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Review

How antibodies use complement to regulate antibody responses

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ABSTRACT

Antibodies, forming immune complexes with their specific antigen, can cause complete suppression or several 100-fold enhancement of the antibody response. Immune complexes containing IgG and IgM may activate complement and in such situations also complement components will be part of the immune complex. Here, we review experimental data on how antibodies via the complement system upregulate specific antibody responses. Current data suggest that murine IgG1, IgG2a, and IgG2b upregulate antibody responses primarily via Fc-receptors and not via complement. In contrast, IgM and IgG3 act via complement and require the presence of complement receptors 1 and 2 (CR1/2) expressed on both B cells and follicular dendritic cells. Complement plays a crucial role for antibody responses not only to antigen complexed to antibodies, but also to antigen administered alone. Lack of C1q, but not of Factor B or MBL, severely impairs antibody responses suggesting involvement of the classical pathway. In spite of this, normal antibody responses are found in mice lacking several activators of the classical pathway (complement activating natural IgM, serum amyloid P component (SAP), specific intracellular adhesion molecule-grabbing non-integrin R1 (SIGN-R1) or C-reactive protein. Possible explanations to these observations will be discussed.

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1. Introduction

An important part of our immune system are the antibodies. These large protein molecules are essential in the defense against infectious agents such as bacteria and viruses, but can also be harmful. When reacting against self antigens, antibodies may cause autoimmune diseases and when reacting against non-infectious antigens, such as pollen, allergic disease can be induced. The consequences of recognition of a target structure by an antibody depends on which effector functions the antibody can initiate, and this in turn depends on its isotype or subclass. An antibody's effector functions are primarily mediated via binding to various Fc-receptors or by activation of the complement system. In addition to their role in attacking pathogens, antibodies can regulate the immune response against the antigen they bind to. This is evidenced by the observations that antigens administered to animals together with specific antibodies can induce a dramatically different immune response than when the antigen is administered alone. The phenomenon is known as antibody feedback regulation, and can result in complete suppression or several hundred-fold enhancement of the specific antibody responses (reviewed in Heyman, 2000, 2003, 2013; Hjelm et al., 2006; Nimmerjahn and Ravetch, 2010). Not only passively transferred, but also endogenously produced antibodies affect immune responses. In the experimental situation, specific antibodies are administered in close temporal relationship to the antigen. This leads to enhanced or suppressed responses, usually to the entire antigen, independently of which epitopes the antibodies bind to. Although antibody feedback regulation was discovered already in the end of the 19th century (von Behring and Wernicke, 1892), the underlying mechanisms are incompletely understood. Instead of being presented with a "naked" antigen, the immune system is presented with an immune complex consisting of antigen, antibody and often complement components. Since an immune complex can bind not only to BCRs but also to Fc- and complement receptors, it will have many possibilities to affect immune responses.

Lack of certain factors of the complement system is known to cause severely impaired antibody responses both in animals and humans (Botto et al., 2009; Carroll and Isenman, 2012). Very little is known about how specific antibodies regulate human immune responses, but an interesting example is the so called Rhesus

Abbreviations: BCR, B cell receptor; BSA, bovine serum albumin; CR1/2, complement receptor 1 and 2 (CD35/CD21); CRP, C-reactive protein; FDCs, follicular dendritic cells; KLH, keyhole limpet hemocyanine; MBL, mannose binding lectin; MZ, marginal zone; OVA, ovalbumin; RhD, Rhesus D blood group; SAP, serum amyloid P component; SIGN-R1, specific intracellular adhesion molecule-grabbing non-integrin R1; SRBC, sheep red blood cells; TNP, trinitrophenyl.

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prophylaxis. Pregnant women with the RhD negative blood group can become immunized against fetal RhD positive erythrocytes, acquired via transplacental hemorrhage. This can lead to production of IgG anti-RhD in the mother and passage of these antibodies over the placenta may damage fetal erythrocytes. Administration of small doses of therapeutic IgG anti-RhD during pregnancy or immediately after delivery prevents maternal immunization and has brought the incidence of hemolytic disease of the newborn down dramatically (Clarke et al., 1963; Urbaniak and Greiss, 2000). In contrast, administration of IgM anti-RhD led to an enhanced production of anti-RhD antibodies (Clarke et al., 1963). These findings are paralleled by the ability of IgG to suppress and of IgM to enhance antibody responses to erythrocytes in experimental models, and suggest that the similarities between antibody feedback regulation in humans and animals are considerable.

The focus of the present review will be on current understanding of antibody-mediated feedback regulation in experimental models where complement may be involved. Therefore, only regulation by IgM and IgG is discussed although also IgE has potent immunoregulatory effects (Getahun et al., 2005).

2. Complement and antibody responses to uncomplexed antigens

A role for complement in antibody responses was first recognized when animals depleted of C3 by treatment with cobra venom factor showed a markedly impaired antibody response (Pepys, 1974). Now it is known that hereditary deficiencies or gene targeting leading to lack of C1qA and thereby the entire C1q molecule (Cutler et al., 1998; Rutemark et al., 2011), C2 (Bitter-Suermann et al., 1981), C3 (Fischer et al., 1996; OíNeil et al., 1988), C4 (Fischer et al., 1996; Jackson et al., 1979; Ochs et al., 1983), or CR1/2 (Ahearn et al., 1996; Carlsson et al., 2009; Croix et al., 1996; Fang et al., 1998; Molina et al., 1996; Rutemark et al., 2011, 2012; Thiel et al., 2012), lead to severely impaired antibody responses in many species, including humans (reviewed in (Botto et al., 2009; Carroll, 2004; Carroll and Isenman, 2012). Primary responses, memory responses, thymus dependent and thymus independent responses are affected. The influence of complement is more pronounced against suboptimal antigen doses, but also responses to antigens administered in adjuvants can be impaired.

2.1. Primary importance of the classical pathway for antibody responses

The complement cascade leading to cleavage of C3 can be activated via three pathways: the classical, lectin, and alternative pathways. Antibody responses in mice lacking Factor B of the alternative pathway (Matsumoto et al., 1997; Mehlhop and Diamond, 2006) are normal. Mice lacking mannose-binding lectin (MBL) of the lectin pathway have either normal, moderately higher or moderately lower antibody responses than wildtype mice (Carter et al., 2007; Guttormsen et al., 2009; Lawrence et al., 2009; Ruseva et al., 2009). Noteworthy is that the defect occasionally seen in MBL-deficient animals is not nearly as severe as the defect in C1q-deficient mice (Cutler et al., 1998; Rutemark et al., 2011). Also ficolins can activate the lectin pathway. Mice deficient in ficolin A, ficolin B, both ficolin A and B, or MASP-2 (which completely lack lectin pathway activation but retain activation via the classical and alternative pathways) are more susceptible to *Streptococcus pneumoniae* infection than wildtype mice (Ali et al., 2012; Endo et al., 2012). To our knowledge, no studies of antibody responses have been performed in these animals and therefore firm conclusions on whether the lectin pathway affects antibody responses cannot be drawn. However, the fact that antibody responses are severely impaired in C1q-deficient mice, argues against a major role of either the lectin or the alternative pathway. An overview over the importance of complement factors for antibody responses can be found in Fig. 1.

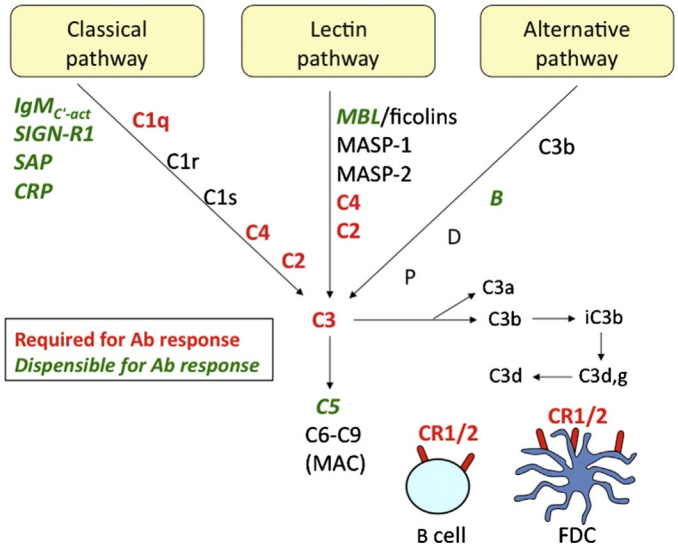


Fig. 1. Involvement of the complement system in antibody responses. Antibody responses in animals lacking the factors and receptors in red (bold) are severely impaired (C1q, C4, C2, C3, CR1/2) whereas antibody responses in animals lacking factors in green (bold italics) are largely normal (complement-activating IgM, SIGN-R1, SAP, CRP, MBL, C5, Factor B). Noteworthy is (i) that disruption of the classical pathway alone leads to impaired antibody responses, although all pathways generate CR1/2 ligands and (ii) that lack of C1q, but not of any of the tested classical pathway activators, leads to impaired antibody responses (adapted from Rutemark et al., 2011).

2.2. Linear relationship between C1q, C2, C4, C3, and CR1/2 in antibody responses?

The phenotype with regard to antibody responses is similar in animals lacking C1q, C2, C4, C3, or CR1/2. Therefore, it is generally assumed that the complement system exerts its regulatory effects via CR1/2, and that the other factors are required merely to generate the C3 split products constituting the ligands for these receptors (iC3b, C3d(g), and C3b for CR1; iC3b and C3d(g) for CR2). Since all three pathways for complement activation are able to generate CR1/2 ligands, it is puzzling that only one is required for antibody responses. One possibility is that there is a quantitative difference in the ability of the activation pathways to cleave C3 and that only the classical pathway generates sufficient amounts of CR1/2-ligands. Another possibility is that C1q plays two parallel roles which are both crucial for antibody responses, one complement-activating and one non-complement activating (Nayak et al., 2012). For the remainder of the discussion we will however assume that the roles of C1q and CR1/2 in antibody responses are linked.

2.3. CR1 versus CR2 in antibody responses

Complement receptors 1 and 2 are primarily expressed on B cells and FDCs. In mice, both receptors are derived from one gene, Cr2, by alternative splicing (Kurtz et al., 1990). Because of this, Cr2 knockout mice lack both receptors and most studies demonstrating the crucial role of CR1/2 on antibody responses have been performed in such animals. The initial observations that CR1/2 were involved in antibody responses were made with blocking monoclonal antibodies (Heyman et al., 1990; Thyphronitis et al., 1991). Treatment of mice with antibodies binding to both CR1 and CR2, but not to CR1

alone, led to severely impaired antibody responses upon immunization (Heyman et al., 1990). Treatment of mice with a soluble CR2 molecule, competing with surface CR2 for ligand, also led to impaired antibody responses (Hebell et al., 1991). This suggested that CR2 played a more important role than CR1. Recently, mice selectively lacking CR1 have been generated by deleting the exons unique to CR1 from the Cr2 locus (Donius et al., 2013). In such animals, the antibody responses to thymus dependent antigens were impaired, although not as dramatically as in CR1/2-deficient mice (Donius et al., 2013). In summary, it appears that both CR1 and CR2 are involved in antibody responses. Their relative importance may depend on antigen type and dose as well as on administration route.

2.4. How can primary antibody responses depend on the classical pathway alone when only low titers of specific antibodies are present to form immune complexes in naïve mice?

Lack of C1q leads to severely impaired primary responses (Cutler et al., 1998; Rutemark et al., 2011). Since the major activators of the classical pathway are specific antibodies forming immune complexes with antigen, this raises the question of how such complexes can be generated in naïve mice where presumably very low titers of specific antibodies are available. One possibility suggested to explain this enigma is that natural IgM, obviously present in naïve mice, binds to antigen with low affinity and activates complement, thereby forming the missing link explaining the requirement for classical complement activation in primary antibody responses (Ehrenstein et al., 1998).

3. Natural IgM and enhancement of antibody responses

Natural IgM is defined as IgM circulating prior to antigen challenge and is mainly produced by a subset of peritoneal B cells, the B-1 B-cells (Baumgarth et al., 2005; Thurnheer et al., 2003). Immune IgM is mainly produced by conventional B-2 B cells, although both B-1 B cells and MZ B cells contribute to the production (Baumgarth et al., 2000; Carey et al., 2008; Choi and Baumgarth, 2008; Holodick et al., 2014; Kretschmer et al., 2003). Natural IgM can bind to a wide range of antigens with low affinity and plays an important role in early defense against bacteria, viruses, and parasites (Ehrenstein and Notley, 2010; Gommerman et al., 2000; Ochsenbein et al., 1999). Virus-like particles were unable to localize to B cell follicles in mice lacking C1q, C3, CR1/2, or secretory IgM and localization was restored by IgM from normal mouse serum (Link et al., 2012). Natural IgM works together with C1q to initiate innate control of *S. pneumoniae* infection (Brown et al., 2002), to neutralize virions (Jayasekera et al., 2007) and to help in clearance of dying cells (Notley et al., 2011; Quartier et al., 2005), suggesting that the effector function of natural IgM in the defense against pathogens relies on complement activation.

3.1. Is natural IgM the missing link explaining the role of the classical pathway in primary antibody responses?

IgM present in naïve mice plays a role in antibody responses. Mice lacking secretory IgM have impaired antibody responses to hapten-conjugated KLH and to influenza virus (Baumgarth et al., 2000; Boes et al., 1998; Ehrenstein et al., 1998). The responses were restored by transfer of serum IgM from naïve mice prior to immunization, thus ascertaining that it was lack of IgM and not other effects on the immune system in these mice that caused the impaired antibody responses (Baumgarth et al., 2000; Ehrenstein et al., 1998). These results, together with the observations that immune IgM in complex with its antigen enhances antibody responses only if it can activate complement (Heyman et al., 1988a; Youd et al., 2002), formed the basis for the hypothesis that natural IgM was the missing link explaining the requirement for classical complement activation in primary antibody responses (Ehrenstein et al., 1998).

3.2. Cμ13 knock-in mice, which lack complement-activating IgM, have normal antibody responses

To test whether natural IgM, in analogy with immune IgM, must activate complement in order to enhance antibody responses, a knock-in mouse strain (Cμ13) was generated. A point mutation was inserted in the gene encoding the third constant domain of the IgM heavy chain, resulting in a serine to proline substitution in position 436 of the μ chain. The same substitution had previously been shown to result in production of IgM which was unable to bind C1 and to induce hemolysis (Shulman et al., 1987; Wright et al., 1988) as well as to enhance specific antibody responses (Heyman et al., 1988a). As expected, IgM produced by Cμ13 mice was also unable to activate complement (Rutemark et al., 2011) but bound as well as wildtype IgM to the FμR (Ding et al., 2013). Immunization of these mice with various doses of SRBC, KLH, or NP-KLH, surprisingly revealed that their primary as well as secondary antibody responses were very similar to those in wildtype controls (Rutemark et al., 2011). In contrast, CR1/2- and C1q-deficient mice had severely reduced responses (Rutemark et al., 2011). These observations suggested that natural IgM was not the classical pathway activator explaining the role of C1q for primary antibody responses. Subsequently, mice lacking three other known classical pathway activators, SIGN-R1, CRP, or SAP were also shown to have normal antibody responses to SRBC (Rutemark et al., 2011). In conclusion, the involvement of natural IgM in antibody responses seems to be independent of its ability to activate complement. Possibly, natural IgM could act via the recently discovered FcμR, also shown to play a role in antibody responses (Choi et al., 2013; Honjo et al., 2012; Ouchida et al., 2012).

The observations that Cμ13 mice have normal antibody responses (Rutemark et al., 2011) leaves open the question of what does indeed activate the classical pathway, allowing its profound role in the generation of normal antibody responses. It cannot be excluded that a hitherto unknown classical pathway activator is involved. Another possibility is that two or more of the known classical pathway activators act redundantly and that single deficiencies will not reveal an effect. Alternatively, the spontaneous low-level activation of C1 may suffice and no additional activation be required (Manderson et al., 2001).

3.3. Summary

Lack of C1q, C2, C4, C3, or CR1/2 leads to severely impaired primary as well as secondary antibody responses to antigens administered alone. In spite of the crucial role of C1q, neither lack of complement-activating natural IgM, CRP, SIGN-R1, nor SAP, all of which are classical pathway activators, leads to the severely impaired antibody responses seen in C1q-deficient mice. What activates C1 and generates C1q in this situation is currently not understood. Through which mechanisms CR1/2, expressed on B cells and FDCs, may influence the antibody responses will be discussed in relation to enhancement of antibody responses by specific IgM (Section 4.2). A possible scenario is depicted in Fig. 2A.

4. Specific IgM and enhancement of antibody responses

One of the cornerstones of the hypothesis that complement activation by natural IgM explains why C1q is of crucial importance for primary antibody response, was the earlier finding that specific IgM is able to enhance antibody responses in a complement

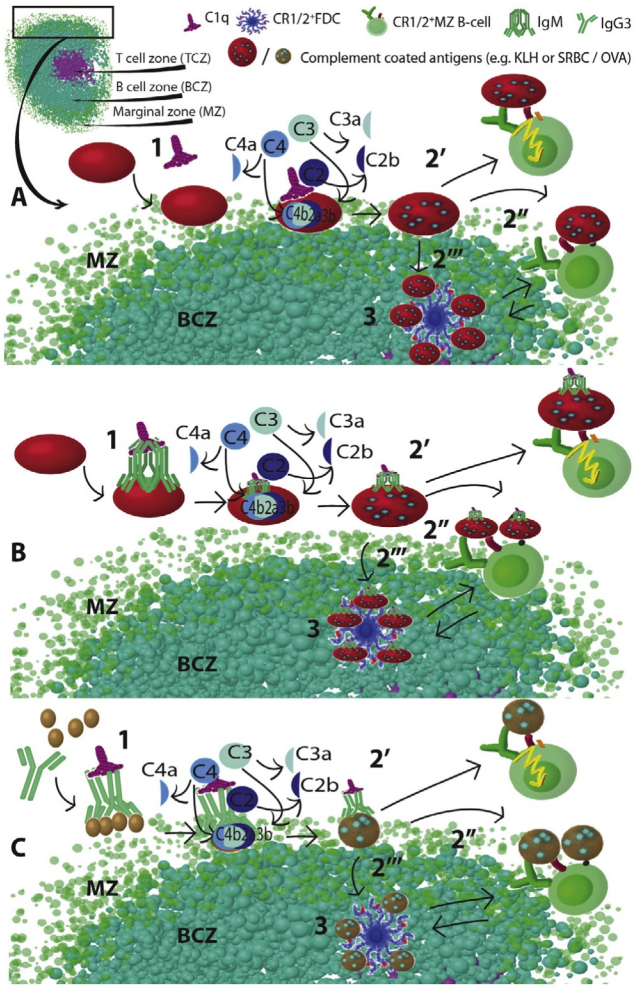


Fig. 2. Scenario for complement involvement in antibody responses to antigen alone, IgM-antigen complexes, or IgG3-antigen complexes. (A) Antigen alone. C1 is activated by an unknown factor, leading to complement deposition on the antigen (1). The antigen-complement complex may then co-crosslink the BCR and the CR2/CD19/TAPA-1 coreceptor complex on B cells, lowering the threshold for B-cell activation (2′). Complexes can also be transported on MZ B cells into the follicle (2″) and delivered to CR1/2$^+$ FDCs (3). To explain the observation that antibody responses are initiated also in the absence of CR1/2 on B cells (Rutemark et al., 2012), it can be hypothesized that MZ B cells bind complexes via FcμRs, that complexes are transported by other cell types, or that they are directly "fished" out from the marginal zone by FDC dendrites (2‴). (B) IgM-antigen complexes. IgM binds to the antigen and C1q is activated, leading to complement deposition (IgM-IC) (1). IgM-IC may then co-crosslink the BCR and the CR2/CD19/TAPA-1 coreceptor complex on B cells, lowering the threshold for B-cell activation (2′). IgM-IC can also be transported on MZ B cells into the follicle (2″) where they are delivered to CR1/2$^+$ FDCs (3). For responses to IgM-IC, CR1/2$^+$ B cells clearly play a role. However, low responses are seen even when B cells lack CR1/2 (Rutemark et al., 2012). Possibly MZ B cells bind IgM-IC via FcμRs, IgM-IC are transported by other cell types, or they are directly "fished" out from the marginal zone by FDC dendrites (2‴). (C) IgG3-antigen complexes. At least two IgG3 molecules bind the antigen and C1q is activated, leading to complement deposition (IgG3-IC) (1). The IgG3-IC may then co-crosslink the BCR and the CR2/CD19/TAPA-1 coreceptor complex on B cells, lowering the threshold for B-cell activation (2′). Complexes can also be transported on CR1/2$^+$ MZ B cells into the follicle (2″) and be delivered to CR1/2$^+$ FDCs (3). For responses to IgG3-IC, CR1/2$^+$ B cells clearly play a role. However, low responses are seen even though B cells lack CR1/2 (Zhang et al., 2014) and it cannot be excluded that IgG3-IC may also be transported into the follicle via FcμR$^+$ MZ B-cells or be directly "fished" out from the marginal zone by FDC dendrites (2‴).

dependent fashion. Already in the 1960s, it was discovered that IgM anti-SRBC, administered together with SRBC, enhanced the specific antibody response (Henry and Jerne, 1968). IgM upregulates primary IgM, IgG, and IgE responses as well as priming for memory responses (Heyman, 2000). In order to have this effect, IgM must be administered before or simultaneously with the antigen.

4.1. Specific IgM must activate complement in order to enhance antibody responses

IgM is an efficient activator of the classical pathway and several observations demonstrate that this ability is required for its enhancing capacity. A monoclonal IgM anti-TNP antibody with the previously mentioned (Section 3.2) point mutation in the Cμ heavy chain resulting in inability to activate complement, lost the enhancing effect of the wildtype IgM (Heyman et al., 1988a). Both SRBC- and KLH-specific IgM, obtained from Cμ13 mice carrying the same mutation, were poor enhancers of antibody and germinal center responses while IgM from wildtype mice worked well (Ding et al., 2013). IgM from Cμ13 mice bound equally well to the FcμR as did wildtype IgM (Ding et al., 2013). This excludes that the loss of an enhancing effect was due to loss of binding to FcμR, a receptor recently shown to play a role for antibody responses (Choi et al., 2013; Honjo et al., 2012; Ouchida et al., 2012). Another line of investigation demonstrated that monomers of IgM, which cannot activate complement (Chen et al., 1997), lost the enhancing capacity of the corresponding pentameric IgM (Youd et al., 2002). The dependence on complement may explain why IgM can only enhance responses to particulate antigens, like erythrocytes (Clarke et al., 1963; Ding et al., 2013; Henry and Jerne, 1968; Heyman et al., 1988a) or malaria parasites (Harte et al., 1983), or to KLH (Ding et al., 2013; Youd et al., 2002) which is a protein with a molecular weight of over 1000 kDa. Presumably, binding to an antigen larger than IgM itself is necessary to allow the conformation change which is required for C1q binding (Czajkowsky and Shao, 2009). Further supporting a role for complement activation are studies in mice immunized with IgM anti-SRBC and SRBC, where a C1q-dependent deposition of C3 on SRBC in the blood could be detected as early as 10 s after immunization (Fig. 3). IgM from Cμ13 mice failed to induce C3 deposition in a similar assay (Ding et al., 2013).

Suggestive of a role for complement are also observations that antibody responses to IgM-antigen complexes are impaired in C3-depleted mice and in mice lacking CR1/2 (Applequist et al., 2000; Heyman et al., 1988a; Rutemark et al., 2012).

4.2. Mechanisms behind enhancement of antibody responses by specific IgM

One of the most well known functions of complement is to lyse cells. However, it seems unlikely that this would explain IgM-mediated enhancement of antibody responses since it works well in mice lacking C5 of the terminal pathway (Heyman et al., 1988a). Moreover, complement activation is required for IgM to enhance of responses to the proteins KLH and NP-KLH which cannot be lysed (Ding et al., 2013; Youd et al., 2002).

Antibody responses to antigens administered alone is similar in animals lacking C1q, C2, C4, C3, or CR1/2. Likewise, antibody responses to antigens administered together with specific IgM are dependent on complement activation and are abrogated in C3-depleted and CR1/2-deficient mice (Applequist et al., 2000; Heyman et al., 1988a; Rutemark et al., 2012). Therefore, it seems reasonable that the effects of specific IgM on antibody responses are also mediated via CR1/2, with IgM serving as an initiator of the classical pathway generating receptor ligands.

4.2.1. Both B cells and FDCs must express CR1/2 for optimal responses to IgM-antigen

In order to understand the mechanism behind IgM-mediated enhancement of antibody responses, it is of interest to find out on which cell type CR1/2 must be expressed to allow IgM to enhance

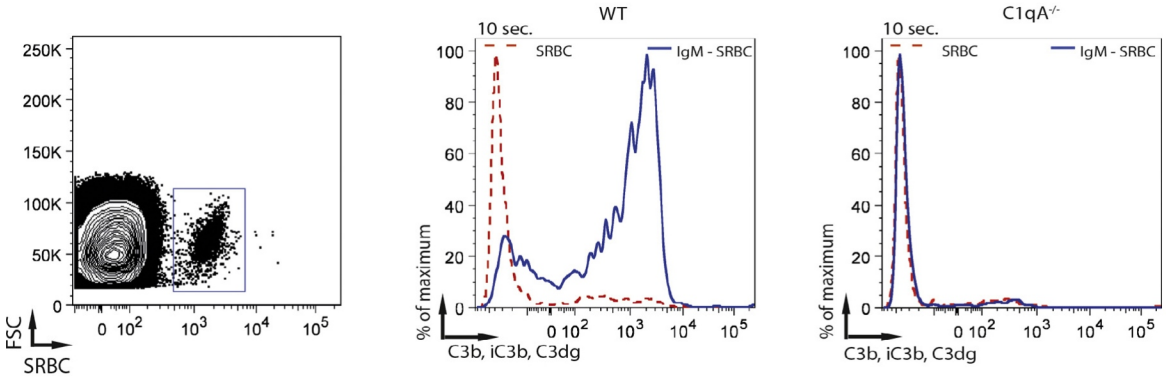


Fig. 3. C3 split products are deposited on IgM-SRBC complexes *in vivo* ten seconds after immunization. Wildtype and C1qA$^{-/-}$ mice were immunized intravenously with SRBC alone or with IgM anti-SRBC followed after 30 min by SRBC. Peripheral blood samples were taken after 10 s and rabbit anti-SRBC used to identify SRBC in the blood (left panel). Deposition of C3 split products on SRBC from mice immunized with SRBC alone (red, dashed line) or together with IgM (blue, solid line) was analyzed in wildtype mice (middle panel) and C1qA$^{-/-}$ mice (right panel). For details, see Ding et al. (2013) (For interpretation of the color information in this figure legend, the reader is referred to the web version of the article.).

antibody responses. One way to differentiate between the involvement of CR1/2-expressing FDCs and B cells is to study responses in bone marrow chimeric mice. FDCs are radioresistant stromal cells developing from vascular mural cells (Krautler et al., 2012) whereas B cells are derived from radiosensitive hematopoietic stem cells. Wildtype or CR1/2-deficient mice can be irradiated and reconstituted with bone marrow from either strain. In this way, chimeras where both cell types, neither cell type, only B cells, or only FDCs express CR1/2 can be obtained. After immunization of such chimeras with IgM and SRBC, no response was seen in the total absence of CR1/2 whereas an optimal response required presence of CR1/2 on both B cells and FDCs (Rutemark et al., 2012). In mice where only FDCs expressed CR1/2, a lower response was seen and when only B cells expressed the receptors an even lower, but still significant response, was detected (Rutemark et al., 2012).

Studies in chimeric mice which instead of being immunized with IgM-antigen were immunized with antigen alone, have given discrepant results. Some report that CR1/2 on FDCs play a predominant role for antibody responses (Brockman et al., 2006; Fang et al., 1998; Mattsson et al., 2011; Rutemark et al., 2012) and others that CR1/2 on B cells are more important (Ahearn et al., 1996; Croix et al., 1996). Mice selectively lacking CR1 have impaired antibody responses, albeit not as low as mice lacking both CR1 and CR2, thus implying that both receptors are important (Donius et al., 2013).

Several mechanisms explaining the crucial role of CR1/2 in antibody responses have been discussed. CR1/2$^+$ FDCs may serve to focus complement-coated antigen in the germinal centers. CR1/2$^+$ B cells may have at least three different functions: transport of antigen into follicles, lowering of the threshold for B cell activation, or endocytosis and presentation of antigen to T helper cells.

4.2.2. The role of FDCs

FDCs play an important role in retaining complement-coated antigens and have been shown to present antigen on their dendrites several months after immunization (Aguzzi et al., 2014; Mandel et al., 1981). An explanation to this amazing capacity was suggested by data showing that complement-coated antigen is recycled in FDCs and while it is present within the cell is located to a non-degradative compartment (Heesters et al., 2013). Antigen administered together with specific IgM localizes to FDCs (Ferguson et al., 2004; Link et al., 2012; Youd et al., 2002) and localization was decreased in the absence of CR1/2, C3, or C1q (Link et al., 2012). Few investigators have followed the antibody response in parallel to antigen localization on FDCs. However, the findings above together with the observation that CR1/2 on FDCs are required for optimal antibody responses to IgM-antigen complexes (Rutemark et al., 2012), strongly suggest that capture of antigen by FDCs is an important mechanism in IgM-mediated enhancement of antibody responses.

4.2.3. The role of MZ B cells

MZ B cells continuously shuttle between the marginal zone and the B cell follicle (Arnon et al., 2013; Cinamon et al., 2008). The exchange rate is surprisingly high with approximately 20% of the cells moving between the two compartments every hour (Arnon et al., 2013). Treatment of mice with an antagonist to the sphingosine 1-phosphate receptors S1P1 and S1P3 (FTY720) results in displacement of MZ B cells from the marginal zone. In such animals, the thymus independent antigen TNP-Ficoll cannot be delivered to FDCs (Cinamon et al., 2008). Studies of how IgM affects this transport showed that pentameric, but not monomeric, specific IgM induced localization of antigen onto FDCs (Youd et al., 2002). In mice lacking C3 and CR1/2, the IgM-antigen complexes were trapped in the marginal zone and did not move further on to FDCs (Ferguson et al., 2004; Youd et al., 2002). Moreover, FDCs in mice with reduced numbers of MZ B cells trap IgM-antigen less efficiently whereas FDCs in mice with reduced numbers of follicular B cells trap IgM-antigen normally (Ferguson et al., 2004). Whether MZ B cells, in addition to facilitating localization of antigen to FDCs also affect the ability of IgM-antigen to induce antibody responses, has not been directly studied. However, a role for MZ B cells is compatible with data showing that CR1/2-expressing B cells are required for optimal antibody responses against IgM-antigen (Rutemark et al., 2012).

4.2.4. The role of facilitated B cell signalling after co-crosslinking of CR2/CD19/TAPA-1 and BCR

In vitro, co-crosslinking of BCR and CR2/CD19/TAPA-1 lowers the threshold for B cell activation (Carter et al., 1988). Whether this pathway plays a role also *in vivo* is difficult to assess since the mere demonstration that CR1/2$^+$ B cells are required can also be explained by MZ B cell-mediated antigen transport into follicles. In bone marrow chimeras immunized with either IgM-SRBC or SRBC alone, a weak antibody response was seen in mice where only B cells expressed CR1/2 (Rutemark et al., 2012). This indicates that extrafollicular antibody production by CR1/2$^+$ B cells, in the absence of CR1/2$^+$ FDCs, benefits from the facilitated signalling resulting from co-crosslinking of BCR and CR2/CD19/TAPA-1. In a study of mixed allotype chimeric mice, B cells from wildtype (Ig allotype b) and CR1/2-deficient (Ig allotype a) mice produced equal amounts of antibodies (Rutemark et al., 2012). This suggests that when FDCs

Table 1
Antibody feedback regulation by specific IgM and IgG.

Regulating isotype	Effect on Ab/CD4$^+$ T cell responses	Effective against	Complement dependent	Fc-receptor dependent	Suggested mechanism(s)
IgM	Enhancement/none	Particulate Ags (erythrocytes, malaria parasites) large proteins (KLH)	Yes	No	FDC capture; MZB transport; B cell signalling (?)
IgG3	Enhancement/none	Proteins (OVA, BSA)	Yes	No	FDC capture; MZ B cell transport; B cell signalling (?)
IgG1, IgG2a, IgG2b	Enhancement/enhancement	Small and large proteins (OVA, BSA, KLH)	No (?)	Yes	Increased Ag presentation to CD4$^+$ T cells by dendritic cells
IgG1, IgG2a, IgG2b, IgG3	Suppression/none	Particulate Ags (erythrocytes, malaria parasites)	No	No	Epitope masking and/or increased Ag clearance

express CR1/2, co-crosslinking of BCR and the CR2-receptor complex is not further amplifying the antibody response.

4.2.5. *The role of antigen presentation to CD4$^+$ T cells*

Several laboratories have shown that B cells can endocytose complement-containing immune complexes via CR1/2 and present the antigen to T cells *in vitro* (Boackle et al., 1997; Carlsson et al., 2009; Cherukuri et al., 2001; Hess et al., 2000; Prechl et al., 2002; Thornton et al., 1994, 1996; Villiers et al., 1996). Early *in vivo* studies showed that blocking of CR1/2 with anti-CR1/2 antibodies prior to immunization abrogated the antibody response to antigen administered alone whereas priming of T helper cells was normal (Gustavsson et al., 1995). Subsequent studies in CR1/2-deficient mice have confirmed these findings (Carlsson et al., 2009; Da Costa et al., 1999). These studies were all performed after immunization with antigen alone. To investigate whether specific IgM, administered together with antigen, affected T cell responses, the DO11.10 system was used. Transgenic CD4$^+$ T cells, specific for an OVA-peptide presented in MHC-II I-A^d, were transferred to naïve recipients which were subsequently immunized with IgM anti-SRBC and SRBC-OVA. These animals had a higher antibody response to both SRBC and OVA than mice given SRBC-OVA alone (Ding et al., 2013). In spite of this, IgM did not increase the proliferation or activation of OVA-specific T cells over the level seen in controls (Ding et al., 2013).

4.3. Summary

Antigen-specific IgM which cannot activate complement, either because of a point mutation or because of its monomeric form, does not enhance antibody responses (Ding et al., 2013; Heyman et al., 1988a; Youd et al., 2002) and wildtype IgM does not enhance in mice lacking C3 or CR1/2 (Applequist et al., 2000; Heyman et al., 1988a; Rutemark et al., 2012). Thus, the ability of specific IgM to upregulate antibody responses depends on its ability to activate complement. In order to generate an optimal response to IgM-antigen, CR1/2 must be expressed both on FDCs and B cells. This suggests that retention of antigen on FDCs together with transport of antigen from the marginal zone to the follicle by MZ B cells, and possibly increased B cell signalling via co-crosslinking of BCR and CR2/CD19/TAPA-1, explains how IgM upregulates antibody responses (Fig. 2B). There is no evidence for a role of enhanced presentation of IgM-antigen-complement complexes to T cells by CR1/2$^+$ B cells *in vivo*.

5. Specific IgG and regulation of antibody responses

The regulatory effects of IgG antibodies are complex for several reasons. Most IgG subclasses can both activate complement and bind Fc-receptors. There is also evidence that these two systems directly interact (Schmidt and Gessner, 2005), a recent example being that IgG immune complexes and FcγRIIB suppress C5a receptor functions (Karsten et al., 2012). Fc-receptors can be either activating or inhibitory, are co-expressed on many of the cells in the immune system and a given Fc-receptor usually binds more than one IgG subclass (Nimmerjahn and Ravetch, 2006). Moreover, IgG can act both to suppress and to enhance antibody responses. The immunoregulatory effects of the IgG subclasses will be discussed below and are summarized in Table 1.

5.1. Specific IgG3 and enhancement of antibody responses

Mice have four IgG subclasses, IgG1, IgG2a, IgG2b (in some strains IgG2c), and IgG3. Murine IgG3 constitutes a small fraction of the total IgG response to T cell-dependent antigens but is the predominant IgG subclass in responses against T cell-independent type 2 antigens (Perlmutter et al., 1978; Rubinstein and Stein, 1988) and plays an important role in defenses against pneumococcal sepsis (McLay et al., 2002). IgG3 was initially thought to activate only the alternative pathway, but is now known to activate also the classical pathway (da Silveira et al., 2002). IgG3 binds to FcγRI, but not to any of the other FcγRs (Barnes et al., 2002; Gavin et al., 1998).

Relatively little is known about the immunoregulatory functions of IgG3, as compared to the other murine IgG subclasses. IgG3 administered together with proteins such as BSA or OVA enhances the antibody responses to these antigens, often several 100-fold (Diaz de Ståhl et al., 2003; Hjelm et al., 2005). In analogy with IgM (Ding et al., 2013), IgG3 does not enhance CD4$^+$ T cell responses (Hjelm et al., 2005). IgG3-mediated enhancement of antibody responses is impaired in mice lacking CR1/2 and in mice depleted of C3 (Diaz de Ståhl et al., 2003) but is unperturbed in mice lacking FcγRI (Diaz de Ståhl et al., 2003; Hjelm et al., 2005). These observations strongly suggest that IgG3, again in analogy with IgM, uses the complement system for its immunoregulatory effects.

All IgG subclasses can enhance antibody responses against BSA and OVA and bind to FcγRs. With the exception of IgG1, all subclasses can also activate complement. In spite of these similarities, IgG3 enhances antibody responses via complement (Diaz de Ståhl et al., 2003; Hjelm et al., 2005) whereas IgG1, IgG2a, and IgG2b use FcγRs (see Section 5.2). How can this be explained? Unlike the other subclasses, IgG3 acts as a cryoglobulin, precipitating in the cold (Abdelmoula et al., 1989). This is probably explained by the intermolecular cooperativity between the Fc regions of IgG3 molecules, where binding of one molecule to, e.g. bacterial surfaces facilitates binding of other IgG3 molecules via Fc–Fc interactions (Cooper et al., 1991; Greenspan and Cooper, 1992). The current understanding is that two or more IgG molecules must bind to neighboring epitopes on the same antigen in order to bind C1q, and the intermolecular cooperativity between IgG3 molecules probably increases the chances for this to happen. The probability that IgG2a and IgG2b, which are not reported to exhibit intermolecular cooperativity, bind to the same antigen close enough to bind C1q appears

smaller and may explain why they preferentially use Fc-receptors (Getahun et al., 2004; Wernersson et al., 1999). Interestingly, all human IgG subclasses form hexamers at cell surfaces (Diebolder et al., 2014), and should this turn out to be true also for murine IgG, the explanation above is less likely.

5.1.1. Mechanisms of IgG3-mediated enhancement of antibody responses

Studies in bone marrow chimeras have shown that both B cells and FDCs need to express CR1/2 for an optimal response to IgG3-antigen (Zhang et al., 2014). IgG3-antigen binds to MZ B cells and can be found in follicles two hours after immunization (Zhang et al., 2014). These observations, as well as the dependence of complement and the failure to induce T helper cell responses, resembles the observations in IgM-mediated enhancement of antibody responses. Therefore, it is likely that the same mechanisms to upregulate antibody responses are used by IgG3 and IgM, i.e. antigen focusing on FDCs, antigen transport by MZ B cells and possibly enhanced B cell signalling owing to co-crosslinking of BCR and CR2/CD19/TAPA-1 (see Section 4.2) (Fig. 2C).

A noteworthy difference between the ability of IgM and IgG3 to enhance antibody responses is that IgM can only work with large antigens like erythrocytes, malaria parasites, and KLH whereas IgG3 enhances responses against OVA and BSA (KLH has not been studied). A likely explanation is their different modes of complement activation. IgM depends on a large antigen to assume the conformation change necessary for C1q binding (Czajkowsky and Shao, 2009). IgG3 on the other hand, depends on intermolecular cooperativity, where probably binding of one IgG3 molecule to a small antigen suffices to attract other IgG3 molecules. The biological significance of this may be that these isotypes complement each other in amplifying antibody responses, IgM taking care of large and IgG3 of small antigens.

5.1.2. Summary

IgG3 enhances antibody, but not T helper cell responses to proteins such as OVA and BSA. An optimal effect requires expression of CR1/2 on both B cells and FDCs. The mechanism is most likely a combination of efficient transport of complement-coated antigen from the marginal zone to the follicles by MZ B cells followed by increased retention of these immune complexes by FDCs.

5.2. Specific IgG1, IgG2a, and IgG2b and enhancement of antibody responses

Regulation of antibody responses by IgG1, IgG2a, and IgG2b has been studied in much more detail than regulation by IgG3. These isotypes, administered together with proteins such as OVA, BSA, or KLH, enhance primary antibody responses and induction of memory (Heyman, 2000). IgG2a administered with OVA also enhances the proliferation of specific $CD4^+$ T cells *in vivo* (de Jong et al., 2006; Getahun et al., 2004; Hamano et al., 2000).

Monoclonal antibodies made it possible to study the effect of individual IgG subclasses in detail. Experiments with a panel of TNP-specific IgG1, IgG2a, and IgG2b monoclonals administered together with TNP-KLH, demonstrated that IgG2a and IgG2b were able to enhance the carrier-specific response whereas IgG1 was a poor enhancer (Coulie and Van Snick, 1985; Wiersma et al., 1989). Since the IgG1 subclass is also a poor complement activator, these results provided indirect evidence that IgG-mediated enhancement of antibody responses depended on complement. This idea was challenged by observations that wildtype and mutant (non-complement activating) IgG2a antibodies were equally efficient enhancers of antibody responses *in vivo* (Nose and Leanderson, 1989; Wiersma et al., 1990), that IgG2a and IgG2b enhance in C3-depleted mice (Wiersma et al., 1990) and in mice lacking CR1/2 (Applequist et al., 2000). In contrast, enhancement was abrogated in mice deficient in the common Fc-receptor γ chain (FcRγ), which leads to lack of expression of activating Fc-receptors (FcγRI, FcγRIII, and FcγRIV) (Getahun et al., 2004; Wernersson et al., 1999). These observations demonstrate that FcγRs are required for the ability of IgG1, IgG2a, and IgG2b to enhance antibody responses and that complement and complement receptors, present in the FcγR-deficient mice, were not sufficient to allow enhancement. A possible explanation to the findings that monoclonal IgG1 antibodies were generally poor suppressors, may be that IgG1 binds better to inhibitory FcγRs than does IgG2a and IgG2b (Nimmerjahn and Ravetch, 2005). Since inhibitory FcγRs negatively regulate activating FcγRs, this, rather than poor complement activation, may explain the observed lack of enhancement by IgG1 (Coulie and Van Snick, 1985; Wiersma et al., 1989).

5.2.1. Summary

Most experimental evidence point towards a role for FcγRs rather than complement in IgG1-, IgG2a- and IgG2b-mediated enhancement of antibody responses. The likely mechanism is that IgG-antigen is endocytosed by FcγR-expressing dendritic cells and subsequently presented to $CD4^+$ T helper cells (de Jong et al., 2006; Getahun et al., 2004; Hamano et al., 2000).

5.3. Specific IgG1, IgG2a, IgG2b, and IgG3 and suppression of antibody responses

Although all IgG subclasses are able to enhance responses to proteins, they suppress antibody responses to erythrocytes (reviewed in (Heyman, 2000, 2003). The same individual hapten-specific monoclonal IgG antibody can enhance responses against haptenated proteins and suppress responses against haptenated erythrocytes, demonstrating that suppressive and enhancing properties of IgG co-exist in the same molecule (Enriquez-Rincon and Klaus, 1984; Wiersma et al., 1989). The dual effect of IgG may seem paradoxical, but considering the enormous size difference between e. g. OVA and SRBC, it is less surprising. The “length” of an IgG antibody is approximately 10 nm and the diameter of an erythrocyte 7 μm (7000 nm), which means that roughly 700 IgG molecules in a row are required to span the diameter of an erythrocyte. In contrast, IgG is three times heavier than OVA (150 kDa for IgG versus 45 kDa for OVA). In spite of the successful application of IgG-mediated suppression in clinical Rhesus prophylaxis (Urbaniak and Greiss, 2000), it is not clear how IgG mediates its suppressive effects. Both complement- and Fc-receptor-mediated mechanisms have been considered, but available data argue against involvement of either of the two. The background to this conclusion will be briefly described below.

Fc-receptor binding as well as complement activation requires an intact Fc-portion of IgG. However, trying to determine whether $F(ab')_2$ fragments of IgG can induce suppression have resulted in conflicting results, some reporting that the Fc-portion indeed is required and others that it is dispensable (for references, see Heyman, 2003). Using SRBC as model antigen, it has been shown that IgG suppresses well in mice lacking all known Fc-receptors for IgG (Karlsson et al., 2001, 1999).

All murine IgG subclasses are able to suppress antibody responses provided they have sufficient affinity for the antigen (Brüggemann and Rajewsky, 1982). Although most monoclonal IgG1 antibodies are unable to activate complement, exceptions have been described. When the suppressive ability of one IgG1 antibody which could, and one which could not, activate complement was compared, both were equally efficient (Wiersma et al., 1990). No studies using point mutated IgG antibodies have been performed *in vivo*. *In vitro*, an IgG2a anti-TNP antibody with a point mutation abrogating its ability to activate the classical pathway,

suppressed equally well as the wildtype (Heyman et al., 1988b). These two monoclonals were both unable to suppress *in vivo*, probably owing to poor affinity. Thus, direct comparison of suppression by mutant and wildtype IgG antibodies *in vivo* is awaiting a suitable monoclonal IgG antibody.

A different approach to elucidate the role of IgG-mediated complement activation is to test suppression by wildtype IgG in mice lacking various complement components. An inherent problem with such studies is that the complement defect *per se* results in very low antibody responses also to SRBC administered alone. Nevertheless, we have found that IgG suppresses the already low antibody responses to SRBC in $Cr2^{-/-}$, $C3^{-/-}$, and $C1qA^{-/-}$ mice (unpublished data).

5.3.1. Summary

In conclusion, available experimental data suggest that neither complement activation nor Fc-receptor binding is required for the suppressive capacity of IgG antibodies. So far, no mouse strain has been identified in which IgG-mediated suppression is abrogated and the underlying mechanism awaits an explanation. Two not mutually exclusive possibilities are epitope masking and rapid (non-Fc-receptor-, non-complement-dependent) clearance of antigen (Na et al., 2006).

6. Concluding remarks

Although all murine antibody isotypes are able to feedback enhance antibody responses, only specific IgM and IgG3 preferentially utilize the complement system. Presence of CR1/2 is required, and these receptors must be expressed both on FDCs and B cells for optimal responses. Probably, transport of antigen by MZ B cells and subsequent retention of antigen on FDCs are the mechanisms behind IgM- and IgG3-mediated enhancement of the antibody response. The explanation for how complement regulates antibody responses to antigen administered alone, i. e. without specific antibodies, is likely to be the same. Whether co-crosslinking of BCR and CR2/CD19/TAPA-1 plays a significant role *in vivo* is not clear. We do not yet understand why only the classical pathway, i.e. C1q, is required, since ligands for CR1/2 can be generated also via the lectin and alternative pathways. One possibility is that a non-complement activating function of C1q is required in addition to its complement activating function (Nayak et al., 2012). Finally, we do not understand what activates the classical pathway, generating the C1q obviously required for normal antibody responses: IgM, SIGN-R1, SAP, and SAP all seem dispensable.

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References

Abdelmoula, M., Spertini, F., Shibata, T., Gyotoku, Y., Luzuy, S., Lambert, P.H., Izui, S., 1989. IgG3 is the major source of cryoglobulins in mice. J. Immunol. 143, 526–532.

Aguzzi, A., Kranich, J., Krautler, N.J., 2014. Follicular dendritic cells: origin, phenotype, and function in health and disease. Trends Immunol. 35, 105–113.

Ahearn, J.M., Fischer, M.B., Croix, D., Goerg, S., Ma, M., Xia, J., Zhou, X., Howard, R.G., Rothstein, T.L., Carroll, M.C., 1996. Disruption of the Cr2 locus results in a reduction in B-1a cells and in an impaired B cell response to T-dependent antigen. Immunity 4, 251–262.

Ali, Y.M., Lynch, N.J., Haleem, K.S., Fujita, T., Endo, Y., Hansen, S., Holmskov, U., Takahashi, K., Stahl, G.L., Dudler, T., Girija, U.V., Wallis, R., Kadioglu, A., Stover, C.M., Andrew, P.W., Schwaeble, W.J., 2012. The lectin pathway of complement activation is a critical component of the innate immune response to pneumococcal infection. PLos Pathogens, 8.

Applequist, S.E., Dahlström, J., Jiang, N., Molina, H., Heyman, B., 2000. Antibody production in mice deficient for complement receptors 1 and 2 can be induced by IgG/Ag and IgE/Ag, but not IgM/Ag complexes. J. Immunol. 165, 2398–2403.

Arnon, T.I., Horton, R.M., Grigorova, I.L., Cyster, J.G., 2013. Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress. Nature 493, 684–688.

Barnes, N., Gavin, A.L., Tan, P.S., Mottram, P., Koentgen, F., Hogarth, P.M., 2002. FcγRI-deficient mice show multiple alterations to inflammatory and immune responses. Immunity 16, 379–389.

Baumgarth, N., Herman, O.C., Jager, G.C., Brown, L.E., Herzenberg, L.A., Chen, J., 2000. B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J. Exp. Med. 192, 271–280.

Baumgarth, N., Tung, J.W., Herzenberg, L.A., 2005. Inherent specificities in natural antibodies: a key to immune defense against pathogen invasion. Springer Semin. Immunopathol. 26, 347–362.

Bitter-Suermann, D., Hoffmann, T., Burger, R., Hadding, U., 1981. Linkage of total deficiency of the second component of the complement system and of genetic C2-polymorphism to the major histocompatibility complex of the guinea pig. J. Immunol. 127, 608–612.

Boackle, S.A., Holers, V.M., Karp, D.R., 1997. CD21 augments antigen presentation in immune individuals. Eur. J. Immunol. 27, 122–129.

Boes, M., Esau, C., Fischer, M.B., Schmidt, T., Carroll, M., Chen, J., 1998. Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted IgM. J. Immunol. 160, 4776–4787.

Botto, M., Kirschfink, M., Macor, P., Pickering, M.C., Wurzner, R., Tedesco, F., 2009. Complement in human diseases: lessons from complement deficiencies. Mol. Immunol. 46, 2774–2783.

Brockman, M.A., Verschoor, A., Zhu, J., Carroll, M.C., Knipe, D.M., 2006. Optimal long-term humoral responses to replication-defective herpes simplex virus require CD21/CD35 complement receptor expression on stromal cells. J. Virol. 80, 7111–7117.

Brown, J.S., Hussell, T., Gilliland, S.M., Holden, D.W., Paton, J.C., Ehrenstein, M.R., Walport, M.J., Botto, M., 2002. The classical pathway is the dominant complement pathway required for innate immunity to Streptococcus pneumoniae infection in mice. Proc. Natl. Acad. Sci. U.S.A. 99, 16969–16974.

Brüggemann, M., Rajewsky, K., 1982. Regulation of the antibody response against hapten-coupled erythrocytes by monoclonal anti-hapten antibodies of various isotypes. Cell. Immunol. 71, 365–373.

Carey, J.B., Moffatt-Blue, C.S., Watson, L.C., Gavin, A.L., Feeney, A.J., 2008. Repertoire-based selection into the marginal zone compartment during B cell development. J. Exp. Med. 205, 2043–2052.

Carlsson, F., Getahun, A., Rutemark, C., Heyman, B., 2009. Impaired antibody responses but normal proliferation of specific CD4+ T cells in mice lacking complement receptors 1 and 2. Scand. J. Immunol. 70, 77–84.

Carroll, M.C., 2004. The complement system in regulation of adaptive immunity. Nat. Immunol. 5, 981–986.

Carroll, M.C., Isenman, D.E., 2012. Regulation of humoral immunity by complement. Immunity 37, 199–207.

Carter, R.H., Spycher, M.O., Ng, Y.C., Hoffmann, R., Fearon, D.T., 1988. Synergistic interaction between complement receptor type 2 and membrane IgM on B-lymphocytes. J. Immunol. 141, 457–463.

Carter, T., Sumiya, M., Reilly, K., Ahmed, R., Sobieszczuk, P., Summerfield, J.A., Lawrence, R.A., 2007. Mannose-binding lectin A-deficient mice have abrogated antigen-specific IgM responses and increased susceptibility to a nematode infection. J. Immunol. 178, 5116–5123.

Chen, F.H., Arya, S.K., Rinfret, A., Isenman, D.E., Shulman, M.J., Painter, R.H., 1997. Domain-switched mouse IgM/Ig2b hybrids indicate individual roles for C mu 2 C mu 3, and C mu 4 domains in the regulation of the interaction of IgM with complement C1q. J. Immunol. 159, 3354–3363.

Cherukuri, A., Cheng, P.C., Pierce, S.K., 2001. The role of the CD19/CD21 complex in B cell processing and presentation of complement-tagged antigens. J. Immunol. 167, 163–172.

Choi, S.C., Wang, H.S., Tian, L.J., Murakami, Y., Shin, D.M., Borrego, F., Morse, H.C., Coligan, J.E., 2013. Mouse IgM Fc receptor FCMR, promotes B cell development and modulates antigen-driven immune responses. J. Immunol. 190, 987–996.

Choi, Y.S., Baumgarth, N., 2008. Dual role for B-1a cells in immunity to influenza virus infection. J. Exp. Med. 205, 3053–3064.

Cinamon, G., Zachariah, M.A., Lam, O.M., Foss Jr., F.W., Cyster, J.G., 2008. Follicular shuttling of marginal zone B cells facilitates antigen transport. Nat. Immunol. 9, 54–62.

Clarke, C.A., Donohoe, W.T.A., Woodrow, J.C., Finn, R., Krevans, J.R., Kulke, W., Lehane, D., Sheppard, P.M., 1963. Further experimental studies on the prevention of Rh haemolytic disease. Br. Med. J. 1, 979–984.

Cooper, L.J., Schimenti, J.C., Glass, D.D., Greenspan, N.S., 1991. H chain C domains influence the strength of binding of IgG for streptococcal group A carbohydrate. J. Immunol. 146, 2659–2663.

Coulie, P., Van Snick, J., 1985. Enhancement of IgG anti-carrier responses by IgG2-anti-hapten antibodies in mice. Eur. J. Immunol. 15, 793–798.

Croix, D.A., Ahearn, J.M., Rosengard, A.M., Han, S., Kelsoe, G., Ma, M., Carroll, M.C., 1996. Antibody response to a T-dependent antigen requires B cell expression of complement receptors. J. Exp. Med. 183, 1857–1864.

Cutler, A.J., Botto, M., van Essen, D., Rivi, R., Davies, K.A., Gray, D., Walport, M.J., 1998. T cell-dependent immune response in C1q-deficient mice: defective interferon γ production by antigen-specific T cells. J. Exp. Med. 187, 1789–1797.
Czajkowsky, D.M., Shao, Z., 2009. The human IgM pentamer is a mushroom-shaped molecule with a flexural bias. Proc. Natl. Acad. Sci. U.S.A. 106, 14960–14965.
Da Costa, X.J., Brockman, M.A., Alicot, E., Ma, M., Fischer, M.B., Zhou, X., Knipe, D.M., Carroll, M.C., 1999. Humoral response to herpes simplex virus is complement-dependent. Proc. Natl. Acad. Sci. U.S.A. 96, 12708–12712.
da Silveira, S.A., Kikuchi, S., Fossati-Jimack, L., Moll, T., Saito, T., Verbeek, J.S., Botto, M., Walport, M.J., Carroll, M., Izui, S., 2002. Complement activation selectively potentiates the pathogenicity of the IgG2b and IgG3 isotypes of a high affinitiy anti-erythrocyte autoantibody. J. Exp. Med. 195, 665–672.
de Jong, J.M., Schuurhuis, D.H., Ioan-Facsinay, A., Welling, M.M., Camps, M.G., van der Voort, E.I., Huizinga, T.W., Ossendorp, F., Verbeek, J.S., Toes, R.E., 2006. Dendritic cells, but not macrophages or B cells, activate major histocompatibility complex class II-restricted CD4+ T cells upon immune-complex uptake in vivo. Immunology 119, 499–506.
Diaz de Ståhl, T., Dahlström, J., Carroll, M.C., Heyman, B., 2003. A role for complement in feedback-enhancement of antibody responses by IgG3. J. Exp. Med. 197, 1183–1190.
Diebolder, C.A., Beurskens, F.J., de Jong, R.N., Koning, R.I., Strumane, K., Lindorfer, M.A., Voorhorst, M., Ugurlar, D., Rosati, S., Heck, A.J.R., van de Winkel, J.G.J., Wilson, I.A., Koster, A.J., Taylor, R.P., Saphire, E.O., Burton, D.R., Schuurman, J., Gros, P., Parren, P.W.H.I., 2014. Complement is activated by IgG hexamers assembled at the cell surface. Science 343, 1260–1263.
Ding, Z.J., Bergman, A., Rutemark, C., Ouchida, R., Ohno, H., Wang, J.Y., Heyman, B., 2013. Complement-activating IgM enhances the humoral but not the T cell immune response in mice. PLos One, 8.
Donius, L.R., Handy, J.M., Weis, J.J., Weis, J.H., 2013. Optimal germinal center B cell activation and T-dependent antibody responses require expression of the mouse complement receptor Cr1. J. Immunol. 191, 434–447.
Ehrenstein, M.R., Notley, C.A., 2010. The importance of natural IgM: scavenger, protector and regulator. Nature Rev. Immunol. 10, 778–786.
Ehrenstein, M.R., O'Keefe, T.L., Davies, S.L., Neuberger, M.S., 1998. Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. Proc. Natl. Acad. Sci. U.S.A. 95, 10089–10093.
Endo, Y., Takahashi, M., Iwaki, D., Ishida, Y., Nakazawa, N., Kodama, T., Matsuzaka, T., Kanno, K., Liu, Y., Tsuchiya, K., Kawamura, I., Ikawa, M., Waguri, S., Wada, I., Matsushita, M., Schwaeble, W.J., Fujita, T., 2012. Mice deficient in ficolin, a lectin complement pathway recognition molecule, are susceptible to streptococcus pneumoniae infection. J. Immunol. 189, 5860–5866.
Enriquez-Rincon, F., Klaus, G.G.B., 1984. Differing effects of monoclonal anti-hapten antibodies on humoral responses to soluble or particulate antigens. Immunology 52, 129–136.
Fang, Y., Xu, C., Fu, Y.-X., Holers, V.M., Molina, H., 1998. Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response. J. Immunol. 160, 5273–5279.
Ferguson, A.R., Youd, M.E., Corley, R.B., 2004. Marginal zone B cells transport and deposit IgM-containing immune complexes onto follicular dendritic cells. Int. Immunol. 16, 1411–1422.
Fischer, M.B., Ma, M., Goerg, S., Zhou, X., Xia, J., Finco, O., Han, S., Kelsoe, G., Howard, R.G., Rothstein, T.L., Kremmer, E., Rosen, F.S., Carroll, M.C., 1996. Regulation of the B cell response to T-dependent Ags by classical pathway complement. J. Immunol. 157, 549–556.
Gavin, A.L., Barnes, N., Dijstelbloem, H.M., Hogarth, P.M., 1998. Identification of the mouse IgG3 receptor: Implications for antibody effector function at the interface between innate and adaptive immunity. J. Immunol. 160, 20–23.
Getahun, A., Dahlström, J., Wernersson, S., Heyman, B., 2004. IgG2a-mediated enhancement of Ab- and T-cell responses and its relation to inhibitory and activating FcγRs. J. Immunol. 172, 5269–5276.
Getahun, A., Hjelm, F., Heyman, B., 2005. IgE enhances antibody and T cell responses in vivo via CD23$^+$ B Cells. J. Immunol. 175, 1473–1482.
Gommerman, J.L., Oh, D.Y., Zhou, X., Tedder, T.F., Maurer, M., Galli, S.J., Carroll, M.C., 2000. A role for CD21/CD35 and CD19 in responses to acute septic peritonitis: a potential mechanism for mast cell activation. J. Immunol. 165, 6915–6921.
Greenspan, N.S., Cooper, L.J.N., 1992. Intermolecular cooperativity: a clue to why mice have IgG3? Immunol. Today 13, 164–168.
Gustavsson, S., Kinoshita, T., Heyman, B., 1995. Antibodies to murine complement receptor 1 and 2 can inhibit the antibody response in vivo without inhibiting T-helper cell induction. J. Immunol. 154, 6524–6528.
Guttormsen, H.K., Stuart, L.M., Shi, L., Carroll, M.C., Chen, J., Kasper, D.L., Ezekowitz, R.A., Takahashi, K., 2009. Deficiency of mannose-binding lectin greatly increases antibody response in a mouse model of vaccination. Clin. Immunol. 130, 264–271.
Hamano, Y., Arase, H., Saisho, H., Saito, T., 2000. Immune complex and Fc receptor-mediated augmentation of antigen presentation for in vivo Th cell responses. J. Immunol. 164, 6113–6119.
Harte, P.G., Cooke, A., Playfair, J.H.L., 1983. Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. Nature 302, 256–258.
Hebell, T., Ahearn, J.M., Fearon, D.T., 1991. Suppression of the immune response by a soluble complement receptor of B lymphocytes. Science 254, 102–105.
Heesters, B.A., Chatterjee, P., Kim, Y.A., Gonzalez, S.F., Kuligowski, M.P., Kirchhausen, T., Carroll, M.C., 2013. Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell antigen binding and activation. Immunity 38, 1164–1175.
Henry, C., Jerne, N., 1968. Competition of 19S and 7S antigen receptors in the regulation of the primary immune response. J. Exp. Med. 128, 133–152.
Hess, M.W., Schwendinger, M.G., Eskelinen, E.L., Pfaller, K., Pavelka, M., Dierich, M.P., Prodinger, W.M., 2000. Tracing uptake of C3dg-conjugated antigen into B cells via complement receptor type 2 (CR2 CD21). Blood 95, 2617–2623.
Heyman, B., 2000. Regulation of antibody responses via antibodies, complement, and Fc receptors. Annu. Rev. Immunol. 18, 709–737.
Heyman, B., 2003. Feedback regulation by IgG antibodies. Immunol. Lett. 88, 157–161.
Heyman, B., 2013. Antibody mediated regulation of humoral immunity. In: Nimmerjahn, F. (Ed.), Molecular and Cellular Mechanisms of Antibody Activity. Springer Science + Business Media, New York, pp. 221–248.
Heyman, B., Pilström, L., Shulman, M.J., 1988a. Complement activation is required for IgM-mediated enhancement of the antibody response. J. Exp. Med. 167, 1999–2004.
Heyman, B., Wiersma, E., Nose, M., 1988b. Complement activation is not required for IgG-mediated suppression of the antibody response. Eur. J. Immunol. 18, 1739–1743.
Heyman, B., Wiersma, E.J., Kinoshita, T., 1990. In vivo inhibition of the antibody response by a monoclonal complement receptor specific antibody. J. Exp. Med. 172, 665–668.
Hjelm, F., Carlsson, F., Getahun, A., Heyman, B., 2006. Antibody-mediated regulation of the immune response. Scand. J. Immunol. 64, 177–184.
Hjelm, F., Carlsson, F., Verbeek, S., Heyman, B., 2005. IgG3-mediated enhancement of the antibody response is normal in Fc gammaRI-deficient mice. Scand. J. Immunol. 62, 453–461.
Holodick, N.E., Vizconde, T., Rothstein, T.L., 2014. B-1a cell diversity: nontemplated addition in B-1a cell Ig is determined by progenitor population and developmental location. J. Immunol. 192, 2432–2441.
Honjo, K., Kubagawa, Y., Jones, D.M., Dizon, B., Zhu, Z., Ohno, H., Izui, S., Kearney, J.F., Kubagawa, H., 2012. Altered Ig levels and antibody responses in mice deficient for the Fc receptor for IgM (FcmuR). Proc. Natl. Acad. Sci. U.S.A. 109, 15882–15887.
Jackson, C.G., Ochs, H.D., Wedgwood, R.J., 1979. Immune response of a patient with deficiency of the fourth component of complement and systemic lupus erythematosus. N. Engl. J. Med. 300, 1124–1129.
Jayasekera, J.P., Moseman, E.A., Carroll, M.C., 2007. Natural antibody and complement mediate neutralization of influenza virus in the absence of prior immunity. J. Virol. 81, 3487–3494.
Karlsson, M.C.I., Getahun, A., Heyman, B., 2001. FcγRIIB in IgG-mediated suppression of antibody responses: different impact in vivo and in vitro. J. Immunol. 167, 5558–5564.
Karlsson, M.C.I., Wernersson, S., Diaz de Ståhl, T., Gustavsson, S., Heyman, B., 1999. Efficient IgG-mediated suppression of primary antibody responses in Fc-gamma receptor-deficient mice. Proc. Natl. Acad. Sci. U.S.A. 96, 2244–2249.
Karsten, C.M., Pandey, M.K., Figge, J., Kilchenstein, R., Taylor, P.R., Rosas, M., McDonald, J.U., Orr, S.J., Berger, M., Petzold, D., Blanchard, V., Winkler, A., Hess, C., Reid, D.M., Majoul, I.V., Strait, R.T., Harris, N.L., Kohl, G., Wex, E., Ludwig, R., Zillikens, D., Nimmerjahn, F., Finkelman, F.D., Brown, G.D., Ehlers, M., Kohl, J., 2012. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgammaRIIB and dectin-1. Nat. Med. 18, 1401–1406.
Krautler, N.J., Kana, V., Kranich, J., Tian, Y.H., Perera, D., Lemm, D., Schwarz, P., Armulik, A., Browning, J.L., Tallquist, M., Buch, T., Oliveira-Martins, J.B., Zhu, C.H., Hermann, M., Wagner, U., Brink, R., Heikenwalder, M., Aguzzi, A., 2012. Follicular dendritic cells emerge from ubiquitous perivascular precursors. Cell 150, 194–206.
Kretschmer, K., Jungebloud, A., Stopkowicz, J., Kleinke, T., Hoffmann, R., Weiss, S., 2003. The selection of marginal zone B cells differs from that of B-1a cells. J. Immunol. 171, 6495–6501.
Kurtz, C.B., O' Toole, E., Christensen, S.M., Weis, J.H., 1990. The murine complement receptor gene family IV: alternative splicing of Cr2 gene transcripts predicts two distinct gene products that share homologous domains with both human CR2 and CR1. J. Immunol. 144, 3581–3591.
Lawrence, R.A., Carter, T., Bell, L.V., Else, K.J., Summerfield, J., Bickle, Q., 2009. Altered antibody responses in mannose-binding lectin-A deficient mice do not affect Trichuris muris or Schistosoma mansoni infections. Parasite Immunol. 31, 104–109.
Link, A., Zabel, F., Schnetzler, Y., Titz, A., Brombacher, F., Bachmann, M.F., 2012. Innate immunity mediates follicular transport of particulate but not soluble protein antigen. J. Immunol. 188, 3724–3733.
Mandel, T.E., Phipps, R.P., Abbot, A.P., Tew, J.G., 1981. Long-term antigen retention by dendritic cells in the popliteal lymph-node of immunized mice. Immunology 43, 353–362.
Manderson, A.P., Pickering, M.C., Botto, M., Walport, M.J., Parish, C.R., 2001. Continual low-level activation of the classical complement pathway. J. Exp. Med. 194, 747–756.
Matsumoto, M., Fukuda, W., Circolo, A., Goellner, J., Strauss-Schoenberger, J., Wang, X., Fujita, S., Hidvegi, T., Chaplin, D.D., Colten, H.R., 1997. Abrogation of the alternative complement pathway by targeted deletion of murine factor B. Proc. Natl. Acad. Sci. U.S.A. 94, 8720–8725.
Mattsson, J., Yrlid, U., Stensson, A., Schon, K., Karlsson, M.C., Ravetch, J.V., Lycke, N.Y., 2011. Complement activation and complement receptors on follicular dendritic cells are critical for the function of a targeted adjuvant. J. Immunol. 187, 3641–3652.
McLay, J., Leonard, E., Peterson, S., Shapiro, D., Greenspan, N.S., Schreiber, J.R., 2002. γ3 gene-disrupted mice selectively deficient in the dominant IgG subclass made

to bacterial polysaccharides. II. Increased susceptibility to fatal pneumococcal sepsis due to absence of anti-polysaccharide IgG3 is corrected by induction of anti-polysaccharide IgG1. J. Immunol. 168, 3437–3443.
Mehlhop, E., Diamond, M.S., 2006. Protective immune responses against West Nile virus are primed by distinct complement activation pathways. J. Exp. Med. 203, 1371–1381.
Molina, H., Holers, V.M., Li, B., Fang, Y.-F., Mariathasan, S., Goellner, J., Strauss-Schoenberger, J., Karr, R.W., Chaplin, D.D., 1996. Markedly impaired humoral immune responses in mice deficient in complement receptors 1 and 2. Proc. Natl. Acad. Sci. U.S.A. 93, 3357–3361.
Na, D., Kim, D., Lee, D., 2006. Mathematical modeling of humoral immune response suppression by passively administered antibodies in mice. J. Theor. Biol. 241, 830–851.
Nayak, A., Pednekar, L., Reid, K.B., Kishore, U., 2012. Complement and non-complement activating functions of C1q: a prototypical innate immune molecule. Innate Immun. 18, 350–363.
Nimmerjahn, F., Ravetch, J.V., 2005. Divergent immunoglobulin G subclass activity through selective Fc receptor binding. Science 310, 1510–1512.
Nimmerjahn, F., Ravetch, J.V., 2006. Fcgamma receptors: old friends and new family members. Immunity 24, 19–28.
Nimmerjahn, F., Ravetch, J.V., 2010. Antibody-mediated modulation of immune responses. Immunol. Rev. 236, 265–275.
Nose, M., Leanderson, T., 1989. Substitution of asparagine 324 with aspartic acid in the Fc portion of mouse antibodies reduces their capacity for C1q binding. Eur. J. Immunol. 19, 2179–2181.
Notley, C.A., Brown, M.A., Wright, G.P., Ehrenstein, M.R., 2011. Natural IgM is required for suppression of inflammatory arthritis by apoptotic cells. J. Immunol. 186, 4967–4972.
OíNeil, K.M., Ochs, S.R., Heller, S.R., Cork, L.C., Morris, J.M., Winkelstein, J.A., 1988. Role of C3 in humoral immunity. Defective antibody production in C3-deficient dogs. J. Immunol. 140, 1939–1945.
Ochs, H.D., Wedgwood, R.J., Frank, M.M., Heller, S.R., Hosea, S.W., 1983. The role of complement in the induction of antibody responses. Clin. Exp. Immunol. 53, 208–216.
Ochsenbein, A.F., Fehr, T., Lutz, C., Suter, M., Brombacher, F., Hengartner, H., Zinkernagel, R.M., 1999. Control of early viral and bacterial distribution and disease by natural antibodies. Science 286, 2156–2159.
Ouchida, R., Mori, H., Hase, K., Takatsu, H., Kurosaki, T., Tokuhisa, T., Ohno, H., Wang, J.Y., 2012. Critical role of the IgM Fc receptor in IgM homeostasis, B-cell survival, and humoral immune responses. Proc. Natl. Acad. Sci. U.S.A. 109, E2699–E2706.
Pepys, M.B., 1974. Role of complement in induction of antibody production in vivo: effect of cobra factor and other C3-reactive agents on thymus-dependent and thymus-independent antibody responses. J. Exp. Med. 140, 126–145.
Perlmutter, R.M., Hansburg, D., Briles, D.E., Nicolotti, R.A., Davie, J.M., 1978. Subclass restriction of murine anti-carbohydrate antibodies. J. Immunol. 121, 566–572.
Prechl, J., Baiu, D.C., Horvath, A., Erdei, A., 2002. Modeling the presentation of C3d-coated antigen by B lymphocytes: enhancement by CR1/2-BCR co-ligation is selective for the co-ligating antigen. Int. Immunol. 14, 241–247.
Quartier, P., Potter, P.K., Ehrenstein, M.R., Walport, M.J., Botto, M., 2005. Predominant role of IgM-dependent activation of the classical pathway in the clearance of dying cells by murine bone marrow-derived macrophages in vitro. Eur. J. Immunol. 35, 252–260.
Rubinstein, L.J., Stein, K.E., 1988. Murine immune response to the *N. meningitidis* group C capsular polysaccharide: ontogeny. J. Immunol. 141, 4352–4356.
Ruseva, M., Kolev, M., Dagnaes-Hansen, F., Hansen, S.B., Takahashi, K., Ezekowitz, A., Thiel, S., Jensenius, J.C., Gadjeva, M., 2009. Mannan-binding lectin deficiency modulates the humoral immune response dependent on the genetic environment. Immunology 127, 279–288.
Rutemark, C., Alicot, E., Bergman, A., Ma, M., Getahun, A., Ellmerich, S., Carroll, M.C., Heyman, B., 2011. Requirement for complement in antibody responses is not explained by the classic pathway activator IgM. Proc. Natl. Acad. Sci. U.S.A. 108, E934–E942, http://dx.doi.org/10.1073/pnas.1109831108.
Rutemark, C., Bergman, A., Getahun, A., Hallgren, J., Henningsson, F., Heyman, B., 2012. Complement receptors 1 and 2 in murine antibody responses to IgM-complexed and uncomplexed sheep erythrocytes. PLoS One 7, e41968.
Schmidt, R.E., Gessner, J.E., 2005. Fc receptors and their interaction with complement in autoimmunity. Immunol. Lett. 100, 56–67.
Shulman, M.J., Collins, C., Pennell, N., Hozumi, N., 1987. Complement activation by IgM: evidence for the importance of the third constant domain of the μ heavy chain. Eur. J. Immunol. 17, 549–554.
Thiel, J., Kimmig, L., Salzer, U., Grudzien, M., Lebrecht, D., Hagena, T., Draeger, R., Volxen, N., Bergbreiter, A., Jennings, S., Gutenberger, S., Aichem, A., Illges, H., Hannan, J.P., Kienzler, A.K., Rizzi, M., Eibel, H., Peter, H.H., Warnatz, K., Grimbacher, B., Rump, J.A., Schlesier, M., 2012. Genetic CD21 deficiency is associated with hypogammaglobulinemia. J. Allergy Clin. Immunol. 129, 801-U299.
Thornton, B.P., Vetvicka, V., Ross, G.D., 1994. Natural antibody and complement-mediated antigen processing and presentation by B-lymphocytes. J. Immunol. 152, 1727–1737.
Thornton, B.P., Vetvicka, V., Ross, G.D., 1996. Function of C3 in a humoral response: iC3b/C3dg bound to an immune complex generated with natural antibody and a primary antigen promotes antigen uptake and the expression of co-stimulatory molecules by all B cells, but only stimulates immunoglobulin synthesis by antigen-specific B cells. Clin. Exp. Immunol. 104, 531–537.
Thurnheer, M.C., Zuercher, A.W., Cebra, J.J., Bos, N.A., 2003. B1 cells contribute to serum IgM, but not to intestinal IgA, production in gnotobiotic Ig allotype chimeric mice. J. Immunol. 170, 4564–4571.
Thyphronitis, G., Kinoshita, T., Inoue, K., Schweinle, J.E., Tsokos, G.C., Metcalf, E.S., Finkelman, F.D., Balow, J.E., 1991. Modulation of mouse complement receptors 1 and 2 suppresses antibody responses in vivo. J. Immunol. 147, 224–230.
Urbaniak, S.J., Greiss, M.A., 2000. RhD haemolytic disease of the fetus and the newborn. Blood Rev. 14, 44–61.
Villiers, M.B., Villiers, C.L., Jacquier-Sarlin, M.R., Gabert, F.M., Journet, A.M., Colomb, M.G., 1996. Covalent binding of C3b to tetanus toxin: influence on uptake/internalization of antigen by antigen-specific and non-specific B cells. Immunology 89, 348–355.
von Behring, E., Wernicke, E., 1892. Über Immunisierung und Heilung von Versuchstieren bei der Diphterie. Z. Hyg. Infektionskrankheit 12, 10–44.
Wernersson, S., Karlsson, M., Dahlström, J., Mattsson, R., Verbeek, J.S., Heyman, B., 1999. IgG-mediated enhancement of Ab responses is low in FcRγ chain deficient mice and increased in FcγRII deficient mice. J. Immunol. 163, 618–622.
Wiersma, E.J., Coulie, P.G., Heyman, B., 1989. Dual immunoregulatory effects of monoclonal IgG-antibodies: suppression and enhancement of the antibody response. Scand. J. Immunol. 29, 439–448.
Wiersma, E.J., Nose, M., Heyman, B., 1990. Evidence of IgG-mediated enhancement of the antibody response in mice without classical pathway complement activation. Eur. J. Immunol. 20, 2585–2589.
Wright, J.F., Shulman, M.J., Isenman, D.E., Painter, R.H., 1988. C1 binding by murine IgM. The effect of a Pro-to-Ser exchange at residue 436 of the mu-chain. J. Biol. Chem. 263, 11221–11226.
Youd, M.E., Ferguson, A.R., Corley, R.B., 2002. Synergistic roles of IgM and complement in antigen trapping and follicular localization. Eur. J. Immunol. 32, 2328–2337.
Zhang, L., Ding, Z., Xu, H., Heyman, B., 2014. Marginal zone B cells transport IgG3-immune complexes to splenic follicles. J. Immunol., in press.