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Isotype-dependent pathogenicity of autoantibodies: analysis in experimental autoimmune hemolytic anemia

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Introduction

Autoantibodies are the essential factors for several clinical manifestations associated with a number of autoimmune diseases. The direct binding of certain autoantibodies such as anti-erythrocyte and anti-platelet autoantibodies to their targets can cause autoimmune cellular damage. Other autoantibodies such as anti-DNA autoantibodies can provoke tissue lesions as a result of their deposition in renal glomeruli and small vessels. However, because of the occasional lack of correlation between elevated serum levels of autoantibodies and clinical manifestations, it has long been suggested that only a subset of autoantibodies generated during the course of autoimmune diseases is indeed pathogenic, and that the qualitative aspects of autoantibodies are important for the pathogenesis of autoantibody-mediated cellular and tissue injuries.

The structure of the Fab region defines the specificity and affinity with which autoantibodies bind to self-antigen, and these characteristics are clearly of primary importance for pathogenic activities. In addition, the Fc region is also likely to play a critical role in autoantibody pathogenicity by activating IgG Fc-receptor(FcγR)-bearing effector cells, by activating the complement cascade and by inducing IgM multivalency-dependent agglutination of target cells. Thus, the pathogenic potential of autoantibodies is most straightforwardly attributable to the combined action of the self-antigen-binding properties of the Fab region and the effector functions associated with the Fc region of the different immunoglobulin (Ig) isotypes. Therefore, it is conceivable that a change of Ig isotype may result in a remarkable change in the pathogenic potential of autoantibodies, because Ig class switching can alter Fc-dependent effector functions and can be accompanied by concomitant changes in autoantibody affinity (affinity maturation). Until recently this question has not been directly studied, because of the lack of suitable experimental models that easily reproduce some of the main pathological manifestations observed in autoimmune diseases.

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For this reason, we have recently assessed the pathogenic activity of murine anti-erythrocyte monoclonal autoantibodies derived from autoimmune-prone NZB mice. This experimental system presents several advantages. First, the binding of the anti-red blood cell (anti-RBC) autoantibodies in vivo, i.e., the presence of opsonized RBC, can be easily monitored by flow-cytometric analysis of circulating RBC after injection of anti-RBC autoantibodies into mice. Second, the pathogenic effect of the anti-RBC autoantibodies, i.e., the development of anemia, can be followed by a simple measurement of hematocrit values. Finally, the histological analysis of spleen and liver can identify the pathogenic mechanisms of anemia, and its severity can be evaluated by analyzing the extent of erythrophagocytosis by Kupffer cells in the liver. In this article, we will discuss the molecular and cellular basis for pathogenic activities of anti-RBC autoantibodies with regard to their specificity and RBC-binding affinity, as well as the effector functions associated with the Fc region of the different Ig isotypes.

Autoimmune hemolytic anemia in NZB mice

NZB mice spontaneously develop autoimmune hemolytic anemia as a result of production of Coombs' autoantibodies reacting with their own RBC [26]. Although the molecular nature of the target autoantigens responsible for the induction of this autoimmune response has not been well characterized, the fact that autoantibodies eluted from RBC of Coombs-positive NZB mice react only with mouse RBC but not with RBC from various other species [30] has suggested the importance of the specificity of these autoantibodies for their pathogenicity. A more detailed analysis of a panel of anti-RBC monoclonal antibodies (mAb) derived from NZB mice has shown that the pathogenic autoantibodies capable of inducing anemia recognize only species-specific antigens on mouse RBC, while non-pathogenic autoantibodies cross-react with determinants present on RBC from many different species [9, 48]. The importance of the anti-mouse RBC specificity is further documented by the finding that, among five different IgM anti-RBC mAb, only the three pathogenic mAb (4C8, 1E10 and G8) are capable of inducing a marked hemagglutination, which is responsible for the development of anemia by this isotype. It may be that the surface density of antigenic determinants recognized by non-pathogenic IgM autoantibodies is too low to promote agglutination of mouse RBC in vivo. Thus, differences in the distribution and the nature of target antigens likely account for the difference in the pathogenic activities of these IgM anti-RBC mAb.

The specificity of these autoantibodies reacting with exposed surface determinants of intact RBC is distinct from that of another category of erythrocyte autoantibodies which react with a cryptic surface antigen disclosed after proteolytic treatment with bromelain [30]. However, none of the autoantibodies specific for bromelain-treated mouse RBC (BrMRBC) is able to induce anemia, excluding the possibility that they play a primary role in the immune destruction of intact RBC, causing anemia. It is, however, possible that pathogenic anti-RBC autoantibodies may damage the RBC membrane sufficiently to expose the cryptic antigens, and consequently, anti-BrMRBC autoantibodies may additionally contribute to the hemolytic process [8]. Alternatively, these antibodies may represent physiological constituents of serum, serving as transporters of catabolic products, as proposed by Grabar [22], as they are detectable at significant levels in non-autoimmune mice.

High pathogenic potential of low-affinity anti-RBC autoantibodies promoting efficient Ig-isotype-dependent effector functions

It has long been believed that the high-affinity binding of autoantibodies to self-antigens is critical for the expression of their pathogenic activities in vivo. This notion has been supported by the demonstration that the affinity maturation of autoantibodies resulting from somatic hypermutations in the Ig genes in association with IgM to IgG class switching coincides with progression of the clinical development of autoimmune diseases such as systemic lupus erythematosus [24, 49, 57]. However, somatic hypermutations do not always result in affinity maturation of autoantibodies, as observed in clonally related anti-IgG2a rheumatoid factors isolated from an autoimmune-prone MRL-*lpr/lpr* mouse [29]. More significantly, it has been shown that a low-affinity anti-IgG2a rheumatoid factor is able to induce lupus-like glomerulonephritis and immune-complex-mediated vasculitis when provided with a cryoglobulin activity uniquely associated with murine IgG3 isotype [20]. Thus, it is possible that low-affinity anti-RBC autoantibodies could become highly pathogenic, if combined with appropriate Fc-dependent effector functions of a given Ig isotype.

In fact, the analysis of an IgG2a class-switch variant of 4C8 IgM anti-RBC mAb reveals its high pathogenicity despite its low-affinity binding to mouse RBC (Table 1). The low-affinity feature of the 4C8 mAb is documented by a markedly limited RBC-binding activity of the 4C8 IgG2a variant, 1,000 times lower than that of a high-affinity 34-3C IgG2a mAb [19]. As expected, opsonized RBC is hardly detectable in the circulating blood from mice injected with even 1 mg 4C8 IgG2a, in marked contrast to the presence of bound antibodies on circulating RBC following the injection of as little as 1 μg 34-3C IgG2a mAb [19]. Nevertheless, to our surprise, the 4C8 IgG2a variant with low binding affinity was able to induce a severe anemia as a result of FcγR-mediated erythrophagocytosis by hepatic Kupffer cells. This indicates that a remarkably efficient ability of the IgG2a isotype to interact with FcγR renders even low-affinity anti-RBC autoantibodies highly pathogenic. Most strikingly, the amount of the low-affinity 4C8 IgG2a variant required to induce anemia, despite a more than 1,000 times weaker RBC-binding activity, is comparable to that of the high-affinity 34-3C IgG2a mAb (Table 1). Thus, RBC-binding affinities of Coombs'

Table 1. Pathogenic activities of low-affinity 4C8 Ig class-switch variants in comparison with those of high-affinity 34-3C and 105-2H mAb in autoimmune hemolytic anemia. The original isotype of the mAb isolated from NZB mice [48] is in roman type, while genetically produced switch variants are in italics. The anemic dose is the quantity of mAb required for inducing anemia, where hematocrit values below 40% are considered as anemic

mAb	Affinity	Isotype	Anemic dose (μg)	Pathogenicity
4C8	Low	IgM	50 μg	High
		IgG2a	50 μg	High
		IgG1	1000 μg	Intermediate
		IgG2b	5000 μg	Low
		IgG3	–	None
34-3C	High	IgG2a	25 μg	High
		IgG1	500 μg	Intermediate
105-2H	High	IgG1	500 μg	Intermediate

autoantibodies apparently play a relatively minor role in the in vivo hemolytic activities of the IgG2a isotype.

It is, however, worth noting that when the pathogenic activities are compared in mice receiving higher doses of these two IgG2a anti-RBC mAb, the 34-3C mAb is clearly more pathogenic than the 4C8 IgG2a mAb [19]. This may be related in part to differential usage of two different classes of FcγR, high-affinity FcγRI and low-affinity FcγRIII, involved in erythrophagocytosis. In fact, we have observed that both FcγRI and FcγRIII contribute to the development of anemia induced by a high dose (200 μg) of the 34-3C IgG2a mAb, while only the low-affinity FcγRIII is implicated in anemia provoked with the same amount of the 4C8 IgG2a mAb [18]. This suggests that the contribution of the high-affinity FcγRI to Kupffer-cell-mediated erythrophagocytosis is more dependent on the antigen-binding affinity of the IgG2a anti-RBC autoantibody. The usage of FcγRI by phagocytes is apparently limited in vivo because of the competition between IgG2a autoantibody bound to RBC and excess amounts of monomeric, unbound IgG2a having a high-affinity interaction with FcγRI [60]. However, highly increased densities of the 34-3C IgG2a bound on RBC could efficiently compete with circulating monomeric IgG2a for the binding to FcγRI on phagocytes, thereby promoting erythrophagocytosis and hence accelerating the development of anemia. On the other hand, this is not the case for the binding to the low-affinity FcγRIII, which lacks significant affinity to monomeric IgG2a. In addition, markedly opsonized RBC in mice receiving higher doses of the 34-3C mAb could efficiently activate complement, thereby aggravating hemolytic anemia. In fact, this has been supported by our recent finding that the injection of 200 μg 34-3C IgG2a, but not 4C8 IgG2a, induces a significant anemia in mice lacking both phagocytic FcγRI and FcγRIII (unpublished observation). Thus, an enhanced pathogenicity of the high-affinity 34-3C mAb at higher doses, as compared with the low-affinity 4C8 IgG2a mAb, is likely due to the combined effect of a more efficient interaction with the high-affinity FcγRI and a more efficient activation of the complement system.

It should also be stressed that the pathogenic potential of the low-affinity 4C8 IgG1 variant is almost comparable to that of the high-affinity 105-2H IgG1 and an IgG1 switch variant of the high-affinity 34-3C mAb (Table 1), all of which induce anemia through the activation of FcγR-dependent erythrophagocytosis [18, 31]. However, IgG2b and IgG3 class-switch variants of the 4C8 mAb are poorly pathogenic, because of limited interaction of these isotypes with FcγR (Tables 1, 2). This clearly indicates that the pathogenic activity of low-affinity IgG anti-RBC autoantibodies is dramatically enhanced, if combined with an appropriate Ig heavy-chain effector function, namely the capacity to interact efficiently with FcγR involved in erythrophagocytosis. However, it is still possible that high-affinity anti-RBC autoantibodies of IgG2b and IgG3 isotypes could become more pathogenic, owing to their enhanced interaction with FcγR and/or activation of complement as a result of a markedly increased opsonization, as compared with their low-affinity counterparts.

In addition to the remarkable contribution of the IgG Fc region to the pathogenic activities of the low-affinity 4C8 IgG2a variant, it is worth noting that the low-affinity 4C8 IgM isotype is also highly pathogenic to an extent comparable to that of its IgG2a variant and the high-affinity 34-3C IgG2a mAb (Table 1). The strong pathogenic activity of the 4C8 IgM isotype is dependent on its pentameric form, since it promotes a high-avidity binding, which is more than 1,000 times stronger than that of its IgG2a variant and comparable to that of the 34-3C IgG2a mAb [19], and induc-

es the agglutination of RBC in vivo [48]. Consequently, the IgM isotype of the 4C8 mAb induces a different form of anemia, resulting from massive agglutination of RBC in spleen and liver, which does not involve FcγR-mediated phagocytosis and complement activation [12, 48]. The importance of the pentameric form of IgM for the in vivo agglutination of RBC is further supported by the finding that an IgG3 variant of the 4C8 mAb, which is unable to mediate FcγR-dependent erythrophagocytosis, fails to induce agglutination of RBC in vivo [18].

The demonstration of the high pathogenic potency of low-affinity autoantibodies suggests that the affinity maturation of autoantibodies may not necessarily be a critical process for the generation of autoantibodies with immunopathological consequences. It has also been shown that a low-affinity anti-IgG2a rheumatoid factor of IgG3 isotype is able to induce lupus-like glomerulonephritis and immune-complex-mediated skin vasculitis, owing to the IgG3-dependent cryoglobulin activity [20]. These results provide new insight into the cellular basis of the generation of pathogenic autoantibodies. It has already been shown that a fraction of B cells expressing the low-affinity 4C8 autoantibody can escape clonal deletion in the bone marrow and can be activated to produce pathogenic autoantibodies in the periphery, as a result of nonspecific B cell activation [33, 34]. These autoantibodies may even be switched to IgG classes under certain conditions, possibly through the action of cytokines and independently of the presence of autoantigen-specific T helper cells, as in the case of T-independent type II immune responses [50]. Genetic abnormalities present in certain autoimmune-prone mice may favor the switching of IgM to IgG isotypes, as shown by a spontaneous class switch from IgM to IgG2a autoantibody in B cells derived from lupus-prone (NZB × NZW)F1 mice in the absence of functional $CD4^+$ T helper cells [42, 43]. It has been hypothesized that autoreactive B cells can be generated as a result of somatic hypermutations in the germinal centers during immune responses against environmental antigens, and that such B cells may persist, if they are defective in the process of apoptosis, which is likely to be one of the genetic defects present in autoimmune-prone mice [13, 58]. This suggests that pathogenic autoantibodies can be generated at least partly by a mechanism that is independent of the activation of autoreactive B cells by autoreactive T helper cells and self-antigens.

Differential contributions of high-affinity FcγRI and low-affinity FcγRIII to IgG-isotype-dependent anti-RBC pathogenicity

An initial analysis of pathogenic anti-RBC mAb bearing different Ig isotypes has shown that complement activation plays a minimal role in the development of anemia [48]. In contrast, FcγR-mediated erythrophagocytosis by IgG anti-RBC mAb and splenic and hepatic sequestration of RBC agglutinated by IgM anti-RBC mAb have been recognized as the major pathogenic mechanisms responsible for autoimmune hemolytic anemia in mice [12, 31, 48]. Murine phagocytic cells express two different classes of phagocytic FcγR: the high-affinity FcγRI and the low-affinity FcγRIII (for reviews, see [27, 37, 39]). The high-affinity FcγRI is capable of binding monomeric IgG2a [60], while the low-affinity FcγRIII binds polymeric forms of different IgG isotypes except IgG3 [62]. Both FcγR are hetero-oligomeric complexes, in which the respective ligand-binding α chains are associated with the common γ chain, which is critical for the assembly and cell-surface expression of functional FcγRI and FcγRIII. The FcR γ chain carries a cytoplasmic ITAM (immunoreceptor tyrosine-based acti-

Table 2. Pathogenic activities of the 4C8 Ig class-switch variants, respective contributions of FcγRI and FcγRIII to the development of 4C8-induced anemia, and their relative affinities to polymeric forms of the four IgG isotypes. The relative pathogenic activity of individual Ig isotypes of the 4C8 anti-RBC mAb is expressed as a percentage of the capacity of the 4C8 IgM mAb to induce anemia (Table 1). The relative in vivo affinity of FcγRI and FcγRIII to polymeric forms of the four different IgG isotypes is arbitrarily graded on the basis of in vivo evidence for erythrophagocytosis by Kupffer cells in the liver of different FcγR-deficient mice

Isotype	Pathogenicity (%)	FcγR contribution	Affinity of	
			FcγRI	FcγRIII
IgM	100	–	–	–
IgG1	5	FcγRIII	–	++
IgG2a	100	FcγRIII>FcγRI	++	+++
IgG2b	1	FcγRIII/FcγRI	+	+
IgG3	0	–	–	–

vation motif) sequence required for the recruitment and activation of the Src and Syk family of protein tyrosine kinases [15]. Thus, the FcR γ chain is an essential component for the triggering of various effector functions of both FcγRI and FcγRIII, including phagocytosis by macrophages, degranulation by mast cells and antibody-dependent cell-mediated cytotoxicity by natural killer cells [54].

The in vivo pathogenicity of anti-RBC autoantibodies of different IgG isotypes may be critically dependent on the relative affinities of FcγRI and FcγRIII to the polymeric form of each IgG isotype, in view of the major role of FcγR-mediated erythrophagocytosis in the pathogenesis of autoimmune hemolytic anemia [12, 31, 48]. This question has been recently addressed by assessing the pathogenic potency of four different IgG class-switch variants (IgG1, IgG2a, IgG2b and IgG3) of the low-affinity 4C8 anti-RBC mAb in relation to their utilization of the two classes of phagocytic FcγR [18]. Our analysis has demonstrated that the four IgG isotypes of this autoantibody display striking differences in pathogenicity. The IgG2a isotype exhibits the highest pathogenicity, approximately 20- and 100-fold more potent than that of its IgG1 and IgG2b variants respectively, while the IgG3 variant is not pathogenic at all (Tables 1, 2). These differences are apparently determined by the capacity of individual IgG isotypes to interact in vivo with FcγRI and FcγRIII. In fact, the IgG2a isotype interacts most efficiently with both FcγRI and FcγRIII, the IgG1 isotype binds only with the low-affinity FcγRIII, and the IgG2b isotype apparently interacts only weakly with both receptors (Table 2). In contrast, the IgG3 isotype displays little interaction with these phagocytic FcγR.

The complete dependence of the 4C8 IgG1-mediated erythrophagocytosis on FcγRIII is consistent with the results obtained with the 105-2H IgG1 anti-RBC mAb [31] and 34-3C IgG1 switch variant (unpublished observation). On the basis of in vitro studies using macrophages or transfected cell lines, it has been proposed that FcγRIII has a comparable affinity to IgG1, IgG2a and IgG2b, but little affinity to IgG3 [23, 62]. However, our in vivo analysis in different FcγR-deficient mice has clearly demonstrated marked differences in the relative affinity of FcγRIII to these three IgG isotypes; highest for IgG2a, intermediate for IgG1 and lowest for IgG2b, which directly reflects the different pathogenicity of these different IgG isotypes (Table 2).

It is striking to observe that mice lacking FcγRIII are totally resistant to the pathogenic effect of lower doses (50–200 μg) of the 4C8 IgG2a variant, while FcγRI additionally contributes to IgG2a-dependent erythrophagocytosis only when higher doses (above 1 mg) of the anti-RBC mAb are injected [18]. This indicates that FcγRIII plays a major role in the 4C8-IgG2a-induced anemia, which is also the case for anemia induced by the 34-3C IgG2a mAb. This demonstration is somehow unexpected, since it has been thought that IgG2a immune complexes preferentially interact with FcγRI because of its high-binding affinity to the IgG2a isotype [60]. However, as discussed above, a limited utilization of FcγRI for phagocytosis of IgG2a-opsonized RBC, due to competition with excess amounts of circulating monomeric IgG2a, could explain this observation. In fact, our recent demonstration of an enhanced utilization of FcγRI in IgG2a-dependent erythrophagocytosis in Ig-deficient mice confirms this idea (manuscript in preparation).

It has long been believed that the IgG2b isotype is unable to interact with the high-affinity FcγRI [28]. However, studies with the 4C8 IgG2b variant reveal that FcγRI apparently has a significant affinity to IgG2b immune complexes, though much lower than that to IgG2a immune complexes [18]. This conclusion is drawn from the observation that 4C8-IgG2b-dependent erythrophagocytosis by Kupffer cells is little affected by the absence of either FcγRI or FcγRIII, but completely protected in FcRγ-deficient mice lacking both FcγRI and FcγRIII, indicating a significant contribution of FcγRI as well as FcγRIII to IgG2b-induced autoimmune hemolytic anemia (Table 2). Notably similar results are obtained with in vitro studies using peritoneal macrophages isolated from mice deficient in FcγRI and/or FcγRIII. Therefore, the lack of detectable binding of IgG2b-opsonized RBC to FcγRI in previous studies may be in part related to the fact that these results were obtained by using COS cells transfected with FcγRIα cDNA, thereby expressing FcγRI α chains but not γ chains [17, 28]. Thus, the "FcγRI" expressed on those transfected cells do not appear to behave like the native receptors, the expression of which on phagocytic effector cells is totally dependent on FcR γ chains [54]. In fact, the affinity of the "FcγRI" expressed on COS cells is 2- to 5-fold lower than that of the native FcγRI [32], which would explain the lack of binding of IgG2b-opsonized RBC to the "FcγRI", because of a weak binding affinity of FcγRI to IgG2b immune complexes.

It has been controversial whether there exists any receptor for murine IgG3 isotype. Diamond and Yelton proposed some time ago that a new, previously unidentified IgG3-specific phagocytic FcγR might be expressed on macrophages [16]. More recently, following work using bone-marrow-derived macrophages from FcγRI-deficient mice, FcγRI has been claimed to be the sole receptor for the IgG3 isotype [21]. However, the complete absence of erythrophagocytosis by Kupffer cells in mice, even after the implantation of 4C8-IgG3-secreting cells, argues against any significant affinity of both FcγRI and FcγRIII to murine IgG3 isotype in vivo [18]. One should nevertheless consider that RBC are very poorly opsonized by the 4C8 IgG3 variant, and that the affinity, if any, of FcγRI for the IgG3 isotype is apparently lower than that for the IgG2a isotype [21]. Therefore, one cannot exclude the possibility that the interaction of FcγRI with a limited amount of the 4C8 IgG3 mAb present on RBC can be readily interfered with by an excess of circulating monomeric IgG2a. However, the finding that a high-affinity IgG3 mAb against cryptococcal capsular polysaccharide also fails to provoke phagocytosis through both FcγRI and FcγRIII in vitro and in vivo [63] strongly argues against this possibility, further supporting the lack of interaction of FcγRI and FcγRIII with the IgG3 isotype.

Lack of contribution of the inhibitory FcγRIIB to autoimmune hemolytic anemia

Murine phagocytic effector cells express an additional FcγR, FcγRIIB, which is a single α chain receptor, in which the extracellular domain is highly homologous with its activatory counterpart, while its cytoplasmic domain contains the inhibitory ITIM (immunoreceptor tyrosine-based inhibition motif) sequence [2]. Upon coligation of the inhibitory FcγR to an ITAM containing activatory FcγR, cell activation is inhibited by the recruitment of an inositol-polyphosphate phosphatase, SHIP, which mediates the inhibition of calcium flux [35]. Murine FcγRIIB exist in two major isoforms, FcγRIIB1 and FcγRIIB2 [40]. The B1 isoform is not efficiently internalized upon binding of IgG immune complexes, because of the presence of a 47-amino-acid insertion in the cytoplasmic region, but mediates inhibition of B cell activation after coligation with the B cell antigen receptor (BCR) [2]. In contrast, the FcγRIIB2 isoform is capable of promoting the endocytosis of IgG immune complexes, thereby facilitating antigen processing and presentation by macrophages [2]. Nevertheless, the complete dependence of erythrophagocytosis on FcγRI and/or FcγRIII, as observed in studies with different 4C8 IgG class-switch variants, clearly indicates a lack of phagocytosis-inducing capacity of FcγRIIB [18].

It has been demonstrated that FcγRIIB-deficient mice exhibit higher humoral, anaphylactic and inflammatory immune responses, which underlines the importance of FcγRIIB for the negative regulation of BCR-, FcεRI- and FcγRIII-dependent effector functions in vivo [11, 46, 55, 59]. However, we have recently shown that FcγRIIB is unable to down-regulate FcγRI- and FcγRIII-mediated phagocytosis of RBC opsonized with 34-3C IgG2a (unpublished observation) or 105-2H IgG1 anti-RBC mAb in vivo [46]. In this regard, it is somehow unexpected that the development of immune thrombocytopenia induced by an IgG2a anti-platelet mAb is apparently down-regulated by FcγRIIB [44]. This may raise the possibility that the development of thrombocytopenia following the injection of IgG2a anti-platelet autoantibodies may be mediated not only by FcγR-mediated phagocytosis but also by another mechanism as a consequence of FcγR-triggered activation of macrophages. This latter process may be efficiently counter-regulated by the co-engagement of FcγRIIB, while it is not relevant to the development of autoimmune hemolytic anemia.

Possible role of complement in autoimmune hemolytic anemia

The complement components interact most efficiently with mouse IgM immune complexes and to a lesser extent with IgG2a immune complexes, which trigger the classical pathway of complement activation by binding of C1q. Therefore, it is somehow surprising to see that none of the anti-RBC mAb, even of the IgM isotype, is able to induce hemolysis of mouse RBC in vitro in the presence of complement from any species [48]. In addition, the development of anemia induced by 4C8 IgM and 34-3C IgG2a mAb is hardly affected in C5-deficient DBA/2 mice as well as in BALB/c mice depleted of C3 by the treatment with cobra venom factor [31, 48]. These data thus indicate limited, if any, activation of complement by anti-RBC autoantibodies in mice. However, non-complement-fixing IgM antibodies with diverse specificities have been described [36, 61], and the structure of the antigen appears to influence the capacity of IgM antibodies to fix and/or activate

complement [14]. Therefore, it is possible that the limited activation of complement by anti-mouse RBC monoclonal autoantibodies may be related to the particular structure of the target autoantigen. In addition, it is known that the reaction of complement-binding antibodies with RBC does not always lead to complement activation. This is due to the activity of a number of complement-inhibitory proteins present on RBC membranes, and these regulatory proteins likely play an important role in preventing excessive damage to autologous cells. This would explain why the development of anemia caused by polyclonal rabbit IgG anti-mouse RBC antibodies, which are expected to activate complement, is hardly affected in mice genetically deficient in C3, but almost completely prevented in mice lacking both FcγRI and FcγRIII [51].

These results, however, cannot totally exclude a possible role of C4 in autoimmune hemolytic anemia, since the C4b fragment is recognized by complement receptors, which stimulate phagocytosis (for reviews, see [1, 6]). In addition, in vivo clearance experiments of RBC sensitized with polyclonal rabbit IgG anti-RBC antibodies in C4-deficient and control guinea pigs have shown that erythrophagocytosis can be mediated by the synergistic cooperation of FcγR and complement receptors expressed on Kupffer cells [47]. Such a synergistic cooperation has also been demonstrated in a study using human peripheral blood monocytes in vitro [7]. This mechanism could be operative under certain conditions, depending on the extent of opsonization and the IgG isotypes of anti-RBC autoantibodies. In fact, our recent results suggest an additional role of complement in the accelerated development of anemia in mice receiving higher doses of the high-affinity 34-3C IgG2a mAb (manuscript in preparation). Clearly, more extensive analysis in C3- and C4-deficient mice in relation to the IgG isotypes of anti-RBC autoantibodies, their RBC-binding affinities and the extent of RBC opsonization could help to define more precisely the role of complement in the development of autoimmune hemolytic anemia.

In addition to autoimmune hemolytic anemia, the study with an IgG2a anti-platelet monoclonal autoantibody has also shown a minimal role of complement in immune elimination of platelets [51]. Moreover, it has been reported that C3- and C4-deficient mice respond normally to the presence of pathogenic immune complexes by generating the Arthus reaction [51], in which FcγR-dependent activation of mast cells appears to be the major pathogenic mechanism [52]. In contrast, recent studies on such complement-deficient mice have clearly shown that complement is essential in innate immunity to bacterial pathogens, in which the interaction of natural IgM antibodies with bacteria apparently plays a critical role in the activation of the classical pathway of complement (for review, see [38]). On the basis of these findings, Ravetch and Clynes have proposed the following hypothesis: complement activation is essential in protection through natural IgM antibodies during innate immune responses, but not in immune-complex-triggered inflammation, while FcγR relay the action of IgG antibodies to effector cells and initiate the inflammatory cascade, thereby playing the dominant role in autoantibody-triggered autoimmune diseases. However, more recent studies have shown that complement is indeed required for the full-blown expression of immune-complex-mediated inflammatory reactions in several experimental models including the Arthus reaction, immune complex alveolitis and anti-glomerular basement membrane nephritis [4, 5, 25, 56]. As discussed above, more extensive analysis in complement- or complement-receptor-deficient mice is awaited to elucidate what role complement really plays in autoantibody-triggered autoimmune cellular and tissue injuries.

Concluding remarks

The use of four different anti-RBC IgG switch variants bearing identical variable regions has provided a unique opportunity to define the pathogenic potency of individual murine IgG isotypes in relation to the respective roles of high-affinity FcγRI and low-affinity FcγRIII. Given the role of FcγR in phagocytosis, cellular cytotoxicity and release of inflammatory mediators by different types of inflammatory effector cells, it is important to understand the respective roles of the two different classes of activatory FcγR, which interact differentially with individual IgG isotypes. Strikingly, the capacity of each IgG isotype to interact with the low-affinity FcγRIII is the critical factor determining the pathogenic potency of individual IgG isotypes, while the high-affinity FcγRI apparently plays a relatively limited role, probably because of the competition with circulating monomeric IgG2a.

The demonstration of the highest pathogenic potency of the IgG2a isotype highlights the importance of the regulation of IgG isotype responses in both autoantibody-mediated diseases and immune-complex-mediated inflammatory disorders. A recent study has shown that FcγR-mediated inflammatory responses play an important role in the pathogenesis of lupus-like glomerulonephritis [10], supporting the possibility of a higher nephritogenic potential for autoantibodies of the IgG2a isotype. Although anti-RBC autoantibodies of the IgG3 isotype appear to be less pathogenic, nephritogenic activities of IgG3 autoantibodies have also been well established, on the basis of a cryoglobulin activity uniquely associated with the IgG3 heavy-chain constant region [20, 41]. These findings are consistent with the observation that the progression of murine lupus-like autoimmune syndrome is correlated with the relative dominance of Th1 autoimmune responses promoting the production of IgG2a and IgG3 autoantibodies [3, 45, 53]. Clearly, further studies on the pathogenic role of autoantibodies of different Ig isotypes in relation with the Th subset response would help establish new strategies for the development of therapeutic approaches in autoantibody-mediated autoimmune diseases.

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References

1. Ahearn JM, Fearon DT (1989) Structure and function of the complement receptors of CR1 (CD35) and CR2 (CD21). Adv Immunol 46:183
2. Amigorena S, Bonnerot C, Drake JR, Choquet D, Hunziker W, Guillet JG, Webster P, Sautes C, Mellman I, Fridman WH (1992) Cytoplasmic domain heterogeneity and functions of IgG Fc receptors in B lymphocytes. Science 256:1808
3. Balomenos D, Rumold R, Theofilopoulos AN (1998) Interferon-γ is required for lupus-like disease and lymphoaccumulation in MRL-*lpr* mice. J Clin Invest 101:364
4. Baumann U, Köhl J, Tschernig T, Schwerter-Strumpf K, Verbeek JS, Schmidt RE, Gessner JE (2000) A codominant role of FcγRI/III and C5aR in the reverse Arthus reaction. J Immunol 164:1965
5. Bozic CR, Lu B, Höpken UE, Gerard C, Gerard NP (1996) Neurogenic amplification of immune complex inflammation. Science 273:1722
6. Brown EJ (1991) Complement receptors and phagocytosis. Curr Opin Immunol 3:76
7. Brown EJ, Bohnsack JF, Gresham HD (1988) Mechanism of inhibition of immunoglobulin G-mediated phagocytosis by monoclonal antibodies that recognize the Mac-1 antigen. J Clin Invest 81:365

8. Cabral AR, Cabiedes J, Alarcon-Segovia D (1990) Hemolytic anemia related to an IgM autoantibody to phosphatidylcholine that binds in vitro to stored and to bromelain-treated human erythrocytes. J Autoimmun 3:773
9. Caulfield MJ, Stanko D, Calkins C (1989) Characterization of the spontaneous autoimmune (anti-erythrocyte) response in NZB mice using a pathogenic monoclonal autoantibody and its anti-idiotype. Immunology 66:233
10. Clynes R, Dumitru C, Ravetch JV (1998) Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. Science 279:1052
11. Clynes R, Maizes JS, Guinamard R, Ono M, Takai T, Ravetch JV (1999) Modulation of immune complex-induced inflammation in vivo by the coordinate expression of activation and inhibitory Fc receptors. J Exp Med 189:179
12. Clynes R, Ravetch JV (1995) Cytotoxic antibodies trigger inflammation through Fc receptors. Immunity 3:21
13. Cohen PL, Eisenberg RA (1991) *Lpr* and *gld*: single gene models of systemic autoimmunity and lymphoproliferation disease. Annu Rev Immunol 9:243
14. Cunniff RV, Stollar BD (1968) Properties of 19 S antibodies in complement fixation. I. Temperature dependence and role of antigen structure. J Immunol 100:7
15. Daeron M (1997) Fc receptor biology. Annu Rev Immunol 15:203
16. Diamond B, Yelton DE (1981) A new Fc receptor on mouse macrophages binding IgG3. J Exp Med 153:514
17. Ernst LK, Duchemin AM, Anderson CL (1993) Association of the high-affinity receptor for IgG (FcγRI) with the γ subunit of the IgE receptor. Proc Natl Acad Sci USA 90:6023
18. Fossati-Jimack L, Ioan-Facsinay A, Reininger L, Chicheportiche Y, Watanabe N, Saito T, Hofhuis FMA, Gessner JE, Schiller C, Schmidt RE, Honjo T, Verbeek JS, Izui S (2000) Markedly different pathogenicity of four IgG isotype-switch variants of an anti-erythrocyte autoantibody is based on their respective capacity to interact in vivo with the low-affinity FcγRIII. J Exp Med 191:1293
19. Fossati-Jimack L, Reininger L, Chicheportiche Y, Clynes R, Ravetch JV, Honjo T, Izui S (1999) High pathogenic potential of low-affinity autoantibodies in experimental autoimmune hemolytic anemia. J Exp Med 190:1689
20. Fulpius T, Spertini F, Reininger L, Izui S (1993) Immunoglobulin heavy chain constant region determines the pathogenicity and the antigen-binding activity of rheumatoid factor. Proc Natl Acad Sci USA 90:2345
21. Gavin AL, Barnes N, Dijstelbloem HM, Hogarth PM (1998) Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. J Immunol 160:20
22. Grabar P (1983) Autoantibodies and the physiological role of immunoglobulins. Immunol Today 4:337
23. Heusser CH, Anderson CL, Grey HM (1977) Receptors for IgG: subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a macrophage cell line. J Exp Med 145:1316
24. Hirose S, Wakiya M, Kawano-Nishi Y, Yi J, Sanokawa R, Taki S, Shimamura T, Kishimoto T, Tsurui H, Nishimura H, Shirai T (1993) Somatic diversification and affinity maturation of IgM and IgG anti-DNA antibodies in murine lupus. Eur J Immunol 23:2813
25. Höpken UE, Lu B, Gerard NP, Gerard C (1997) Impaired inflammatory responses in the reverse Arthus reaction through genetic deletion of the C5a receptor. J Exp Med 186:749
26. Howie JB, Helyer BJ (1968) The immunology and pathology of NZB mice. Adv Immunol 9:215.
27. Hulett MD, Hogarth PM (1994) Molecular basis of Fc receptor function. Adv Immunol 57:1
28. Hulett MD, Osman N, McKenzie IFC, Hogarth PM (1991) Chimeric Fc receptors identify functional domains of the murine high affinity receptor for IgG. J Immunol 147:1863
29. Jacobson BA, Sharon J, Shan H, Shlomchik M, Weigert MG, Marshak-Rothstein A (1994) An isotype switched and somatically mutated rheumatoid factor clone isolated from a MRL-*lpr/lpr* mouse exhibits limited intraclonal affinity maturation. J Immunol 152:4489
30. Linder E, Edgington TS (1972) Antigen specificity of anti-erythrocyte autoantibody responses of NZB mice: identification and partial characterization of two erythrocyte surface autoantigens. J Immunol 108:1615
31. Meyer D, Schiller C, Westermann J, Izui S, Hazenbos WLW, Verbeek JS, Schmidt RE, Gessner JE (1998) FcγRIII (CD16)-deficient mice show IgG isotope-dependent protection to experimental autoimmune hemolytic anemia. Blood 92:3997

32. Miller KL, Duchemin AM, Anderson CL (1996) A novel role for the Fc receptor γ subunit: enhancement of FcγR ligand affinity. J Exp Med 183:2227
33. Murakami M, Nakajima K, Yamazaki K-I, Muraguchi T, Serikawa T, Honjo T (1997) Effects of breeding environments on generation and activation of autoreactive B-1 cells in anti-red blood cell autoantibody transgenic mice. J Exp Med 185:791
34. Murakami M, Tsubata T, Shinkura R, Nisitani S, Okamoto M, Yoshioka H, Usui T, Miyawaki S, Honjo T (1994) Oral administration of lipopolysaccharides activates B-1 cells in the peritoneal cavity and lamina propria of the gut and induces autoimmune symptoms in an autoantibody transgenic mouse. J Exp Med 180:111
35. Ono M, Okada H, Boooand S, Yanagi S, Kurosaki T, Ravetch JV (1997) Deletion of SHIP of SHP-1 reveals two distinct pathways for inhibitory signaling. Cell 90:293
36. Plotz PH, Colten H, Tala N (1968) Mouse macroglobulin antibody to sheep erythrocytes: a non-complement-fixing type. J Immunol 100:752
37. Ravetch JV (1994) Fc receptors: rubor redux. Cell 78:553
38. Ravetch JV, Clynes RA (1998) Divergent roles for Fc recptors and complement in vivo. Annu Rev Immunol 16:421
39. Ravetch JV, Kinet JP (1991) Fc receptors. Annu Rev Immunol 9:457
40. Ravetch JV, Luster AD, Weinshank R, Kochan J, Pavlovec A, Portnoy DA, Hulmes J, Pan Y-CE, Unkeless JC (1986) Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. Science 234:718
41. Reininger L, Berney T, Shibata T, Spertini F, Merino R, Izui S (1990) Cryoglobulinemia induced by a murine IgG3 rheumatoid factor: Skin vasculitis and glomerulonephritis arise from distinct pathogenic mechanisms. Proc Natl Acad Sci USA 87:10038
42. Reininger L, Radaszkiewicz T, Kosco M, Melchers F, Rolink AG (1992) Development of autoimmune disease in SCID mice populated with long-term in vitro proliferating (NZB x NZW)F1 pre-B cells. J Exp Med 176:1343
43. Reininger L, Winkler TH, Kalberer CP, Jourdan M, Melchers F, Rolink AG (1996) Intrinsic B cell defects in NZB and NZW mice contribute to systemic lupus erythematosus in (NZB x NZW)F1 mice. J Exp Med 184:853
44. Samuelsson A, Towers TL, Ravetch JV (2001) Anti-inflammatory activity of IVIG mediated through the inhibitory receptor. Science 291:484
45. Santiago ML, Fossati L, Jacquet C, Müller W, Izui S, Reininger L (1997) Interleukin-4 protects against a genetically linked lupus-like autoimmune syndrome. J Exp Med 185:65
46. Schiller C, Janssen-Graalfs I, Baumann U, Schwerter-Strumpf K, Izui S, Takai T, Schmidt RE, Gessner JE (1999) Mouse FcγRII is a negative regulator of FcγRIII in IgG immune complex triggered inflammation but not in autoantibody induced hemolysis. Eur J Immunol 30:481
47. Schreiber AD, Frank MM (1972) Role of antibody and complement in the immune clearance and destruction of erythrocytes. I. In vivo effects of IgG and IgM complement-fixing sites. J Clin Invest 51:575
48. Shibata T, Berney T, Reininger L, Chicheportiche Y, Ozaki S, Shirai T, Izui S (1990) Monoclonal anti-erythrocyte autoantibodies derived from NZB mice cause autoimmune hemolytic anemia by two distinct pathogenic mechanisms. Int Immunol 2:1133
49. Shlomchik M, Mascelli M, Shan H, Radic MZ, Pisetsky DS, Marshak-Rothstein A, Weigert M (1990) Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. J Exp Med 171:265
50. Snapper CM, Mond JJ (1993) Towards a comprehensive view of immunoglobulin class switching. Immunol Today 14:15
51. Sylvestre DL, Clynes R, Ma M, Warren H, Carroll MC, Ravetch JV (1996) Immunoglobulin G-mediated inflammatory responses develop normally in complement deficient mice. J Exp Med 184:2385
52. Sylvestre DL, Ravetch JV (1996) A dominant role for mast cells Fc receptors in the Arthus reaction. Immunity 5:387
53. Takahashi S, Fossati L, Iwamoto M, Merino R, Motta R, Kobayakawa T, Izui S (1996) Imbalance towards Th1 predominance is associated with acceleration of lupus-like autoimmune syndrome in MRL mice. J Clin Invest 97:1597
54. Takai T, Li M, Sylvestre D, Clynes R, Ravetch JV (1994) FcR γ chain deletion results in pleiotrophic effector cell defects. Cell 76:519
55. Takai T, Ono M, Hikida M, Ohmori H, Ravetch JV (1996) Augmented humoral and anaphylactic responses in FcγRII-deficient mice. Nature 379:346

56. Tang T, Rosenkranz A, Assmann KJM, Goodman MJ, Gutierrez-Ramos JC, Carroll MC, Cotran RS, Mayadas TN (1997) A role of Mac-1(CD11b/CD18) in immune complex-stimulated neutrophil function in vivo: Mac-1 deficiency abrogates sustained Fcg receptor-dependent neutrophil adhesion and complement-dependent proteinuria in acute glomerulonephritis. J Exp Med 186:1853
57. Tillman DM, Jou N-T, Hill RJ, Marion TN (1992) Both IgM and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB × NZW)F1 mice. J Exp Med 176:761
58. Tsubata T, Murakami M, Honjo T (1994) Antigen-receptor cross-linking induces peritoneal B-cell apoptosis in normal but not autoimmunity-prone mice. Curr Biol 4:8
59. Ujike A, Ishikawa Y, Ono M, Yuasa T, Yoshino T, Fukumoto M, Ravetch JV, Takai T (1999) Modulation of immunoglobulin (Ig)E-mediated systemic anaphylaxis by low-affinity Fc receptors for IgG. J Exp Med 189:1573
60. Unkeless JC, Eisen HN (1975) Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. J Exp Med 142:1520
61. Van Snick JL, Masson P (1978) The effects of complement on the ingestion of soluble antigen-antibody complexes and IgM aggregates by mouse peritoneal macrophages. J Exp Med 148:903
62. Weinshank RL, Luster AD, Ravetch JV (1988) Function and regulation of a murine macrophage-specific IgG Fc receptor, FcγR-α. J Exp Med 167:1909
63. Yuan R, Clynes R, Ravetch JV, Scharff MD (1998) Antibody-mediated modulation of *Cryptococcus neoformans* infection is dependent on distinct Fc receptor functions and IgG subclasses. J Exp Med 187:641