Abstract

Complement amplification in blood takes place not only on activating surfaces, but in plasma as well, where it is maintained primarily by C3b\textsubscript{2–}IgG complexes. Regular products of C3 activation in serum, these complexes are inherently very efficient precursors of the alternative pathway C3 convertase. Moreover, they can bind properdin bivalently, thus creating preferred sites for convertase formation. C3b\textsubscript{2–}IgG complexes have a half-life that is substantially longer than that of free C3b, since both C3b molecules are partially protected from inactivation by factor H and I. These complexes are preferentially generated on certain naturally occurring and induced antibodies that exhibit a paratope-independent affinity for C3/C3b. Such antibodies are known to stimulate alternative complement pathway activation. We have assembled the evidence for the generation and the functional potency of the C3b\textsubscript{2–}IgG complexes, which have been studied during the last two decades. We illustrate their roles in immune complex solubilization, phagocytosis, immune response, and their ability to initiate devastating effects in ischemia/reperfusion and in aggravating inflammation.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Complement; Antibodies; C3; Alternative complement pathway; C3 convertase

1. Introduction

Our understanding of complement amplification as it occurs in systemic inflammation (Hietala et al., 2004; Ward, 2004) and in ischemia-reperfusion (Chan et al., 2003) is incomplete. Strong evidence suggests that spontaneous initiation of the alternative complement pathway occurs in the fluid phase (Isenman et al., 1981; Pangburn and Müller-Eberhard, 1980; Pangburn and Muller-Eberhard, 1980), on which bound C3b is not or is only partially inactivated by fluid phase factors H and I (Pangburn, 1983; Pangburn et al., 1981). Its amplification, however, is thought to take place exclusively on "activating surfaces" (Pangburn and Müller-Eberhard, 1980), on which bound C3b is not or is only partially inactivated by fluid phase factors H and I (Pangburn, 1983; Pangburn et al., 2000). Here we portray a potent precursor of the alternative pathway C3 convertase, C3b\textsubscript{2–}IgG, which is generated in blood from nascent C3b and IgG primarily because C3/C3b have an affinity for IgG molecules (Kulics et al., 1983). We summarize the evidence gathered over the last two decades in trying to understand its generation and its molecular and functional properties as a fluid phase and cell surface precursor of the amplification loop. C3b\textsubscript{2–}IgG differs from free or surface-bound C3b in several aspects. The two C3b molecules are sequentially ester bonded to each other and to one IgG heavy chain, most likely as a dimer (Jelezarova et al., 2003). Since both C3b molecules are covalently bound to a large molecule (Sahu and Pangburn, 1994), either the next C3b or the IgG, they are equally partially protected from inactivation by factors H and I. This accounts for the long half-life of C3b\textsubscript{2–}IgG complexes in blood (≈40 min in 2% serum (Fries et al., 1984); 3–4 min in 20% serum (Lutz et al., 1996)). C3b\textsubscript{2–}IgG complexes are inherently potent C3 convertase precursors, because C3 convertase assembly is 7–10 times more efficient on complexes than on immobilized C3b (Jelezarova et al., 2001, 2000). Both properties together render C3b\textsubscript{2–}IgG complexes the most potent C3 convertase precursor, because C3 convertase assembly is 7–10 times more efficient on complexes than on immobilized C3b (Jelezarova et al., 2001, 2000).

Evidence suggests that C3b\textsubscript{2–}IgG complex formation may occur preferentially on certain types of IgG molecules (Lutz et al., 1993b), although the prerequi-
sites for C3b2-IgG complex formation are not yet known. Nevertheless, it is of interest to recall that several naturally occurring antibodies (NAbs), which opsonize zymosan (Schenkein and Ruddy, 1981a), pathogens (Winkelstein and Shin, 1974), and red blood cells (RBC) (Nelson and Ruddy, 1979), stimulate alternative complement pathway upon binding to their antigen.

2. Fluid phase and solid phase complement amplification

To illustrate the role of C3b2-IgG complexes as C3 convertase precursors and to discuss the evidence for their generation, we suggest a revised scheme for the AP (Fig. 1).

This scheme gives credit to the well known sequence of events in serum, where the initial AP C3 convertase is generated by the interaction of spontaneously “activated” C3, C3(H2O) (Pangburn et al., 1981), which has C3b-like properties, with factor B, which is activated by factor D in its bound state. This neoenzyme activates C3 which in turn forms C3bBb (reviewed in: Pangburn, 1983; Pangburn and Müller-Eberhard, 1984). The C3 convertase is stabilized by properdin that exists only in oligomeric form (Farries et al., 1988). This amplifying C3 convertase is active in the fluid phase, but is extremely short lived due to spontaneous decay and inactivation of C3b. Consequently, free C3b remains undetectable in serum upon complement activation (Lutz et al., 1996). A parallel pathway is shown in which nascent C3b reacts covalently with fluid phase IgG and generates C3b2-IgG complexes. These complexes facilitate bivalent binding of oligomeric properdin, which favors factor B binding. Bound factor B is activated by factor D whereby the assembled and already properdin-stabilized C3 convertase is generated. Since their half-life is at least 100 times longer than that of free C3b, these complexes are the predominant species that perform C3b-like functions in blood.

A second scheme (Fig. 2) illustrates complement amplification on surfaces. Nascent C3b binds covalently to surface components in the vicinity of the C3 convertase, forming clusters (Pangburn et al., 2000, 1983). Monomeric C3b, ester bonded to a surface component, is also partially protected from inactivation and therefore endowed with a longer half-life than fluid phase C3b. In addition, C3b may also allow bivalent binding of properdin, if the spatial arrangement of two C3bs is favorable. Binding of properdin is independent of factors H and B and thus independent of whether the C3b-accepting surface is an “activating or non-activating” one. Complement amplification on surfaces may occur by yet another mean, since many NAbs in blood associate with self and non-self and are known to stimulate amplification. Such NAbs have low affinities (Avrameas and Ternynck, 1993) and their efficient binding to cells requires that their target antigen is clustered (Lutz et al., 1987; Tuorti et al., 1991). Once bound, they are presumably among the first to react with nascent C3b and form C3b2-IgG complexes (Lutz et al., 1993b).
3. From AP-stimulating naturally occurring antibodies to C3b−IgG complexes, a historical view

3.1. Naturally occurring antibodies that stimulate AP may preferentially form C3b−IgG complexes

Immune complexes formed from induced IgG2 antibodies did not activate the classical pathway in guinea pig serum, but stimulated complement amplification (Sandberg and Oeder, 1971). Likewise, opsonization of pneumococci by the IgG2 fraction of non-immunized guinea pig serum augmented alternative complement pathway activity in C4 deficient serum (Winkelstein and Shin, 1974). The latter finding suggested that NAbs specific for pneumococci stimulated AP activity when bound to their target. The same phenomenon was found for NAbs with specificity for zymosan (Schenkein and Ruddy, 1981b), glycolipids (Okada et al., 1983), and red blood cells (RBC) (Nelson and Ruddy, 1979; Polhali et al., 1978). In trying to elucidate how these NAbs may stimulate AP, Schenkein and Ruddy studied for the first time C3 convertase generation in the presence or absence of AP-stimulating NAbs. The AP-stimulating NAbs did not enhance the activity of a preformed C3 convertase similar to properdin, but increased the rate of uptake of C3 and B onto zymosan and thus were involved in generation of C3 convertase precursors (Schenkein and Ruddy, 1981b). While added NAbs doubled the amount of C3b that bound to zymosan, C3b interaction with IgG was not investigated in detail. This question was addressed at the same time by Gadd and Reid on immune complexes generated from rabbit IgG antibodies in human serum in the presence of EGTA and magnesium (Gadd and Reid, 1981). These authors demonstrated for the first time that preformed immune complexes took up 2–3% (Gadd and Reid, 1981). These authors demonstrated for the first time that preformed immune complexes took up 2–3% of the total C3b activated under AP-conditions. They found two complexes with MW of 360 and 580 kDa corresponding presumably to one or two C3b molecules bound to one molecule of IgG. Fries et al. (1984) showed that tryptic-cleaved C3b generated hydroxylamine-sensitive complexes containing C3b and IgG, provided that a 250-fold molar excess of IgG was added to C3 and trypsin. The generated complexes were termed “C3b−IgG”, though their MW corresponded to a C3b−IgG complex (for a detailed technical discussion see Jelezarova and Lutz, 1999). More importantly, these complexes served as a very potent C3 convertase precursor, when added to EGTA serum and therefore could maintain complement amplification. This suggested that complement amplification can occur not only on activating surfaces, but also on C3b−IgG complexes in the fluid phase.

C3b-IgG complexes had yet additional, functionally important advantages as compared to the corresponding IgG antibody. C3b−IgG complexes artificially generated from an induced bactericidal rabbit antibody to the O antigen of Escherichia coli were three- to four-fold more effective in desensitizing bacteria for serum dependent killing via the terminal complement pathway than the IgG antibody alone (Joiner et al., 1985). Interestingly, this effect was neither induced by an augmented binding of IgG nor by a significantly higher number of C5b-9 per cell. Instead, C3b−IgG complexes most likely altered the molecular arrangement of the C5b-9 complexes or had a favorable effect on their insertion.

C3b−IgG complex formation is a general phenomenon in serum and plasma in which complement is activated by an immune complex or high MW heat-aggregated IgG (Jacobs and Reichlin, 1983). Jacobs and Reichlin used differently labeled IC and IgG and found that low molecular weight C3b-containing complexes (e.g. C3b−IgG) were generated in large amounts from serum IgG rather than from aggregated IgG. The fact that nascent C3b did not deposit exclusively onto the immune complex, but over hours to fluid phase IgG was an important finding, because it illustrated how local complement activation could result in systemic complement amplification. C3b-containing complexes were also detected in normal human serum and their amount increased with the IC concentration and time. The exact composition of the “low molecular weight C3b-containing complexes” was not an issue at that time and could not be solved, since the incubation times were long and factor H and I active. Takata et al. (1984) then studied the composition of the C3b-containing complexes that were generated during IC solubilization by the six components of the alternative pathway. The C3b-containing complexes were separated by 2D SDS-PAGE, using a hydroxylamine treatment between the dimensions. The complexes were cleavable by hydroxylamine and in their inactivated form (iC3b-containing) released exclusively the 6-5 kDa fragment from α-C3 and IgG heavy chain. The complexes were termed “C3b−IgG” complexes, despite the apparent MW of the reduced iC3b-containing form was admittedly too high as to contain only one 6-5 kDa fragment and one heavy chain (see p. 2536 in Takata et al., 1984). Thus, in the early 1980s, the C3b-containing complexes were considered to represent C3b−IgG complexes. Their unique potency in activating AP was attributed to the reduced binding of factor H as compared to that to C3b-ceruloplasmin complexes (Fries et al., 1984). Whether the partial protection from inactivation was the only reason for the potency of these complexes was not investigated, since AP activation by C3b−IgG and C3b-ceruloplasmin was not compared.

3.2. The covalent attachment site of C3b on IgG

Ten years later the docking site of nascent C3b within the IgG molecule was studied by several groups. C3b binds to the CH1 domain of the heavy chain within a stretch of about 20 amino acids, with six OH group-containing residues from Val114 to Lys120 (Shohet et al., 1993). While Sahu and Pangburn (1994) found Thr144 as the predominant site on peptides mimicking portions of the given stretch of amino acids, Vidarte et al. (2001) identified Ser152 as the major site on CH1 on intact IgG1, by studying mutants. This serine residue is exposed in the native molecule and located close to the hinge region. Furthermore, the serine residues on the
two heavy chains are so close to each other that only one C3b can bind covalently to one IgG molecule (see structure of IgG1 in Fig. 7 in Vidarte et al., 2001). In any case, several putative attachment sites exist in a short stretch of the CH1 domain.

3.3. Preferential generation of C3b-IgG by a naturally occurring antibody

We repeated the generation of C3b-containing complexes from whole human IgG and C3b by trypsin (Fries et al., 1984), in order to have a model complex at hand (Lutz et al., 1993b). Immunoblotting data suggested that two distinct complexes were formed, the major one having two C3b molecules bound to IgG heavy chain, as suggested by its MW (see Fig. 1 in Lutz et al., 1993b). The minor complex was assumed to represent a C3b-IgG complex. As the evidence was indirect we continued to use the term “C3b–IgG” for the major complex in the title of the paper. Based on these premises we studied whether a naturally occurring antibody (anti-band 3 NAbs) overcame its low affinity by forming C3b2-IgG complexes (Lutz et al., 1993b). Anti-band 3 NAbs preferentially formed C3b2-IgG complexes as compared to whole IgG and anti-spectrin NAbs, irrespective of whether the complexes were generated by trypsin or by serum C3 convertase. In vivo aged RBC also carried C3b2-IgG complexes, but in inactivated form. The IgG molecules released from these complexes by hydroxylamine bound to RBC band 3 and to C3 (Lutz et al., 1993b), validating the suggestion that anti-band 3 NAbs had an affinity for C3 (Lutz et al., 1989). Their affinity for C3 was low (2–3 × 10^5 L/mol) and involved a site independent of the antigen binding domain (Lutz et al., 1993a). Thus, the preferred formation of C3b2-IgG complexes from C3 and anti-band 3 NAbs may be related to this affinity. Consequently, it is possible that the known affinity of whole human IgG for C3 (Kulics et al., 1983) corresponds to the sum of affinities of NAbs for C3 and reflects the existence of an additional, presumably framework-located, binding site for C3 in many NAbs. These NAbs should not be confused with anti-C3 NAbs that recognize C3 via their paratope (Price et al., 1975), since they do not share the extra binding site (Lutz et al., 1993a) and have other roles (Jelezarova and Lutz, 2005).

3.4. Generation of C3b2–IgG complexes and of C3b dimers in human serum

Immune aggregate-induced complement activation in factor I deficient serum generated primarily C3b2–IgG complexes from serum IgG (Lutz et al., 1996). The reduced form of these complexes with an apparent MW of 263 kDa released upon hydroxylamine treatment of C3 and IgG heavy chain. Addition of factor I generated C3b2–IgG complexes that had a MW of 185 kDa in reduced form and released the 65-kDa fragment of α-C3 and IgG heavy chain. The use of factor I deficient serum allowed to unequivocally identify the major complex as one containing two C3b molecules bound to one IgG heavy chain. SDS-PAGE run with C3b2–IgG preparations, generated by C3 convertase in serum or by trypsin, confirmed that half the amount of IgG heavy chains was released from the C3b2–IgG complex upon reduction, while the other half was present as C3b–HC (Jelezarova et al., 2000). This implied that each complex contained two C3b molecules on one heavy chain, while the other heavy chain was not modified at all. This finding was in accordance with the position of the most likely docking point for C3b in the CH1 domain (Vidarte et al., 2001).

The second, less abundant complex in the C3b2–IgG preparations, with an apparent MW of 160–180 kDa in reduced form, appeared to represent a C3b–IgG complex (Lutz et al., 1996), as originally suggested by Gadd and Reid (1981). This assignment was, however, not fully established, because the intensity of the heavy chain spot underneath the inactivated and reduced form of the C3b-containing complex did not increase in parallel to the specific activity of the added IgG (Fig. 8 in Lutz et al., 1996). Jelezarova et al. provided a definite answer as to the structure of these complexes by studying the composition of complexes that had been generated with either component in labeled form compared to that of purified complexes that were labeled as a whole. The autoradiograms of these 2D SDS-PAGE gels with a hydroxylamine treatment between the dimensions clearly identified the two complexes as C3b2–IgG and C3b–C3b dimers, since only the C3b2–IgG complexes released labeled IgG heavy chain (Jelezarova et al., 2003). C3b2–IgG and C3b dimers were reproducibly generated under a variety of conditions, including factor I-deficient and normal serum, purified AP proteins, or C3/trypsin and IgG and thus were not merely the result of a particular experimental condition.

3.5. Arrangement of the two C3b molecules within C3b2–IgG complexes

With the notion that the major complex is comprised of two C3b molecules bound to one IgG heavy chain, the arrangement of the components within the complex was not yet established. Jelezarova et al. (2003) then distinguished between four possible arrangements of two C3b molecules on one heavy chain by applying factors H and I or CR1 and I to generate partially or fully inactivated complexes. The actual composition of the complexes was analyzed by studying immunoblots or stained 2D SDS-PAGE with a hydroxylamine treatment between the dimensions. The main findings were: (1) C3b2–IgG complexes retain two C3 fragments at all levels of inactivation, with the reduced form of the fully inactivated complex releasing exclusively C3dg and heavy chain. (2) The unreduced C3dg2–HC complex appeared as a doublet on SDS-PAGE only at low gel porosity, suggesting the presence of two conformers of the same composition rather than a mixture of two chemically different entities, representing C3dg bound individually or sequentially to the
heavy chain. Thus, C3b-IgG complexes are composed of a C3b dimer ester-bonded to CH1 of one heavy chain, with the two C3b molecules most likely connected by a C3d-C3d ester bond.

4. Open questions as to how C3b2–IgG complexes are generated

As outlined above, C3b2–IgG complex formation is strictly coupled to generation of C3b dimers. Indeed, spontaneous formation of C3b dimers appears to be a rather common event upon C3 activation as was established already by Amassou et al. (1981) who characterized trypsin-generated C3b dimers. The C3b dimer bound to IgG heavy chain differs from a C4b–C3b dimer that is generated from classical pathway C3 convertase (Kozono et al., 1990; Meri and Pangburn, 1990; Takata et al., 1987). Takata et al. convincingly demonstrated covalent association of C3b with C4b within the classical pathway C5 convertase for the first time (Takata et al., 1987). The formation of the C4b–C3b dimer was quite an efficient reaction, since half of the bound C3b was associated with C4b. C4b-C3b dimer formation proceeded at a rate similar to or even faster than C3b monomer deposition (Takata et al., 1987). There was evidence for a faint band at the 600-kDa position, probably representing a trimer consisting of one molecule C4b and two molecules of C3b. Formation of C4b-C3b complexes was only seen when a large amount of C3b was bound and the trimers comprised a minute fraction of the total. Thus, C4b-C3b complex formation appears to happen via the acquisition of one C3b molecule by C4b. The C3b binding site on C4b was localized to a 74 amino acid residue fragment of the α chain (Kozono et al., 1990) and was identified as Ser-1217 (Kim et al., 1992). This amino acid residue belongs to one of two C4 regions that are not found in C3, despite the high homology between the two proteins. Evidently, the formation of a C3b dimer proceeds by a different mechanism and uses a separate attachment site.

In fact, nascent C3b that was generated by a C3 convertase assembled from purified AP proteins on monomeric C3b did not deposit to the initiating C3b, but to other sites and did so to a large extent as C3b dimers (Kinoshita et al., 1988). Hence, C3b forms dimers immediately upon activation and dimer deposition might require an acceptor other than C3b or C4b. Cells that carried monomeric C3b and C3b dimers had high affinity binding sites for C5 (Kinoshita et al., 1988). The interpretation was that C3b dimers themselves provided the high affinity binding site for C5 in analogy to C4b-C3b dimers. In view of the fact that the C3b dimer was de novo generated, whereas the C4b-C3b complex originated from addition of one C3b, the two dimeric structures differ considerably. Hence, the high affinity binding sites for C5 may have originated from the simultaneous presence of C3b monomers and C3b dimers in immediate vicinity and thus from three C3b molecules. Later, Hong et al. (1991) proposed that formation of covalent C3b dimers was a general phenomenon associated with the AP activation. These authors incubated an insoluble glucan with human serum to generate C3b dimers and then isolated them by solubilizing the glucan with glucosidases. Larger covalent C3b oligomers of unclear composition were present both on the glucan surface and in the C3b dimer preparation. The oligomers released α’C3 chain and a band of approximately 50 kDa after reduction and hydroxylamine cleavage of the ester bonds. Those oligomers might have been C3b trimers or C3b2–IgG complexes formed by nascent C3b with IgG molecules associated or specifically bound to the glucan. Fractions enriched in oligomers had higher activity in reconstituting C5 convertase activity than fractions containing predominantly C3b dimers. The question whether a C3b dimer or a C3b2–IgG complex alone is sufficient to create a high affinity C5 binding site is still unclear. As discussed by Rawal and Pangburn (2001), with increasing C3b deposition on the surface, covalent C3b complexes are formed, which bind C5 multivalently and thereby convert the low affinity C3/C5 convertase precursor to one with high affinity. Association of dimers or C3b2–IgG with additional C3b molecules on the cell surface may reflect the actual situation.

Formation of C3b2–IgG complexes does not proceed by a stepwise addition of C3b to IgG, because generation of covalent C3b-containing complexes under variety of conditions has never yielded a C3b-IgG or a complex having C3b molecules attached to both IgG heavy chains. A structural constraint must exist that prevents nascent C3b from binding covalently to both potential binding sites on IgG. On the contrary, de novo C3b dimer formation may preferentially occur in contact with IgG. Suggestive evidence comes from the ability of certain NAbs to generate C3b2–IgG complexes preferentially. The low affinity C3 binding region is presumably close to the covalent docking site on each CH1 domain. In the IgG structure, the two CH1 regions face opposite sides of the IgG molecule. Thus, one must assume that two C3 molecules can simultaneously occupy, non-covalently, the two binding sites of one IgG molecule. However, the two associated C3 molecules would not be able to interact with each other to form an ester bond. A plausible explanation is that a preformed C3b dimer attaches covalently to one of the putative C3b binding sites. A recent solution study revealed that both recombinant and serum-derived C3d possessed an unexpected tendency to dimerize at high concentrations, e.g. at sites of deposition (Gilbert et al., 2005). The physiological significance of the phenomenon remains unclear, but it may reflect a potential that becomes effective in the process of C3 activation. Alternatively, C3 may occupy one binding region as a non-covalent dimer. Upon its activation the C3 molecule bound to CH1 forms an ester bond with this heavy chain. Once the dimer is bound covalently, a conformational reorganization may prevent the second docking site from accepting another large ligand and the non-covalently bound C3 molecule would then dissociate.
5. The roles of C3b2–IgG complexes

5.1. C3b2–IgG complexes as C3 convertase precursors

C3b2–IgG complexes can form a functional C3 convertase in the absence of properdin under conditions at which C3 convertase generated with monomeric C3b is barely detectable (Jelezarova et al., 2000). Thus, C3b2–IgG complexes are intrinsically much more potent C3 convertase precursors than free C3b. In addition, the presence of dimeric C3b on C3b2–IgG complexes facilitates binding of oligomeric properdin which in turn augments factor B binding (Jelezarova et al., 2001, 2000). These properties of the complexes enhance C3 convertase assembly by 7–10-fold on a molar basis as compared to immobilized C3b. Indeed, properdin induces an increase of the affinity of factor B for C3b, as is known already from work of DiScipio (1981). In fact, the phenomenon that an efficient C3 convertase formation requires two C3b molecules to facilitate binding of oligomeric properdin is by no means new. Already Medicus et al. (1976) suggested that attachment of properdin to surface-bound C3b would require two C3b molecules (as the affinity of properdin for monomeric C3b was barely measurable) and, consequently, expression of C3 convertase activity would require at least three C3b molecules. For the sake of simplicity, the reference to the C3b multiplicity required for properdin binding to C3b within the assembled C3 convertase was omitted ever since.

5.2. Immune complex solubilization

C3b2–IgG complexes do not only serve as the most efficient C3 convertase precursor, but have additional physiological functions as well. Solubilization of immune complexes by AP-dependent complement activation may be more efficient in case nascent C3b binds to the IC-forming antibody in dimeric rather than monomeric form. While some NAbs have an inherent, paratope-independent, low affinity for C3 (Lutz et al., 1993a), other IgG molecules may acquire such a binding property once they have formed a structured IC in which the high flexibility within the IgG molecule (Saphire et al., 2002) which varies with the subclass (Roux et al., 1997) is restricted. This would result in C3b2–IgG complex formation on IgG molecules forming the IC as indeed observed during C3 activation (Gadd and Reid, 1981; Takata et al., 1984). ICs generated from F(ab’)2 IgG antibodies, in which the accessibility for C3b is further increased by the lack of the Fc portion, are known to form C3b2–F(ab’)2 complexes (Takata et al., 1984) that are very potent stimulators of AP (Akagaki and Inai, 1983; Fujita et al., 1977; Reid, 1971). Immune complexes that have been opsonized by C3b are captured by CR1 on RBC in the periphery (Schifferli and Taylor, 1989). The affinity of one C3b molecule for CR1 is weak (10^{-6} M^{-1}) (Pascual and Schifferli, 1992). However, the binding of C3b-containing IC to RBC occurs multivalently and thus more avidly (Schifferli et al., 1988), since CR1 has two to five C3b binding sites and is clustered on RBC (Pascual et al., 1988).

Thus, C3b2–IgG complexes fulfill the requirements for an efficient binding to CR1 on RBC.

5.3. Stimulation of phagocytosis

Efficient phagocytosis of opsonized target cells is highly dependent on recognition of target cell-bound IgG and of covalently bound complement components generated from C3 (Ehleifer and Nussenzweig, 1977). Bound C3b alone, generated on the surface of sheep RBC (SRBC) by adding trypsin, allowed opsonized SRBC to form rosettes with neutrophiles, but ingestion of opsonized SRBC was dependent on IgG and bound C3b (Newman and Johnston, 1979). In vitro phagocytosis of SRBC by human PMN or macrophages was at least 10-fold more effective, when SRBC were opsonized with C3b2–IgG (called C3b–IgG) than when SRBC were opsonized with the IgG Ab alone (Fries et al., 1987). C3b2–IgG complexes were yet two times more effective than the same number of randomly deposited C3b and IgG molecules. Most importantly, phagocytosis of C3b2–IgG-carrying target cells was inhibited to only 40–50% by physiological concentrations of the same IgG subclass, while phagocytosis of cells carrying only IgG1 was fully inhibited. The reason is that physiological IgG concentrations strongly inhibit phagocytosis mediated exclusively by Fc-receptors (Fries et al., 1987; Malbran et al., 1987).

Thus, IgG antibodies that effectively form C3b2–IgG complexes and thus recruit complement receptors are considerably more effective than IgG antibody alone in inducing phagocytosis. C3b2–IgG complexes that have already been inactivated by factors H and I to iC3b2–IgG retain the ability to bind bivalently and may co-cap FcRs and CR3 on phagocytes (Krauss et al., 1994; Petty and Todd, 1993). For example, iC3b-carrying IC were phagocytosed nine times more effectively than IC alone (Olkuro et al., 1995).

5.4. Phagocytosis and bactericidal/fungicidal effects of NAbs and mAbs stimulating AP

As mentioned earlier, sera from vertebrates contain NAbs that bind to pathogens and stimulate AP-dependent C3b deposition. Work along these lines continued with emphasis on NAbs to various yeast species (Boes et al., 1998; Kozel et al., 1991) and the idea to generate vaccines (Han and Cutler, 1995). Some of the generated mAbs were protective (Han and Cutler, 1995), while others had the opposite effect and their bivalent binding to encapsulated cells of Cryptococcus neoformans prevented the NAbs in serum from binding and from activating AP-dependent C3b deposition to the cells (Kozel et al., 1998). The mechanism by which the protective NAbs activated AP on yeast cells has not been further investigated. We think that NAbs and corresponding mAbs against bacteria and fungi which conveyed protection against infection, are the molecules that stimulate AP by forming C3b2–IgG complexes. With this assumption in
due to IgM NABs that recognize a lipooligosaccharide epitope and additional IgG NABs that stimulate AP (Griffiss et al., 1991). In order to deplete AP-stimulating NABs, normal human sera were absorbed three times for 2 h at 0 °C on acetone-powdered Neisseria strains. The authors could deplete most of the specific NABs, but found that absorption removed properdin as well, and with a high correlation: the higher the binding to Neisseria variants, the larger was the depletion of properdin and the loss of AP-activity in the absorbed sera, which could be restored by adding properdin. The authors explained the phenomenon by the presence of a 39 kDa protein found in one species which appeared to interact with properdin. An alternative explanation is that the prolonged absorption of NABs by solid phase antigens generated antigen-bound C3b–IgG NABs complexes that attracted properdin. This explanation implies that C3b–IgG complex formation occurred even at low temperatures. This possibility has not been investigated thoroughly, but is not unexpected, because preformed IgG anti-albumin complexes fixed complement, when incubated with serum at 4 °C overnight under AP-conditions (Reid, 1971) and even normal EDTA plasma contained C3b-containing complexes upon storage for 6 months at −29 °C, but not at −70 °C (Jacobs and Reichlin, 1983).

5.5. Ischemia/reperfusion damage

Ischemia results in local tissue damage and partial lysis of cells, whereby autoantigens are either exposed or released in form of protein complexes. In some cases, the complement-dependent ischemia/reperfusion injuries are initiated by mannose binding lectin via the lectin pathway (Jordan et al., 2001), in others by CRP and classical pathway (Grüssel et al., 1999), while in the majority of cases injuries are induced by complement activation on NABs associating with released or exposed self antigens (Chan et al., 2003; Weiser et al., 1996). The involvement of NABs is best illustrated by the fact that the absence of specific NABs is protective (Fleming et al., 2002; Reid et al., 2002; Williams et al., 1999). Classical pathway activation and amplification eventually result in systemic complement activation. Some damage-inducing NABs have been identified in mice, where NABs are almost exclusively of the IgM class that activates classical pathway (Austen et al., 2004; Zhang et al., 2004). Little is known on whether IgG NABs that make up a large portion of NABs in humans can have similarly dangerous effects. As damage is generally observed only after reperfusion, it can be postulated that the local reservoir of specific NABs is too limited to activate complement sufficiently. Thus, upon reperfusion additional NABs form immune complexes that activate complement by the classical pathway either locally or on liberated antigens in the fluid phase. Irrespective of the class of NABs that initiates the complement cascade, complement amplification on immune complexes may again result in the formation of C3b–IgG complexes from fluid phase IgG.

Be motile, a number of papers on this issue and analyzed whether the assumption could help to understand the data. Han et al. (2001) analyzed protective and non-protective mAbs against C. albicans, which evidently differed in their binding sites within the phosphomannoprotein (Han et al., 2000; Zhang et al., 1998). Han et al. (2001) found that the protective mAbs, whether of the IgG or the IgM class, were ineffective in C3-knockouts and required either a small amount of mouse serum or mouse C3 that could yet be activated, since inactivated C3 did not protect. These data are in accordance with the assumption that the IgG mAb (C3.1) preferentially formed C3b2–IgG complexes on the surface of C. albicans, similarly to known mannanspecific IgG NABs that stimulate AP (Zhang and Kozel, 1998). The similarly protective IgM mAb (B6.1) was, however, a poor stimulator of AP at 50 μg/ml (Zhang et al., 1998). Hence, it is doubtful whether the IgM mAb formed C3b2–IgM complexes. It may, however, have activated classical pathway C3b deposition to the yeast, which upon amplification, has recruited serum IgG as described earlier (Jacobs and Reichlin, 1983).

Interestingly, Han et al. (2001) observed yet another phenomenon that can best be explained on the basis that AP-activating C3b2–IgG complexes may have been involved: when animals were challenged with 1000 times the conventional dose of C. albicans, the “protective” mAbs of both classes given at a high dose induced a more rapid killing of the mice (<30 min) than the non-protective mAbs (2–3 h). Again the effect was C3 dependent. The protective mAbs at high dose may have exerted an overall effect by competing with C3b2–IgG complexes that were already associated with C. albicans. This is likely because, when applied in vitro to C. albicans, these mAbs increased the overall C3b deposition to the cells up to 100 μg/ml, but induced a marked decline of bound C3b molecules at higher concentrations. If the material released from cells had been free C3b, it would have been inactivated within seconds. However, if comparatively long-lived C3b2–IgG complexes containing IgG mAb and preexisting IgG NABs had been liberated, they would have induced a deadly systemic complement amplification. The authors did not consider this possibility, but wrote: “the protective Ab may cause death through rapid classical pathway complement activation and release of pharmacologically active products.” It was, however, impossible that C. albicans released pharmacologically active products, because the same results were found with formalin-fixed C. albicans. Hence, induced antibodies that recognized structures on C. albicans initiated the deadly process that was fully dependent on endogenous substances, above all C3 and IgG NABs capable of forming AP-stimulating C3b2–IgG complexes.

Neisseria species causing gonorrhoeae and meningitis have gained a myriad of means to evade complement-mediated killing, e.g. by attracting factor H (Kam et al., 1999) and vertebrates have developed a multitude of NABs against various species of Neisseria. Lysis appears to be mind we reread a number of papers on this issue and analyzed whether the assumption could help to understand the data. Han et al. (2001) analyzed protective and non-protective mAbs against C. albicans, which evidently differed in their binding sites within the phosphomannoprotein (Han et al., 2000; Zhang et al., 1998). Han et al. (2001) found that the protective mAbs, whether of the IgG or the IgM class, were ineffective in C3-knockouts and required either a small amount of mouse serum or mouse C3 that could yet be activated, since inactivated C3 did not protect. These data are in accordance with the assumption that the IgG mAb (C3.1) preferentially formed C3b2–IgG complexes on the surface of C. albicans, similarly to known mannanspecific IgG NABs that stimulate AP (Zhang and Kozel, 1998). The similarly protective IgM mAb (B6.1) was, however, a poor stimulator of AP at 50 μg/ml (Zhang et al., 1998). Hence, it is doubtful whether the IgM mAb formed C3b2–IgM complexes. It may, however, have activated classical pathway C3b deposition to the yeast, which upon amplification, has recruited serum IgG as described earlier (Jacobs and Reichlin, 1983).

Interestingly, Han et al. (2001) observed yet another phenomenon that can best be explained on the basis that AP-activating C3b2–IgG complexes may have been involved: when animals were challenged with 1000 times the conventional dose of C. albicans, the “protective” mAbs of both classes given at a high dose induced a more rapid killing of the mice (<30 min) than the non-protective mAbs (2–3 h). Again the effect was C3 dependent. The protective mAbs at high dose may have exerted an overall effect by competing with C3b2–IgG complexes that were already associated with C. albicans. This is likely because, when applied in vitro to C. albicans, these mAbs increased the overall C3b deposition to the cells up to 100 μg/ml, but induced a marked decline of bound C3b molecules at higher concentrations. If the material released from cells had been free C3b, it would have been inactivated within seconds. However, if comparatively long-lived C3b2–IgG complexes containing IgG mAb and preexisting IgG NABs had been liberated, they would have induced a deadly systemic complement amplification. The authors did not consider this possibility, but wrote: “the protective Ab may cause death through rapid classical pathway complement activation and release of pharmacologically active products.” It was, however, impossible that C. albicans released pharmacologically active products, because the same results were found with formalin-fixed C. albicans. Hence, induced antibodies that recognized structures on C. albicans initiated the deadly process that was fully dependent on endogenous substances, above all C3 and IgG NABs capable of forming AP-stimulating C3b2–IgG complexes.

Neisseria species causing gonorrhoeae and meningitis have gained a myriad of means to evade complement-mediated killing, e.g. by attracting factor H (Kam et al., 1999) and vertebrates have developed a multitude of NABs against various species of Neisseria. Lysis appears to be
5.6. Clearance of senescent and apoptotic cells

The clearance of effete cells, like senescent and oxidatively damaged RBC (for a recent review, see Lutz, 2004) or apoptotic cells requires phagocytes, the complement system and complement initiators like CRP (Gershov et al., 2000; Mold et al., 1999), lectins or NAbs (Hart et al., 2004; Lutz, 2004). While apoptotic blebs may bind C1q directly (Korb and Ahearn, 1997), NAbs are required in clearance of a variety of apoptotic cells (Kim et al., 2002; Mevorach et al., 1998; Price et al., 1996), but so far little is known whether these NAbs generate C3b–IgG complexes and thereby stimulate AP: C3b deposition to apoptotic HUVEC cells occurs under AP-conditions and is followed by generation of high MW C3b-containing complexes that are cleavable by hydroxylamine (Tsuiji et al., 1994). Interestingly, two-thirds of the effect was observed by adding purified AP proteins and only the remainder was clearly NAb-dependent.

5.7. C3b2–IgG complexes and their inactivated forms in the adaptive immune response

Dempsey et al. (1996) found that recombinitely generated complexes comprised of 2 and more C3d molecules linked to hen egg lysozyme acted like an adjuvant and induced a B cell response that was 10,000-fold more effective than the antigen alone and 100 times more effective than antigen with a concomitant binding of the complexes to CR2 and CD19 (Boackle et al., 2004; Tedder et al., 2005). The reason may be that self-specifying sites are NAb-dependent and may prevent antigen presentation to self-reactive B cell antigen receptors (Lutz, 1999). The upregulation of CD19 may, however, represent an epiphenomenon, because the absence of fluid phase IgM NAbs, which comprise the majority of NAbs in mice, also results in enhanced formation of autoantibodies to double stranded DNA and autoimmunity (Boes et al., 2000). Hence, these findings support the hypothesis that fluid phase NAbs to self may prevent antigen presentation to self-reactive B cell antigen receptors (Lutz, 1999).

6. Concluding remarks

It was the potency of C3b-containing complexes that had attracted the many scientists, who have contributed to a better understanding of the structure and functions of C3b2–IgG complexes. The predominant interest in their features prevented all of us to spend additional time years ago in trying to fully understand their makeup and assembly. While the arrangement of the components appears settled by now, the exact mode of generation of these complexes remains to be investigated. C3b2–IgG complexes, representing an interface between the complement system and antibodies, have crucial roles in the innate and adaptive immune system. Their ability to activate AP not only on cell surfaces, but also in the fluid phase will be an area of intense studies in the near future. The possibility that C3b2–IgG complex formation can be arrested in complement-dependent autoimmune diseases by a therapy with high dose whole human IgG (Lutz et al., 2004), will eventually result in the development of new and alternate means to fight inflammatory processes.

Acknowledgements

Discussed work that originated from the authors’ laboratory was supported by the Swiss National Science Foundation (2-77104-01 and 3100-064916) and grants from the ETH; the Jubiläumsstiftung of Swiss-Life, Zurich; Novartis Stiftung, Basle; KIDNEEDS dedicated to research in MPGN type II, Iowa City.

References


Baskin, R.J., Hefers, V.M., 2004. CRUCR2 deficiency alters IgG3 autoantibody...
body production and IgA fomellar deposition in the MRL/lpr model of SLE. Autoimmunity 37, 111–123.


Han, Y., Rieselman, M.H., Callier, J.E., 2002. Protection against candidiasis by an immunoglobulin G3 (IgG3) monoclonal antibody specific for the same mannose epitope as an IgM protective antibody. Infect. Immun. 68, 1649–1654.


Kozel, T.R., MacGill, R.S., Wall, K.K., 1998. Bivalency is required for anticapsular monoclonal antibodies to optimally suppress activation of
the alternative complement pathway by the Cryptococcus neoformans capsule. Infect. Immun. 66, 1547–1553.

Kozel, T.R., Wilson, M.A., Murphy, J.W., 1991. Early events in initia-
tion of alternative complement pathway activation by the capsule of

Koren, H., Kinoshita, T., Kim, Y.U., Takada-Korenzo, Y., Tsumita, A.,
Okada, Y., Noda, T., Tsuneto, T., Okada, H., 1983. Activation of
the alternative complement pathway by natural antibody to glycolipids in
guinea-pig serum. Immunology 50, 115–121.

Krauss, J.C., Poo, H.Y., Xue, W., Mayordomo, T., Todd, R.F., Petty, H.R.,
1994. Reconstitution of antibody-dependent phagocytosis in fibro-
blasts expressing Fc gamma receptor IIIb and the complement receptor


Latz, L.U., 1999. How pre-existing, germline-derived antibodies and com-
plement may help induce a primary immune response to nonself.

Latz, L.U., 2004. Innate immune and non-immune mediators of erythro-

Lutz, H.U., 1999. How pre-existing, germline-derived antibodies and com-
plement may help induce a primary immune response to nonself.

Lutz, H.U., 1983. IgG bearing covalently bound C3b with polymorphonuclear leuco-

Mold, C., Gevertz, H., Dell, T.W., 1991. Regulation of complement
activation by C-reactive protein. Immunopharmacology 24, 101–106.

Mold, C., Gevertz, H., Dell, T.W., 1991. Regulation of complement
activation by C-reactive protein. Immunopharmacology 24, 101–106.

Mold, C., Gevertz, H., Dell, T.W., 1991. Regulation of complement
activation by C-reactive protein. Immunopharmacology 24, 101–106.

Mold, C., Gevertz, H., Dell, T.W., 1991. Regulation of complement
activation by C-reactive protein. Immunopharmacology 24, 101–106.

Mold, C., Gevertz, H., Dell, T.W., 1991. Regulation of complement
activation by C-reactive protein. Immunopharmacology 24, 101–106.

Obikoro, M., O隔urumakii, M., Kobayashi, K., Sakai, M., Takahashi, K.,
Nakamura, S., 1995. Effect of C3b binding to immune complexes
upon the phagocytic response of human neutrophils: synergistic func-

Okada, Y., Noda, T., Tsuneto, T., Okada, H., 1983. Activation of
the alternative complement pathway by natural antibody to glycolipids in
guinea-pig serum. Immunology 50, 75–84.

the clustered nature of complement receptors type 1 in the erythrocyte

Pangburn, M.K., 1983. Activation of complement via the alternative path-


