critical link to the adaptive immune system by serving as an effector arm of the acquired immune system supplementing antibodies. Complement activation by this manner is part of the physiologic immune response and plays an important role in fighting infections; however, it is not always beneficial and can play a role in different debilitating diseases.

In the current review, an overview of complement activation by (auto)antibodies in both human and mouse models is given and the role of this process in disease is discussed.

Complement activation by antibodies IgG and IgM

It is well known that antibodies can activate the complement system through activation of the classical pathway. However, new insights indicate that antibodies also play a role in activating and influencing both the lectin and alternative pathways.

The classical pathway is activated when C1q binds to the Fc portions of immunoglobulins. In humans the different immunoglobulin isotypes differ in their ability to bind C1q and cause subsequent activation of the classical pathway. In this respect IgM, IgG1 and IgG3 are very effective in this, while IgG2 fixes complement relatively poorly and IgG4, IgA, IgD and IgE are not capable of activating the classical pathway (Bindon et al., 1988).

Binding of C1q to IgG occurs within the CH2 domain of the immunoglobulin and is sensitive to ionic strength (Duncan and Winter, 1988; Hughes-Jones and Gardner, 1978). The C1q binding site is localized to three side chains, namely, Glu 318, Lys 320 and Lys 322. These sequence motifs seem highly conserved through evolution and are found on IgGs in all species, indicating this binding site to be universal (Burton et al., 1980; Duncan and Winter, 1988). However, this motif is also present on the IgG4 isotype, which is not able to bind C1q and activate the CP, suggesting that other features in or near the CH2 domain are important for C1q binding. It was shown that the amino acid residue at position 331, which is in close proximity to the indicated binding site of C1q, is important for proper binding and subsequent activation. Both IgG1 and IgG3 carry a proline residue at this location, in contrast to IgG4, which carries a serine residue (Tao et al., 1991). In IgM the CH3 domain is

important for C1q binding and Asp 417, Glu 418 and Hys 420 are thought to be involved in this process. Additionally, proline residues at position 436 seem to be critical for CP activation. Since the structure of the C1q binding sites between IgG and IgM are not identical, it is thought that the recognition structure of C1q may vary between different molecules (Miletic and Frank, 1995).

Additionally, comparative analysis has shown that hexameric IgM is ten to twenty times more cytolitically active than pentameric IgM, which may be due to a better symmetry of the IgM molecule to bind a C1q molecule (Collins et al., 2002; Davis et al., 1988).

Upon binding of C1q to either IgG- or IgM-containing ICs, auto-activation of C1r occurs, followed by activation of C1s. This subsequently leads to activation of C4 and C2 and to the formation of the CP C3 convertase.

Next to the well known and well studied activation by IgG and IgM of the CP, more recent studies indicate a role for these antibodies in both the AP, as well as in the LP of the complement system.

Activation of the LP occurs when mannose-binding lectin (MBL) binds to certain sugar residues. Upon binding of MBL, MBL-associated serine proteases (MASP-1 and MASP-2) are activated, which subsequently leads to activation of C4 and C2, similar to the CP. Immunoglobulins are present in the human body in different glycosylated variants and it has been shown that MBL can bind especially to antibodies with certain patterns of glycosylation.

IgG contains a single N-linked glycosylation site on each heavy chain of the antibody and the glycans that occupy this site vary in the number of terminal galactoses attached to them. MBL can bind the IgG-G0 form, in which the glycans lack a terminal galactose residue, but instead have terminal GlcNAc residues. Interaction of one carbohydrate recognition domain head of the MBL molecule with a glycan is relatively poor and therefore, most likely, only transient in serum. However, in immune complexes containing multiple IgG-G0 glycans, numerous recognition domains of MBL can engage these antibodies, generate high avidity interactions and subsequently activate complement through the LP (Arnold et al., 2005; Malhotra et al., 1995).

Although IgM has five N-linked glycosylation sites it is not able to bind MBL and activate the LP, because the GlcNAc-structures are not exposed when IgM is bound to antigen (Arnold et al., 2005).

In the AP, activation is not necessarily dependent on immunoglobulins and the first complement components are bypassed in this process. Antibodies however, do play a role and influence the activation of this pathway in different ways. IgG heavy chains can bind nascent C3b in a covalent manner, thereby enable the generation of C3-convertases on the surface of sensitized targets. Additionally, the rate of cleavage of IgG bound C3b by factor H and factor I is slowed remarkably, when compared to unbound C3b. This reduced degree of cleavage is mainly due to a diminished affinity for factor H and therefore the amplification loop can be sustained for a longer period of time (Fries et al., 1984). Additionally, it

has been shown that C3b(2)-IgG complexes in plasma are the major substrate of C3-convertase and properdin provides these complexes with partial protection from inactivation, maintaining the amplification loop longer as compared to that of free C3b (Jelezarova and Lutz, 1999; Jelezarova et al., 2000).

Complement activation by IgA

Not only in traditional antibody-mediated diseases such as systemic lupus erythematosus (SLE) and poststreptococcal glomerulonephritis, but also in IgA-dominated diseases such as primary IgA nephropathy and Henoch Schonlein purpura (Endo et al., 1998, 2000; Hisano et al., 2001; Roos et al., 2006), complement has been clearly recognized as a component in the inflammatory process. These findings have led to numerous studies into mechanisms of complement activation by IgA, revealing that IgA can activate the AP (Hiemstra et al., 1987) and LP (Roos et al., 2001) but not the CP.

AP activation by IgA has been shown with immobilized IgA purified from human plasma, resulting in activation of complement in a calcium-independent way (Hiemstra et al., 1987,1988). Moreover, IgA bound to a bacterial surface in an antigen-specific way can also mediate activation of C3 (Fasching et al., 2007; Janoff et al., 1999). The mechanism of AP activation has not been precisely defined. Most likely it involves stabilization of the C3 convertase on the IgA molecule. Since properdin has recently been recognized as a recognition molecule of the AP (Spitzer et al., 2007), it cannot be excluded that properdin may directly interact with IgA.

IgA-mediated complement activation, both via the AP and via the LP, is a function of polymeric but not monomeric IgA (Hiemstra et al., 1987; Roos et al., 2001). Both IgA subclasses in the human circulation, IgA1 and IgA2, consist of polymeric and monomeric IgA. From circulating human IgA, the majority is IgA1 and generally less than 20% is polymeric. Whereas monomeric IgA is a clearly defined molecule, polymeric IgA may consist of different molecular complexes which are incompletely defined (Papista et al., 2011).

Purified human polymeric serum IgA has been shown to interact with human MBL resulting in activation of the LP (Roos et al., 2001). This interaction involves the lectin domain of MBL and likely involves a carbohydrate ligand. Whereas IgG, IgM and IgA2 are glycosylated via N-linked sugars only, the IgA1 heavy chain also has O-linked glycosylation sites. The structure of IgA heavy chain glycans is highly variable and is determined by the cellular source of IgA. Glycosylation may be different between secretory IgA and plasma IgA (Royle et al., 2003), and between monomeric- and polymeric IgA (Oortwijn et al., 2006). Moreover, also components present in polymeric IgA complexes may be (heavily) glycosylated, such as J-chain and secretory component (Royle et al., 2003). In secretory IgA, the glycans of secretory component mask MBL-binding sites on the IgA heavy chain (Royle et al., 2003). Until now, the precise identity of the ligand involved in interaction between MBL and polymeric IgA has not been established.

In a recent study from Terai et al. (2006) purified polymeric serum IgA did not bind to MBL unless the glycan structure was modified or disrupted (Terai et al., 2006). As also stated

by these authors, it is impossible to explain this discrepancy, but most likely it is related to the isolation procedure.

The pathophysiological significance of the interaction between IgA and MBL is underlined by studies in patients with IgA nephropathy. This renal disease is characterized by deposition of IgA in the mesangial area of the glomerulus, which is accompanied by complement activation, activation of the inflammatory cascade, renal injury, and, on the longer term, end stage renal disease. Several reports showed deposition of MBL together with IgA in the mesangium (Endo et al., 1998, 2000; Hisano et al., 2001; Matsuda et al., 1998; Roos et al., 2006). This MBL deposition was associated with deposition of MASP-2 and C4 (Roos et al., 2006), supporting its role in glomerular complement activation. Moreover, the subpopulation of IgA nephropathy patients with mesangial MBL deposition has a more severe glomerular disease than IgA nephropathy patients who only showed evidence for complement activation via the AP (Roos et al., 2006). These data were confirmed in a later study reporting that C4d-positive IgA nephropathy patients have a significantly more rapid progression to end-stage renal disease than C4d-negative patients (Espinosa et al., 2009). Taken together, complement activation by IgA may support humoral host defense, but, in diseased conditions, may also amplify inflammation and tissue injury. There is evidence for involvement of the AP and the LP, and not only MBL but also ficolins may be involved (Roos et al., 2006). Since MBL deficiency is the most common human complement deficiency, this effector function of the IgA immune system differs between individuals. Furthermore, given the large variety of molecular forms of human IgA, the molecular interactions are complex and need multidisciplinary studies, with respect to the identity of the protein components as well as the structure of the glycans on these molecules.

Complement activation by murine autoantibodies

Several mouse models are available in which autoantibodies play a clear role. Using these established models it was possible to unravel the complex contribution of complement activation into the overall process of tissue damage. Although many informative models are available, in this review we have focused on mouse models of rheumatoid arthritis (RA), SLE and subepidermal blistering diseases. The role of autoantibodies in complement activation in mouse models of auto-immune diseases suggests a prominent and in some cases essential role of the AP in their pathogenesis.

Murine models of arthritis

Several auto-antibody-mediated mouse models of arthritis are available to study RA. Initially, the role of complement in Collagen Induced Arthritis (CIA) was suggested from studies where mice treated with cobra venom to deplete complement were found to be refractory to arthritis until their complement levels were restored (Backlund et al., 2002). Two additional mouse models have been extensively used to explore the role of complement in arthritis: the anti-G6PI (K/B x N) and CAIA (anti-collagen antibody induced) models. Interestingly, in the K/B x N model disease develops spontaneously in the offspring of TCR-transgenic KRN mice crossed with NOD mice carrying the MHC class II Ag7 allele (Ji

et al., 2002). Disease is dependent on KRN TCR recognition of G6PI (residues 282-294) when presented by MHC class II Ag7. It has been shown that G6PI autoantibodies are present in these mice and transfer of either serum or affinity-purified G6PI autoantibodies will induce arthritis in healthy mice in a lymphocyte independent fashion (Ji et al., 2002; Matsumoto et al., 1999). These antibodies act through FcγIII receptors and C5a (Ji et al., 2002). Importantly, the AP and not the CP is required for the development of arthritis. Furthermore, in mice it has been shown that anti-G6PI IgG localizes specifically to the joints where arthritis occurs and that localization is dependent on mast cells, neutrophils, FcRs, and ICs (Mandik-Nayak and Allen, 2005).

Traditional views regarding the role of properdin in the activation of the AP has also been changed, since it has now been shown that properdin can bind to target surfaces and initiate AP activation (Hourcade, 2006). It was shown that Cfp-/- mice were significantly protected from development of arthritis induced by serum transfer from K/B x N mice (Kimura et al., 2010).

Complement activation is also critical for the effector phase of arthritis in the CAIA model (Hietala et al., 2004). It was demonstrated that have shown, using this model, that the AP is necessary and sufficient for the development of arthritis (Banda et al., 2006). Consistent with this observation, it was found that fD-/-, C1q-/-/Df-/-, and MBL-/-/Df-/- mice were also resistant to CAIA while C1q-/-/MBL-/- were highly susceptible (Banda et al., 2007, 2010a) (Fig. 1). Interestingly MASP-1/3-/- mice are resistant to CAIA for these mice only have an inactive form of factor D called pro-factor D (pro-Df), and MASP-1 protein is required for the cleavage of inactive pro-Df into active factor D (Banda et al., 2010b; Takahashi et al., 2010). These studies indicate that the AP can be activated by anti-collagen autoantibodies directly and independently from the CP and LP.

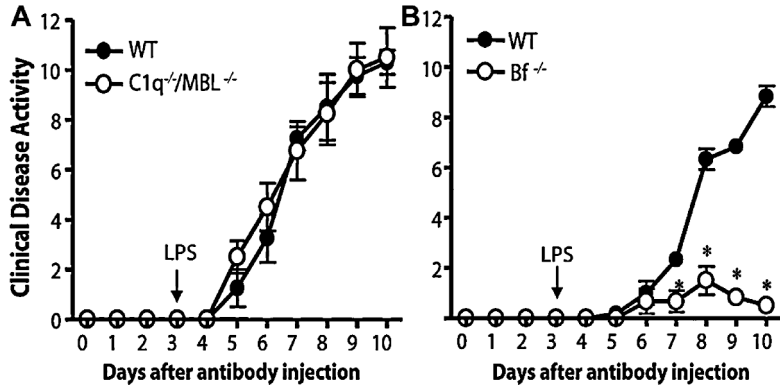

Fig. 1. Antibody mediated arthritis is depending on the AP and not the CP or LP. Clinical disease activity of anti-collagen type II mAb-induced arthritis in WT and Clq-/-/MBL-/- and Bf-/— C57 BL/6 mice (Banda et al., 2006, 2007). Mice were given a mixture of anti-collagen type II mAbs at day 0. All mice were injected with LPS at day 3. The clinical disease activity was assessed every day using a 3-point score for each paw with four paws per animal (maximum score of 12). The data are expressed as the clinical disease activity scores. (A) WT (solid black circles) *(n* = 4) and C1q-/-/MBL-/- (empty white circles) *(n* = 4) mice vs. days after the initial injection of the anti-collagen type II mAb mixture. (B) WT (solid black circles) (n = 6) and Bf-/- (empty white circles) (n = 6) mice vs. days after the initial injection of the anti-collagen type II mAb mixture. These data are represented as mean ± SEM. *All of the p-values for clinical disease activity in Bf-/- mice in comparison with WT were statistically significant at $p < 0.001$.

About a decade ago it has been reported that anti-citrullinated protein antibodies (ACPA) are present in RA patients that can be detected years before disease onset (Rantapaa-Dahlqvist et al., 2003). ACPA can be of IgG, IgM, IgE or IgA isotype (Schuerwegh et al., 2010; Verpoort et al., 2006) and recognize proteins that are post-translationally modified by the conversion of arginine to citrulline by the enzyme peptidyl arginine deiminase (PAD) (Vossenaar et al., 2003). As ACPA are found long before disease onset, it was hypothesized that they could be pathogenic. Human ACPA when tested in vitro have the capacity to activate the CP and AP but not the LP (Trouw et al., 2009).

Injection of one purified mouse IgM ACPA monoclonal antibody against citrullinated fibrinogen was not pathogenic by itself but exacerbated established disease induced by anti-collagen antibodies (Kuhn et al., 2006). In another study five monoclonal autoantibodies to citrullinated collagen type (CII), ACC1-5, were generated (Uysal et al., 2009). Two of these five autoantibodies, ACCI (IgG2c) and ACC5 (IgM), when injected into B10.RIII mice, directly induced arthritis and the severity of disease was increased after the injection of LPS. Importantly, another clone, AAC4 (IgG1), did not induce arthritis. However, when this AAC4 antibody was mixed with an anti-collagen antibody M2139 (IgG2b) it did cause severe arthritis. Since in mice IgG1 does not activate complement while IgG2b is a strong activator of complement this may explain the difference between these monoclonal antibodies in inducing arthritis.

Banda et al. (2008) have shown that the AP of complement is capable of initiating C3 activation induced by ICs of collagen and anti-collagen autoantibodies, and that it requires the presence of N-glycans on the IgG. IgG G0 anti-collagen antibodies activated both the CP and AP more than the LP. The glycosylation status of IgG has also been implicated in the pathogenesis of RA. EndoS, an endoglycosidase isolated from Streptococcus pyogenes, specifically cleaves the terminal sialic acid residues from glycan groups and has been used extensively in recent years as a tool to modify the glycosylation status of pathogenic antibodies. For one EndoS-treated anti-collagen autoantibody, M2139-IgG2b, it was shown to have lost its capacity to induce arthritis in B10.RIII mice (Nandakumar et al., 2007). Interestingly, in these studies EndoS treatment did not affect the binding of anti-collagen antibodies to CII and their capacity to activate complement as there was no difference in C1q deposition on anti-collagen antibodies with and without EndoS treatment bound to CII, but it reduced IgG binding to FcγR and the formation of stable ICs (Table 1). Collectively, these mouse models show that the AP is essential for tissue damage induced by auto-antibodies.

Murine models of SLE

SLE is an auto-immune disease in which complement plays a prominent role. It is characterized by autoantibody production, immune complex formation, complement activation and systemic tissue damage. The deposition of ICs in glomeruli of patients with SLE causes lupus-nephritis via complement activation, generation of C3a and C5a, and MAC formation (Biesecker et al., 1981; Falk et al., 1983; Wyatt et al., 1979). Complement activation increases clearance of ICs but deposition of complement activation products contributes to inflammation, fibrosis and local tissue injury (Couser et al., 1985).

Table 1. *Overview of auto-antibodies directed towards complement components.*

Antibody	Disease association
Anti-C1q	Hypocomplementaemic Urticarial Vasculitis Syndrome; Felty's syndrome; SLE; Rheumatoid vasculitis; Classic polyarthritis nodosa; Sjogren's syndrome; Mixed connective tissue disease; Polygondritis; Temporal arthritis; Mixed cryoglobulinaemia; MPGN; IgA nephropathy; Anti-GBM glomerulonephritis; Membranous glomerulopathy
Anti-C1s	SLE
Anti-C1-INH	SLE
Anti-MBL	SLE
Anti-H ficolin	SLE
Anti-factor B	Dense deposit disease
Anti-factor H	MPGN
Anti-C4	SLE
C3NeF	Post streptococcal glomerulonephritis; MPGN; partial lipodystrophy; SLE
C4NeF	MPGN
Immunoconglutinins	SLE; rheumatoid arthritis; paroxysmal nocturnal hemoglobinuria; chronic liver disease
Anti-CR1	Colitis; SLE; liver cirrhosis
Anti-CR2	HIV
Anti-CR3	Rheumatoid arthritis; auto-immune neutropenia
Anti-CD46	HIV
Anti-CD59	Multiple sclerosis

Adapted from (Norsworthy and Davies, 2003; Seelen et al., 2003a; Trouw et al., 2001), with addition of anti-H ficolin (Yae et al., 1991), anti-MBL (Seelen et al., 2003b), anti-factor H (Skerka et al., 2009) and anti-factor B (Strobel et al., 2010).

Antibodies to double-stranded DNA (dsDNA) represent the hall-mark of SLE and their capacity to fix complement has been associated with disease activity (Mackworth-Young et al., 1986; Rothfield and Stollar, 1967). By using antigen microarray technology to analyze serum derived from lupus prone MRL/lpr mice it has been shown that C3 deposition on nucleic acids is profoundly increased by the presence of mAbs specific for dsDNA (Papp et al., 2010). Observational studies have shown that the inflamed glomeruli of MRL-lpr mice stain positive for markers of CP, LP and AP activation (Trouw et al., 2004b, 2005). MRL/lpr mice lacking either fB or fD developed less renal disease than WT mice (Elliott et al., 2004; Watanabe et al., 2000). Auto-antibodies to dsDNA, did not differ between fD+/+, fD+/- and fD -/- MRL/lpr mice at any age. Thus lack of AP activation protects against proliferative renal disease in MRL/lpr mice. However, C3-/- MRL/lpr mice are not protected from renal disease and there is no difference in autoantibody titer compared with WT mice. This indicates that complement activation is not required for the production of auto-antibodies in MRL/lpr mice. Nonetheless inhibition of the AP is beneficial in lupus nephritis despite glomerular IgG deposition mediated effects. Blocking the AP has profound effects next

to SLE also in many inflammatory and renal diseases underscoring the generality of this pathogenic mechanism (Fung et al., 2001; Kalli et al., 1994; Moore, 1994; Pascual et al., 1993; Tanhehco et al., 1999). In these mouse models of SLE also anti-C1q autoantibodies play a role, which will be discussed in below.

Murine models of subepidermal blistering disease

Epidermolysis bullosa acquisita (EBA) is an auto-immune disease with chronic subepidermal blisters of skin and mucous membranes and is characterized by autoantibodies to type VII collagen (COL7) (Lapiere et al., 1993; Woodley et al., 1988). Experimental EBA can be induced in mice by injecting rabbit or human anti-mouse COL7 IgG (Sitaru et al., 2006; Woodley et al., 2005, 2006). The pathogenic relevance of COL7 auto-antibodies has been shown by passively transferring these antibodies in mice and by injecting mice with autologous COL7 (Sitaru et al., 2006). Anti-COL7 antibodies induced blisters in normal mice but failed to induce blisters in C5-deficient mice, supporting the role of complement activation in the pathogenesis of EBA. Additionally, using genetic manipulations similar to those described above, it has been shown that complement activation by the AP is required to elicit disease (Mihai et al., 2007).

Bullous pemphigoid (BP) is another example of an auto-immune subepidermal blistering disease (Gudi et al., 2005; Langan et al., 2008). Serum anti-human COL17 autoantibodies target human type XVII collagen (Robson et al., 2003). Deposition of anti-human COL17 auto-antibodies at the basement membrane zone triggers a sequence of inflammatory cascades including complement activation, infiltration of neutrophils and eosinphils, and degranulation of dermal mast cells (Chen et al., 2001; Liu et al., 2008; Nishie et al., 2007; Oikarinen et al., 1983; Robson et al., 2003; Stahle-Backdahl et al., 1994). Recently, an anti-hCOL17 IgG1 monoclonal antibody was shown to effectively reproduce a BP-like phenotype in mice (Li et al., 2010). Interestingly, a human IgG1 monoclonal antibody, mutated at residue P331 in the Fc region, lost its capacity to activate complement and also lost its pathogenicity. This is the first report that IgG1 antibodies to hCOL17 can induce blister formation in mice and it raises the possibility that an IgG1 monoclonal with an Fc modification may be used to block pathogenic epitopes in auto-immune disease (Li et al., 2010).

Although the initiation of the complement activation normally takes place by the CP or by the LP, potentially it is the AP amplification that causes most tissue damage. In addition the AP itself can be activated by auto-antibodies in the absence of CP and LP to cause tissue damage.

Why is the absence of any component of the AP so protective for tissue damage induced by autoantibodies? The major part of the pro-inflammatory C5a is generated by the AP (Banda et al., 2010a) and each component of the AP uniquely participates in this activation process. Whether properdin binds to autoantibodies and initiates AP of the complement is a subject of future research.

To what extent this is also true for humans is not clear. One in vitro study, compared the C3a and C5a generation by either the CP or AP induced by anti-collagen antibodies using

mouse and human sera. The level of C5a generated by the AP alone (79.1%) in mouse sera was significantly higher than the level generated by the CP alone (46.6%). However, the opposite was observed with human sera (Banda et al., 2010a,b). Also of note is the observation that C3 deficient persons can develop SLE, indicating that in the absence of complement activation, immunecomplex mediated damage can occur. However, in complement sufficient SLE patients complement activation clearly contributes to damage.

Autoantibodies to complement components

Next to the fact that several complement components like MBL, C1q and Properdin can interact with antibodies and result in complement activation, complement itself may be a target of autoantibodies (Norsworthy and Davies, 2003; Trouw et al., 2001). In this regard the best known autoantibodies are directed against for instance C1q and the amplification convertase C3bBb. In this section we provide a very brief overview of autoantibodies against complement.

Autoantibodies against several complement components have been described including autoantibodies against C1q and MBL(Seelen et al., 2005; Siegert et al., 1991; Trendelenburg et al., 1999; Trouw and Daha, 2005). Both of these autoantibodies have been described in patients with systemic lupus erythematodus (SLE) but may also be found in other diseases. Generally the auto-antibodies are directed against neoepitopes and this is evident especially concerning autoantibodies against C1q that react mainly with immune-complex-bound C1q.

Anti-C1q autoantibodies are present in 30-50% of SLE patients (Monova et al., 2002; Siegert et al., 1991), and are strongly associated with the development of lupus nephritis. The absence of these antibodies seems to exclude active renal disease (Trendelenburg et al., 1999). However high titers of anti-C1q autoantibodies have also been reported in hypocomplementaemic urticarial vasculitis syndrome (HUVS), suggesting that there may be a contextual element to the deposition of this antibody (Wisnieski and Naff, 1989).

There is strong evidence that anti-C1q autoantibodies may result in direct interaction with C1q that is transiently present in the glomerular basement membrane (GBM) leading to a low degree of inflammation accompanied by infiltration of polymorphonuclear leukocytes. However for a pronounced induction of renal injury and proteinuria more than one hit seems to be required (Trouw et al., 2004a). Anti-C1q autoantibodies are present in many strains of auto-immune mice (Hogarth et al., 1996; Trinder et al., 1995). In MRL-lpr mice a temporal association between the occurrence of anti-C1q autoantibodies and nephritis was observed (Trouw et al., 2004b) whereas in another model of lupus nephritis no such correlation was found (Bigler et al., 2010). In search of the involved mechanistics, mice were pretreated with a subnephritogenic dose of anti-GBM antibody followed by an injection of anti-C1q, and a complement-dependent glomerular inflammation was induced (Trouw et al., 2004a). Mice treated with anti-GBM antibody or anti-C1q antibody alone developed no renal disease. Anti-C1q antibodies, were dependent on their antigen C1q, as this effect was not seen in C1q-/- mice. The combination of anti-GBM and anti-C1q

antibodies caused no glomerular damage in C4-/-, C3-/- and FcɣR receptor-deficient mice, demonstrating that the mechanism of glomerular damage was dependent on the CP and on FcɣR receptors.

Auto-antibodies against MBL have been described also in patients with SLE (Seelen et al., 2003b; Trendelenburg et al., 1999; Trouw and Daha, 2005) but seem to occur only in patient that are not deficient for MBL. In SLE patients, MBL deficiency, is associated with increased presence of autoantibodies against cardiolipin and against C1q (Seelen et al., 2005). The hypothesis is that an enhanced presence of autoantibodies may be related to disturbed clearance of apoptotic cells due to impaired MBL function.

Several autoantibodies against complement regulators have been reported including autoantibodies against factor H and C1-inhibitor. Anti-factor H autoantibodies are strongly related with the occurrence of atypical hemolytic uremic syndrome and C3-nephropathies (Dragon-Durey et al., 2010a). The presence of anti factor H autoantibodies induces systemic AP activation resulting in low plasma levels of C3 and factor B. The antigenic level of factor H may be subnormal but more importantly the binding of these autoantibodies causes a functional defect in factor H imparing its capacity to interact with endothelial surfaces (Dragon-Durey et al., 2010b). The age at onset of the anti factor H auto-antibodies varies between the very young of less than 1 year up to 13 years in age (Dragon-Durey et al., 2010a).

The presence of anti-C1-inhibitor autoantibodies is a hallmark of acquired C1-inhibitor deficiency (Donaldson et al., 1996; Meszaros et al., 2010). A recent study in a large cohort of

SLE patients revealed that the level of anti-Cl-inhibitor-autoantibodies was significantly higher in SLE patients than in controls, which was associated with disease activity. Acquired Cl-inhibitor deficiency in elderly patients is in most cases associated with clonal B cell diseases, most likely due to production of monoclonal antibodies against C1 inhibitor. Several studies have indicated that the anti-Cl-inhibitor-autoantibodies result both in inhibition of functional activity of Cl-inhibitor and in reduction in Cl-inhibitor levels and as a consequence in deregulation of both the CP and LP.

Two convertases determine the degree of activation of the central component of complement C3. These are the CP/LP induced C3-convertase C4b2a and the amplification convertase C3bBb. Autoantibodies against C4b2a (C4NeF) were described in patients with SLE (Daha et al., l980; Gigli et al., l985) and shown to functionally stabilize the otherwise labile enzyme resulting in enhanced activation of C3, low levels of C3 and potentially in inflammation. Unfortunately C4NeF had received little attention in later years.

An activity present in serum of patients with membranoproliferative glomerulonephritis (MPGN) that was able to induce activation of complement in normal human serum via the AP was recognized for the first time by West et al. (l965) in the early sixties. This activity was indicated as C3-nephritic factor (C3NeF). Since then C3NeF has received broad attention. It was demonstrated that C3NeF is an auto-antibody directed against C3bBb and that

it stabilizes the otherwise labile C3bBb convertase giving it a longer half-life and thereby the potential to activate more C3 resulting in reduced circulating levels of C3. C3NeF is highly associated with the occurrence of MPGN. Stabilization of the C3bBb convertase can also occur by Properdin (Fearon and Austen, l975) but there is a main difference between Properdin and C3NeF. While the stabilization by C3NeF gives a biphasic decay characteristic of the C3bBb convertase sites (Daha et al., l976), the Properdin stabilization results in a linear decay pattern. This suggests that C3NeF only stabilizes the C3bBb convertase sites to which it is bound while Properdin seems to interact with all convertase sites dependent on the concentration of properdin used and presumably by moving from one site to another.

Results obtained with a large number of C3NeF preparations suggest that C3NeF is an auto-antibody directed against antigenic determinants on Bb, which are exposed after interaction of B with C3b (Daha and Van Es, l98l). Taken together C3NeF seems to be responsible for hyper catabolism of C3 with the consequence that activated C3 is deposited in the kidney resulting subsequently in injury to the kidney. One study provided the proof-of-concept showing that three mouse IgM monoclonal antibodies had C3Nef-like activity (Daha et al., l984). More recent studies indicate that several mechanisms including deregulation of convertase function as can be seen in patients with atypical hemolytic syndrome, gain of function mutations in C3 and B, or the occurrence of anti-H and anti-B auto-antibodies are all associated with hypercatabolism of C3 and induction of vascular injury (Skerka et al., 2009).

Therapeutical antibodies and complement activation

With the approval by the US Food and Drug Administration of 28 monoclonal antibodies for use in humans in 20ll, these antibodies have become a critical component of clinical treatment regimens in a large variety of indications. Many therapeutic monoclonal antibodies exert their killing activity through several mechanisms of action. Whereas several antibodies kill via apoptosis, receptor down modulation or by altering signal transduction, others use the immune system to induce efficient killing of target cells. Indeed, successful therapeutic antibodies like alemtuzumab and rituximab seem to be well able to eradicate target cells via antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) (Teeling et al., 2004, 2006; Waldmann and Hale, 2005). Alemtuzumab targets CD52 on B- and T-cell cancers, while rituximab targets CD20 on B-cells and is used in the treatment of many lymphomas, leukemias, transplant rejection and some autoimmune disorders (Olszewski and Grossbard, 2004). A large body of evidence supports the role of complement activation in antibody immunotherapy in patients (Cragg et al., 2003; Di Gaetano et al., 2003; Golay et al., 2000, 2006; Idusogie et al., 2000; Manches et al., 2003; Reff et al., l994; Zent et al., 2008). Furthermore, under certain complement limiting conditions, rituximab can become ineffective (Glennie et al., 2007; Taylor and Lindorfer, 2008). Fresh frozen plasma could enhance or restore complement titers and rituximab therapy in CLL patients, which was probably due to the exhaustion of the limiting factor C2 (Kennedy et al., 2004; Klepfish et al., 2008; Taylor, 2007).

The role of complement in immunotherapy is further supported by the observation that polymorphisms in C1q were associated with response rates to rituximab treatment in follicular lymphoma (Racila et al., 2008).

The fact that therapeutic monoclonal antibodies are able to use the complement system to induce target cell killing is intriguing. First of all, they need to overcome the inhibitory effects of a set of plasma proteins and membrane-bound complement regulatory proteins, which prevent unintended injury by our own activated complement (Atkinson et al., l99l; Junnikkala et al., 2000; Kim and Song, 2006; Kirkitadze et al., l999; Liszewski and Atkinson, l996; Morgan et al., l998). Especially tumor cells, in comparison to normal cells, can be difficult to kill since complement inhibitors may be upregulated such as via cytokines, streamers or be the result of selection (Beum et al., 2008; Blok et al., 2003; Cruz et al., 2007; Teeling et al., 2004).

Secondly, binding to very small or compact surface proteins, like CD20, CD52 and CD38 seems to be a prerequisite for killing via CDC (de Weers et al., 20ll; Polyak et al., l998; Xia et al., l993). Possibly, binding close to the cell surface facilitates effective capture and concentration of active complement components proximating to the cell membrane, potentially shielding activated complement components (C4 and C3) from inactivation by fluid phase inhibitors, overcoming inhibition by mCRPs and promoting efficient generation of the MAC complex (Beum et al., 2008; Bindon et al., l988; Michaelsen et al., l990). This is further exemplified by atumumab that binds an epitope encompassing both the small and large loop on the CD20 molecule very close to the cell membrane, which results in killing of B cells with low CD20 densities, and reduced sensitivity to complement inhibitors (Beum et al., 2008; Pawluczkowycz et al., 2009; Teeling et al., 2004, 2006). Also other factors, like binding avidity to C1q and clustering of surface antigens into lipid rafts may further influence CDC and subsequent therapeutic activity (Cragg et al., 2003; Mone et al., 2006; Pawluczkowycz et al., 2009; Taylor and Lindorfer, 20l0; Teeling et al., 2006; Wierda et al., 20l0).

Recent work has shed light on the role of complement factors, C1q, and C3 in opsonization of target cells (Gelderman et al., 2004; Racila et al., 2008). A split product of the initiation of the MAC complex, C5a, is thought to contribute to the complement mechanism of action. C5a is a potent chemoattractant, stimulator and survival factor of effector cells which are involved in the elimination of malignant cells (Monk et al., l994; Perianayagam et al., 2002). It also modulates IgG Fc receptor expression via upregulation of activating and downregulation of inhibitory IgG Fc receptors (Shushakova et al., 2002). By using fusion proteins containing C5a, enhanced ADCC of breast cancer cells was shown (Fuenmayor et al., 20l0).The importance ofCR3 in tumor cell killing was underscored by Boross et al. (20ll) who recently showed that antibodies can kill tumor cells in vivo after binding of the complement receptor CR3 to C3 on target cells.

Recent evidence shows that CDC can be further augmented by conferring more potent CDC activity of IgG. This was done by exchanging segments between IgG isotypes to

generate a variety of chimeric IgG molecules or by amino acid substitutions (Dall'Acqua et al., 2006; Idusogie et al., 2001; Michaelsen et al., 2009; Moore et al., 2010; Natsume et al., 2009). Optimization of IgG1 antibodies for complement has enormous potential for improving the next generation of therapeutic antibodies which could bear great promise to increase the chances to cure patients in the future.

CONCLUSIONS

Collectively we conclude that antibodies, as a traditional hallmark of adaptive immunity, contribute to host protection and to tissue damage by utilizing additional mediators of the innate immune system and especially complement activation. All three pathways of complement can be initiated directly by antibodies. However, clearly not all antibodies activate each pathway. Different isotypes have a different capacity to trigger complement activation in vitro. In-vivo however, the relative contribution of each isotype is less clear. Even the relatively weak activators may still activate sufficient complement as to result in tissue pathology as for instance seen in IgA nephropathy. The molecular composition, sequence, density and glycosylation status of the different antibody isotypes determine, to a certain extend, their ability to activate each of the pathways. Such modifications provide us with the possibility to interfere with the pathogenic effects of autoantibodies and serve as a tool for optimization of therapeutic antibodies. Obviously complement activation by antibodies is not just bad news, it provides an important effector mechanism to clear pathogens and may be used therapeutically to kill tumor cells or autoreactive immune cells.

The fact that autoantibodies not directed against a tissue antigen but rather against a complement factor or a convertase may still induce a tissue specific inflammatory response, makes it difficult to dissect the relative contribution of each autoantibody to the overall disease process. The traditional view that only the CP is directly activated by antibodies and that only IgG and IgM can activate complement are both replaced by the concept that all pathways of complement can be activated by antibodies directly and that no general rules apply to isotypes and or subtypes regarding their in vivo complement activating potential. Additionally, once tissue injury has been initiated, the tissue itself, as exemplified by the activation of all three pathways by cell debris, may directly contribute to enhanced tissue damage.

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