Markedly Different Pathogenicity of Four Immunoglobulin G Isotype-Switch Variants of an Antierythrocyte Autoantibody Is Based on Their Capacity to Interact In Vivo with the Low-Affinity Fcγ Receptor III

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Abstract

Using three different Fcγ receptor (FcγR)-deficient mouse strains, we examined the induction of autoimmune hemolytic anemia by each of the four immunoglobulin (Ig)G isotype-switch variants of a 4C8 IgM antierythrocyte autoantibody and its relation to the contributions of the two FcγRs, FcγRI, and FcγRIII, operative in the phagocytosis of opsonized particles. We found that the four IgG isotypes of this antibody displayed striking differences in pathogenicity, which were related to their respective capacity to interact in vivo with the two phagocytic FcγRs, defined as follows: IgG2a > IgG2b > IgG3/IgG1 for FcγRI, and IgG2a > IgG1 > IgG2b > IgG3 for FcγRIII. Accordingly, the IgG2a autoantibody exhibited the highest pathogenicity, ~20–100-fold more potent than its IgG1 and IgG2b variants, respectively, while the IgG3 variant, which displays little interaction with these FcγRs, was not pathogenic at all. An unexpected critical role of the low-affinity FcγRIII was revealed by the use of two different IgG2a anti-red blood cell autoantibodies, which displayed a striking preferential utilization of FcγRIII, compared with the high-affinity FcγRI. This demonstration of the respective roles in vivo of four different IgG isotypes, and of two phagocytic FcγRs, in autoimmune hemolytic anemia highlights the major importance of the regulation of IgG isotype responses in autoantibody-mediated pathology and humoral immunity.

Key words: autoantibody • autoimmune hemolytic anemia • Fc receptor • IgG isotype • knockout mouse

Introduction

NZB mice spontaneously develop an autoimmune hemolytic anemia as a result of production of Coombs’ anti-RBC autoantibodies (1). Although the specificity of anti-RBC autoantibodies is of primary importance in the expression of their pathogenic activities in vivo, effector functions associated with the Fc regions of the different Ig isotypes are also likely to play a critical role. Among the various effector functions mediated by the Ig heavy-chain constant regions, it is striking to see that the complement activation plays a minimal, if any, role in the development of anemia induced by anti-RBC antibodies (2, 3). In con-
selectively interact with FcRg has been recognized as the major pathogenic mechanism responsible for autoimmune hemolytic anemia in mice (2, 4–7).

Murine phagocytic effector cells express three different classes of FcRγ: a high-affinity receptor, FcRγI, and two low-affinity receptors, FcRγII and FcRγIII (for reviews, see references 8–10). FcRγI and FcRγIII are heterooligomeric complexes, in which the respective ligand-binding α-chains are associated with the common γ chain (FcRγ). FcRγ is required for their assembly and for the triggering of their various effector functions, including phagocytosis by macrophages, degranulation by mast cells, and antibody-dependent cell-mediated cytotoxicity by NK cells (11). In contrast, FcγRII is a single α-chain receptor, with two major isoforms, FcγRIIb1 and FcγRIIb2 (12), both of which apparently lack phagocytosis-inducing capacity (11). The macrophage-specific isoform, FcγRIIb2, is capable of mediating the binding and endocytosis of IgG immune complexes (ICs), thereby facilitating antigen processing and presentation, whereas the b1 isoform, mainly expressed in B lymphocytes, is not efficiently internalized upon binding of IgG ICs but mediates inhibition of surface IgM-triggered B cell activation after coligation (13–15). The high-affinity receptor, FcγRI, is capable of binding monomeric IgG2a (16–18), and the two low-affinity receptors, FcγRII and FcγRIII, bind polymeric IgG of different IgG isoatypes except IgG3 (19). Thus, it has been proposed that IgG2a ICs interact preferentially with the high-affinity FcγRI, IgG1, and IgG2b ICs with the low-affinity FcγRII. In addition, a recent in vitro study has claimed that FcγRIII ICs selectively interact with FcγRI (20). By the use of FcγRI-deficient mice, it has now been well established that FcγRIII is the sole receptor mediating IgG1-dependent phagocytosis in vivo (6, 7, 21). However, the precise contribution of each of these two FcγR to phagocytize opsonized particles with IgG2a, IgG2b, or IgG3 antibodies remains to be defined.

In view of the major role of FcγR-mediated erythrophagocytosis in the pathogenesis of autoimmune hemolytic anemia (4–7), the in vivo pathogenicity of anti-RBC autoantibodies of different IgG isoatypes may be critically dependent on the relative affinities of two different phagocytic FcγRs (FcγRI and FcγRIII) to the polymeric form of each IgG isotype. This question cannot be explored through the use of a random panel of monoclonal anti-mouse-RBC autoantibodies differing in IgG isoatypes, as they may also differ in antigen-binding specificities and affinities. We have recently prepared an IgG2a class-switch variant from the NZB-derived 4C8 anti-RBC IgM monomolucytic autoantibody (2), and found it highly pathogenic as the result of its efficient interaction with phagocytic FcγR (22). Therefore, we have generated three other IgG class-switch variants (IgG1, IgG2b, and IgG3) of this mAb, and compared their pathogenic potency with that of the IgG2a variant in relation to their utilization of the two classes of phagocytic FcγR, as explored by the use of three different strains of FcγRII-deficient mice. We observed remarkable differences in the pathogenic potentials of these IgG variant autoantibodies. These differences appear to be determined by the capacity of individual IgG isoatypes to interact in vivo with the low-affinity FcγRIII. Thus, the results have defined the respective roles of the two different phagocytic FcγRs in vivo, providing a rational for the IgG isotype-dependent pathogenicity observed in autoimmune hemolytic anemia.

**Materials and Methods**

Mice. FcγRIII-deficient (FcγRIII−/−) mice lacking the α-chain of FcγRIII with a mixed genetic background between C57BL/6 and 129 strains, FcγRII-deficient (FcγRII−/−) mice lacking the α-chain of FcγRII, and FcγRI-deficient (FcγRI−/−) mice lacking the α-chain of FcγRI were generated in the laboratory of J. S. Verbeek (Leiden University) by homologous recombination, backcrossed for four generations with BALB/c mice, and bred to homozygosity at the fgl1 null allele (our unpublished results). BALB/c mice were purchased from Gl. Bomholtgard Ltd.

**DNA Constructions.** The VDJH4C8-Cγ1, -Cγ2, and -Cγ3 plasmids containing the complete 4C8 IgG heavy-chain gene of the respective IgG subclass were constructed using the following DNA fragments the rearranged VDJ region isolated from cDNA encoding the V region of the heavy chain of the 4C8 mAb (24), the promoter region isolated from pSV-Vµ1 (25), the heavy chain enhancer region isolated from pSVE2-neo (26), and the Cγ1, Cγ2b, or Cγ3 region derived from the respective genomic clones, pEVH/Cγ1 (26), plgh 22 (27), and pW7 (28). mAb. The 4C8 IgG1, IgG2b, and IgG3 class-switch variants were obtained by transfecting 4C8 heavy-chain-loss mutant cells by electroporation with the VDJH4C8-Cγ1, -Cγ2, and -Cγ3 plasmids together with a pSV2-neo plasmid containing the neomycin-resistant gene, as described for the generation of the 4C8 IgG2a variant (22). Clones secreting ~2–5 μg/ml were selected and used in this study. The 4C8 IgG class-switch variants exhibited a comparable mouse RBC-binding activity in vitro, as assessed by a flow cytometric analysis using a biotinylated rat anti-mouse-k-chain mAb (H139.52.1.5), followed by PE-conjugated streptavidin (22). Notably, the VH4C8 and Vκ4C8 sequences of the 4C8 heavy- and light-chain cDNA derived from a reverse transcriptase PCR amplification of mRNA isolated from the cells secreting 4C8 IgG switch variants were identical to the original published sequence (24). Hybridoma secreting 34-3C IgG2a anti-mouse RBC mAb was derived from nonmanipulated NZB mice (2), S54 IgG1 anti-4C8 idiotype mAb recognizing the combination of both the heavy and light chains of the 4C8 mAb was obtained as described (29). Other mAbs in use were: IgG2a anti-TNP (Hy1.2), IgG1 anti-4C8 idiotype mAb, IgG2b anti-4C8 idiotype mAb, IgG2b anti-TNP (Hy1.2), and IgG2b anti-CD4 (GK1.5), and rat IgG2b anti-CD8 (H35-17.2) mAbs. IgG mAbs were purified from culture supernatants by protein A or protein G column chromatography. The purity of IgG was ~90% as documented by SDS-PAGE.
Results

Marked Differences in Pathogenic Activities among the 4C8 IgG Class-switch Variants. The role of the IgG heavy-chain C region of anti-mouse RBC autoantibodies on the development of anemia was first analyzed by a single intraperitoneal injection of 1 mg of the four different IgG class-switch variants of the 4C8 mAb into BALB/c mice. The IgG2a variant induced the most severe form of anemia (a decrease in mean Ht values to 21% at day 4), the IgG1 variant induced a mild anemia (average Ht of 36%), and the IgG2b and IgG3 variants were unable to significantly decrease Ht levels (Fig. 1 A). Quantitative analysis revealed that 50 μg of the 4C8 IgG2a mAb was sufficient to induce anemia (mean Ht values of three mice 4 d after the injection: 36 ± 3%) at a level comparable to that observed with 1 mg of the 4C8 IgG1 isotype. For the 4C8 IgG2b variant, a dose as high as 5 mg caused a significant, though marginal, drop in Ht values (means of four mice: 46 ± 1% at day 0; 41 ± 2% at day 4; P < 0.01), whereas up to 5 mg of the 4C8 IgG3 variant had no detectable effects (means of four mice: 47 ± 2% at day 0; 45 ± 2% at day 4), as was the case of mice injected with a control IgG2a anti-TNP mAb (data not shown).

Thus, the pathogenic potency of the 4C8 IgG2a isotype was ~20-fold, 100-fold, and still much higher than that of the IgG1, IgG2b, and IgG3 isotypes, respectively.

To compare more precisely the potency of the two less pathogenic isotypes, IgG2b- and IgG3-secreting transfectomas were implanted intraperitoneally into BALB/c mice. The 4C8 IgG2b transfectoma cells provoked severe anemia, with a decrease in mean Ht values to 31 and 18% at day 6 and 8, respectively, whereas Ht values remained within normal limits (>40%) in mice transplanted with 4C8 IgG3 or Hy1.12 IgG2a anti-TNP hybridoma cells (Fig. 1 B). The secretion of excess amounts of the 4C8 IgG3 mAb in vivo was documented by the presence of substantial amounts of free antibodies bearing the 4C8 idiotype (data not shown).

With the three anemia-inducing isotypes, histological examinations showed that the most remarkable pathological change was erythrophagocytosis by hepatic Kupffer cells. The extent of erythrophagocytosis, documented by iron deposits in Kupffer cells, correlated with the level of anemia induced by these three different IgG switch variants (Fig. 2).
It should be mentioned that although Ht values in mice receiving 5 mg 4C8 IgG2b remained within normal limits, significant iron deposits in Kupffer cells were observed; this was markedly augmented in mice implanted with the 4C8 IgG2b transfectoma. In contrast, Kupffer cell-mediated erythrophagocytosis and iron deposits were totally absent in the 4C8 IgG3-injected mice, even following the transplantation of 4C8 IgG3 cells (Fig. 2). Moreover, these mice failed to show a massive accumulation of agglutinated RBCs in spleen and liver, unlike mice injected with the 4C8 IgM mAb (2).

**Table I.** Development of Anemia in FcyR-deficient and WT Mice after the Injection of the 4C8 IgG1 and IgG2a Variants

| Isotype   | Dose | Mice   | Ht* (%) |
|-----------|------|--------|---------|
| IgG1      | 1 mg | WT (4) | 36.3 ± 2.9 |
|           |      | FcγRIII−/− (5) | 46.6 ± 0.9 |
| IgG2a     | 200 μg | WT (4) | 31.0 ± 2.0 |
|           | 1 mg  | FcγRIII−/− (4) | 45.7 ± 2.1 |
|           |      | WT (7)  | 21.5 ± 3.7 |
|           |      | FcγRIII−/− (7) | 37.2 ± 2.3 |
|           |      | FcγRI−/− (5) | 27.6 ± 3.9 |
|           |      | FcγRγ−/− (4) | 44.6 ± 1.8 |

*Ht values (mean ± 1SD) were determined 4 d after the intraperitoneal injection of purified 4C8 IgG variants. Ht values before the injection of anti-RBC mAb in WT and FcyR-deficient mice were in the 44-48% range.

*Numbers of mice studied are indicated in parentheses.
jected with 1 mg 4C8 IgG1 mAb was completely prevented in FcγR III−/− mice (Table I), which failed to exhibit erythrophagocytosis, as documented by the lack of iron deposits in their Kupffer cells (Fig. 2). The far more severe erythrophagocytosis observed in WT mice after the transplantation of the 4C8 IgG1 transfectedoma was also abolished in FcγR III−/− mice (Fig. 2), indicating that FcγR III is the sole receptor involved in the 4C8 IgG1-mediated erythrophagocytosis by Kupffer cells.

Although FcγR III−/− mice were also totally resistant to the pathogenic effect of 200 μg 4C8 IgG2a variant, a less severe, but significant anemia with a lower extent of erythrophagocytosis was still induced in these mice by a higher dose (1 mg) of the 4C8 IgG2a (P < 0.001) (Table I, and Fig. 2). This indicated that FcγR III plays a major role in the 4C8 IgG2a-induced anemia, but that FcγR I is also involved in the severe anemia caused by higher amounts of this isotype. This conclusion was confirmed by the use of two other strains of FcγR−/− mice, in which the level of protection from the pathogenic effect of 1 mg 4C8 IgG2a mAb was found to be more limited in FcγR I−/− mice than in FcγR III−/− mice (P < 0.005), but complete in FcR γ−/− mice lacking both receptors (Table I and Fig. 2).

In contrast to the IgG1 and IgG2a variants, the development of a severe anemia provoked by the transplantation of the 4C8 IgG2b transfectedoma was almost indistinguishable in kinetics and histological changes among FcγR III−/−, FcγR I−/−, and WT mice (Fig. 2 and Fig. 3). However, FcR γ−/− mice deficient in both FcγR I and FcγR III were resistant to the pathogenic effect of the 4C8 IgG2b, as erythrophagocytosis by Kupffer cells was no longer visible in these mice (Fig. 2 and Fig. 3). Notably, serum levels of antibodies bearing the 4C8 idiotype, measured at killing (6–8 d after the transplantation of the 4C8 IgG2b cells), were comparable between FcγR−/− mice and their corresponding WT mice (data not shown). It should also be stressed that both FcγR I−/− and FcγR III−/− mice injected with 5 mg of the 4C8 IgG2b exhibited a modest hepatic erythrophagocytosis at levels comparable to that of WT mice (data not shown). These results indicated that the involvement of both FcγR I and FcγR III in hemolytic anemia is induced by very high doses of the 4C8 IgG2b variant.

Major contribution of FcγR III to the Development of Anemia by 34-3C IgG2a Anti-Mouse RBC mAb. Previous studies have demonstrated the contribution of both FcγR I and FcγR III to the development of anemia after the injection of a highly pathogenic dose of the 34-3C IgG2a anti-mouse RBC mAb (5, 7). As the present studies revealed a critical role of FcγR III in the development of anemia induced by a lower dose of the 4C8 IgG2a variant, we reassessed the pathogenic effect of the 34-3C IgG2a mAb in FcγR III−/− mice compared with WT mice. The development of anemia was partially prevented in FcγR III−/− mice after the injection of a highly pathogenic dose (200 μg) of the 34-3C mAb, which caused a severe anemia in WT mice (P < 0.05). However, it was completely prevented after the injection of a lower dose (50 μg) that still caused anemia in WT mice (P < 0.005; Fig. 4). These results confirmed the prominent role of FcγR III over FcγR I in the IgG2a anti-RBC-induced autoimmune hemolytic anemia.

Contribution of Both FcγR I and FcγR III to Phagocytosis of IgG2b-opsonized RBCs by Peritoneal Macrophages In Vitro. An intriguing observation made in this study was the sig-

![Figure 3](image3.png)

**Figure 3.** Development of anemia in FcγR−/− deficient and WT mice after the transplantation of the 4C8 IgG2b transfectedoma. 10⁷ transfectedoma cells were injected into (A) FcγR III−/− (○) or WT littermates (●); (B) FcγR I−/− (△) or WT littermates (●); and (C) FcR γ−/− (△) or WT littermates (●) on day 0. Ht values of individual mice measured every 2 d are shown.

![Figure 4](image4.png)

**Figure 4.** Development of anemia in FcγR III−/− and WT mice after the injection of the 34-3C IgG2a mAb. 200 or 50 μg of the mAb was injected intraperitoneally into FcγR III−/− (○) or WT littermates (●) on day 0. Ht values of individual mice measured 4 d after the mAb injection are shown. Note the complete prevention of anemia in FcγR III−/− mice injected with 50 μg of the 34-3C mAb.
sige macrophages that had ingested more than two SRBCs. The IgG3 isotype lacking significant binding to these FcγRs displayed no pathogenicity at all. Furthermore, our results have demonstrated preferential utilization of FcγRI by the IgG2a isotype in vivo, despite their low-affinity interaction compared with FcγRI, revealing the major role of the low-affinity FcγRIII in autoimmune hemolytic anemia.

Differential Contributions of FcγRI and FcγRIII to IgG Iso-type-dependent Anti-RBC Pathogenicity. It was striking to observe how different the utilization by individual IgG isotypes of the two different types of FcγR involved in erythrophagocytosis in vivo is, as reflected in the pathogenesis of autoimmune hemolytic anemia (Table II). The complete absence of erythrophagocytosis by the 4C8 IgG1 variant in FcγRIII−/− mice confirmed the critical role of FcγRII in the IgG1-mediated erythrophagocytosis, as shown recently by using another 105-2H IgG1 anti–mouse RBC monoclonal autoantibody (6, 7). Significantly, our study disclosed that the contribution of FcγRIII to the development of IgG2a-induced autoimmune hemolytic anemia is more prominent than that of FcγRI. This was somehow unexpected, as the high-affinity binding of FcγRI to the IgG2a isotype has been well established (16–18, 33). However, it should be stressed that FcγRI contributes to IgG2a-dependent erythrophagocytosis but only when higher doses of IgG2a anti–mouse RBC mAb were injected. A limited utilization of FcγRI for phagocytosis of IgG2a-opsonized RBCs may be due to the competition by excess amounts of unbound circulating monomeric IgG2a having a high-affinity interaction with FcγRI. In this regard, it may be worth noting that a significant anemia was still observed in FcγRIII−/− mice injected with 200 μg of the 34-3C IgG2a, but not with the same amount of the 4C8 IgG2a. Thus, it appears that the contribution of the high-affinity FcγRI to pathogenicity is more influenced by the antigen-binding properties of the IgG2a antibodies. In agreement with this conclusion, we have recently shown that in vivo bindings to circulating RBCs were much stronger with the 34-3C mAb than with the 4C8 IgG2a, reflecting marked differences in RBC-binding affinities of these two antibodies (22). Thus, it is likely that higher densities of the 34-3C IgG2a bound on RBCs may efficiently compete with circulating monomeric IgG2a for FcγRI binding on phagocytes. An alternative, or additional possibility is that the IgG2a anti–RBC mAb at higher doses could mediate erythrophagocytosis by their direct binding to FcγRI, followed by subsequent interaction of cell-bound antibodies with circulating RBCs. Owning to its higher RBC-binding capacity, the FcγRI-bound 34-3C mAb on the surface of phagocytes may be much more efficient to capture circulating RBCs, causing erythrophagocytosis, compared with the low-affinity 4C8 IgG2a.

An additional and unexpected observation was that of a significant role of FcγRI in the pathogenesis of 4C8

**Figure 5.** In vitro phagocytosis of IgG2b- and IgG1-opsonized SR BCs by macrophages from FcγR-deficient and WT mice. Adherent thioglycollate-elicited peritoneal macrophages from WT, FcγRIII−/−, FcγRI−/−, and FcγRIγ−/− mice were incubated with SR BCs opsonized with N-S.8.1 IgG2b (A) or Sp3HL IgG1 (B) anti–SR BC mAb. Phagocytosis was determined after lysing extracellular SR BCs by a hypotonic shock. Results are expressed as the percentage (means ± SEM of triplicate cultures) of positive macrophages that had ingested more than two SR BCs.
IgG2b-induced autoimmune hemolytic anemia. Indeed, it has long been believed that the IgG2b isotype is unable to interact with the high-affinity FcγRI (33). However, it should be emphasized that the latter conclusion was based on the results obtained with COS cells expressing FcγRI α chains in absence of the FcR γ chain (33, 34). The Fc receptors expressed on those transfected cells exhibit binding to monomeric IgG2a and IgG2a-coated RBCs. However, these receptors do not appear to behave as do heterooligomeric complexes of the native receptor normally borne on phagocytic effector cells, as FcγRI is functionally absent in Fcγ-deficient peritoneal macrophages and Kupffer cells (5, 11). It has also been noted that the affinity of the FcγRI expressed on the transfected COS cells is two- to fivefold lower than that of the native receptor, but is restored at normal levels through their association with FcγRI on normal levels through their association with FcγRI (33). This could account for the lack of detectable binding of IgG2b-opsonized RBCs to COS cells transfected with murine FcγRI γ cDNA (33), because of a weak affinity of FcγRI to IgG2b ICs, as discussed below. On the other hand, it has been demonstrated that FcγRI-dependent erythrophagocytosis by Kupffer cells is little affected by FcγRI -/- mice lacking both FcγRI and FcγRI (6, 21). However, it should be emphasized that Kupffer cell-mediated erythrophagocytosis in WT mice injected with 5 mg of purified antibodies or transplanted with the 4C8 IgG2b-secreting cells was not affected in FcγRI -/- mice, but completely protected in FcγRI -/- mice lacking both FcγRI and FcγRI. The presence of very high concentrations of the 4C8 IgG2b mAb in these experimental conditions may promote the utilization of FcγRI, as discussed above for the IgG2a isotype. Consequently, FcγRI and FcγRI are able to efficiently compensate each other to mediate the phagocytosis of IgG2b-opsonized RBCs in vivo. As we could not assess the respective role of both receptors at lower concentrations of the IgG2b isotype, in contrast with the situation with the 4C8 IgG2a isotype, our present conclusion that the comparable contribution of FcγRI and FcγRI to the IgG2b-induced anemia, is still tentative (Table II). Only experiments with a highly pathogenic IgG2b anti-RBC mAb would provide definitive conclusions on this issue.

Table II. Pathogenetic Activities of the 4C8 IgG Class-Switch Variants, Respective Contributions of FcγRI and FcγRI to the Development of Anemia Induced by the 4C8 IgG Variants, and Their Relative Affinities to ICs of the Four IgG Isotypes

| Isotype | Pathogenicity* | Contribution of FcγRI to 4C8 IgG-induced anemia | FcγRI† | FcγRI‡ |
|---------|----------------|-----------------------------------------------|--------|--------|
| IgG1    | ++ (1 mg)      | FcγRI III                                    | −      | ++     |
| IgG2a   | +++ (50 µg)    | FcγRI III > FcγRI I                          | ++     | +++    |
| IgG2b   | + (>5 mg)      | FcγRI I/FcγRI III                           | +      | +      |
| IgG3    | −              | None                                         | −      | −      |

*Minimum amounts of mAb required for inducing anemia (decreasing Ht values <40%) are indicated in parentheses.
†The relative affinity of FcγRI and FcγRI to ICs of the four different IgG isotypes is arbitrarily graded on the base of in vivo evidence of erythrophagocytosis by Kupffer cells.
polysaccharide failed to provoke phagocytosis through Fc-γR I and FcγR III in vitro and in vivo (40). Together with the complete protection from the pathogenic effect of the 4C8 IgG1 variant in FcγR III−/− mice, we propose that the relative in vivo binding activity of FcγRI to antigen-antibody complexes of different murine IgG isotypes is in the order of IgG2a > IgG2b > IgG3/IgG1 (Table II).

Based on in vitro studies using macrophages or transfected cell lines (17, 19), it has been proposed that FcγR III has a comparable affinity to IgG1, IgG2b, and IgG2a, but little affinity to IgG3. Although we confirmed that FcγR III is capable of mediating phagocytosis of RBCs opsonized with IgG1, IgG2b, and IgG2a, but not with IgG3, our analysis has clearly demonstrated marked differences in the relative affinity of FcγR III to these three IgG isotypes, highest for IgG2a, intermediate for IgG1, and lowest for IgG2b (Table II). This conclusion is based on the finding that FcγRIII-dependent erythrophagocytosis was inducible at a dose of 50 μg, 1 mg, and 5 mg of the 4C8 IgG2a, IgG1, and IgG2b isotypes, respectively. However, it should be mentioned that IgG2b ICs are potent to induce passive cutaneous anaphylaxis upon triggering FcγR III expressed on mast cells (6). An efficient activation of mast cells by IgG2b ICs may be related to the fact that unlike macrophages, mast cells express a unique form of FcγRIII associated with the FcR β chain, which functions as an amplifier of FcγRIII responses by enhancing FcRγ-mediated signaling (41).

Does Complete Play Any Role in Autoimmune Hemolytic Anemia? The present results are consistent with the previous conclusion that complement-mediated hemolysis and complement receptor-dependent erythrophagocytosis may play a minor, if any, role in this model of autoimmune hemolytic anemia. The development of 34-3C IgG2a-induced anemia in C3-deficient DBA/2 mice or in C3-depleted mice by the treatment with cobra venom factor (2, 7). This is further supported by the recent demonstration that mice genetically deficient in C3 were not protected from anemia caused by polyclonal rabbit IgG anti-mouse RBC antibodies, whereas loss of both FcγRI and FcγR III prevented the anemia (3). However, these results cannot completely exclude the possible role of C4 in autoimmune hemolytic anemia, as the C4b fragment is recognized by the complement receptor type I (CR1), which stimulates phagocytosis (for a review, see reference 42). In addition, in vivo clearance experiments of RBCs sensitized with polyclonal rabbit IgG anti-RBC antibodies in C4-deficient guinea pigs have shown that erythrophagocytosis can be mediated by the synergistic cooperation of FcγR and complement receptors expressed on Kupffer cells (43). Such a mechanism could be operative under certain conditions, depending on the extent of opsonization and the IgG isotypes of anti-RBC autoantibodies. If so, the differential ability of individual IgG isotypes to activate the complement pathway may additionally contribute to the remarkable differences in the pathogenicity observed in this report. Clearly, more detailed analysis on 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 a role for complement in the development of autoimmune hemolytic anemia.

Concluding Remarks. The use of the four different anti-RBC IgG switch variants bearing identical VH and Vk regions has provided a unique opportunity to define the respective roles of two different phagocytic FcγRs in IgG isotype-dependent effector functions, and hence, the pathogenic potency of individual murine IgG isotypes. Strikingly, the capacity of each IgG isotype to interact with the low-affinity FcγR III is the critical factor determining the pathogenic potency of individual IgG isotypes, as the high-affinity FcγR I apparently plays a relatively limited role, probably because of the competition by circulating monomeric IgG2a. In addition, our results should provide useful guiding principles for the engineering of mAbs for in vivo applications.

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 pathology and IC-mediated inflammatory disorders. A recent study has shown that FcγR-mediated inflammatory responses play an important role in the pathogenesis of lupus-like glomerulonephritis (44), supporting the possibility of a higher nephritogenic potential for autoantibodies of the IgG2a isotype. Although anti-RBC autoantibodies of the IgG3 isotype are poorly 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 C region (45-48). 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 (49-53). Clearly, further studies on the pathogenic role of autoantibodies according to their Ig isotypes and in relation with the Th subset responses would help establish new strategies for the development of therapeutic approaches in autoimmune-mediated autoimmune diseases.

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