



# How antibodies act as natural adjuvants

Andrew Getahun\*, Birgitta Heyman

*Department of Genetics and Pathology, Uppsala University, SE-751 85 Uppsala, Sweden*

Received 4 November 2005; received in revised form 5 November 2005; accepted 8 November 2005

## Abstract

Antibodies can act like adjuvants. They can potently enhance the antibody response, and in the case of IgG and IgE also the T cell response, to the very antigen they are specific for. In this review we will discuss the recent advances made in our understanding of the underlying mechanisms of antibody-mediated feedback enhancement. The immuno-stimulatory properties of IgM, IgG1, IgG2a, IgG2b, IgG3 and IgE will be reviewed in relationship to the complement system and Fc receptors and the physiological relevance will be discussed.

© 2005 Elsevier B.V. All rights reserved.

**Keywords:** Fc-receptors; Complement; Antibody response; Immune complexes; T helper cells; IgM; IgG; IgE; Review

## 1. Introduction

Antigens provoke different immune responses when they are introduced to the immune system bound by antibodies, as compared to when they are introduced alone. Antibodies have the ability to regulate the antibody response to the antigen they are specific for, in a process called antibody feedback regulation [1,2]. The outcome of antibody feedback regulation can either be an almost completely inhibited antibody response or a strongly enhanced antibody response, depending mainly on the nature of the antigen. Large particulate antigens, such as sheep red blood cells, are efficiently suppressed by IgG. On the other hand, the same IgG antibodies will enhance the antibody response to soluble protein antigens [3]. In this review we will concentrate on antibody-mediated enhancement of antibody responses, discussing recent advances in our understanding of the underlying mechanisms.

## 2. Enhancement of antibody responses by IgG1, IgG2a and IgG2b/antigen complexes

IgG antibodies efficiently enhance antibody responses to the very antigen they are specific for, provided it is a soluble protein antigen. Both primary IgM [4] and IgG [3,5,6] responses are

enhanced. IgG immune complexes are also potent inducers of immunological memory [7], giving early rise to high affinity memory B cells [8].

Antibody/antigen complexes can interact with three different types of cellular receptors: the B cell receptor, Fc receptors and in the case of complement activating Ig subclasses also complement receptors. Although murine IgG2a and IgG2b can activate complement, neither these subclasses nor IgG1 require a functional complement system in order to be able to enhance [9,10]. These observations suggest involvement of Fc receptors for IgG (FcγR). In mice there are five types of Fc receptors for IgG: the neonatal FcR (FcRn), FcγRI (CD64), FcγRIIB (CD32), FcγRIII (CD16) and the newly characterized FcγRIV [11,12]. FcγRI, FcγRIII and FcγRIV are activating receptors, differing in affinity, subclass-specificity and cellular expression. They all require the common gamma chain (FcγRγ) for functional expression and for conferring an activating signal upon crosslinking by antigen-bound IgG. FcRγ contains an immunoreceptor tyrosine-based activation motif (ITAM), which gets phosphorylated upon receptor crosslinking, initiating an activating signaling cascade [13]. FcγRIIB is an inhibitory receptor which has an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic tail. Co-crosslinking of FcγRIIB with ITAM-bearing receptors, such as activating Fc receptors and the B cell receptor, results in an inhibition of cellular activation by the ITAM-bearing receptor [14].

Experiments in mice deficient of FcRγ (lacking functional FcγRI, FcγRIII and FcγRIV) revealed that antibody enhance-

\* Corresponding author. Tel.: +46 18 611 11 75; fax: +46 18 55 89 31.  
E-mail address: [andrew.getahun@genpat.uu.se](mailto:andrew.getahun@genpat.uu.se) (A. Getahun).

ment mediated by IgG1, IgG2a and to a lesser extent IgG2b is dependent on the presence of activating Fc $\gamma$ Rs [6]. Activating Fc $\gamma$ Rs are expressed on a number of cell types, with macrophages, dendritic cells (DCs) and follicular dendritic cells (FDCs) being the most relevant for antibody responses. Bone marrow transfer experiments showed that FDCs do not need to express Fc receptors for efficient IgG-mediated enhancement to occur [7]. In vitro studies have shown that antigen presenting cells which acquire IgG-bound antigen via Fc receptors, present antigen at concentrations over a 100-fold lower compared to those exposed to free antigen [15,16]. Crosslinking of activating Fc $\gamma$ Rs by IgG/antigen complexes also induce DC maturation, turning DCs into better antigen presenting cells [17]. This raises the possibility that IgG-mediated antigen presentation plays a major role in enhancing antibody responses, by inducing stronger T cell responses, which provide better help to antigen-specific B cells.

To directly visualize the effect of IgG2a/antigen complexes on the antigen-specific T cell response in vivo, we adoptively transferred OVA-specific DO11.10 T cells (collected from mouse transgenic for a T cell receptor recognizing an OVA peptide on A<sup>d</sup>) into normal BALB/c mice [18]. The effects of immunization on the antigen-specific T cell population can then be followed by flow cytometry using a clonotypic monoclonal antibody specific for the DO11.10 T cell receptor. As expected, immunization with IgG2a/OVA complexes induced enhanced IgG anti-OVA serum titers in the recipients. The enhanced antibody response was preceded by a rapid expansion of OVA-specific CD4<sup>+</sup> cells, peaking at day 3 post immunization. A 3.2–9.5-fold increase in population size was observed when compared to the response induced by antigen alone. Similar experiments in Fc $\gamma$ -deficient mice showed that, like IgG2a-mediated enhancement of the antibody response, enhancement of the T cell response is dependent on activating Fc $\gamma$ Rs [18].

Fc $\gamma$ RIIB negatively influences antibody responses and IgG-mediated inflammation (reviewed in [19]). When Fc $\gamma$ RIIB-deficient mice are immunized with IgG/antigen complexes, antibody responses are greatly enhanced (250–470-fold compared to 10-fold in wildtype mice) [6,18]. Fc $\gamma$ RIIB<sup>-/-</sup> mice also developed stronger secondary responses after priming with IgG2a/antigen complexes. The higher antibody titers in Fc $\gamma$ RIIB<sup>-/-</sup> mice are reflected in higher numbers of B cells producing specific IgG and an earlier onset of germinal center formation [18]. Fc $\gamma$ RIIB is widely expressed on haematopoietic cells, lacking only on erythrocytes, T cells and NK cells. Theoretically Fc $\gamma$ RIIB could affect antibody responses by negatively regulating B cell activation and/or antigen presentation. Co-crosslinking of Fc $\gamma$ RIIB with the B cell receptor inhibits B cell activation [20,21] and antigen presentation via the B cell receptor [22,23]. Fc $\gamma$ RIIB has also been reported to negatively influence Fc $\gamma$ R-mediated DC maturation [24], although others did not observe this effect [25]. To get an indication of whether Fc $\gamma$ RIIB negatively influences IgG-mediated T cell activation, wildtype and Fc $\gamma$ RIIB<sup>-/-</sup> mice were compared in their ability to induce delayed-type hypersensitivity reactions. No difference between the two strains was found [18].

Taken together our current state of knowledge suggests that activating and inhibitory Fc $\gamma$  receptors act on different levels when regulating the antibody response to IgG-bound antigen.

IgG-bound antigen is efficiently taken up by activating Fc $\gamma$ Rs on antigen presenting cells and presented to antigen-specific T cells, provoking stronger T helper cell responses. This will increase the likelihood of antigen-specific B cells getting the T cell help they require.

Antigen-specific B cells will receive activating signals after clustering of B cell receptors by the antigen present in IgG/antigen complexes. However, the Fc domains of the IgG present in the complex will interact with Fc $\gamma$ RIIB on the B cell, attenuating activating signals. It should be stressed that Fc $\gamma$ RIIB only attenuates antibody responses and does not completely inhibit them, as IgG/antigen complexes provoke strong antibody responses in wildtype mice (with functional Fc $\gamma$ RIIB) [5,6]. To reduce Fc $\gamma$ RIIB-mediated attenuation, germinal center B cells express reduced levels of Fc $\gamma$ RIIB [26]. Furthermore, it has been suggested that the high levels of Fc $\gamma$ RIIB expression of FDCs within germinal centers will further reduce B cell inhibition by competing for the Fc $\gamma$ RIIB ligands within IgG/antigen complexes [27]. A moderate level of Fc $\gamma$ RIIB-mediated attenuation will result in appropriate antibody responses. Defects result in unwanted antibody responses, as demonstrated in autoimmune-prone mouse strains which have a promoter polymorphism resulting in reduced Fc $\gamma$ RIIB expression in germinal center B cells and higher antibody responses [28,29]. In addition, mice deficient of Fc $\gamma$ RIIB develop spontaneous immune complex-mediated autoimmune diseases [30,31].

### 3. Enhancement of antibody responses by IgG3/antigen, IgM/antigen and complement

Murine IgG3 is the dominating IgG subclass in responses to bacterial polysaccharides. This implies that IgG3, together with IgM, plays a major role in antibody responses to thymus independent type II antigens. Since IgG1, IgG2a and IgG2b play important roles in feedback enhancement, we decided to investigate whether also IgG3 has an effect. Monoclonal TNP-specific IgG3 antibodies were generated, and mice were immunized intravenously with purified IgG3 and TNP-conjugated protein antigens in physiological salt solutions. IgG3 enhances the carrier-specific antibody response in all normal mouse strains tested, often several 100-fold [32]. There has been some uncertainty about whether there is an Fc-receptor that binds IgG3 or not. Murine macrophages were early on shown to bind monoclonal IgG3/sheep red blood cells [33] and more recently Fc $\gamma$ RI was reported to bind murine IgG3 [34]. However, IgG3 efficiently enhances antibody responses in mice lacking Fc $\gamma$ RI [35] as well as in mice lacking the common Fc $\gamma$ -chain or Fc $\gamma$ RIIB [32]. This suggests an effector mechanism independent of Fc $\gamma$ Rs.

Apart from using FcRs, antibodies can feedback enhance antibody responses via the complement system. IgM is dependent on a functional complement system to be able to enhance antibody responses. Monoclonal IgM, which owing to a point mutation is unable to activate complement, is unable to enhance

[36]. Polyclonal IgM cannot enhance antibody responses in mice depleted of C3 by treatment with cobra venom factor [36] or in knock-out mice lacking complement receptors 1 and 2 (CR1/2) [10]. Moreover, monomeric IgM, which is unable to activate complement, lost its ability to enhance antibody responses [37]. To investigate whether complement is involved in IgG3-mediated enhancement of antibody responses, mice were treated with cobra venom factor to deplete them of C3. Such animals had an impaired, although not completely absent, response to IgG3/BSA–TNP [32]. A more pronounced effect was seen when the ability of IgG3 to enhance antibody responses was tested in mice lacking complement receptors 1 and 2 [32]. Together, these findings suggest that IgG3, like IgM, primarily uses the complement system to feedback enhance antibody responses.

There is a well known connection between the complement system and antibody responses. Initially, Pepys demonstrated that de complementation of mice with cobra venom factor, led to impaired antibody responses to thymus-dependent antigens [38]. These findings were extended to show that responses to suboptimal doses of both thymus-dependent and thymus-independent antigens were inhibited by cobra venom factor-treatment [39–41]. A similar phenotype was observed in animals with hereditary deficiencies in C4, C3 or C2 [42–46] and in humans lacking C4 [47]. Subsequently, it was shown that targeting of the genes for C3 and C4 [48] as well as CR1/2 [49,50] led to impaired antibody responses. The fact that deficiency of alternative pathway factor B [51] was compatible with normal antibody responses, suggested that the link between complement and antibody responses is to be found in classical pathway activation.

Whereas IgM and IgG3 require complement in order to enhance antibody responses, IgG1, IgG2a and IgG2b can enhance without complement. IgM is a very efficient activator of complement. Once it has bound an antigen and assumed the spider shape allowing it to fix C1q, one single IgM molecule can lyse an erythrocyte [52,53]. The requirement for conformation change before IgM can bind C1q may explain why it only enhances antibody responses to large antigens like erythrocytes [36], keyhole limpet hemocyanine [37] and malaria parasites [54], all of which would allow IgM to attach with several of its antigen-binding domains and expose its C1q binding sites. In contrast, IgM does not seem to enhance responses to small proteins as BSA and OVA (B. Heyman, unpublished observations). These are all smaller than the IgM molecule and would not be appropriate for allowing conformation changes. In contrast to IgM, one single IgG molecule has too low affinity for C1q to allow sufficient binding times for activation of C1r and C1s. Therefore, two or more IgG molecules must aggregate before a sufficient affinity for C1q is reached. The probability that two IgG molecules end up close enough on the surface of an antigen to cooperate in the binding of C1q is relatively small, perhaps explaining why IgG1, IgG2a and IgG2b preferentially use FcγRs to enhance antibody responses. Why then is IgG3 able to utilize complement in feedback enhancement? One possibility is that this subclass has the capacity for cooperative binding: once one IgG3 molecule has bound to, e.g. a bacterial surface, it facilitates

the binding of other IgG3 molecules via Fc–Fc interactions [55]. Therefore, the probability that two IgG3 molecules end up close enough on the cell surface to bind C1q may be greater than that of the other IgG subclasses which rely on high antibody concentrations and epitope densities.

What then is the molecular mechanism behind involvement of complement in immune responses? The answer to this question is not yet clear, but several hypotheses have received experimental support, and it is not unlikely more than one mechanisms operate simultaneously. First, complement plays a role in localization of antigen on FDC in splenic follicles and may thereby increase the effective concentration of antigen available to stimulate B cells [37,56]. Second, antigen-opsonized by complement may be taken up efficiently by antigen-presenting cells via complement receptors, and cause increased activation of T helper cells and indirectly enhanced antibody responses. There are many examples of CR2-mediated antigen uptake and efficient presentation in *in vitro* studies [57–60] and also from one *in vivo* study using antigen coupled to anti-CR1/2 antibodies [61]. However, in other *in vivo* studies where antibody responses were impaired owing to blockade of CR1/2 [62] or deficiency of C3 and C4 [48], T cell priming was normal. Moreover, IgG3/OVA–TNP complexes [35], as opposed to IgG2a/OVA–TNP- and IgE/OVA–TNP complexes [18,63], were poor inducers of CD4<sup>+</sup> T cell proliferation *in vivo*. Since IgG3, but not IgG2a or IgE, is complement-dependent in its feedback enhancing role, this indicates that antigen presentation plays a minor role for antibody responses involving complement. CR2 (CD21) is associated with CD19 on the B cell surface, and co-crosslinking of CR2/CD19 with the B cell receptor lowers the threshold for B cell signalling *in vitro* [64,65]. Therefore, a third possibility for why animals lacking CR1/2 or C4, C2 and C3 have poor antibody responses to suboptimal doses of antigen, is that this amplification step is abrogated in the absence of the receptor itself or the C3 split products required for its ligation. This idea is supported by the observation that CR2 expressed on B cells is required for normal antibody responses [66]. Complement plays a role in primary antibody responses. Since classical, but not alternative, complement activation is involved, one must ask the question of how sufficient amounts of antigen-specific antibodies in a naive animal can be present to activate complement. An interesting possibility, suggested by studies in mice lacking secretory IgM [67,68], is that the presence of natural IgM antibodies are required for normal antibody responses to thymus-dependent antigens.

#### 4. Enhancement of antibody responses by IgE/antigen complexes

Introducing antigen bound to IgE to the immune system has dramatic effects on the antigen-specific antibody response, increasing titers up to a 1000-fold. Primary IgM, IgG and IgE responses are enhanced [69–71] and more efficient immunological memory is induced [70]. A rapid increase in specific IgG-producing B cells is seen, peaking 6 days after primary immunization [71]. Serum titers of specific IgG peak at day 7 and remain high for weeks [71].

The first clue as to how IgE facilitates such enhanced antibody responses came from the observation that IgE-mediated antibody enhancement is completely dependent on CD23, the low affinity Fc receptor for IgE. IgE cannot enhance in mice treated with anti-CD23 mAbs [69,70] or in CD23-deficient mice [72,73]. Lack of involvement of the high affinity receptor for IgE, Fc $\epsilon$ RI, was confirmed in FcR $\gamma$ -deficient mice, which have no surface expression of Fc $\epsilon$ RI [6].

CD23 is a C-type lectin-like receptor, present on the cell surface both as monomers and trimers, with the latter having a higher affinity for IgE ( $1.45 \times 10^8 \text{ M}^{-1}$ ) [74,75]. CD23 exists in two isoforms (CD23a and CD23b) and additional alternative splice variants of CD23b. Mice constitutively express CD23a on B cells [76] and FDCs [77]. Upon stimulation with IL-4 and lipopolysaccharide, CD23b mRNA can be detected in murine B cells and other haematopoietic cells [78]. CD23b and CD23b alternative splice variants have also been detected on intestinal epithelial cells [79]. CD23 has several physiological functions, besides facilitating IgE-mediated enhancement of antibody responses [69,72]. Under specific conditions, i.e. immunization with antigen in alum, CD23 acts as a negative regulator of IgE, and to a lesser extent, IgG1 production [80,81]. This apparent paradox is probably explained by the involvement of a different ligand for CD23 than IgE, as antigen-specific IgE concentrations will be very low during primary immunization. In animal models for food allergy, CD23 expressed in the intestine mediates transepithelial transport of free IgE and IgE/allergen complexes [82,83].

Four potential mechanisms could explain how IgE-bound antigen provokes stronger antibody responses via CD23: (1) decreasing the threshold for B cell activation by involving CR2 (CD21); (2) targeting to FDCs, making the antigen more immunostimulatory; (3) co-crosslinking of CD23 with the B cell receptor, facilitating B cell activation; (4) enhancing antigen presentation by CD23<sup>+</sup> B cells, resulting in better T cell help to antigen-specific B cells. The first two alternatives are unlikely to be involved. Although human CD23 has been shown to interact with CR2, influencing B cell activation [84], no such interaction has been observed in mice [85]. This is probably due to the low amino acid homology between the part of human CD23 shown to interact with human CD21 and the corresponding part of murine CD23 [86]. Deposition of IgE/antigen complexes on FDCs has been shown to occur [77] and FDCs efficiently present immune complexes, immune complex-coated bodies, to B cells. However, chimeric mice, in which FDCs were CD23<sup>−</sup> and bone marrow-derived cells CD23<sup>+</sup>, showed no defects in their response to IgE/antigen complexes [73].

The latter study strongly suggests a role for CD23<sup>+</sup> B cells, since B cells are the only bone marrow-derived cells to constitutively express CD23. Inducible CD23b is not likely to be involved since IgE-mediated enhancement is unperturbed in IL-4-deficient mice [87]. The effects of crosslinking CD23 with the B cell receptor or with other CD23 molecules on B cells are relatively unclear, although they seem to be of a negative nature [88,89]. On the other hand, *in vitro* experiments have shown that CD23 expressed on B cells mediates efficient uptake of antigen in complex with IgE, followed by

efficient presentation of antigenic peptides on B cells to T cells [90–92].

In a recent study [63] we tested whether IgE-mediated antigen presentation by B cells occurs *in vivo* and if this results in enhanced antibody responses. To examine the effect of immunization with IgE/antigen complexes on the antigen-specific T helper cell response the DO11.10 adoptive transfer system, described above, was used. Immunization with IgE/OVA complexes, but not OVA alone, resulted in an enhanced IgG anti-OVA response, which was preceded by a potent and rapid CD4<sup>+</sup> T cell response. The number of OVA-specific T cells was significantly increased at day 2 post immunization and peaked at day 3 with a 6–21-fold expansion. The observed expansion in cell numbers was due to T cell activation as we demonstrated that the T cells went through several rounds of division and changed the expression of cell surface markers from a naive to an activated phenotype. In analogy with the enhanced antibody responses observed after immunization with IgE/antigen complexes, enhanced T cell responses required CD23 but not Fc $\epsilon$ RI.

To verify that the CD23<sup>+</sup> effector cells indeed were B cells as proposed, we performed cell transfer studies in which purified CD23<sup>+</sup> or CD23<sup>−</sup> B cells were transferred to CD23<sup>−/−</sup> recipients. As expected, IgE/OVA complexes only enhanced T cell responses in mice receiving CD23<sup>+</sup> B cells, strongly suggesting that the effector cell is a B cell. Binding of IgE/antigen complexes to CD23 on B cells could, besides facilitate efficient antigen presentation, also affect the B cell in other ways to promote enhanced antibody responses.

To investigate this, we used allotype chimeras in which CD23<sup>+</sup> spleen cells from C.B-17 mice (expressing Ig of allotype b) were transferred to CD23<sup>−/−</sup> recipients on a BALB/c background (expressing Ig of allotype a). Immunization with IgE/antigen complexes resulted in enhanced antibody production by CD23<sup>−</sup> B cells [63]. This demonstrated that enhanced antigen presentation is sufficient to enhance antibody responses. The observation also makes it unlikely that signaling involving CD23 on the antigen-specific B cell is required for enhanced IgE-mediated antibody production by a B cell.

Taken together all evidence supports the model where IgE-bound antigen is targeted to CD23 on B cells, taken up, processed and efficiently presented to T cells. The enhanced activation of antigen-specific T helper cells then provide better help to antigen-specific B cells, resulting in enhanced antibody responses. The central role of B cells in IgE-mediated enhancement would explain the rapid kinetics of the enhanced antibody response [71]. The majority of freshly isolated splenic B cells, regardless of antigen specificity, bind IgE immune complexes via CD23 [93], this should increase the likelihood of antigen-specific B cells getting activated. Whether or not B cells have the ability to activate naïve T cells *in vivo* is a matter of debate [94,95] although antigen-specific B cells have indeed been reported to do so [96,97]. IgE mediated T cell activation by CD23<sup>+</sup> B cells is an interesting model for B cell mediated antigen presentation since the B cells acquire antigen via an “unspecific” receptor and are most likely antigen-unspecific.

IgE-mediated enhancement of antigen-specific antibody and T cell responses results in a positive feedback loop strengthening

Table 1  
Antibody feedback enhancement: requirements and proposed mechanisms

| Antibody isotype present in immune complex | Antigen                   | Impaired in                           | Mechanism?                        |
|--------------------------------------------|---------------------------|---------------------------------------|-----------------------------------|
| IgM                                        | Particulate antigens, KLH | CR2 <sup>-/-</sup> , CVF-treated mice | B cell activation                 |
| IgG3                                       | Soluble protein antigens  | CR2 <sup>-/-</sup> , CVF-treated mice | B cell activation                 |
| IgG1, IgG2a, IgG2b                         | Soluble protein antigens  | FcRγ <sup>-/-</sup> mice              | Antigen presentation (by DC)      |
| IgE                                        | Soluble protein antigens  | CD23 <sup>-/-</sup> mice              | Antigen presentation (by B cells) |

immune responses to antigens which provoke IgE responses, such as helminthic parasites. However, in the western world it is more likely to be involved in the development of allergic disease [98,99]. Support for a role for IgE-mediated antigen presentation in allergy comes from animal studies [100,101] and studies on patient material [92,102,103].

## 5. Concluding remarks

Antibodies arising early in an immune response such as IgM and IgG3, use complement to enhance antibody responses and are most likely to act directly on the antigen-specific B cell, lowering the threshold for B cell activation by interacting with CR2 (see Table 1). This will stimulate the development of a potent antibody response and probably reflects the way “natural” antibodies help initiating antibody responses [67,68]. Enhancement by antibodies being produced later in an immune response, such as IgG1, IgG2a, IgG2b and IgE, will play a role in the immune response to persisting antigens and during secondary responses. They enhance antibody responses by enhancing CD4<sup>+</sup> T cell responses, increasing the likelihood of antigen-specific B and T cells interacting. Interestingly, IgG can also induce CD8<sup>+</sup> T cell responses [17,104,105]. By affecting T cell responses the beneficiary effects of antibodies will go beyond opsonizing and neutralization of antigen. Indeed recent observations within the field of bacteriology [106], virology [107] and tumor biology [104,108] are in support of this notion.

## Acknowledgements

This work was supported by: Lisa Eriksson's memory Foundation; Ellen, Walter and Lennart Hesselman's Foundation; Hans von Kantzow's Foundation; Ankarstrand's Foundation, King Gustaf V:s 80 Years Foundation; Lilly and Ragnar Åkerhams's Foundation; the Swedish Foundation for Health Care Science and Allergy Research; the Swedish Medical Research Council; the Swedish Association against Rheumatism; Ollie and Elof Ericsson's Foundation; the “Network for Inflammation research” funded by the Swedish Foundation for Strategic Research, the Swedish Society for Medical Research and Uppsala University.

## References

- [1] Uhr JW, Möller G. Regulatory effect of antibody on the immune response. *Adv Immunol* 1968;8:81–127.
- [2] Heyman B. Regulation of antibody responses via antibodies, complement, and Fc receptors. *Annu Rev Immunol* 2000;18:709–37.
- [3] Enriques-Rincon F, Klaus GGB. Differing effects of monoclonal anti-hapten antibodies on humoral responses to soluble or particulate antigens. *Immunology* 1984;52:129–36.
- [4] Wiersma EJ. Enhancement of the antibody response to protein antigens by specific IgG under different experimental conditions. *Scand J Immunol* 1992;36:193–200.
- [5] Coulie P, Van Snick J. Enhancement of IgG anti-carrier responses by IgG2-anti-hapten antibodies in mice. *Eur J Immunol* 1985;15:793–8.
- [6] Wernersson S, Karlsson M, Dahlström J, Mattsson R, Verbeek JS, Heyman B. IgG-mediated enhancement of Ab responses is low in FcRγ chain deficient mice and increased in FcγRII deficient mice. *J Immunol* 1999;163:618–22.
- [7] Diaz de Ståhl T, Heyman B. IgG2a-mediated enhancement of antibody responses is dependent of FcRγ<sup>+</sup> bone marrow-derived cells. *Scand J Immunol* 2001;54:1–7.
- [8] Kunkl A, Klaus GGB. The generation of memory cells. IV. Immunization with antigen-antibody complexes accelerates the development of B-memory cells, the formation of germinal centres and the maturation of antibody affinity in the secondary responses. *Immunology* 1981;43:371–7.
- [9] Wiersma EJ, Nose M, Heyman BB. Evidence of IgG-mediated enhancement of the antibody response in mice without classical pathway complement activation. *Eur J Immunol* 1990;20:2585–9.
- [10] Applequist SE, Dahlström J, Jiang N, Molina H, Heyman B. 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* 2000;165:2398–403.
- [11] Hulett MD, Hogarth PM. Molecular basis of Fc receptor function. *Adv Immunol* 1994;57:1–127.
- [12] Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcγRIV: a novel FcR with distinct IgG subclass specificity. *Immunity* 2005;23(1):41–51.
- [13] Daëron M. Fc receptor biology. *Annu Rev Immunol* 1997;15:203–34.
- [14] Daëron M, Latour S, Malbec O, Espinosa E, Pina P, Pasmans S, et al. The same tyrosine-based inhibition motif, in the intracytoplasmic domain of FcγRIIB, regulates negatively BCR-, TCR-, and FcR-dependent cell activation. *Immunity* 1995;3:635–46.
- [15] Manca F, Fenoglio D, Li Pira G, Kunkl S, Celada F. Effect of antigen/antibody ratio on macrophage uptake, processing, and presentation to T cells of antigen complexed with polyclonal antibodies. *J Exp Med* 1991;173:37–48.
- [16] Serre K, Machy P, Grivel JC, Jolly G, Brun N, Barbet J, et al. Efficient presentation of multivalent antigens targeted to various cell surface molecules of dendritic cells and surface Ig of antigen-specific B cells. *J Immunol* 1998;161(11):6059–67.
- [17] Regnault A, Lankar D, Lacabanne V, Rodriguez A, Thery C, Rescigno M, et al. Fcγ-receptor-mediated induction of dendritic cell maturation and major histocompatibility complex class I-restricted antigen presentation after immune complex internalization. *J Exp Med* 1999;189:371–80.
- [18] Getahun A, Dahlström J, Wernersson S, Heyman B. IgG2a-mediated enhancement of Ab- and T-cell responses and its relation to inhibitory and activating FcγRs. *J Immunol* 2004;172:5269–76.
- [19] Takai T. Roles of Fc receptors in autoimmunity. *Nat Rev Immunol* 2002;2:580–92.
- [20] Phillips NE, Parker DC. Crosslinking of B lymphocyte Fcγ receptors and membrane immunoglobulin inhibits anti-immunoglobulin-induced blastogenesis. *J Immunol* 1984;132:627–32.

- [21] Bijsterbosch MK, Klaus GGB. Crosslinking of surface immunoglobulin and Fc receptors on B lymphocytes inhibits stimulation of inositol phospholipid breakdown via the antigen receptors. *J Exp Med* 1985;162:1825–36.
- [22] Minskoff SA, Matter K, Mellman I. FcγRII-B1 regulates the presentation of B cell receptor-bound antigens. *J Immunol* 1998;161:2079–83.
- [23] Wagle NM, Faassen AE, Kim JH, Pierce SK. Regulation of B cell receptor-mediated MHC class II antigen processing by FcγRIIB1. *J Immunol* 1999;162:2732–40.
- [24] Kalergis AM, Ravetch JV. Inducing tumor immunity through the selective engagement of activating Fcγ receptors on dendritic cells. *J Exp Med* 2002;195:1653–9.
- [25] Yada A, Ebihara S, Matsumura K, Endo S, Maeda T, Nakamura A, et al. Accelerated antigen presentation and elicitation of humoral response in vivo by FcγRIIB- and FcγRI/III-mediated immune complex uptake. *Cell Immunol* 2003;225(1):21–32.
- [26] Rao SP, Vora KA, Manser T. Differential expression of the inhibitory IgG Fc receptor FcγRIIB on germinal center cells: implications for selection of high-affinity B Cells. *J Immunol* 2002;169(4):1859–68.
- [27] Tew JG, Wu J, Fakher M, Szakal AK, Qin D. Follicular dendritic cells: beyond the necessity of T-cell help. *Trends Immunol* 2001;22(7):361–7.
- [28] Jiang Y, Hirose S, Abe M, Sanokawa-Akakura R, Ohtsuji M, Mi X, et al. Polymorphisms in IgG Fc receptor IIB regulatory regions associated with autoimmune susceptibility. *Immunogenetics* 2000;51(6):429–35.
- [29] Xiu Y, Nakamura K, Abe M, Li N, Wen XS, Jiang Y, et al. Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. *J Immunol* 2002;169(8):4340–6.
- [30] Bolland S, Ravetch JV. Spontaneous autoimmune disease in FcγRIIB-deficient mice results from strain-specific epistasis. *Immunity* 2000;13:277–85.
- [31] Yajima K, Nakamura A, Sugahara A, Takai T. FcγRIIB deficiency with Fas mutation is sufficient for the development of systemic autoimmune disease. *Eur J Immunol* 2003;33(4):1020–9.
- [32] Diaz de Ståhl T, Dahlström J, Carroll MC, Heyman B. A role for complement in feedback-enhancement of antibody responses by IgG3. *J Exp Med* 2003;197:1183–90.
- [33] Diamond B, Yelton DE. A new Fc receptor on mouse macrophages binding IgG3. *J Exp Med* 1981;153:514–9.
- [34] Gavin AL, Barnes N, Dijkstra HM, Hogarth PM. Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. *J Immunol* 1998;160:20–3.
- [35] Hjelm F, Carlsson F, Verbeek JS, Heyman B. IgG3-mediated enhancement of the antibody response is normal in FcγRI-deficient mice. *Scand J Immunol* 2005;62:453–61.
- [36] Heyman B, Pilström L, Shulman MJ. Complement activation is required for IgM-mediated enhancement of the antibody response. *J Exp Med* 1988;167:1999–2004.
- [37] Youd ME, Ferguson AR, Corley RB. Synergistic roles of IgM and complement in antigen trapping and follicular localization. *Eur J Immunol* 2002;32:2328–37.
- [38] Pepys MB. Role of complement in induction of the allergic response. *Nat N Biol* 1972;237(74):157–9.
- [39] Martinelli GP, Matsuda T, Osler AG. Studies of immunosuppression by cobra venom factor. I. On early IgG and IgM responses to sheep erythrocytes and DNP-protein conjugates. *J Immunol* 1978;121:2043–7.
- [40] Böttger EC, Hoffmann T, Metzger S, Hadding U, Bitter-Suermann D. The role and mechanism of cobra venom factor-induced suppression of the humoral immune response in guinea pigs. *J Immunol* 1986;137:1280–5.
- [41] Matsuda T, Martinelli GP, Osler AG. Studies on immunosuppression by cobra venom factor. II. On responses to DNP-Ficoll and DNP-Polyacrylamide. *J Immunol* 1978;121:2048–51.
- [42] Ochs HD, Wedgwood RJ, Frank MM, Heller SR, Hosea SW. The role of complement in the induction of antibody responses. *Clin Exp Immunol* 1983;53:208–16.
- [43] Böttger EC, Hoffmann T, Hadding U, Bitter-Suermann D. Influence of genetically inherited complement deficiencies on humoral immune response in guinea pigs. *J Immunol* 1985;135:4100–7.
- [44] Böttger EC, Metzger S, Bitter-Suermann D, Stevenson G, Kleindienst S, Burger R. Impaired humoral immune response in complement C3-deficient guinea pigs: absence of secondary antibody response. *Eur J Immunol* 1986;16:1231–5.
- [45] Bitter-Suermann D, Hoffmann T, Burger R, Hadding U. 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* 1981;127:608–12.
- [46] O'Neil KM, Ochs SR, Heller SR, Cork LC, Morris JM, Winkelstein JA. Role of C3 in humoral immunity. Defective antibody production in C3-deficient dogs. *J Immunol* 1988;140:1939–45.
- [47] Jackson CG, Ochs HD, Wedgwood RJ. Immune response of a patient with deficiency of the fourth component of complement and systemic lupus erythematosus. *N Engl J Med* 1979;300:1124–9.
- [48] Fischer MB, Ma M, Goerg S, Zhou X, Xia J, Finco O, et al. Regulation of the B cell response to T-dependent Ags by classical pathway complement. *J Immunol* 1996;157:549–56.
- [49] Ahearn JM, Fischer MB, Croix D, Goerg S, Ma M, Xia J, et al. 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* 1996;4:251–62.
- [50] Molina H, Holers VM, Li B, Fang Y-F, Mariathasan S, Goellner J, et al. Markedly impaired humoral immune responses in mice deficient in complement receptors 1 and 2. *Proc Natl Acad Sci USA* 1996;93:3357–61.
- [51] Matsumoto M, Fukuda W, Circolo A, Goellner J, Strauss-Schoenberger J, Wang X, et al. Abrogation of the alternative complement pathway by targeted deletion of murine factor B. *Proc Natl Acad Sci USA* 1997;94:8720–5.
- [52] Borsos T, Rapp HJ. Hemolysin titration based on fixation of the activated first component of complement: evidence that one molecule of hemolysin suffices to sensitize an erythrocyte. *J Immunol* 1965;95:559–66.
- [53] Burton DR. Is IgM-like dislocation a common feature of antibody function? *Immunol Today* 1986;7:165–7.
- [54] Harte PG, Cooke A, Playfair JHL. Specific monoclonal IgM is a potent adjuvant in murine malaria vaccination. *Nature* 1983;302:256–8.
- [55] Greenspan NS, Cooper LJJ. Intermolecular cooperativity: a clue to why mice have IgG3? *Immunol Today* 1992;13:164–8.
- [56] Papamichail M, Gutierrez C, Embling P, Johnson P, Holborow EJ, Pepys MB. Complement dependence of localisation of aggregated IgG in germinal centers. *Scand J Immunol* 1975;4:343–7.
- [57] Villiers MB, Villiers CL, Jacquier Sarlin MR, Gabert FM, Journet AM, Colomb MG. Covalent binding of C3b to tetanus toxin: influence on uptake/internalization of antigen by antigen-specific and non-specific B cells. *Immunology* 1996;89:348–55.
- [58] Boackle SA, Morris MA, Holers VM. Complement opsonization is required for presentation of immune complexes by resting peripheral blood B cells. *J Immunol* 1998;161:6537–43.
- [59] Hess MW, Schwendinger MG, Eskelinen EL, Pfaller K, Pavelka M, Dierich MP, et al. Tracing uptake of C3dg-conjugated antigen into B cells via complement receptor type 2 (CR2, CD21). *Blood* 2000;95(8):2617–23.
- [60] Prechl J, Baiu DC, Horvath A, Erdei A. 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* 2002;14(3):241–7.
- [61] Baiu DC, Prechl J, Tchorbanov A, Molina HD, Erdei A, Sulica A, et al. Modulation of the humoral immune response by antibody-mediated antigen targeting to complement receptors and Fc receptors. *J Immunol* 1999;162:3125–30.

- [62] Gustavsson S, Kinoshita T, Heyman B. Antibodies to murine complement receptors 1 and 2 can inhibit the antibody response in vivo without inhibiting T-helper cell induction. *J Immunol* 1995;154: 6524–8.
- [63] Getahun A, Hjelm F, Heyman B. IgE enhances antibody and T cell responses in vivo via CD23<sup>+</sup> B Cells. *J Immunol* 2005;175(3):1473–82.
- [64] Carter RH, Spycher MO, Ng YC, Hoffmann R, Fearon DT. Synergistic interaction between complement receptor type 2 and membrane IgM on B-lymphocytes. *J Immunol* 1988;141:457–63.
- [65] Carter RH, Fearon DT. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* 1992;256:105–7.
- [66] Croix DA, Ahearn JM, Rosengard AM, Han S, Kelsoe G, Ma M, et al. Antibody response to a T-dependent antigen requires B cell expression of complement receptors. *J Exp Med* 1996;183:1857–64.
- [67] Ehrenstein MR, O’Keefe TL, Davies SL, Neuberger MS. Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. *Proc Natl Acad Sci USA* 1998;95:10089–93.
- [68] Boes M, Esau C, Fischer MB, Schmidt T, Carroll M, Chen J. Enhanced B-1 cell development, but impaired IgG antibody responses in mice deficient in secreted IgM. *J Immunol* 1998;160:4776–87.
- [69] Heyman B, Liu T, Gustavsson S. In vivo enhancement of the specific antibody response via the low affinity receptor for IgE. *Eur J Immunol* 1993;23:1739–42.
- [70] Gustavsson S, Hjulström S, Liu T, Heyman B. *J Immunol* 1994;152:4793–800.
- [71] Westman S, Gustavsson S, Heyman B. Early expansion of secondary B cells after primary immunization with antigen complexed with IgE. *Scand J Immunol* 1997;46:10–5.
- [72] Fujiwara H, Kikutani H, Suematsu S, Naka T, Yoshida K, Yoshida K, et al. The absence of IgE antibody-mediated augmentation of immune responses in CD23-deficient mice. *Proc Natl Acad Sci USA* 1994;91:6835–9.
- [73] Gustavsson S, Wernersson S, Heyman B. Restoration of the antibody response to IgE/antigen complexes in CD23-deficient mice by CD23<sup>+</sup> spleen or bone marrow cells. *J Immunol* 2000;164:3990–5.
- [74] Dierks SE, Bartlett WC, Edmeades RL, Gould HJ, Rao M, Conrad DH. The oligomeric nature of the murine FcεRII/CD23. Implications for function. *J Immunol* 1993;150:2372–82.
- [75] Bartlett WC, Kelly AE, Johnson CM, Conrad DH. Analysis of murine soluble FcεRII: sites of cleavage and requirements for dual affinity interaction with IgE. *J Immunol* 1995;154:4240–9.
- [76] Rao M, Lee WT, Conrad DH. Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J Immunol* 1987;138:1845–51.
- [77] Maeda K, Burton GF, Padgett DA, Conrad DH, Huff TF, Masuda A, et al. Murine follicular dendritic cells and low affinity Fc receptors for IgE (FcεRII). *J Immunol* 1992;148:2340–7.
- [78] Kondo H, Ichikawa Y, Nakamura K, Tsuchiya S. Cloning of cDNAs for new subtypes of murine low-affinity Fc receptor for IgE (FcεRII/CD23). *Int Arch Allergy Immunol* 1994;105:38–48.
- [79] Montagnac G, Molla-Herman A, Bouchet J, Yu LC, Conrad DH, Perdue MH, et al. Intracellular trafficking of CD23: differential regulation in humans and mice by both extracellular and intracellular exons. *J Immunol* 2005;174(9):5562–72.
- [80] Yu P, Kosco-Vilbois M, Richards M, Köhler G, Lamers MC. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 1994;369:753–6.
- [81] Payet ME, Woodward EC, Conrad DH. Humoral response suppression observed with CD23 transgenics. *J Immunol* 1999;163:217–23.
- [82] Yang P-C, Berin MC, Yu LCH, Conrad D, Perdue MH. Enhanced intestinal transepithelial antigen transport in allergic rats is mediated by IgE and CD23 (FcεRII). *J Clin Invest* 2000;106:879–86.
- [83] Montagnac G, Yu LC, Bevilacqua C, Heyman M, Conrad DH, Perdue MH, et al. Differential role for CD23 splice forms in apical to basolateral transcytosis of IgE/allergen complexes. *Traffic* 2005;6(3):230–42.
- [84] Aubry J-P, Pochon S, Graber P, Jansen KU, Bonnefoy J-Y. CD21 is a ligand for CD23 and regulates IgE production. *Nature* 1992;358: 505–7.
- [85] Davey EJ, Bartlett WC, Kikutani H, Fujiwara H, Kishimoto T, Conrad DH, et al. Homotypic aggregation of murine B lymphocytes is independent of CD23. *Eur J Immunol* 1995;25:1224–9.
- [86] Hibbert RG, Teriete P, Grundy GJ, Beavil RL, Reljic R, Holers VM, et al. The structure of human CD23 and its interactions with IgE and CD21. *J Exp Med* 2005;202(6):751–60.
- [87] Hjulström S, Landin A, Jansson L, Holmdahl R, Heyman B. No role of interleukin-4 in CD23/IgE-mediated enhancement of the murine antibody response in vivo. *Eur J Immunol* 1995;25:1469–72.
- [88] Luo H, Hofstetter H, Bancherau J, Delespesse G. Cross-linking of CD23 by its natural ligand (IgE) or by anti-CD23 antibody prevents B lymphocyte proliferation and differentiation. *J Immunol* 1991;146:2122–9.
- [89] Campbell KA, Studer EJ, Kilmon MA, Lees A, Finkelman F, Conrad DH. Induction of B cell apoptosis by co-cross-linking CD23 and sIg involves aberrant regulation of c-myc and is inhibited by bcl-2. *Int Immunol* 1997;9:1131–40.
- [90] Kehry MR, Yamashita LC. Low-affinity IgE receptor (CD23) function on mouse B cells: role in IgE-dependent antigen focusing. *Proc Natl Acad Sci USA* 1989;86:7556–60.
- [91] Bheekha E, Escura R, Wasserbauer E, Hammerschmid F, Pearce A, Kidd P, Mudde GC. Regulation and targeting of T-cell immune responses by IgE and IgG antibodies. *Immunology* 1995;86:343–50.
- [92] van der Heijden FL, van Neerven RJJ, van Katwijk M, Bos JD, Kapsenberg ML. Serum-IgE-facilitated allergen presentation in atopic disease. *J Immunol* 1993;150:1–8.
- [93] Gustavsson S, Chomez S, Heyman B. Low responsiveness to immunization with immunoglobulin E/antigen and immunoglobulin G/antigen complexes in H-2A<sup>b</sup> mice. *Scand J Immunol* 1999;50:45–51.
- [94] Ronchese F, Hausmann B. B lymphocytes in vivo fail to prime naive T cells but can stimulate antigen-experienced T lymphocytes. *J Exp Med* 1993;177:679–90.
- [95] Jenkins MK, Khoruts A, Ingulli E, Mueller DL, McSorley SJ, Reinhardt RL, et al. In vivo activation of antigen-specific CD4 T cells. *Annu Rev Immunol* 2001;19:23–45.
- [96] Constant S, Schweitzer N, West J, Ranney P, Bottomly K. B lymphocytes can be competent antigen-presenting cells for priming CD4<sup>+</sup> T cells to protein antigens in vivo. *J Immunol* 1995;155:3734–41.
- [97] Attanavanich K, Kearney JF. Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. *J Immunol* 2004;172(2):803–11.
- [98] Mudde GC, Bheekha R, Bruijnzeel-Koomen CAFM. Consequences of IgE/CD23-mediated antigen presentation in allergy. *Immunol Today* 1995;16:380–3.
- [99] Heyman B. IgE-mediated enhancement of antibody responses: the beneficial function of IgE? *Allergy* 2002;57:577–85.
- [100] Coyle AJ, Wagner K, Bertrand C, Tsuyuki S, Bews J, Heusser C. Central role of immunoglobulin (Ig) E in the induction of lung eosinophil infiltration and T helper 2 cell cytokine production: inhibition by a non-anaphylactogenic anti-IgE antibody. *J Exp Med* 1996;183:1303–10.
- [101] Dasic G, Juillard P, Graber P, Herren S, Angell T, Knowles R, et al. Critical role of CD23 in allergen-induced bronchoconstriction in a murine model of allergic asthma. *Eur J Immunol* 1999;29(9):2957–67.
- [102] van Neerven RJJ, Wikborg T, Lund G, Jacobsen B, Brich-Nielsen Å, Arnved J, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4<sup>+</sup> T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 2003;163:2944–52.
- [103] Wachholz PA, Kristensen Soni N, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;112:915–22.

- [104] Rafiq K, Bergtold A, Clynes R. Immune complex-mediated antigen presentation induces tumor immunity. *J Clin Invest* 2002;110(1):71–9.
- [105] den Haan JM, Bevan MJ. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(–) dendritic cells in vivo. *J Exp Med* 2002;196(6):817–27.
- [106] Tobar JA, Gonzalez PA, Kalergis AM. Salmonella escape from antigen presentation can be overcome by targeting bacteria to Fcγ receptors on dendritic cells. *J Immunol* 2004;173(6):4058–65.
- [107] Villinger F, Mayne AE, Bostik P, Mori K, Jensen PE, Ahmed R, et al. Evidence for antibody-mediated enhancement of simian immunodeficiency virus (SIV) Gag antigen processing and cross presentation in SIV-infected rhesus macaques. *J Virol* 2003;77(1):10–24.
- [108] Dhodapkar KM, Krasovsky J, Williamson B, Dhodapkar MV. Anti-tumor monoclonal antibodies enhance cross-presentation of cellular antigens and the generation of myeloma-specific killer T cells by dendritic cells. *J Exp Med* 2002;195(1):125–33.