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Anti-idiotypes against anti-neutrophil cytoplasmic antigen autoantibodies in normal human polyspecific IgG for therapeutic use and in the remission sera of patients with systemic vasculitis

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SUMMARY

Anti-neutrophil cytoplasmic antigen (ANCA) activity was inhibited in 15 out of 21 sera from patients with acute systemic vasculitis following incubation with normal polyspecific IgG for therapeutic use (IVIg). ANCA antibodies reacted with IVIg through idiotypic–anti-idiotypic interactions, as shown in competitive binding assays using $F(ab')_2$ fragments from IVIg and affinity chromatography of ANCA IgG on Sepharose-bound $F(ab')_2$ fragments from IVIg. Co-incubation of sera from patients with acute systemic vasculitis with paired autologous remission stage sera also resulted in inhibition of ANCA activity in acute sera. Remission sera contain IgM and IgG capable of interacting with β and/or α idiotypes of ANCA IgG from acute sera. Anti-idiotypic IgM may account for the lack of expression of ANCA activity in whole serum from patients in remission from systemic vasculitis, which were found to contain high titres of ANCA IgG. These observations suggest that remission of systemic vasculitis is associated with the generation of anti-idiotypes against autoantibodies rather than the suppression of production of ANCA autoantibodies. IVIg may modulate the activity of systemic vasculitis *in vivo*.

Keywords Systemic vasculitis idiotypes intravenous immunoglobulins autoimmunity

INTRODUCTION

Sera from patients with systemic vasculitis contain IgG autoantibodies directed against antigens of neutrophil cytoplasm (ANCA) (van der Woude *et al.*, 1985; Falk & Jennette, 1988; Nölle *et al.*, 1989). ANCA activity is not detected, or is present at a lower level in the serum of patients in remission from acute vasculitis.

Therapeutic preparations of normal polyspecific IgG (IVIg) contain anti-idiotypic antibodies capable of neutralizing autoantibody activity in serum and in purified IgG or $F(ab')_2$ fragments obtained from patients with a variety of autoimmune diseases. Thus, IVIg contain anti-idiotypes against anti-factor VIII (Sultan *et al.*, 1984; Sultan, Rossi & Kazatchkine, 1987; Rossi, Sultan & Kazatchkine, 1988) anti-DNA, anti-thyroglobulin, anti-intrinsic factor (Rossi & Kazatchkine, 1989; Dietrich & Kazatchkine, 1990) and anti-peripheral nerve (Van Doorn *et al.*, 1990) autoantibodies from pathological sera. IVIg also contain anti-idiotypes against natural autoantibodies secreted by Epstein–Barr virus (EBV) transformed peripheral blood B cells from healthy individuals (Rossi *et al.*, 1990). The evidence for the presence of anti-idiotypes against autoantibodies in IVIg includes (Rossi, Dietrich & Kazatchkine, 1989) the dose-dependent neutralization of autoantibody activity in $F(ab')_2$ fragments from autoantibodies by $F(ab')_2$ fragments from IVIg; the specific retention of autoantibody activity on affinity columns of Sepharose-bound $F(ab')_2$ fragments from IVIg; the lack of anti-allotypic activity in IVIg against the commonest allotypes expressed in the $F(ab')_2$ region of human IgG; and the presence in IVIg of anti-idiotypes against idiotypic determinants expressed on autoantibodies defined by heterologous anti-idiotypic antibodies (Dietrich & Kazatchkine, 1990).

The present study demonstrates that IVIg contain anti-idiotypes against ANCA. Our results also indicate that remission sera from patients with systemic vasculitis contain anti-idiotypes that recognize ANCA autoantibodies in acute autologous sera. The anti-idiotypes are predominantly of the IgM isotype. These observations emphasize further the analogy that may be drawn between therapeutic suppression of autoantibodies with IVIg and spontaneous regulation of autoantibody activity by anti-idiotypes in autoimmune diseases.

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MATERIALS AND METHODS

Patients

Sera from 21 patients with active, ANCA-positive, systemic vasculitis were collected and stored in aliquots at $-20°C$. The clinical diagnosis was systemic vasculitis in 10, microscopic polyarteritis in eight and undefined in three. Paired ANCA-positive acute sera and ANCA-negative remission sera were obtained in seven cases. Remission was defined according to the criteria of Fauci *et al.* (1983), as complete remission with no evidence of active disease and appropriate reduction in the erythrocyte sedimentation rate. The mean time between acute and remission sera samples were 9 weeks (range 3–13). Normal serum was taken from whole blood donations of healthy non-laboratory controls.

Preparation of IgG $F(ab')_2$ fragments from IgG, and IgM

IgG fractions were prepared from serum samples by chromatography on DEAE–Trisacryl (IBF, Villeneuve la Garenne, France). $F(ab')_2$ fragments were prepared from IVIg and from patients' IgG by pepsin (Sigma Chemicals Corp., St Louis, MO) digestion (2% w/w) and chromatography on Protein A–Sepharose (Pharmacia, Uppsala, Sweden). $F(ab')_2$ fragments were free of detectable Fc fragments as assessed by ELISA. IgG in plasma was quantified by radial immunodiffusion using monospecific antiserum. IgM was purified from remission sera of two patients with Wegener's granulomatosis (patients A and B) by low ionic strength precipitation and chromatography on protein A Sepharose. IgM from serum A contained no detectable contaminating IgG. IgM from serum B contained 4% (wt/wt) IgG.

Quantification of ANCA activity

ANCA activity was determined in serum by radioimmunoassay and/or immunofluorescence (Lockwood *et al.*, 1987; Savage *et al.*, 1987). For the radioimmunoassay, microtitration plates (CML, Nemours, France) were coated with 15 μg/ml of a preparation of cytoplasmic neutrophil antigen (Lockwood *et al.*, 1987) in phosphate-buffered saline (PBS) pH 7·4, for 1 h at 37°C and extensively washed with PBS containing 0·1% gelatin (w/vol) (Sigma) and 0·05% Tween 20 (vol/vol) (Sigma) (PBS-G-T). The plates were incubated with PBS containing 0·1% gelatin for 1 h at room temperature before addition of the test sera diluted 1/16 in PBS. The plates were then incubated for 1 h at 37°C, washed with PBS-G-T and sequentially incubated with an appropriate dilution of mouse monoclonal anti-human IgG (Unipath, Basington, UK) in PBS-G-T and with ^{125}I labelled goat anti-mouse IgG antibodies (Sigma) (3 μCi/μg). After washing the plates with PBS-G-T, individual wells were counted using a Packard Cristal II gamma counter (Packard, Pangbourne, UK). Results of the radioimmunoassay were expressed as per cent of a standard positive serum. For immunofluorescence measurement of ANCA activity, neutrophils were separated (Lockwood *et al.*, 1987) and resuspended at 2×10^6 cells/ml in RPMI 1640 medium (GIBCO, Paisley, UK). Cytocentrifuge preparations (Shandon Cytospin 2) were made on glass microscope slides, air-dried and fixed in absolute ethanol for 5 min at 4°C. Test and control sera diluted 1/20 in PBS (50 μl) were overlaid on the slides for 1 h at room temperature. After three washes in PBS, bound antibody was detected using a FITC-conjugated rabbit anti-human immunoglobulin antiserum (Dakopatts, Copenhagen, Denmark). The slides were dried, mounted in 90% glycerol and viewed under U.V. light. Samples were scored 'blind' for bright cytoplasmic fluorescence on a scale from ++ to − (Savage *et al.*, 1987).

ANCA activity in the IgG fraction from plasma, $F(ab')_2$ fragments and in the IgM fraction was quantified by ELISA. Coating of microtitration plates with antigen was performed as described for the ANCA radioimmunoassay. Plates were incubated with IgG or $F(ab')_2$ fragments appropriately diluted in PBS-G-T for 1 h at 37°C. Bound antibody was revealed using either peroxidase-labelled goat anti-human Fcγ antibodies (Biosys, Compiège, France), peroxidase-labelled goat anti-human Fc μ antibodies (Sigma) or biotinylated goat anti-human $F(ab')_2$ antibodies (Cappel, Cochranville, PA) and peroxidase-labelled streptavidine (Amersham International, Amersham, UK). Anti-human Fcγ and anti-human Fc μ antibodies did not react with human $F(ab')_2$ fragments as assessed by ELISA.

Inhibition of ANCA activity

Inhibition of ANCA activity by IVIg, patients' IgM, IgG, $F(ab')_2$ from IgG and patients' sera was assessed by incubating serial dilutions of inhibitor in PBS with a fixed dilution of ANCA-positive sera or a fixed amount of patients' IgG for 1 h at 37°C and 16 h at 4°C. After completion of the incubations, residual ANCA activity was measured. Concentrations of patients' IgG used in the tests were those from the top of the titration curve in the ANCA assay. Inhibition of ANCA activity was expressed as percent of reduction of OD in the sample incubated in the presence of inhibitor relative to OD in the sample incubated with buffer alone.

Affinity chromatography experiments

$F(ab')_2$ fragments from IVIg or from IgG prepared from remission sera were coupled to cyanogen-bromide-activated Sepharose (Pharmacia) (Sultan *et al.*, 1987). Columns (4 ml) of Sepharose-bound antibodies were equilibrated with PBS. IgG preparations containing ANCA activity were loaded on the columns and circulated for 18 h at 4°C. The columns were washed with PBS until no protein was found in the fall-through and then eluted with glycine–HCl, 0·1 M pH 2·8. Protein content and ANCA activity were measured in the loaded material and in the eluted fractions. Specific ANCA activity in the samples was calculated by dividing the OD values measured at 491 nm in the ANCA ELISA, by μg of protein.

RESULTS

Inhibition of anti-neutrophil cytoplasmic activity by IVIg

Incubation of patients' sera with $F(ab')_2$ fragments from IVIg resulted in inhibition of ANCA activity in the serum of 15 out of 21 patients with systemic vasculitis (Fig. 1). Inhibition of ANCA activity by IVIg was dose dependent. Inhibition curves tended to be bell-shaped as shown for at least for one of two representative patients in Fig. 2. Maximal inhibition range was 25–77% of ANCA activity. Maximal inhibition of autoantibody activity occurred at a specific molar ratio between a patients' IgG and $F(ab')_2$ from IVIg for each patient. Inhibition of ANCA binding was considered significant when residual activity, in ct/min, was more than 15% less (i.e. below 85%) of pre-incubation binding (ct/min) (Savage *et al.*, 1987). Similar inhibition curves were obtained when purified IgG from patients' acute sera were

interacted with $F(ab')_2$ fragments from IVIg (Table 1). $F(ab')_2$ fragments from IVIg contained no detectable ANCA activity within the range of concentrations used in the inhibitory assays.

Affinity chromatography of IgG containing ANCA activity on Sepharose-bound IVIg

Purified IgG from the serum of two patients with active systemic vasculitis were chromatographed on Sepharose-bound $F(ab')_2$ fragments from IVIg. The specific ANCA activity of the loaded material was 1·12 and 2·33 OD/μg for patients B and A, respectively. Acid elution of the columns yiuelded 11·7% and 7·0% of the loaded proteins. The specific ANCA activity in the acid-eluate was enriched 4·83- and 4·90-fold, as compared with that of the loaded IgG for patients A and B, respectively (Table 2).

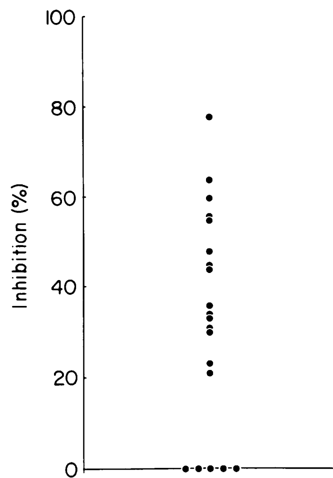

Fig. 1. Inhibition of ANCA activity in whole serum of patients with systemic vasculitis by $F(ab')_2$ from IVIg. Serum (50 μl) was incubated with increasing concentrations of $F(ab')_2$ fragments from IVIg ranging from 400 μg/ml to 12·5 mg/ml for 2 h at 37 C and overnight at 4 C. Residual ANCA activity was measured by radioimmunoassay. Ct/min in the radioimmunoassay of serum samples incubated with buffer alone ranged between 6000 and 8000. Background ct/min values in the assay were $\leq$400. Results are expressed as per cent inhibition of ANCA activity in the serum incubated with buffer alone. Maximal inhibition of ANCA activity that was observed for each serum tested. Maximal inhibition of autoantibody activity occurred at a specific molar ratio between patient IgG and $F(ab')_2$ from IVIg for each serum.

Inhibition of anti-neutrophil cytoplasmic antigen activity in acute sera from patients with systemic vasculitis by serum of patients in remission

Co-incubation of the serum of patients with active systemic vasculitis with autologous serum obtained from the patients in remission resulted in dose-dependent inhibition of ANCA activity in acute serum (Table 3). Inhibition of ANCA activity was observed in five out of seven pairs of sera examined. In the five sera in which inhibition was observed, the inhibition curve was bell shaped. Maximal inhibition was observed at specific molar ratios between prerecovery IgG and remission IgG ranging from 0·125 to 2·000. No ANCA activity was detectable in remission sera. Figure 3 indicates that similar results were obtained when the inhibitory activity of remission on ANCA activity of the acute serum was tested by ELISA and by a conventional indirect immunofluorescence assay. A complex pattern of inhibition is seen (Fig. 3): inhibition of autoantibody activity increased in the reaction mixture as the relative amount of acute to remission serum decreased. Inhibition of ANCA activity subsequently occurred, at low inputs of remission serum.

Inhibition of ANCA activity in IgG from acute serum by remission IgM and IgG

Inhibition experiments described above with the serum of patients in remission could not be reproduced with purified remission IgG or $F(ab')_2$ fragments because of the presence of a relatively high ELISA-reactive ANCA activity in purified IgG and $F(ab')_2$ fragments from remission sera, as assessed by ELISA. ANCA activity was present in IgG and $F(ab')_2$ from patients in remission although it was not detected in whole remission serum tested under the same conditions (Fig. 4). The

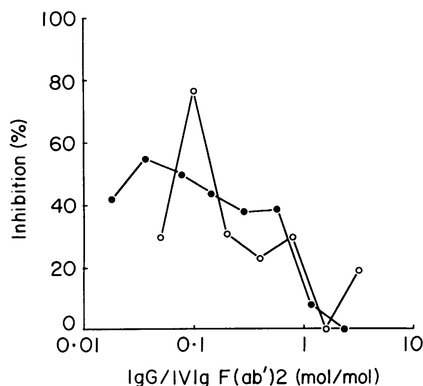

Fig. 2. Inhibition of ANCA activity in acute serum of two patients (● and ○) with systemic vasculitis by $F(ab')_2$ fragments from IVIg. Sera were incubated with IVIg $F(ab')_2$ as described in Fig. 1.

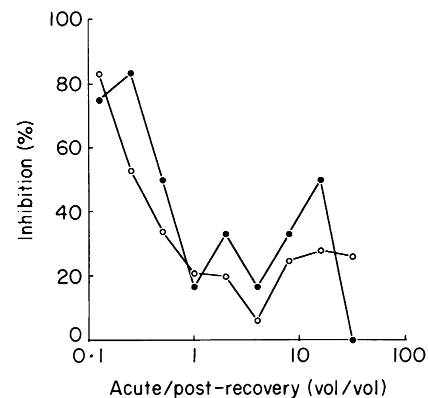

Fig. 3. Inhibition of ANCA activity in the acute serum of a patient with systemic vasculitis (patient B) by autologous remission serum from the patient in remission. ANCA activity was assessed by indirect immunofluorescence (●) and by ELISA (○).

lack of detectable ANCA activity in whole remission serum could be accounted for by the presence in serum of anti-idiotypes that were not of the IgG isotype. We therefore examined the capacity of the IgM fraction obtained from remission sera to inhibit autoantibody activity of ANCA-positive IgG from acute sera. IgM from remission sera exhibited no ANCA activity (Fig. 4). IgM from patients B and A remission sera inhibited ANCA activity of autologous and homologous IgG from acute sera in a dose-dependent manner, as shown in Fig. 5. The results suggest that anti-idiotypic IgM is responsible for the absence of detectable ANCA activity in remission sera.

Table 1. Inhibition of ANCA activity in the IgG fraction from the plasma of patients with systemic vasculitis by $F(ab')_2$ fragments from IVIg

Patient	Inhibition of ANCA activity (%)	mol/mol†	Range of molar ratios tested‡
C	33	1·20	0·30–4·80
D	74	0·08	0·08–6·15
E	52	0·14	0·07–1·12
F	47	1·30	0·34–4·00
B	68	0·155	0·085–48·64

* Maximal values of inhibition of ANCA activity that were observed.

† Molar ratio between IgG containing ANCA activity and $F(ab')_2$ from IVIg at which maximal inhibition was achieved.

‡ Between ANCA IgG and IVIg $F(ab')_2$ that were tested. ANCA activity was tested using an ELISA.

Table 2. Affinity chromatography of IgG containing ANCA activity on Sepharose-bound $F(ab')_2$ fragments from IVIg

	Loaded		Eluted	
	Amount (μg)	Specific activity (OD/μg)	Amount (μg)	Specific activity (OD/μg)
Patient A	2280	2·330	160·3	11·43
Patient B	2000	1·119	257·5	5·41

ANCA activity was measured by ELISA. Specific ANCA activity was calculated as described in Materials and Methods.

We then examined whether IgG from remission sera contained anti-idiotypes against ANCA autoantibodies in addition to antibodies with ANCA activity. Incubation of acute IgG from patients A and B with autologous $F(ab')_2$ fragments from remission IgG resulted in dose-dependent inhibition of ANCA activity (data not shown). The pattern of the inhibition curve was similar to that previously observed when whole acute serum was mixed with whole autologous remission serum. The ability of Sepharose-bound $F(ab')_2$ fragments from remission IgG to specifically bind ANCA-containing IgG from acute autologous sera was then assessed using affinity chromatography. Table 4 indicates the presence of detectable anti-idiotypes against autologous ANCA antibodies in IgG from two out of three sera of patients in remission that were tested. The acid eluate of affinity columns of $F(ab')_2$ fragments from these two sera contained a specific ANCA activity that was increased 2·1- and 4·84-fold, compared with that of IgG that had been loaded on the columns. The results indicated the presence of anti-idiotypes against ANCA in remission IgG from the patients.

Inhibition of ANCA activity by normal human serum

Incubation of systemic vasculitis acute sera with sera from healthy individuals also resulted in inhibition of ANCA activity (Fig. 6). Two out of three normal sera that were tested exhibited an inhibitory activity. One serum did not inhibit ANCA activity. Inhibition curves were dose dependent. The curves were sigmoid for the two individual normal sera that were inhibitory, reaching a plateau of approximately 70% inhibition at ratios of ANCA-containing IgG to normal IgG below 1·5. An inhibitory activity was also found in a large pool of sera of 30 healthy individuals. However, in the latter case, the inhibitory curve was bell shaped. A maximal inhibition of 58% of ANCA activity was obtained at a molar ratio of patients' IgG to normal IgG of 1·2.

Table 3. Inhibition of ANCA activity in prerecovery serum by autologous remission serum.

Patient A		Patient G		Patient H		Patient B		Patient I	
Inhibition (%)	mol/mol	Inhibition (%)	mol/mol	Inhibition (%)	mol/mol	Inhibition (%)	mol/mol	Inhibition (%)	mol/mol
17·0	0·25	25·0	0·1	0	0·2	65·0	0·125	35·0	0·10
15·0	0·50	26·5	0·2	26	0·4	62·0	0·250	47·0	0·2
25·0	1·00	20·5	0·4	26	0·8	51·0	0·500	25·0	0·4
		11·0	0·8	42	1·6	43·0	1·000	39·0	0·8
37·5	2·00	10	1·6	33	3·2	41·0	2·000	38·0	1·6
16·0	4·00	0·0	3·2	37	6·4	12·5	4·000	33·0	3·2
0·0	8·00			26	12·8	25·0	8·000	28·0	6·4
				26	25·6	28·0	16·000	7·0	12·8
				12	51·2	26·0	32·000		

Sera from patients with acute systemic vasculitis were incubated with autologous sera obtained from the patients in remission of disease for 1 h at 37°C and overnight at 4°C. Residual ANCA activity was assessed using an ELISA.

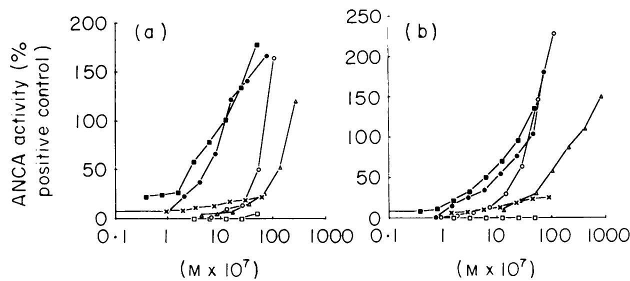

Fig. 4. ANCA activity in acute and remission sera, IgM and IgG from two patients (a, patient A; b, patient B): ■, acute serum; ●, acute IgG; □, remission serum; ○, remission IgG; △, $F(ab')_2$ fragments from remission IgG; +, remission IgM.

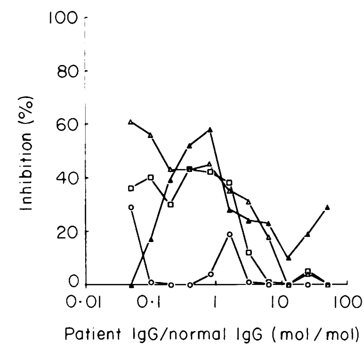

Fig. 6. Inhibition of ANCA activity in acute serum of a patient with systemic vasculitis upon incubation with serum from three healthy individuals (○, □, △) and with a pool of sera from 30 healthy individuals (▲). Experimental conditions were those depicted in Table 3.

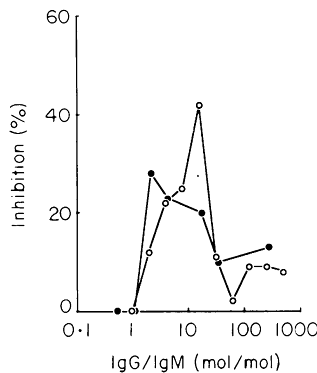

Fig. 5. Inhibition of ANCA activity in IgG from acute serum of patients (○, patient A; ●, patient B) with systemic vasculitis by IgM from autologous remission serum.

Table 4. Affinity chromatography of acute ANCA IgG on Sepharose-bound $F(ab')_2$ fragments of IgG from autologous remission serum

	Loaded		Loaded	
Patient	Amount (μg)	Specific activity (OD/μg)	Amount (μg)	Specific activity (OD/μg)
B	2220	1·17	21·7	0·00
A	3080	2·54	196·7	5·31
I	2570	0·29	77·5	14·28

DISCUSSION

ANCA are present in the sera of patients with systemic vasculitis (van der Woude *et al.*, 1985; Falk & Jennette, 1988; Nölle *et al.*, 1989). Changes in levels of ANCA activity in serum parallel changes in disease activity. Here we demonstrated that anti-idiotypic specificities directed against ANCA autoantibodies are present in poole normal IgG for therapeutic use (IVIg), and in remission IgM and IgG from patients with systemic vasculitis.

Incubation of serum containing ANCA autoantibodies with IVIg resulted in inhibition of ANCA activity in 70% of 21 acute systemic vasculitis sera that were tested. Inhibition was dependent on an interaction between the variable region of antibodies in IVIg and the antigen-binding site of ANCA autoantibodies since $F(ab')_2$ from IVIg inhibited the binding of IgG with ANCA activity to the autoantigen. As previously observed with anti-factor VIII, anti-thyroglobulin, anti-DNA, anti-intrinsic factor and anti-peripheral nerve autoantibodies (Sultan *et al.*, 1984, 1987; Rossi *et al.*, 1988; Rossi & Kazatchkine, 1989; Dietrich & Kazatchkine, 1990; van Doorn *et al.*, 1990), inhibition of ANCA autoantibody activity by IVIg or by $F(ab')_2$ from IVIg was dose dependent, displaying a bell-shaped inhibitory pattern. Maximal inhibition of autoantibody activity occurred at specific molar ratios between patients' IgG and IVIg for each patient ranging between 0·125 and 2·000. Affinity chromatography of patients' IgG containing ANCA activity on Sepharose-bound $F(ab')_2$ from IVIg resulted in the specific retention of ANCA activity. Previous results indicated that IVIg do not contain anti-allotypic specificities against the most common allotypes expressed in the variable region of human IgG (Rossi *et al.*, 1988). Taken together, these data indicate that IVIg contain anti-idiotypes against ANCA autoantibodies. Whereas functional inhibition assays are directed towards the detection of anti-idiotypes that interfere with the autoantibody binding site, affinity chromatography experiments may detect the presence of $AB_2\beta$, and $AB_2\alpha$ anti-idiotypic antibodies. Interestingly, the enrichment in specific ANCA activity that was found in the acid eluates from affinity columns of Sepharose-bound $F(ab')_2$ fragments from IVIg, was found to be the same for IgG of two patients, although the specific ANCA activity in chromatographed IgG differed between the patients. This observation is suggestive of a homogeneous distribution of IVIg-reactive idiotypes in ANCA autoantibodies.

IVIg contain a wide spectrum of anti-idiotypic specificities against autoantibodies from patients with autoimmune diseases and natural autoantibodies that are present in normal individuals (Rossi *et al.*, 1989). Anti-idiotypic activity against autoantibodies in IgG from large pools of normal plasma probably results from the additive contribution of anti-idiotypic specificities that may be present in insufficient quantitative and qualitative amounts in order to be expressed in individually tested normal sera. Co-incubation of acute serum from a patient with systemic vasculitis with serum from normal individuals

resulted in inhibition of ANCA activity with two out of three of the normal sera tested. The experiment does not indicate whether the source of inhibitory activity in the normal sera is IgG and/or IgM. Inhibition of ANCA activity in acute serum was also observed following incubation of the serum with a pool of sera from 30 healthy individuals. Interestingly, in the latter case the pattern of the inhibition curve was similar to that observed upon incubation of acute serum with IVIg. The presence in IVIg of anti-idiotypes against disease-associated autoantibodies provides some evidence of a physiological role of the idiotypic network in preventing the expansion of autoreactive clones (Jerne, 1974). In selected organ-specific autoimmune diseases, IVIg may be effective in suppressing autoantibodies *in vivo* (Morell & Nydegger, 1986; Rossi *et al.*, 1989).

Remission serum from patients with systemic from patients with systemic lupus erythematosus (Abdou *et al.*, 1981; Tanigushi, Chia & Barnett, 1984), Guillain–Barré syndrome (van Doorn *et al.*, 1990) and anti-factor VIII autoimmune disease (Sultan *et al.*, 1987) contain anti-idiotypes against autologous and homologous acute autoantibodies. In the present study, co-incubation of acute serum from patients with systemic vasculitis with autologous remission serum resulted in inhibition of ANCA activity in acute sera. Remission sera contained IgM and IgG capable of specifically interacting with β and/or α idiotypes of ANCA IgG from acute sera. A predominant role for anti-idiotypic IgM in the control of expression of ANCA activity in remission sera was indicated by the presence of ANCA activity in the IgG fraction of the serum from patients in remission and the lack of detectable activity in whole remission serum. Evidence in mice indicates the co-existence of autoantibodies and IgM anti-idiotypes against the autoantibodies in the serum from normal and autoimmune animals (S. Avrameas, personal communication). Our observations emphasize the fact that autoantibody activity which is measured in a pathological serum results from additive and negative contributions of multiple antibody species. The results further stress that the correlation between the clinical activity of an autoimmune disease and the presence of autoantibody activity in serum needs to be investigated by using patients' whole serum rather than purified IgG for quantification of the autoantibody. Our observations suggest that remission from systemic vasculitis is associated with the generation of anti-idiotypes against autoantibodies rather than the suppression of production of ANCA autoantibodies. *In vitro* evidence from our studies also suggests that IVIg could modulate the activity of systemic vasculitis *in vivo*.

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