Normal Immunoglobulin G (IgG) for Therapeutic Use (Intravenous IgG) Contain Antiidiotypic Specificities against an Immunodominant, Disease-associated, Cross-reactive Idiotype of Human Anti–Thyroglobulin Autoantibodies

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Abstract
Pooled normal polyspecific IgG for therapeutic use (IVIg) contain anti-idiotypes against idiotypic determinants expressed by autoantibodies from patients with a variety of autoimmune diseases. In the present study, antiidiotypes in IVIg are shown to recognize a cross-reactive idiotypic site on human anti–thyroglobulin (TG) autoantibodies, that was defined by heterologous antiidiotypic antibodies, termed anti-T44 antibodies. The T44 idiotypic site is located outside the antibody-combining site of anti–TG autoantibodies. F(ab')2 fragments from anti-T44 antibodies inhibited the binding of IVIg to affinity-purified F(ab')2 anti-TG autoantibodies. Anti-T44 antibodies bound to F(ab')2 fragments of patients' antibodies, which were retained on an affinity column of Sepharose-bound F(ab')2 fragments from IVIg, but not to F(ab')2 fragments from the effluent of the column. The T44 idiotypic site was expressed on antibodies that bound to IVIg from eight of nine patients with autoimmune thyroiditis, but not on IVIg-binding Igs from healthy individuals. A small amount of the T44 idiotypic site was also expressed on the fraction of IVIg that bound to itself upon affinity chromatography. The T44 idiotypic site was cross-reactive between antibodies from patients with autoimmune thyroiditis. Thus, IVIg contain antiidiotypic antibodies directed against an immunodominant disease-associated cross-reactive α-idiotypic site expressed by human anti–TG autoantibodies. These results support the concept that IVIg may be beneficial in selected autoimmune diseases by modulating the function of the idiotypic network. (J. Clin. Invest. 1990 85:620–625.) idiotypes • idiotypic network • autoimmune thyroiditis • autoimmunity • intravenous immunoglobulins

Introduction
Intravenous infusion of pooled normal polyspecific IgG for therapeutic use (IVIg) has resulted in clinical improvement and/or decrease in autoantibody titer in a number of human autoimmune diseases (1–8). Several lines of evidence suggest that IVIg contain antiidiotypes against a variety of autoantibodies from patients with autoimmune diseases and against natural autoantibodies from normal individuals (9): (a) F(ab')2 fragments from IVIg inhibit the binding of autoantibodies to their autoantigens (8, 9a, 10, and 11); (b) F(ab')2 fragments with autoantibody activity are specifically retained on affinity columns of Sepharose-bound F(ab')2 fragments from IVIg (10–12); (c) IVIg contain no antibody specificities against the most common allotypes expressed in the F(ab')2 region of human IgG (11). In the present study, IVIg are shown to share antiidiotypic specificities against human anti–thyroglobulin (TG) autoantibodies with heterologous antiidiotypic antibodies. The idiotypic site recognized by IVIg and heterologous antiidiotypic antibodies is an immunodominant cross-reactive α idiotype expressed on anti–TG autoantibodies from patients with autoimmune thyroiditis. These results further support the concept that IVIg may be beneficial in selected autoimmune diseases by modulating the function of the idiotypic network.

Methods
Patients and Igs. Sera were obtained from nine patients (Dem., Ben., Mou., Tan., Dum., Bar., Cat., and Vau.) with circulating anti–TG autoantibodies and a clinical diagnosis of Hashimoto's disease, and from five healthy individuals (Har., Rou., Ro., and Cav.). The IgG fraction was prepared from serum by chromatography on DEAE Trisacryl (IBF, Villeneuve la Garenne, France). IVIg were Sandoglobulin® (Sandoz Ltd., Basel, Switzerland), a preparation of intact human polyspecific IgG for therapeutic use obtained from a large pool of plasma from normal donors. F(ab')2 fragments were prepared from patients' IgG and from IVIg by pepsin digestion and chromatography on protein A Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). F(ab')2 preparations were free of detectable Fc fragments as assayed by ELISA. Protein concentrations were determined spectrophotometrically using an absorption coefficient of 1.4 for IgG and F(ab')2 at 280 nm.

Affinity chromatography. F(ab')2 fragments containing anti-TG activity were chromatographed on Sepharose-bound F(ab')2 fragments from IVIg as previously described (12). F(ab')2 fragments that were eluted from the columns with 0.1 M glycine pH 2.8 are referred to as "acid eluates" in this paper. The fall-through of the affinity columns is designated as "effluent." Acid eluates were pooled and concentrated by ultrafiltration on an Amicon PM 10 membrane (Amicon, Danvers, MA).

Preparation of rabbit antiidiotypic antibodies directed against human anti–TG antibodies. F(ab')2 fragments from patient Dem.'s IgG that had been affinity-purified on Sepharose-bound human TG (α kind gift from Dr. Olivier, INSERM U 283 Paris) were used as immunogens. Purified TG contained no contaminating IgG, as assessed by SDS PAGE analysis and ELISA. A New Zealand white rabbit was immunized by injecting 150 μg of immunizing F(ab')2 fragments emulsified in complete Freund's adjuvant intradermally at multiple sites. The
rabbit was boosted with 110 μg of F(ab')2 in incomplete Freund’s adjuvant every week for 3 wk. Anti-T44 antiserum was the serum obtained 1 wk after the last booster injection. IgG and F(ab')2 fragments were prepared from anti–T44 antiserum as described above. Anti-T44 IgG and F(ab')2 fragments were sequentially adsorbed on Sepharose-bound human myeloma κ and λ light chains (a kind gift from Dr. F. Danois, Hôpital St Louis, Paris). Sepharose-bound human TG and Sepharose-bound F(ab')2 fragments from IV Ig by batch adsorptions overnight at 4°C. F(ab')2 fragments were also prepared from IgG from normal rabbits.

ELISA. The antiidiotypic activity of anti-T44 antibodies was characterized using an ELISA. 96-well ELISA plates (Central European Biotechnology Laboratories, Angers, France) were coated with the immunizing, affinity-purified anti–TG F(ab')2 fragments from patient Dem. (2 μg/ml), or appropriate control human F(ab')2 fragments overnight at 4°C. Uncoated sites were blocked with 1% gelatin (Gibco Laboratories, Grand Island, NY) in PBS for 1 h at 20°C and the plates were washed with PBS. Increasing amounts of anti-T44 IgG in PBS-gelatin were added to wells for 2 h at 20°C. After four washes with PBS, bound IgG was revealed using peroxidase-labeled goat anti-rabbit Fc antibodies (Cappel Laboratories, Cochranville, PA) that had been extensively adsorbed with purified human F(ab')2 fragments.

Competitive binding experiments were performed by incubating increasing concentrations of anti-T44 F(ab')2 fragments or normal rabbit F(ab')2 fragments with 1 mg/ml of IV Ig in PBS-gelatin for 2 h at 20°C in microtiter wells coated with affinity-purified anti–TG F(ab')2 fragments from patient Dem. After washing the plates with PBS containing 0.1% Tween 20 (Sigma Chemical Co., St Louis, MO), bound IV Ig was revealed using peroxidase-labeled goat anti-human Fc gamma antibodies (Biosys, Compiègne, France). Inhibition of the binding of IV Ig was calculated as follows:

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\left[1 - \frac{\text{IV Ig bound in the presence of competitor}}{\text{IV Ig bound in the absence of competitor}}\right] \times 100.
\]

Results

Specificity of anti-T44 antiidiotypic antibodies. The antiidiotypic specificity of anti-T44 IgG was determined by assessing the relative ability of the antibody to bind to immunizing anti–TG F(ab')2 autoantibodies and to control F(ab')2 antibodies. Anti-T44 IgG was first adsorbed with human myeloma κ and λ chains. As shown in Fig. 1, adsorbed anti-T44 IgG strongly bound to the immunizing F(ab')2 fragments from patient Dem. that had been affinity-purified on Sepharose-bound human TG, whereas it weakly bound to patient Dem.’s F(ab')2 fragments from the effluent of the TG affinity column and to F(ab')2 fragments from a normal individual with no detectable anti–TG antibodies. Fig. 2 shows that anti-T44 IgG that had been adsorbed with human TG and IV Ig bound to patient Dem.’s affinity-purified F(ab')2 anti–TG antibodies but exhibited no binding capacity for F(ab')2 fragments from IV Ig. Affinity-purified anti–TG F(ab')2 did not contain anti-rabbit Fc gamma activity as assessed by the lack of ability of normal rabbit IgG that had been preadsorbed with human F(ab')2 to bind to insolubilized anti–TG F(ab')2 from patient Dem. (data not shown). Anti-T44 IgG adsorbed with TG and IV Ig did not bind to patient Dem.’s F(ab')2 fragments from the effluent of the TG affinity column. Thus, adsorbed anti-T44 IgG specifically recognizes F(ab')2 fragments from patient Dem., which exhibit anti-TG activity.

Adsorbed anti-T44 F(ab')2 fragments did not inhibit the binding of patient Dem.’s IgG to insolubilized human TG, indicating that anti-T44 antibodies are preferentially directed against idiotypic determinants located outside the antibody-combining site of patient Dem.’s anti–TG autoantibodies (data not shown).

Shared antiidiotypic specificities between anti-T44 IgG and IV Ig. IV Ig contains antibody species that recognize human anti–TG autoantibodies (10). Preliminary experiments demonstrated that IV Ig bound to affinity-purified anti–TG F(ab')2 fragments from patient Dem. in a dose-dependent manner (data not shown). The experiments depicted in Figs. 3 and 4 indicate that rabbit anti-T44 IgG share antiidiotypic specificities with IV Ig. Fig. 3 shows that anti-T44 antiidiotypic F(ab')2 antibodies exhibited an ~10-fold higher capacity to inhibit the binding of IV Ig to affinity-purified anti–TG F(ab')2 antibodies from patient Dem. than normal rabbit F(ab')2. In

Figure 1. Antiidiotypic specificity of rabbit anti-T44 IgG. Anti-T44 IgG was adsorbed with human κ and λ chains and assessed for its ability to bind to affinity-purified patient Dem.’s F(ab')2 anti–TG antibodies (●), patient Dem.’s F(ab')2 fragments with no anti–TG specificity (○), and F(ab')2 fragments from patient Dem. after the binding of IV Ig to affinity-purified anti–TG antibodies (△), using an ELISA.

Figure 2. Antiidiotypic specificity of rabbit anti-T44 IgG. Binding of anti-T44 IgG that had been adsorbed with human TG and IV Ig to affinity-purified F(ab')2 anti–TG antibodies from patient Dem. (●) and F(ab')2 from IV Ig (○).
Anti-T44 IgG and IVIg recognize a cross-reactive idiotypic expressed by anti-TG autoantibodies from patients with Hashimoto’s disease. F(\(ab')_2\)) fragments from IgG of nine patients with Hashimoto’s disease were chromatographed on Sepharose-bound F(\(ab')_2\) fragments from IVIg. The appearance of the T44 idiotype was assessed in the acid-eluted fractions and in the effluent of the columns. As shown in Fig. 5, acid-eluted F(\(ab')_2\) fragments from eight of nine patients were recognized by antidualtopytic anti-T44 IgG, whereas F(\(ab')_2\) fragments in the effluents of the columns did not express the T44 idiotype. Acid-eluted F(\(ab')_2\) fragments from patients’ IgG did not contain detectable antibodies against rabbit Fc fragments. Thus, IVIg contain antidualtopytic antibodies directed against a cross-reactive idiotype expressed by eight of nine anti-TG autoantibodies from patients with Hashimoto’s disease, which is also recognized by anti-T44 IgG. In contrast, F(\(ab')_2\) fragments of IgG from normal individuals that bind to IVIg-Sepharose did not express the T44 idiotype (Fig. 6). As shown in the figure, IVIg (i.e., a large pool of IgG from normals) contained antibody species expressing the idiotype recognized by both IVIg and anti-T44 IgG.

Cross-reactivity between patients’ autoantibodies expressing the T44 idiotype was further investigated in the following experiment: F(\(ab')_2\) fragments from patient Cai.’s IgG were chromatographed on Sepharose-bound F(\(ab')_2\) fragments from IVIg. Acid-eluted F(\(ab')_2\) fragments expressed relatively high amounts of the T44 idiotype (Fig. 5). The fragments were insolubilized on ELISA plates. The relative ability of F(\(ab')_2\) fragments from IVIg of three other patients and from IVIg that had been acid-eluted from IVIg-Sepharose to inhibit the bind-

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**Figure 3.** Inhibition of the binding of IVIg to insolubilized affinity-purified anti-TG F(\(ab')_2\) autoantibodies from patient Dem. by rabbit anti-T44 F(\(ab')_2\) fragments (●) and F(\(ab')_2\) fragments from normal rabbit IgG (○).

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**Figure 4.** Binding of anti-T44 IgG to F(\(ab')_2\) fragments from patient Dem.’s IgG that had been acid-eluted from Sepharose-bound F(\(ab')_2\) from IVIg (●), F(\(ab')_2\) fragments from the effluent of the IVIg affinity column (○), unchromatographed F(\(ab')_2\) fragments from patient Dem.’s IgG (□), and F(\(ab')_2\) from IVIg (▲). ELISA plates were coated with 40 \(\mu\)g/ml of F(\(ab')_2\) fragments.

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**Figure 5.** Anti-T44 IgG and IVIg recognize a cross-reactive idiotype on anti-TG autoantibodies. F(\(ab')_2\) fragments from IgG of nine patients with anti-TG antibodies were chromatographed on Sepharose-bound F(\(ab')_2\) fragments from IVIg. The acid eluted F(\(ab')_2\) fragments (●) and F(\(ab')_2\) fragments in the effluent (▲) were assessed for their ability to bind anti-T44 IgG. The figure shows the maximal binding of anti-T44 IgG to F(\(ab')_2\) fragments that was achieved. Normal rabbit IgG did not bind to insolubilized F(\(ab')_2\) fragments.
of anti-T44 IgG to insolubilized patient Cai.'s F(ab')2 fragments was then assessed. As shown in Fig. 7, F(ab')2 fragments from IVIg and F(ab')2 fragments from the three patients' IgG inhibited the binding of anti-T44 IgG to patient Cai.'s F(ab')2 fragments in a dose-dependent fashion. Thus, autoantibodies from patients with Hashimoto's disease and some antibody species in IVIg express a cross-reactive idiotype recognized by anti-T44 antibodies and antiidiotypes in IVIg.

Discussion

IVIg contain antiidiotypes against idiotypic determinants expressed by autoantibodies from patients with a variety of autoimmune diseases, including anti-Factor VIII, anti-DNA, anti-TG, antiintrinsic factor, and antiperipheral nerve (9a) autoantibodies (8, 10, and 11). The present study demonstrates that antiidiotypes in IVIg directed against anti-TG autoantibodies recognize an immunodominant α idiotype shared by autoantibodies from patients with autoimmune thyroiditis and not found on antibodies from individually tested healthy subjects.

We prepared rabbit antiidiotypic antibodies (anti-T44 antibodies) that exclusively bound to affinity-purified anti-TG autoantibodies from a patient (Dem.) with autoimmune thyroiditis. Anti-T44 antibodies did not inhibit the binding of the patient's autoantibodies to human TG, indicating that the immunodominant idiotype recognized by the antiserum is an α idiotype, located outside the antibody-combining site of the patient's anti-TG autoantibodies. Anti-T44 antibodies that had been adsorbed with IVIg and TG did not bind to the fraction of patient's antibodies that was devoid of anti-TG activity, nor to F(ab')2 fragments from normal individuals and F(ab')2 fragments from IVIg.

Two lines of evidence indicated that idiotypic determinants within the T44 idiotype are recognized by antiidiotypes in IVIg: (a) F(ab')2 fragments from anti-T44 antibodies inhibited the binding of IVIg to affinity-purified F(ab')2 autoantibodies with anti-TG activity, indicating that anti-T44 antibodies and antiidiotypes in IVIg compete for the binding to idiotypic determinants expressed by the patient's anti-TG autoantibodies; (b) Anti-T44 antibodies bound to F(ab')2 fragments of the patient's antibodies that were retained on an affinity column of Sepharose-bound F(ab')2 fragments from IVIg. Inasmuch as anti-T44 antibodies did not bind to unchromatographed antibodies from the patient, the expression of the T44 idiotype appeared to be associated with the patient's antibody species that were recognized by antiidiotypes in IVIg and that were enriched in the acid-eluted fraction from the IVIg affinity column. Thus, IVIg and anti-T44 antibodies recognize the same idiotypes on anti-TG autoantibodies from patient Dem.

The idiotype recognized by anti-T44 antibodies and by IVIg was found to be expressed by antibodies from eight of nine patients with autoimmune thyroiditis, but not by antibodies from healthy individuals. Thus, the T44 idiotype was detected in the fraction of patients' F(ab')2 fragments that was retained on affinity columns of IVIg, but was not detected in patients' F(ab')2 fragments from the effluent of the columns, nor in F(ab')2 fragments from normal individuals that bound to IVIg. Anti-TG autoantibodies from patients with autoimmune thyroiditis differed in their relative ability to inhibit the binding of anti-T44 IgG to anti-TG F(ab')2 antibodies from one of the patients that had been affinity-purified on IVIg. The lack of retention of detectable T44-positive IgG upon affinity chromatography of normal IgG on IVIg indicates that if normal IgG contains some T44-expressing antibodies, these would be present in very low amounts.

Antiidiotypes to Anti-Thyroglobulin Autoantibodies
It is possible that autoantibodies from different patients differ in the relative amount of expressed cross-reactive idio-
type. Alternatively, differences in the ability of the patients' 
autoantibodies to inhibit the binding of anti-T44 to a specific 
anti–TG autoantibody could reflect the presence in patients' 
IgG of other antibody species that are involved in idiotypic–
antiidiotypic interactions with IVlg. In any case, the exper-
iment indicated that anti–TG autoantibodies from different 
patients express similar and/or overlapping idiotypes that are 
part of the cross-reactive T44 idotype.

IgG from normal individuals and IVlg contain anti-TG 
activity (10, 13). The finding of a specific expression of the T44 
idotype on patients' autoantibodies, but not on antibodies 
from normal subjects, suggest that T44 could be an antigenic 
marker of disease-associated autoantibodies. Cross-reactive 
idiotypes have previously been found on anti–TG autoanti-
bodies from patients with autoimmune thyroiditis (14, 15) that 
were not expressed by antibodies lacking anti-TG specificity 
(14). Some of the cross-reactive idiotypes of anti–TG antibody 
were associated with the antibody-combining site of the 
autoantibodies (14). Expression of a cross-reactive idotype has 
been found on autoantibodies to rat TG of 67% of individual 
animals from the Buffalo strain of rats with spontaneous au-
toimmune thyroiditis (16). Idiotype cross-reactivity has also 
been observed between mouse monoclonal antibodies recogn-
izing highly conserved epitopes of human TG (17). In addi-
tion, spontaneous autoantibodies to TG from rats share an 
interspecies cross-reactive idotype (18) and human autoanti-
bodies from patients with Hashimoto's disease recognize 
interspecies cross-reactive epitopes (19). The finding in IVlg of 
idiotypic antibodies against a disease-associated idotype on anti–TG 
autoantibodies, suggests that the antiidiotypic repertoire of 
IVlg includes antibodies against potential targets for therapeu-
tic immunomodulation. The results also suggest that T44-
expressing anti–TG autoantibodies are clones that expand in 
patients with autoimmune thyroiditis and that may be 
downregulated by specific idiotypic antibodies in healthy individuals.

IVlg are pools of IgG from large numbers of healthy donors. The presence in IVlg of detectable antiidiotypic activity 
against autoantibodies is probably the consequence of addi-
tive contributions of antiidiotypic specificities from normal 
individuals (8). In this context, the probability of finding an-
idiotypic activity against cross-reactive idiotypes is higher 
than that of antiidiotypes against private idiotypic determin-
ants. An interesting observation was that of a small amount 
of T44 idotype expressed in IVlg, which may reflect the coexis-
tence of idotype and antiidotype in the pool. The idotype 
could be expressed on Ab1 or Ab2 antibodies of IgG from a 
fraction of donors.

Several mechanisms have been postulated to explain the 
beneficial effect that has been seen with IVlg in selected au-
toimmune diseases, including inhibition of the clearance of 
autoantibody-coated target cells through Fc receptor blockade 
(20), induction of specific suppressor T cells (21), and antiidio-
typic suppression of autoantibodies (5, 8, 9a, and 22). The 
finding in IVlg of antiidiotypes against cross-reactive, disease-
associated idiotypes of autoantibodies support the hypothesis 
that IVlg could be effective by antiidiotypic suppression and 
by partially restoring defective connectivity within the idio-
typic network of patients with autoimmune diseases.

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References

1. Imbach, P., V. d'Apuzzo, C. Baumgartner, A. Hist, A. Morell, E. 
Rossi, M. Schönli, M. Vest, and H. P. Wagner. 1981. High dose intrave-
nous gammaglobulin for idiopathic thrombocytopenic purpura in 
2. Pollack, S., C. Cunningham-Rundles, E. M. Smithwick, S. Ba-
randun, and R. A. Good. 1982. High-dose intravenous gammaglobu-
1985. Successful response to intravenous immunoglobulin in autoim-
A. Tabillo, and C. Lacombe. 1983. Treatment of pure red cell aplasia 
382.
5. Mc Guire, W. A., H. H. Yong, E. Bruno, J. Brandt, R. Bridell, 
pure red cell aplasia with high dose intravenous gammaglobulin. N. 
6. Gadjos, P. H., H. Outin, D. Elkharrat, D. Brunel, P. de Rohant-
Chabot, J. C. Raphaël, M. Goulon-Goeau, and E. Morel. 1984. High-
dose intravenous immunoglobulin for myasthenia gravis. Lancet. 
7. Vermeulen, M., F. G. A. Van Der Meche, J. D. Speelman, A. 
Weber, and H. F. M. Busch. 1985. Plasma and gammaglobulin infu-
326.
8. Sultan, Y., M. D. Kazatchkine, P. Maisonneuve, and U. E. 
Nydegger. 1984. Anti-idiotypic suppression to autoantibodies of Fac-
tor VIII by high-dose intravenous gammaglobulin. Lancet. ii:765–768.
types against autoantibodies in normal immunoglobulins: evidence for 
Rev. 110:135–149.
10. Van Doorn, P. A., A. Brand, F. Rossi, M. Vermeulen, and 
M. D. Kazatchkine. 1989. On the mechanism of high dose intravenous 
immunoglobulin treatment of patients with chronic inflammatory de-
autoantibodies in pooled normal human polyspecific immunoglobu-
12. Rossi, F., Y. Sultan, and M. D. Kazatchkine. 1988. Anti-idio-
types against autoantibodies and alloantibodies to VIII:C anti-
hemophilic factor) are present in therapeutic polyspecific normal immu-
from anti-VIII:C (anti-hemophilic factor) autoimmune disease is 
dependent on generation of anti-idiotypes against anti-VIII:C autoanti-
occurring antibodies against nine common antigens in human sera. I. 
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