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Recovery from anti-VIII:C (antihemophilic factor) autoimmune disease is dependent on generation of antiidiotypes against anti-VIII:C autoantibodies

(autoimmunity/idiotypic network)

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ABSTRACT **Plasma samples obtained from a patient 6 wk, 6 months, and 4 yr after recovery from anti-VIII:C (antihemophilic factor, where VIII:C = factor VIII procoagulant activity) autoimmune disease were found to contain antibodies that inhibited anti-VIII:C activity in the patient's prerecovery plasma and in the plasma of two other patients with anti-VIII:C autoantibodies. $F(ab')_2$ fragments from postrecovery IgG suppressed anti-VIII:C activity in $F(ab')_2$ fragments from prerecovery IgG within a narrow range of molar ratios. Anti-VIII:C activity in $F(ab')_2$ autoantibodies was also inhibited by $F(ab')_2$ fragments from polyspecific therapeutic immunoglobulins prepared from a large pool of normal donors (IVIg). IgG from prerecovery plasma bound to $F(ab')_2$ from postrecovery IgG and to $F(ab')_2$ from IVIg, as assessed by ELISA. Affinity chromatography experiments demonstrated that $F(ab')_2$ from postrecovery IgG preferentially bound anti-VIII:C antibodies among $F(ab')_2$ fragments from prerecovery plasma containing anti-VIII:C autoantibodies. $F(ab')_2$ from prerecovery plasma bound in higher amounts to postrecovery $F(ab')_2$ than to IVIg. Insolubilized $F(ab')_2$ fragments from postrecovery plasma also bound $F(ab')_2$ fragments prepared from the plasma of another patient with anti-VIII:C autoimmune disease, although in lesser amounts than the patient's own prerecovery anti-VIII:C $F(ab')_2$ antibodies. These observations suggest that human anti-VIII:C autoantibodies share idiotypic determinants and that spontaneous recovery from anti-VIII:C autoimmune disease occurs through idiotypic suppression of autoantibodies. In patients who recover from autoimmune disease and in patients in whom autoantibodies have been suppressed by infusions of IVIg, antiidiotypic antibodies, possibly by providing internal images of the antigen, may have shifted the immune system toward the steady-state equilibrium that prevents autoimmunity in normal individuals.**

The occurrence of spontaneous inhibitors to factor VIII procoagulant activity (VIII:C) in nonhemophilic patients is associated with a high incidence of severe bleeding complications (1). Anti-VIII:C autoantibodies may arise in patients with autoimmune disorders, in patients with allergic drug reactions, in women during pregnancy or postpartum, and in apparently healthy individuals (2). The outcome of anti-VIII:C autoimmune disease is unpredictable. Some patients spontaneously recover from the autoantibody, usually within a year following appearance of the inhibitor. In most patients, however, the autoantibody persists over long periods of time, which results in high morbidity and mortality associated with the disease (1). In these patients, anti-VIII:C activity *in vitro* and the autoimmune response to VIII:C *in vivo* may be suppressed by antiidiotypic antibodies present in polyspecific therapeutic IgG prepared from plasma obtained from large numbers of normal donors (3, 4). In the present study, postrecovery plasma of a patient who spontaneously recovered from anti-VIII:C autoimmune disease was found to contain antibodies that inhibited anti-VIII:C activity in the patient's prerecovery plasma and in the plasma of two other patients with anti-VIII:C autoantibodies. Insolubilized $F(ab')_2$ fragments from postrecovery IgG preferentially bound anti-VIII:C antibodies species among prerecovery $F(ab')_2$ fragments containing anti-VIII:C autoantibody activity. These observations suggest that human anti-VIII:C autoantibodies share recurrent idiotypic determinants and that spontaneous recovery from anti-VIII:C autoimmune disease occurs through idiotypic suppression of autoantibodies.

PATIENTS AND METHODS

Patients. Patient Esk. is a 47-yr-old male with no previous history of bleeding who was referred for hematuria that had lasted for 4 wk and for the rapid onset of an extensive hematoma of both lower limbs. Coagulation was grossly abnormal. Activated partial thromboplastin time (APTT) was prolonged and VIII:C was <1% of normal. An anti-VIII:C inhibitor was found with a titer of 42 Bethesda units (BU) (5). No pathological condition associated with the occurrence of anti-VIII:C autoantibodies was found. The patient underwent nine plasma exchanges and was given infusions of large amounts of factor VIII concentrate, which resulted in significant clinical improvement with a measurable amount of VIII:C in plasma. Treatment was then stopped. Four weeks later, VIII:C levels increased to 340% of normal for several weeks and have since remained at normal levels for up to 5 yr now with undetectable anti-VIII:C activity.

Patient Gra. is a woman of 29 who developed an anti-VIII:C autoantibody with a titer of 10,500 BU during her first pregnancy. Patient Bes. is an otherwise healthy 62-yr-old male who spontaneously developed an anti-VIII:C autoantibody with a titer of 25,000 BU. Patient Rich. is a 27-yr-old hemophiliac known to have an inhibitor for 14 yr and classified as a high responder, in whom the inhibitor titer was 200 BU at the time of testing. All anti-VIII:C antibodies were type I (6, 7).

Methods. VIII:C activity was measured in a one-stage assay by the APTT method, with plasma of a severe hemophiliac as substrate. Anti-VIII:C antibody titer was measured by the method of Kasper *et al.* (5) and expressed in BU. Plasma concentrations of IgG were measured by radial immunodiffusion using rabbit anti-human IgG antibodies

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Abbreviations: APTT, activated partial thromboplastin time; BU, Bethesda units; VIII:C, factor VIII procoagulant activity.

(Behringswerke). The IgG fraction from the patients' plasma was prepared by ammonium sulfate precipitation and chromatography on DE-52 cellulose (Whatman) or DEAE-Trisacryl (IBF, Villeneuve la Garenne, France). Polyspecific monomeric IgG for intravenous infusion (IVIg) (Sandoglobulin) prepared from plasma obtained from a large pool of normal donors was a kind gift from Alfred Hässig (Swiss Red Cross, Bern, Switzerland). $F(ab')_2$ fragments that were free of detectable Fc fragments were prepared from the patient's IgG and from IVIg by pepsin digestion (2%, wt/wt) and chromatography on protein A-Sepharose (Pharmacia).

The neutralizing capacity of IgG and $F(ab')_2$ fragments from patient Esk.'s postrecovery plasma and from IVIg on anti-VIII:C activity *in vitro* was examined as follows: plasma, IgG, or $F(ab')_2$ fragments (100 μl) containing anti-VIII:C activity were incubated with 100 μl of phosphate-buffered saline at pH 7.4 (PBS) alone or containing incremental amounts of IgG or $F(ab')_2$ fragments from postrecovery plasmas or from IVIg for 60 min at 37°C and overnight at 4°C. Residual anti-VIII:C antibody titer was measured and neutralizing activity was expressed as % BU inhibited relative to the number of BU in the sample that had been incubated in buffer alone.

Binding of patient Esk.'s postrecovery IgG to prerecovery $F(ab')_2$ antibodies was examined by ELISA. Multiwell plates (Nunc, Roskilde, Denmark) were coated with prerecovery $F(ab')_2$ antibodies (100 μg/ml) in PBS and incubated with PBS containing 0.1% gelatin for 1 hr at room temperature. The plates were washed with PBS containing 0.1% gelatin and 0.05% Tween 20 (Sigma) (PGT) and incubated with increasing concentrations of postrecovery IgG in PGT for 1 hr at room temperature. After four washes with PGT, peroxidase-labeled goat IgG anti-human Fc antibodies (Cappel Laboratories, Cochranville, PA) that had been preadsorbed with human $F(ab')_2$ fragments were added for 1 hr at room temperature before the reaction was revealed with *o*-phenylethyldiamine (Sigma) and H_2O_2.

For affinity chromatography experiments, $F(ab')_2$ fragments prepared from patient Esk.'s IgG obtained 4 yr after recovery were coupled to glutaraldehyde-activated Sepharose (Act-Ultrogel ACA 22, IBF), and $F(ab')_2$ fragments from IVIg were coupled to cyanogen bromide-activated Sepharose (Pharmacia). Columns (0.8 × 5 cm) were equilibrated in PBS and $F(ab')_2$ fragments containing anti-VIII:C activity (5.4–9.2 mg/2.0 ml of PBS) were circulated on the columns for 48 hr at 4°C. The columns were washed with PBS until no protein could be detected in the effluent and then were eluted with 0.2 M borate/0.1 M glycine buffer at pH 2.8. Eluted fractions were immediately adjusted to pH 7.0 with 1.0 M Tris and were assessed for protein content and anti-VIII:C activity. Specific anti-VIII:C activity in the loaded and eluted material was expressed as BU/mg per ml.

RESULTS

The IgG fraction and $F(ab')_2$ fragments were prepared from plasma samples obtained from patient Esk. prior to the first plasma exchange (prerecovery sample) and 6 wk, 6 months, and 4 yr after recovery from anti-VIII:C autoantibody. The IgG fraction obtained from all postrecovery samples neutralized up to 50% of anti-VIII:C activity in the patient's prerecovery plasma following incubation at molar ratios of prerecovery IgG to postrecovery IgG ranging from 1.66 to 12.0. When $F(ab')_2$ fragments from postrecovery samples were incubated with $F(ab')_2$ fragments prepared from the patient's prerecovery plasma, a dose-dependent neutralization of autoanti-VIII:C activity was observed, with maximal inhibition (100%) occurring at molar ratios of prerecovery $F(ab')_2$ to postrecovery $F(ab')_2$ of 4.35, 2.05, and 2.28 for

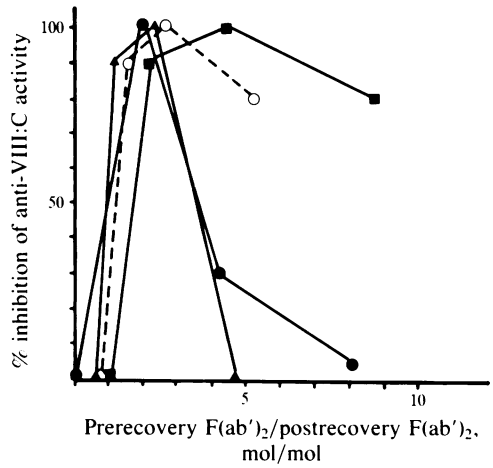

FIG. 1. *In vitro* inhibition of anti-VIII:C activity in $F(ab')_2$ fragments prepared from patient Esk.'s prerecovery plasma following incubation with $F(ab')_2$ fragments prepared from postrecovery plasma samples obtained 6 wk (■), 6 months (●), and 4 yr (▲) after disappearance of the autoantibody and with $F(ab')_2$ fragments prepared from IVIg (○).

samples obtained 6 wk, 6 months, and 4 yr following disappearance of the autoantibody, respectively (Fig. 1). $F(ab')_2$ fragments from IVIg inhibited anti-VIII:C activity in $F(ab')_2$ fragments from prerecovery plasma in a similar fashion. Maximal inhibition (100%) occurred at a molar ratio of prerecovery $F(ab')_2$ to IVIg $F(ab')_2$ of 2.56.

The effect of $F(ab')_2$ fragments obtained from patient Esk.'s postrecovery plasma samples was also examined on anti-VIII:C activity present in $F(ab')_2$ fragments prepared from the plasma of two other patients with anti-VIII:C autoantibody and of a hemophilic patient with anti-VIII:C alloantibody. $F(ab')_2$ fragments from samples obtained 6 wk, 6 months, and 4 yr after recovery inhibited anti-VIII:C activity present in $F(ab')_2$ fragments obtained from the plasma of both patients with autoantibody in a dose-dependent fashion. Molar ratios between $F(ab')_2$ fragments containing anti-VIII:C activity and $F(ab')_2$ fragments prepared from postrecovery samples at which maximal inhibition of autoanti-VIII:C activity was found are shown in Table 1. In contrast, anti-VIII:C activity in $F(ab')_2$ fragments prepared from the plasma of the hemophilic patient with anti-VIII:C alloantibody was not suppressed by $F(ab')_2$ fragments from patient Esk.'s postrecovery plasma samples when tested in a similar range of molar ratios between antibody species.

The capacity of IgG or $F(ab')_2$ fragments prepared from patient Esk.'s postrecovery plasma samples and of IVIg to interact with $F(ab')_2$ autoantibodies containing anti-VIII:C activity was further investigated by using an ELISA and affinity chromatography. By using the ELISA described in *Patients and Methods*, IgGs from patient Esk.'s postrecovery plasma samples and from IVIg were found to bind to patient Esk.'s prerecovery $F(ab')_2$ fragments (Fig. 2). IgG prepared from the plasma obtained 6 wk after recovery bound with higher intensity than that prepared from the plasma obtained 4 yr after recovery and than IVIg. The binding of patient Esk.'s postrecovery IgG to prerecovery $F(ab')_2$ fragments occurred through the antibody-combining site of postrecovery IgG since postrecovery $F(ab')_2$ fragments were found to compete with postrecovery IgG for binding to prerecovery $F(ab')_2$ fragments and since a molar ratio of postrecovery $F(ab')_2$ fragments to postrecovery IgG of 1 resulted in 38%, 40%, and 50% inhibition of the binding of postrecovery IgG for samples obtained 4 yr, 6 wk, and 6 months following recovery, respectively. To demonstrate the presence of antiidiotypic antibody species against anti-

Table 1. Inhibition of anti-VIII:C activity in $F(ab')_2$ fragments prepared from the plasma of patients with anti-VIII:C autoantibody (patients Gra. and Bes.) and of a patient with anti-VIII:C alloantibody (patient Rich.) by $F(ab')_2$ fragments obtained from patient Esk.'s postrecovery plasma and $F(ab')_2$ fragments from IVIg

	$F(ab')_2$ fragments from patient Esk.'s postrecovery plasma							
	6 wk		6 months		4 yr		IVIg $F(ab')_2$	
$F(ab')_2$	mol/mol	% inhibition	mol/mol	% inhibition	mol/mol	% inhibition	mol/mol	% inhibition
Patient Gra.	0.72 (0.36–1.44)	100	0.72 (0.15–2.40)	100	0.39 (0.19–1.52)	100	0.45 (0.45–1.80)	100
Patient Bes.	5.48 (0.67–1.80)	50	1.34 (0.68–10.90)	75	5.12 (1.28–10.24)	54	1.60 (0.4–3.2)	76
Patient Rich.	(0.44–3.52)	0	(0.22–1.76)	0	(0.51–2.04)	0	(0.125–8.00)	0

Indicated are the maximal inhibition of anti-VIII:C activity that was found and the molar ratio between $F(ab')_2$ containing anti-VIII:C activity and $F(ab')_2$ from patient Esk.'s postrecovery plasma or IVIg at which maximal inhibition was achieved. Parentheses indicate the range of molar ratios between anti-VIII:C $F(ab')_2$ and postrecovery $F(ab')_2$ that were tested.

VIII:C autoantibodies within postrecovery IgG, affinity chromatography experiments were performed by using Sepharose-bound $F(ab')_2$ fragments prepared from patient Esk.'s plasma obtained 4 yr after recovery. $F(ab')_2$ fragments from patient Esk.'s prerecovery plasma were circulated on the column before it was washed and eluted at acidic pH. Eluted fractions containing measurable amounts of protein were pooled and screened for anti-VIII:C activity. Specific anti-VIII:C activity was then calculated and expressed as BU/mg per ml. Seven milligrams of protein with a specific anti-VIII:C activity of 0.36 BU/mg per ml was loaded on the column, of which 1.6% could be eluted. The specific activity of the eluted fraction was 28.57 BU/mg per ml and thus increased by 79-fold as compared with the starting material (Table 2). In another experiment, $F(ab')_2$ fragments prepared from the plasma of another patient with anti-VIII:C autoantibody (patient Gra.) were chromatographed on the same affinity column. Eluted material represented 1.23% of the loaded proteins and contained specific anti-VIII:C activity that was 2.7 times that of the starting material. $F(ab')_2$ fragments from patient Gra.'s plasma were also chromatographed on an affinity column of Sepharose to which $F(ab')_2$ from IVIg had been bound. The eluted material represented 5.5% of the loaded protein. The relative increase in specific anti-VIII:C activity was 2.19-fold. When $F(ab')_2$ fragments from patient Rich.'s plasma were chromatographed on Sepharose-bound $F(ab')_2$ from IVIg, 1.16% of the loaded protein were eluted at acid pH, in which no anti-VIII:C activity was detected.

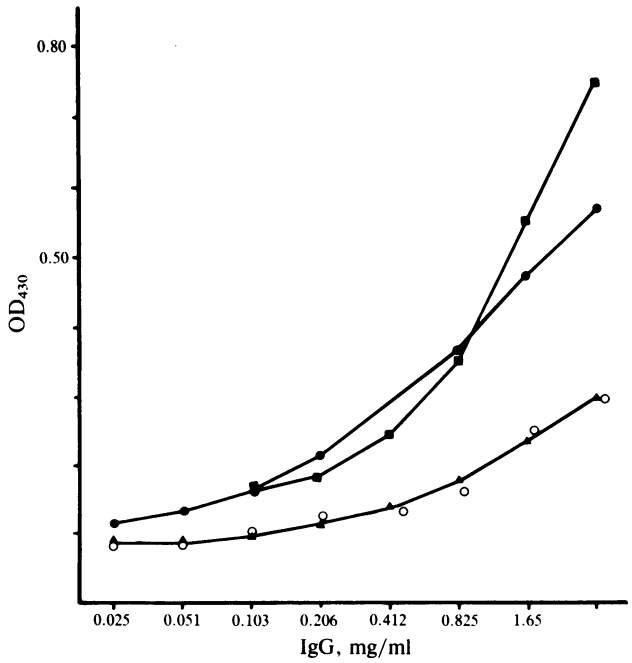

FIG. 2. Binding of IgG prepared from patient Esk.'s plasma obtained 6 wk (■), 6 months (●), and 4 yr (▲) after recovery from autoantibody and of IVIg (○) to insolubilized $F(ab')_2$ fragments obtained from patient Esk.'s prerecovery plasma, as assessed by ELISA.

DISCUSSION

The present study indicates that recovery from anti-factor VIII autoimmune disease in patient Esk. was associated with generation of antiidiotypes against anti-VIII:C autoantibodies. IgG obtained from the patient's plasma 6 wk, 6 months, and 4 yr following disappearance of autoantibody inhibited anti-VIII:C activity in the patient's prerecovery plasma. $F(ab')_2$ fragments prepared from postrecovery plasma samples also inhibited anti-VIII:C activity in $F(ab')_2$ fragments from the patient's prerecovery IgG, indicating that inhibition was mediated by the antibody-combining site of postrecovery IgG. Anti-VIII:C activity in prerecovery $F(ab')_2$ fragments could be totally inhibited by optimal inputs of $F(ab')_2$ fragments prepared from IgG obtained 6 wk and 4 yr following recovery, suggesting that prolonged lack of autoantibody expression in the patient's plasma is associated with persistent autoantibody suppression by antiidiotype. Inhibition of anti-VIII:C activity *in vitro* by antiidiotype was dose-dependent, with maximal inhibition occurring within a relatively narrow range of molar ratios between prerecovery and postrecovery $F(ab')_2$ antibodies. A similar inhibition curve was observed with $F(ab')_2$ fragments from a therapeutic polyspecific immunoglobulin preparation (IVIg), as it had been found *in vitro* for other patients with anti-VIII:C autoantibodies (3).

IgG from postrecovery plasma samples containing suppressive activity against the patient's anti-VIII:C autoantibody bound to insolubilized $F(ab')_2$ fragments from prerecovery IgG, as assessed by ELISA. Binding occurred through the antibody-combining site of postrecovery IgG since ≈50% inhibition of binding was achieved by addition of equimolar amounts of competing $F(ab')_2$ fragments prepared from postrecovery antibodies. Early postrecovery IgG species that bound with the highest affinity to $F(ab')_2$ from prerecovery IgG also exhibited the highest neutralizing capacity toward anti-VIII:C activity since less antibody was required to achieve 100% inhibition of anti-VIII:C activity in prerecovery $F(ab')_2$ fragments. IgG from the patient's plasma obtained 4 yr after recovery bound to prerecovery $F(ab')_2$ with a similar affinity to that of IVIg and resulted in comparable suppression of anti-VIII:C activity. Thus, IgG obtained 4 yr following recovery from autoimmune disease resembles IgG obtained from pooled plasma from normal individuals.

Table 2. Affinity chromatography of $F(ab')_2$ fragments containing anti-VIII:C activity on Sepharose-bound $F(ab')_2$ fragments from postrecovery IgG of patient Esk. and Sepharose-bound $F(ab')_2$ fragments from IVIg

		Loaded		Eluted $F(ab')_2$	
Sepharose-bound $F(ab')_2$	anti-VIII:C $F(ab')_2$	Amount, mg	Specific anti-VIII:C activity, BU/mg per ml	Amount, mg	Specific anti-VIII:C activity, BU/mg per ml
Patient Esk. (postrecovery)	Patient Esk.	7.00	0.36	0.115	28.57
	Patient Gra.	5.67	3.90	0.070	10.71
IVIg	Patient Gra.	5.67	3.90	0.315	8.57
	Patient Rich.	4.80	0.50	0.056	0

Since no information on the specificity of idiotypes recognized by antiidiotypic antibodies could be obtained in ELISA, affinity chromatography experiments were performed to detect the specific presence of anti-VIII:C antibodies within the antibody species recognized by the patient's postrecovery IgG. Less than 2% of $F(ab')_2$ from postrecovery IgG could be eluted at acidic pH from Sepharose-bound prerecovery $F(ab')_2$ fragments. The eluted material was enriched by almost 80-fold in specific anti-VIII:C activity as compared with the initial $F(ab')_2$ preparation. Not all anti-VIII:C antibodies bound with high affinity to the column since $F(ab')_2$ fragments with a specific anti-VIII:C activity similar to that of the loaded material were detected when the column was washed with equilibrating buffer before acid elution. The same affinity column also retained $F(ab')_2$ antibodies from another patient with anti-VIII:C autoimmune disease, patient Gra., in agreement with the observation that postrecovery $F(ab')_2$ antibodies from patient Esk. neutralized anti-VIII:C activity in $F(ab')_2$ antibodies from patient Gra. (Table 1). Thus, similarly to human antithyroid autoantibodies (8), anti-VIII:C autoantibodies share recurrent idiotypic determinants. The existence of additional private idiotopes on anti-VIII:C autoantibodies is indicated by the 17.5-fold higher capacity of postrecovery $F(ab')_2$ antibodies from patient Esk. to enrich his own prerecovery antibodies in specific anti-VIII:C activity as compared with antibodies from patient Gra. (Table 2).

Sepharose-bound $F(ab')_2$ fragments from IVIg were also found to retain autoanti-VIII:C activity. Higher amounts of $F(ab')_2$ from patient Gra. bound to insolubilized $F(ab')_2$ from IVIg than to insolubilized $F(ab')_2$ from patient Esk.'s postrecovery IgG. Thus, IVIg probably contains antiidiotypes reactive with a variety of patient Gra.'s antibody species, which are not found in IgG prepared from the single patient Esk. However, specific anti-VIII:C activity was similarly enriched in material eluted from IVIg $F(ab')_2$ and in that eluted from patient Esk.'s postrecovery $F(ab')_2$; this may suggest that IVIg and patient Esk.'s IgG contain antiidiotypic antibodies that are directed against different idiotopes on anti-VIII:C autoantibodies from patient Gra. Antiidiotypes in patient Esk's postrecovery plasma inhibited anti-VIII:C activity of autoantibodies but not anti-VIII:C activity of an alloantibody from a hemophilic patient (patient Rich.), as it had been found with IVIg (3). Furthermore, whereas 1.16% of $F(ab')_2$ from patient Rich.'s IgG bound with high affinity to $F(ab')_2$ of IVIg, no anti-VIII:C activity was detected in the eluate, indicating that the capacity of antiidiotypic antibodies to inhibit anti-VIII:C activity is correlated with their capacity to adsorb the antibodies on affinity columns and that IVIg contains antiidiotypes against other antibody species than anti-VIII:C antibodies.

Spontaneous recovery from anti-VIII:C autoimmune disease is associated with a modified expression of the immune repertoire that follows the appearance of antiidiotypic antibodies directed against the autoantibodies. This suggests that prolonged down-regulation of autoantibody in patient Esk. is dependent on a suppressive mechanism based on idiotype recognition (9) that modulates expression of anti-VIII:C antibodies. That pooled IgG from normal individuals (IVIg) contains antiidiotypes against anti-VIII:C antibodies suggests the high connectivity of autoantibodies within the immune network (10). In patients who recover from autoimmune disease and in patients in whom autoimmune responses are suppressed by IVIg for prolonged periods of time, antiidiotypic antibodies, possibly by providing a source of internal images of the antigen (10), may have shifted the immune system toward the steady-state equilibrium that prevents autoimmunity in normal individuals (11).

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