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# Natural Anti-Immunoglobulin Autoantibodies: Irrelevant By-Products or Immunoregulatory Molecules?

## Key Words

Rheumatoid factor  
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## Abstract

Anti-immunoglobulin autoantibodies (anti-Ig auto-Abs), particularly IgM-anti-IgG (classical rheumatoid factor), have been studied mainly in the context of rheumatoid arthritis. In this article we focus on other members of the anti-Ig family, including the natural IgG-anti-F(ab')<sub>2</sub> auto-Ab, and look for clinical and experimental findings supporting a role of these antibodies in the regulation of the immune response. We discuss the evidence for suppression of auto- and allo-reactive B cells by IgG-anti-F(ab')<sub>2</sub> auto-Ab and the role of this Ab in the pathogenesis of certain diseases, such as autoimmune hemolytic anemia. The structure of the antibody's antigen-binding site was clarified by isolating the VL and VH gene segments from the cDNA of B cells. Sequence analyses revealed germline gene identity of VL chains and 88% homology with the closest germline gene of VH chains. Finally, we discuss hypothetical models concerning the immunoregulatory role of natural anti-Ig auto-Abs.

## Introduction

Anti-immunoglobulin autoantibodies (anti-Ig auto-Abs) belong to an Ab family known as rheumatoid factor [RF]. The best known member of this family is the IgM-anti-IgG Ab or the classical RF [1]. The vast majority of studies concerning anti-IgG auto-Abs focused on IgM-RF [2] because of implications for the pathogenesis of rheumatic disease. Besides IgM-RF there are auto-Abs of other isotypes [3, 4]. Moreover, the heterogeneity of anti-immunoglobulins concerns not only the isotype but also the binding epitope. In spite of considerable heterogeneity, all RFs have in common a reactivity with constant sequences of Ig. This feature distinguishes them from anti-idiotypic Abs, which bind to the variable part of the Ig molecule and therefore are highly specific biomolecules [5]. In contrast to anti-idiotypes, RFs in-

teract with a broad range of Ig molecules and this has important functional consequences.

There are several excellent reviews on the classical RF [2, 6, 7]. In this article we aim at describing new members of the anti-Ig Ab family with special focus on their physiological relevance.

## Anti-Ig auto-Abs in Animals

Many experiments in animals demonstrated that the immune system is able to produce Abs against its own Igs. Anti-Ig are second-set Abs, appearing during the immune response in addition to Abs directed against foreign antigen. Milgrom and Witebsky [8] and Aho and Wager [9] first described the occurrence of anti-Ig in rabbits immunized with

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soluble protein antigens and with autologous IgG. Certain animal strains have constitutively increased titers of anti-Ig. Andrews et al. [10] described high IgM-anti-IgG Ab titers in lupus-prone mice strains. In mice as in rabbits, activation of the immune system results in a sharp increase of anti-IgG-producing B cells [11, 12]. A fairly strong although transient anti-IgG response was observed in mice immunized and subsequently boosted with various proteins and with sheep red blood cells. With most antigens, IgM-anti-IgG production equaled or exceeded that of antigen-specific IgM [13]. An analysis of hybridomas derived from mice following immunization with foreign proteins showed no anti-IgG B-cell clones during the primary response but up to 10% producing clones during the secondary response [14]. The occurrence of anti-IgG Abs during the secondary response suggested that antigen-antibody complexes were responsible for the induction of autoreactive antibodies [15]. Nemazee and Sato [16] developed a set of mouse monoclonal Abs which arose after repeated immunization with antigen alone but which bound antibody-antigen complexes. These Abs recognized determinants on the Ab portion of the complexes that were expressed as a consequence of antigen binding. Similar Abs were raised in rabbits [17] indicating that there are Abs which react both with free and immune-complexed IgG.

A series of experiments performed in our laboratory focused on anti-IgG Abs appearing during immunization of rats with alloantigens. It is known that transfusion of prospective recipients of organ transplants with allogeneic blood cells prolongs graft survival both in man and animals [18, 19]. We analyzed the relevant factors responsible for suppression of graft rejection following blood transfusions [19]. In addition to allo-Abs, we found anti-Ig auto-Abs in the serum of rats after repeated blood transfusions with untreated cells, or after one single transfusion with antibody-coated cells [20]. This observation was in line with the above mentioned experiments of van Snick and Coulie [14] and Nemazee and Sato [15] showing that anti-Ig Abs are induced by secondary immunization, or by primary immunization with antibody-antigen complexes.

### Anti-Ig auto-Abs in Man

Do similar Abs also exist in humans? The classical RF was first described in patients with rheumatoid arthritis [21]. Later it became evident that the same Ab is associated with various diseases, including autoimmune conditions, viral or parasitic infections, chronic inflammations, neoplasms, and monoclonal gammopathies [22–24].

Subsequently to the demonstration in rats of anti-Ig Ab induction following alloimmunization [25], it was shown that under similar treatment conditions such Abs also appear in humans. Seven out of 9 individuals immunized twice with allogeneic buffy coat cells demonstrated increased IgG-anti-F(ab')<sub>2</sub> Ab titers [26].

Anti-Ig Abs induced by immunization with foreign antigens were described in several studies. Welch et al. [27] examined healthy adults vaccinated with tetanus toxoid and found a threefold rise in the frequency of IgM-anti-IgG B cells. In a similar study, Birdsall and Rossen [28] immunized volunteers with keyhole limpet hemocyanin. Besides Abs against the antigen, an increased concentration of anti-Fab Abs was demonstrated. The same group as well as other groups [29, 30] found anti-IgG auto-Abs specific for Fab or Fc fragments in cultures of mitogen-stimulated lymphocytes derived from healthy individuals. Naturally occurring serum Abs directed against the F(ab')<sub>2</sub> portion of IgG were also described in healthy persons by Nasu et al. [31].

### Specificity of Anti-Ig Abs

Because of the pathogenetic relevance of RF, previous studies focused mainly on the epitope recognized by the classical RF [32]. In a series of studies, chimeric IgG molecules were constructed by fusing human IgG1–IgG4 constant heavy chain regions to murine variable regions [33]. Binding studies showed that some RFs recognized IgG1, IgG2 and IgG4, but that they reacted only weakly with IgG3. Apparently this was related to two residues (His<sup>435</sup>, Tyr<sup>436</sup>) of the CH3 region [32]. The preferential binding of some RFs to certain IgG subclasses was already described earlier [34]. Later it was shown that many RA-derived RFs bind IgG at a discontinuous epitope comprising residues from both the CH2 and CH3 regions [35]. Studies in Waldenström's macroglobulinemia patients revealed that at least three regions, two from CH2 and one from CH3, contribute to the binding epitope [36]. Amongst a panel of monoclonal RFs, Mageed et al. [37] observed four specificity profiles, namely reactivity with an epitope expressed on (a) all IgG subclasses, (b) IgG1, IgG2, IgG4 and certain IgG3 molecules, (c) IgG1, IgG2 and IgG4, but not IgG3, (d) some but not all IgG within the subclasses. Evidently, there is not one but several RFs.

Less has been known about the epitope of auto-IgG Abs binding to the F(ab')<sub>2</sub> region. The vast majority of previously described anti-F(ab')<sub>2</sub> auto-Abs were of anti-idiotypic nature [38–41]. We made the surprising finding that some anti-F(ab')<sub>2</sub> Abs bound strongly to F(ab')<sub>2</sub> but only weakly to

Fab fragments [42, 43]. Pepsin used for production of  $F(ab')_2$  fragments cleaves the human  $\gamma_1$  heavy chain at the Leu<sup>234</sup>–Leu<sup>235</sup> peptide bond, which results in release of the Fc region but almost complete retention of the hinge region. Conversely, papain used for the production of Fab fragments cleaves IgG1 at His<sup>224</sup>–Thr<sup>225</sup>. Thus, Fab are lacking most part of the hinge region [44]. It follows that an anti- $F(ab')_2$  Ab which binds to  $F(ab')_2$  but not to Fab fragments might recognize an epitope located in the hinge region. In order to verify this hypothesis, a series of hinge region peptides were constructed and analyzed by NMR and CD spectroscopy [42, 45, 46]. Binding studies revealed that the anti- $F(ab')_2$  Ab interacts strongly with hinge peptides having an ordered structure but only weakly with peptides without defined conformation [42]. The core region and the entire lower hinge region were required for full expression of the epitope [43]. These data show that one epitope recognized by the anti- $F(ab')_2$  Ab lies in the hinge region of IgG1. Recent experiments performed in our laboratory suggest the existence of additional epitopes located in the CH1 region of IgG and in the constant part of light chains [unpubl. data]. Apparently,  $F(ab')_2$ -reactive anti-Ig contains several Abs.

### Functional Relevance of Anti-IgG auto-Abs

Early experiments in chicken, mice and rabbits showed that heterologous anti-Ig Abs have strong immunoregulatory, mainly suppressive properties [reviewed in 47]. A typical example was the treatment of mice starting at birth with anti-IgM Abs [48]. Under this treatment the IgM production of the animals decreased to an undetectable level, and the other Ig isotypes were also significantly suppressed. Generally, specific Ab responses to antigens administered during the course of anti-Ig treatment were absent, even in animals having substantial quantities of circulating IgG. Consistent with this induced B-cell deficiency is the observation that spleen cells from treated mice fail to transfer either primary or secondary Ab responses. T-cell functions remain generally normal [48]. Altogether, these data demonstrate that heterologous anti-Ig (i.e. Abs against Ig raised in an another species) are very potent immunoregulatory molecules.

The question we would like to address next is whether, similar to heterologous anti-Ig, autologous anti-Ig Abs are also able to regulate the immune response. A series of clinical studies indicated that this might be the case. In one study, IgG-anti- $F(ab')_2$  auto-Abs were determined in the serum of kidney graft recipients on the day of transplantation

[49]. Patients with high Ab titers had a higher rate of immediately functioning grafts and better kidney graft function 1 year after transplantation than patients with low Ab titers, thus suggesting a role of anti- $F(ab')_2$  Ab in the suppression of graft rejection. The findings confirmed similar results of an earlier smaller study [50]. In another study, IgA-anti-Fab Abs were analyzed in patients with head-and-neck cancer, a disease known to be associated with immune defects [51]. The Ab titer was generally higher in patients than in healthy controls, and the Ab concentration gradually increased with the stage of cancer. Of course, neither the transplant nor the cancer study provide direct evidence for a functional role of anti-IgG Abs. However, if autologous anti-IgG Abs, similar to heterologous anti-Ig, have immunosuppressive properties, one would expect that suppression of the humoral anti-graft response would lead to better graft function, or that tumor progression in head-and-neck cancer patients would be more rapid.

The contribution of autoimmune phenomena to the pathogenesis of AIDS has been the objective of numerous studies. Süsal et al. [52] investigated the relationship between IgG-anti-Fab auto-Abs and the main immunologic feature of AIDS, the decrease of CD4<sup>+</sup> lymphocytes. Patients with AIDS had significantly higher anti-Fab activity than HIV<sup>+</sup> patients without AIDS, HIV<sup>−</sup> patients, or controls. Importantly, a striking inverse association was found between anti-Fab and CD4 cell counts. Interestingly, previous studies described a homologous sequence between gp 120 and the CH1 region of Fab-IgG [53]. Thus, anti-Fab Abs might bind to gp 120 on CD4 lymphocytes and contribute to cell destruction.

Silvestris et al. [54] showed that patients with severe systemic lupus erythematosus have high levels of anti-DNA Abs and low levels of anti- $F(ab')_2$  Abs, while patients with quiescent disease present low levels of anti-DNA Abs and high levels of anti- $F(ab')_2$  Abs. A similar study was conducted by our group in patients with cold agglutination [55, 56], an autoimmune disease caused by anti-erythrocyte auto-Abs. It is known that antierythrocyte auto-Ab-producing B cells are part of the physiologic immune repertoire [57]. Under normal conditions these clones are controlled by suppressive mechanisms. Consequently, only low auto-Ab titers are found in the sera of healthy individuals. Factors that disturb this equilibrium induce an increased production of auto-Abs and lead to autoimmune disease. If in patients with cold agglutination anti- $F(ab')_2$  Ab plays a role in the regulation of auto-Ab production, one would expect high titers of the suppressive anti- $F(ab')_2$  Ab to be associated with low titers of antierythrocyte auto-Ab, and vice versa. Our findings confirmed this inverse correlation [55]. Inter-

estingly, the association existed only in patients with noninfectious disease etiology (mainly patients with chronic lymphoproliferation).

### Mechanism of B-Cell Suppression

An important question is whether, in addition to the clinical data, direct evidence for inhibition of antierythrocyte B cells by IgG-anti-F(ab)<sub>2</sub> Ab can be obtained. Upon polyclonal stimulation in vitro, a certain percentage of peripheral B cells derived from healthy donors produce antierythrocyte auto-Abs. We were able to show that by adding IgG-anti-F(ab')<sub>2</sub> Ab to the cultures the auto-Ab production was suppressed [58]. This result was in line with the results of previous experiments in which we studied the effect of a similar anti-Ig auto-Ab in rats [59, 60]. The Ab inhibited autologous B cells at the concentration of 0.9 pg IgG/10<sup>6</sup> cells! Activity at this minuscule concentration demonstrates that anti-Ig auto-Abs are exquisitely active immunoregulatory molecules.

Interestingly, the Abs exert their suppressive effect only when the B cell's antigen receptor (AgR) is occupied by its ligand. That means that only antigen-occupied B cells are susceptible to suppression. Other activated B cells are 'allowed' to produce their Ab. In addition, we defined a second restriction mechanism of anti-Ig-induced suppression. B cells became refractory to suppression soon after activation, indicating that the regulatory Ab 'selects' the B cells according to their stage of activation. Thirdly, the suppressive effect was exerted only at a certain anti-Ig concentration. Either too little or too much Ab was not effective [60]. These results can be extrapolated to the events normally occurring following immunization with foreign antigens. During an immune response, the anti-Ig titer increases, reaches a maximum, and decreases thereafter. If an optimal anti-Ig concentration were also required in vivo, the anti-Ig Ab would suppress antigen-reactive B cells only for a limited period of time during the course of an immune response.

Further studies showed that the Fc receptor (FcR) of B cells is involved in mediating suppression [60]. It was recognized a long time ago that FcRs may deliver inactivating signals to B cells. The first model of FcR-mediated suppression was advanced by Chan and Sinclair [61], postulating negative signaling by cross-linking of AgR with FcR on the B-cell membrane via antibody-antigen complexes. Subsequent work of the same as well as of other groups confirmed the cross-link mechanism [62–64]. The biochemical basis of the FcR-inhibiting effect has been partially elucidated. It is known that an influx of extracellular Ca<sup>2+</sup> is nec-

essary for B-cell proliferation and differentiation. FcR-AgR cross-linking prevents the influx of extracellular Ca<sup>2+</sup> by closing the plasma membrane Ca<sup>2+</sup> channels [65, 66]. A 13-amino-acid motif in the cytoplasmic domain of FcR  $\gamma$  II is necessary and sufficient for this effect [65, 67]. Tyrosine at residue 309 is phosphorylated upon cross-linking and mutation of this residue aborts the inhibitory effect of FcR  $\gamma$  IIb [65].

Several authors addressed the induction of apoptosis by anti-Ig Abs. There is evidence that AgR-FcR cross-linking accelerates the early entry of mouse B cells into apoptosis [68] and reagents which counteract anti-Ig protect the cells from death. Human lymphoma B cells have been shown to enter apoptosis as a consequence of anti-Ig Ab binding [69], a process strongly enhanced by TGF- $\beta$ <sub>1</sub> [70].

Anti-Ig may also induce a state of dormancy in neoplastic B cells [71]. Apart from their potential therapeutic relevance, this finding provides an explanation for results obtained in patients with cold agglutination, which showed that high titers of anti-F(ab')<sub>2</sub> are associated with low titers of antierythrocyte Abs only in patients with B-cell neoplasia [55].

### Gene Structure of the Antigen-Binding Site of Anti-F(ab')<sub>2</sub> Antibody

In order to better understand the Ab's function and to define the relationship with other members of the anti-IgG Ab family, we studied the genetic structure of the antigen-binding domain of recombinant IgG-anti-F(ab')<sub>2</sub> Abs [72]. Genes encoding anti-F(ab')<sub>2</sub> Abs were obtained from cDNA of B cells derived from a patient with high anti-F(ab')<sub>2</sub> titer by amplifying the variable Ig gene segments by PCR. The recombinant Abs expressed in *Escherichia coli* had a similar binding pattern as the natural IgG-anti-F(ab')<sub>2</sub> Ab. They reacted with F(ab')<sub>2</sub> but not with Fab fragments or intact IgG. Moreover, the recombinant Abs had a reasonably high affinity ( $K_d = 3.6 \times 10^{-8}$  M) and were able to competitively inhibit natural serum Ab. Thus, the binding pattern to F(ab')<sub>2</sub>/Fab fragments clearly differed from that of anti-idiotypic Abs. Idiotypes are common to F(ab')<sub>2</sub>, Fab, and IgG molecules. Consequently, an anti-idiotypic Ab reacts with all three molecules. The lack of binding of anti-F(ab')<sub>2</sub> Ab to intact IgG has important functional implications. Anti-IgG Abs suppress B cells through membrane Ig-mediated signaling. This is possible only if the Abs do not bind to free soluble IgG of the serum, but to IgG expressed on the B-cell membrane. Alternatively, the Abs may bind to antigen-bound IgG (immune complexes) but not to free IgG. This

possibility is supported by experiments demonstrating that some natural anti-IgG Abs preferentially bind to antigen-complexed IgG and selectively suppress B cells whose membrane Ig is occupied by its ligand [60, 73].

The VL chains of recombinant Abs were identical with a germline gene sequence. They had therefore not been generated by antigen-driven affinity maturation. In the case of heavy chains, however, there was only 88% homology with the closest germline gene, suggesting that they were mutated [72].

It is an interesting question whether the genes encoding IgG-anti-F(ab')<sub>2</sub> Ab are related to those of IgM-RF. An extensive computer search revealed no relationship to known RF heavy chain genes. The analysis of light chain gene sequences led to a similar conclusion. Studies of IgM-RF sequences obtained from healthy persons after immunization showed that the variable regions undergo extensive hypermutation [74]. In contrast to Abs against exogenous antigens, there is a strong selection against mutations that result in replacement of amino acids in the hypervariable regions. There was no increase in affinity of RFs that paralleled the accumulation of mutations, suggesting that high-affinity variants are inactivated during the hypermutation process. RFs from immunized healthy donors were compared with those of RA patients. There was a difference in the preferential use of VL and VH gene families between the two groups as well as evidence for affinity maturation in the rheumatoid arthritis but not in the healthy group [75–79].

## Hypothesis

The question arises whether anti-IgG auto-Abs are irrelevant by-products of the immune system or whether they are immunoregulatory molecules. Experimental and clinical data point to a participation of IgG-anti-F(ab')<sub>2</sub> auto-Abs in the suppression of self-reactive peripheral B cells [58]. Accordingly, a certain basic anti-F(ab')<sub>2</sub> Ab titer which is present in all individuals can be thought of as providing a means for continuous suppression of self-reactive B cells. During the immune response, stimulatory cytokines are produced which are capable of activating autoreactive B cells. Concomitantly, anti-F(ab')<sub>2</sub>-producing B cells are activated. Anti-F(ab')<sub>2</sub> Abs prevent the stimulation of autoreactive B cells. In fact, we have shown that supernatants of tetanus-toxoid-activated lymphocytes (a model which simulates the immune response against foreign antigens) are able to stimulate auto-Ab-producing B cells of a healthy person [58]. Importantly, the B cells were suppressed by anti-F(ab')<sub>2</sub> Ab derived from the same donor. Apparently, the

immune system has developed contraregulatory mechanisms that prevent autoaggression. Anti-F(ab')<sub>2</sub> Abs might be one such mechanism. In the light of this hypothesis, there are two ways in which anti-F(ab')<sub>2</sub> Abs may participate in the pathogenesis of autoimmune diseases: either by a decrease of suppressive Ab titer below a critical level, or by a loss of susceptibility to suppression of autoreactive B cells.

The anti-F(ab')<sub>2</sub> Abs which we have referred to herein are not specific for a certain B-cell clone. They recognize common sequences of Ig molecules. Accordingly, the Abs are able to regulate both auto- and alloreactive B cells. Suppression of autoreactive clones has been discussed in the previous paragraph. In addition, the Abs may regulate the response to foreign antigens. Upon immunization with antigen, anti-F(ab')<sub>2</sub> Abs, whose serum concentration increases during the immune response, may have several regulatory functions. First, anti-F(ab')<sub>2</sub> down-regulates a successful response to a certain antigen. Secondly, it blocks the recruitment of new B-cell clones to the same antigen. Thirdly, anti-F(ab')<sub>2</sub> prevents the engagement of B cells in a second response to the same antigen. The biochemical basis of this phenomenon relies on the finding that the AgR of activated B cells remains functionally coupled to intracellular signaling pathways and thereby may participate in a second response subsequent to the initial activation [80].

It has been shown many years ago that under certain circumstances the injection of one antigen suppresses the immune response to another antigen administered a few days later [81], a phenomenon called antigenic competition. Suppressive anti-Ig Abs induced by the first immunization may explain the refractoriness to a second challenge. This fits our observation that repeated short-spaced immunizations with foreign blood cells increased the anti-Ig Ab titer and diminished the Ab response to the foreign antigen [20, 82].

In addition to the studies pointing to a regulatory role of natural IgG-anti-F(ab')<sub>2</sub> auto-Abs, there are experiments arguing for a participation of IgM-anti-IgG Abs in the regulation of the immune response. It has been speculated that RF increases the affinity of another Ab to its antigen [16] and facilitates the clearance of immune complexes by the reticuloendothelial system [83]. IgM-RF has also been shown to protect rat pups from infection with *Trypanosoma lewisi* [84]. The mode of protection is unclear but could be related to IgM-RF binding to IgG-antiparasite Abs, thereby inducing more efficient complement activation. In experiments in mice it was shown that during the immune response to influenza virus IgM-anti-IgG2a auto-Abs are produced in addition to the virus-specific Ab [85]. The auto-Abs were able to suppress the secondary IgG2a Ab response to influenza virus and the response to other antigens.

Another function of anti-Igs has been described by Roosnek and Lanzavecchia [86]. IgM-anti-IgG-producing B cells were shown to participate with high efficiency in antigen presentation [86]. They take up IgG immune complexes via their AgR and present the foreign antigen of the complex to T cells.

In conclusion, the experimental evidence discussed in this review indicates that natural anti-Ig auto-Abs are not

irrelevant by-products but highly potent regulatory molecules of the immune system.

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