Analysis of Human Antitopoisomerase-I Idiotypes

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Abstract

Antibodies to topoisomerase-I are present in ~ 26% of patients with scleroderma and are rarely found in patients with other diseases. In the current study, the expression of the antitopoisomerase-I (antitopo-I) idiotype from two scleroderma patients (E.M. and S.G.) and from a healthy individual (N.M.) were studied. Idiotype EM-SCL was restricted to the three classes of antitopo-I, whereas idiotypes SG-SCL and NM were found in all classes of antitopo-I as well as in their non-antitopo-I Igs. Sera from 9 of 10 antitopo-I-positive unrelated scleroderma patients expressed idiotype SG-SCL and some also expressed idiotype NM. Sera from N.M.'s 3 daughters and from 7 of 18 nonrelated normals expressed idiotype NM in the three immunoglobulin classes of non-antitopo-I. Two of the antitopo-I antibodies expressed a cross-reacting idiotype (CRI) that is present in non-antitopo-I antibodies from the same donor. Contrary to the natural CRI, SG-SCL's CRI is closely associated with the antigen binding site. Antitopo-I idiotypes are on the heavy chains. Like many other autoantibodies, Id-SG-SCL use V_H4.2-1, DXP1, and J_H4 in germline configuration. (*J. Clin. Invest.* 1993. 92:1302–1313.) Key words: autoantibodies • immunoglobulins • antigen-driven • scleroderma • isotypes

Introduction

Scleroderma is a rare disease occurring in 19–75 per 100,000 individuals (1). Antitopoisomerase-I (antitopo-I)[1] (2–4) is present in sera from ~ 26% of the scleroderma patients (5, 6). These autoantibodies to topoisomerase-I (topo-I) can be detected by immunoblotting on HeLa nuclei or chromosomes, by double immunodiffusion, by enzyme inhibition (3), or by a recently standardized ELISA (7). The different sensitivities and specificities of these tests may account for the differences in reported prevalence of antitopo-I in scleroderma patients (7). Topo-I catalyzes the breaking/rejoining of single-stranded DNA and relaxes supercoiled DNA in vitro (3, 4, 8–10). The enzymatically active site is expressed in the 67.7-kD carboxy-terminal fragment (8) between amino acids 344 and 483 (11). Topo-I has a native molecular mass of 100 kD, but smaller proteolytic products ranging from 60 to 100 kD are functionally active (2–15). The major protein isolated from calf thymus is 70 kD (2). Antibodies to topo-I bind one or more of six epitopes in the enzyme (13) and inhibit the enzyme's activity in vitro (3, 4, 7, 9–12).

Antitopo-I is highly specific for scleroderma (4–7, 13–15). We have found antitopo-I in only 1 of 264 healthy controls (5, 15). This healthy 64-yr-old female (N.M.) has not developed any sign or symptom related to scleroderma or to any other connective tissue disease over the past 8 yr while the antitopo-I has been in her serum.

In our previous idiotypic analysis of human antitopo-I (15) we described the presence of at least one immunodominant private idiotype (Id) in or near the antigen binding site. In that report we characterized the antitopo-I Id from scleroderma patient EM-SCL. Id-EM-SCL was stable for the period of 9 yr of follow-up and was not expressed in sera from any of 29 antitopo-I-positive patients, 10 anticentromere-positive patients, 10 negative antitopo-I and negative anticentromere patients, or 20 healthy controls (15). The antitopo-I Ids from the SG-SCL and from the healthy individual N.M. did not cross-react with the purified preparations from any of the other two antitopo-I-positive sera (SG and EM), three anticentromere-positive sera, or with a panel of human myeloma and monoclonal Igs.

We report here that (*a*) Ids were expressed in all the Ig classes of antitopo-I EM-SCL, SG-SCL, and NM; (*b*) Id-SG-SCL and Id-NM are also expressed in their non-antitopo-I Igs; (*c*) positive antitopo-I sera from nonrelated scleroderma patients expressed one or both cross-reacting Ids (CRIs); and (*d*) CRI NM was also expressed in the IgG, IgM, and IgA of her 3 daughters' sera and in different Ig classes from 7 of 18 unrelated control sera.

Our results show that antitopo-I antibodies express immunodominant Ids located in the heavy chains that are stable after isotype switch. The private Id from the scleroderma patient, EM-SCL, was specific for antitopo-I antibodies. Both Id-EM-SCL and Id-SG-SCL may be close to the antigen binding site. In contrast the expression of Id-NM is more likely to be contributed by framework region residues and thus is able to bind to anti-Id even after binding to topo-I.

We cloned the variable region heavy chain (V_H) gene bearing the CRI from SG-SCL and found that it uses V_H4.2-1, DXP1, and junctional region heavy chain J_H4 in germline configuration. Addition of one amino acid at each of the diversity (D) region boundaries by N sequences is the only difference from the germline configuration at the complementarity determining region 3 (CDR3). The presence of a private Id in EM-

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1. *Abbreviations used in this paper:* antitopo-I, antitopoisomerase I; C, constant; CA, cold agglutinins; CDR, complimentarity determining region; CRI, cross-reactive idiotype; D, diversity; FT, flow through; HRP, horseradish peroxidase; NHS, normal human serum; PCR, polymerase chain reaction; RF, rheumatoid factor; topo-I, topoisomerase I; V, variable.

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SCL may be the product of somatically mutated heavy chain genes in an individual response to the autoantigen.

Methods

The patients EM-SCL and SG-SCL's clinical features have been described elsewhere (15). N.M. is a healthy 65-yr-old white female with antitopo-I whom we have followed for 8 yr.

Preparation of affinity-purified antitopo-I. Antitopo-I from EM-SCL, SG-SCL, and NM were prepared by affinity purification, as previously described (15). Briefly, whole patient serum from the patients was extensively absorbed with topo-I isolated from calf thymus (7) coupled to Sepharose 4B gel (Pharmacia, Uppsala, Sweden). Eluted fractions were concentrated and flow throughs (FT) were used in final diluted fractions. In total, 10 purified antitopo-I antibodies from EM-SCL, 5 from SG-SCL, and 6 from NM as well as 2 FTs from EM-SCL and 3 from SG-SCL and NM were studied.

Preparation of anti-Ids. Anti-Id sera was prepared as previously described (15). Briefly, two Pasteurella-free New Zealand white rabbits were immunized with 100–150 μg of either SG-SCL-purified antitopo-I, NM antitopo-I, or EM-SCL antitopo-I. Booster immunizations were carried out at week 2 and then at monthly intervals. The animals were bled 15 d after the final boost (total of three for all rabbits except for one of the two EM-SCL rabbits). This EM-SCL rabbit received a total of 700 μg in six booster injections. Igs were precipitated with 40% saturated ammonium sulfate, dialyzed overnight at 4°C with PBS, and exhaustively adsorbed with a mixture of human myeloma IgG, IgM, and IgA (gift from Dr. Blas Frangione, New York University School of Medicine) coupled to Sepharose 4B gel (Pharmacia). After further adsorption with normal human serum (NHS) depleted of Ig (Sigma Chemical Co., St. Louis, MO) coupled to Sepharose 4B (Pharmacia) the FT fractions were absorbed with Staphylococcal protein A (Sigma Chemical Co.) coupled to Sepharose 4B (Pharmacia). Fractions were eluted with a 0.2 M glycine buffer, pH 1.9, and immediately were neutralized, pooled, and concentrated by vacuum dialysis with PBS at 4°C.

Seven anti-Ids from the two rabbits immunized with EM-SCL antitopo-I, five from SG-SCL's rabbits, and six from N.M.'s rabbits were studied. All anti-Ids from the homologous antitopo-I-purified sample behaved and were expressed in the same manner.

Control anti-Ids reagents were prepared by ammonium sulfate precipitation of Igs from each preimmunized rabbits' sera. These were absorbed with Staphylococcal protein A (Sigma Chemical Co.) coupled to Sepharose 4B (Pharmacia) and concentrated by vacuum dialysis at 4°C.

Detection of antitopo-I. SDS-PAGE gels were performed as described (16) using Western blot and either HeLa chromosome extracts or purified topo-I (7, 17). Blots on topo-I were developed with ^{125}I Staphylococcal protein A (Amersham Corp., Arlington Heights, IL) for IgG detection after incubation with the purified samples. For detection of IgM and IgA after incubation with the purified samples, strips were incubated with horseradish peroxidase (HRP) rabbit anti-human, mu chain, or alpha chain–specific antibodies (Dakopatts, Copenhagen, Denmark) and developed with high sensitivity IBI enzygraphic web (Kodak, International Biotechnologies, Inc., New Haven, CT). Blots on HeLa chromosome extracts were blocked 1 h at room temperature, incubated with antitopo-I-positive sera at a dilution of 1:1,000 1 h at room temperature as previously described (3). They were then incubated with HRP anti-human gamma, mu, or alpha chain–specific antibodies (Dakopatts) 1 h at room temperature and developed with the ECL system (Amersham).

The topo-I functional assay was performed on a 1.2% agarose DNA gel as described (3, 7). Using a final concentration of 15 μg/ml of EM-SCL or SG-SCL or 30 μg/ml NM or 50 μg/ml of EM-SCL-FT, SG-SCL-FT, or NM-FT incubated with 1:1 volume of a final concentration of 0.25 μg/ml topo-I or buffer alone overnight at 4°C in a final volume of 20 μl. Samples were then incubated 1 h at 4°C with 0.46 μg per assay of DNA Phi × 174 (Gibco BRL, Gaithersburg, MD) in a final volume of 40 μl per tube. The reaction was stopped by the addition of 5 μl of a solution containing 5% SDS, 50 mM EDTA, 0.6 mg/ml bromophenol blue, and 50% glycerol. Samples were loaded onto the gel and run at 70 V in a buffer containing 40 mM Trizma base (Sigma Chemical Co.), 20 mM acetic acid glacial (J. T. Baker Chemical Co., Phillipsburg, NJ), and 2.5 mM EDTA (Sigma). DNA lines were visualized using ethidium bromide under UV light. For inhibition studies, optimal concentrations of purified antitopo-I and homologous anti-Ids were incubated at 37°C for 1 h and then at 4°C overnight. These samples were then incubated with topo-I and the assay was performed as described.

Detection of Id by Western blot. Purified samples were run in a 10% SDS-PAGE (16–18) as follows: ≥ 10 μg of EM-SCL, SG-SCL, or NM and ≥ 150 μg of EM-SCL-FT, SG-SCL-FT, or NM-FT, previously boiled at 100°C for 5 min in sample buffer containing 10% β_2-mercaptoethanol. As controls, 10 μg each of human IgG kappa (Sigma Chemical Co.), IgM kappa and lambda (Cappel, West Chester, PA), and IgA kappa (Sigma Chemical Co.) were run in the same gel. Transfer to nitrocellulose paper was performed as described above. Each lane was cut in three strips and incubated 1 h at room temperature in blocking agent, 0.2% Triton-X, 0.15 M NaCl, 0.01 M $NaPO_4$, 0.001 M EDTA, 0.05% Tween-20, and 4% BSA. The first two sets of strips were incubated with either preimmune rabbits' purified Ig or homologous anti-Id overnight at room temperature. After thorough washing, strips were incubated for 3 h at room temperature with swine antibody to rabbit IgG conjugated to HRP (Dakopatts). The third set of strips was incubated with HRP-conjugated rabbit anti–human gamma, mu, alpha, kappa, and lambda (polyvalent) antibodies (Dakopatts). Strips were thoroughly washed and developed using TMB membrane peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD).

Solid-phase ELISA. Solid-phase ELISAs were performed as previously described (14). Briefly, 96-well polyvinyl microtiter plates (Fisher Scientific, Medford, MA) were coated 1 h at 37°C with optimal dilution of the protein in 0.9% saline, pH 7.4 (50 μl per well). Incubation of 50 μl per well of samples was carried out at 37°C for 1 h after blocking with 1% ovalbumin (Sigma Chemical Co.) in PBS.

HRP antisera at optimal dilutions in 1% BSA, 2% FCS in PBS 0.05% Tween were incubated 1 h at 37°C on the plates. 50 μl per well of a solution containing 150 μl of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)-$[NH_4]_2$ (Sigma Chemical Co.) in 10 ml citrate buffer (0.1 M citric acid with 0.1 M sodium citrate, pH 4) with 0.012% H_2O_2 was added for 15 min at room temperature in the absence of light. The optical density (OD) was read at 405 nm using a micro ELISA plate reader (MR580, Dynatech, Torrance, CA). Each sample was tested in triplicate.

Characterization of anti-Ids. Plates were coated with four serial dilutions of each purified antitopo-I or with NHS starting at 1.5 μg/ml. This was followed by the addition of optimal concentration of the purified homologous anti-Id or preimmune IgG. HRP-conjugated swine antibodies to rabbit IgG (1:1,000) was added and plates were developed as above. In no instance was there any binding of the control, preimmune purified IgG to the respective purified Id (data not shown).

Quantitation of IgG, IgM, and IgA in antitopo-I. Plates were coated with 5 μg/ml in 10 serial dilutions of IgG (Sigma Chemical Co.), IgM (Cappel), and IgA (Sigma Chemical Co.) or 3–5 μg/ml of EM-SCL, SG-SCL, or NM or 30–50 μg/ml of the respective FTs. HRP-conjugated rabbit antibodies to human gamma chain (1:5,000), anti-human mu, or alpha chains (1:2,000) were added. The amount of IgG, IgM, and IgA in each sample was determined by plotting the mean of each sample's triplicate on the standard curves and adjusting for each sample's dilution factor.

Detection of antitopo-I. Plates were coated with 0.5 μg/ml of topo-I. 10 serial dilutions of 3 μg/ml of EM-SCL, EM-SCL-FT, SG-SCL, SG-SCL-FT, NM, or NM-FT were added. Plates were incubated with HRP rabbit anti–human polyvalent conjugate (1:1,000) and developed as

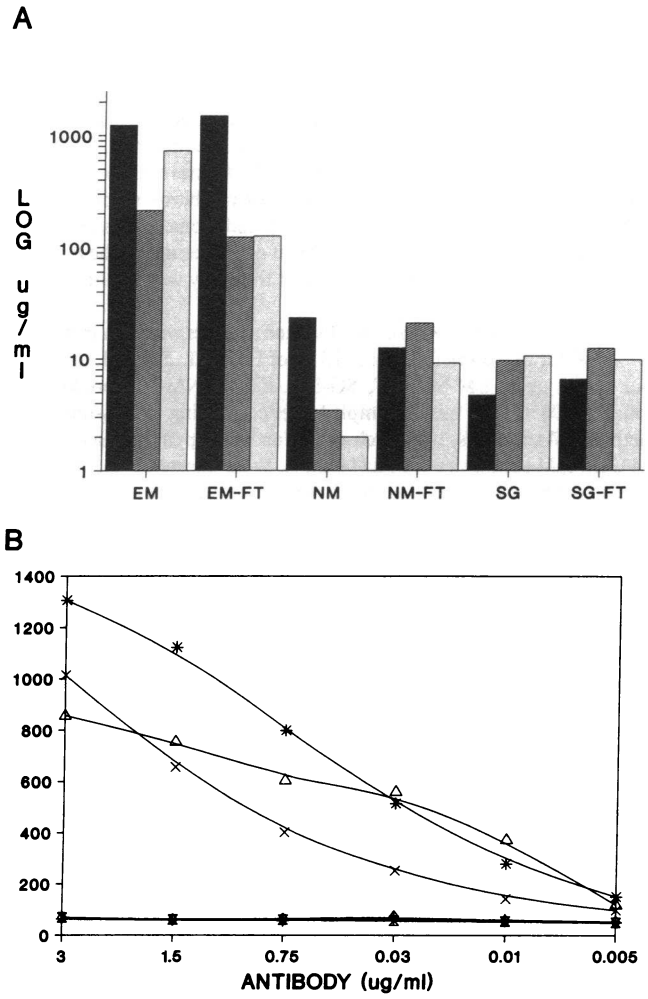

Figure 1. (*A*) Amount of Ig classes by direct binding ELISA. Final amounts of each antibody class (in μg/ml) were calculated by plotting the log OD of serial dilutions of commercial human IgG, IgM, and IgA compared with the OD of known amounts of each purified sample (see Methods). (*y*-axis) Amount of Ig class expressed as logarithm of the calculated (μg/ml) contents of each preparation. ■, IgG; ■, IgM; □, IgA. EM, purified antitopo-I from scleroderma patient EM-SCL; EM-FT, flow through after abortion of EM-SCL's serum on topo-I; NM, purified antitopo-I from the healthy individual; NM-FT, flow through; SG, purified antitopo-I from scleroderma patient SG-SCL; SG-FT, flow through. (*B*) Detection of antitopo-I by direct binding ELISA using plates precoated with topo-I and serial dilutions of each purified sample, detected with polyvalent anti-human HRP-conjugate (see Methods). The final concentration of protein in each dilution is plotted against the OD value × 1,000 of that specific dilution. Purified antitopo-I EM-SCL (— * —), SG-SCL (— △ —), and NM (— × —) follow a dose-response curve. Flow throughs EM-SCL-FT (— ▽ —), SG-SCL-FT (— △ —), and NM-FT (— + —) show no binding to topo-I.

above. The results were plotted as logarithm of concentration in micrograms per milliliter vs. OD reading × 1,000.

ELISA blocking studies. Samples were incubated with each putative blocking protein at optimal concentrations for 1 h at 37°C in 1% BSA, 2% FCS in PBS-Tween and overnight at 4°C before adding to plates.

Detection of IgG, IgM, and IgA antitopo-I. Optimal concentrations of EM-SCL, SG-SCL, or NM or 10 times the respective FT (containing non-antitopo-I Igs) was incubated as described with 2 μg/ml of topo-I or buffer alone and added to plates precoated with 0.5 μg/ml of topo-I. HRP-conjugated rabbit anti-human gamma, mu, or alpha chain–specific antibodies (Dakopatts) was added and plates were developed as described. Percentage inhibition was calculated using the formula: {[nonblocked OD (preincubated with buffer) − blocked OD (preincubated with topo-I)]/(nonblocked OD)} × 100.

Detection of IgG, IgM, and IgA Id. Optimal concentrations of each purified antitopo-I or 10 times the respective FT was incubated with homologous anti-Id. These samples were added to plates precoated with homologous anti-Id. Specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha chain were used as above. Percentage inhibition was calculated as above.

Relationship between Id and antigen binding site. EM-SCL, SG-SCL, NM, or the respective FTs were incubated with homologous anti-Id or preimmune IgG at optimal concentrations and added to plates precoated with topo-I (0.5 μg/ml). Plates were then incubated with specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha and were developed as above. Percentage inhibition was calculated as described above.

The blocking by topo-I of the binding of Id to homologous anti-Id was evaluated by incubating each purified antitopo-I or the respective FT with topo-I or buffer and added to plates precoated with homologous anti-Id. Specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha were added and plates were developed as above. Percentage inhibition was calculated as above.

Blocking studies using varying amounts of anti-Id. EM-SCL, SG-SCL, NM, or the respective FT was incubated with 10 serial dilutions of homologous anti-Id and tested on topo-I precoated plates. After incubation and addition of specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha, plates were developed as above. Percentage inhibition was calculated for each anti-Id concentration and results plotted on a graph as logarithm of the reciprocal dilution in micrograms per milliliter vs. percent inhibition.

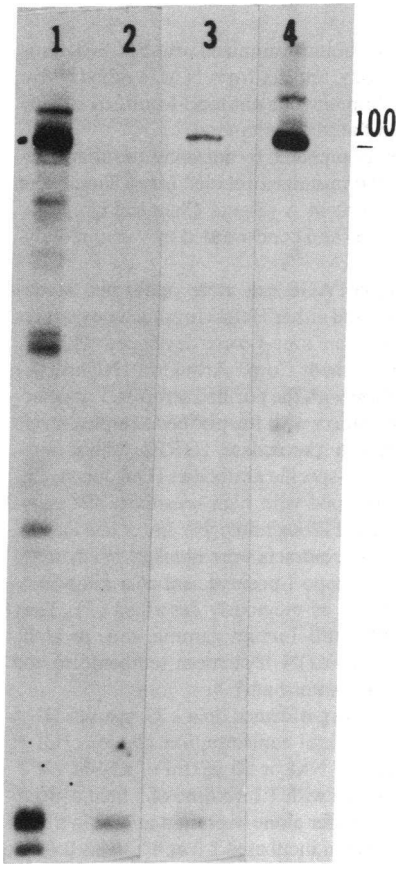

Figure 2. Detection of antitopo-I on HeLa chromosome extracts blot using rabbit antibodies coupled to HRP at a dilution of 1:15,000 and the ECL system (see Methods). Topo-I runs as a major 100-kD band. Lane *1*, after incubation with 1 μg/ml of SG-SCL and anti-human gamma chain; lane *2*, after incubating with 4 μg/ml of SG-SCL-FT and anti-human gamma, mu, and alpha chains; lane *3*, after incubation with 1 μg/ml of SG-SCL and anti-human mu chain; and lane *4*, after incubation with 1 μg/ml of SG-SCL and anti-human alpha chain. Lanes *1*, *3*, and *4* show the binding to topo-I (100-kD band) of SG-SCL IgG, IgM, and IgA, respectively. Lane *2* shows no binding of any SG-FT Ig to topo-I.

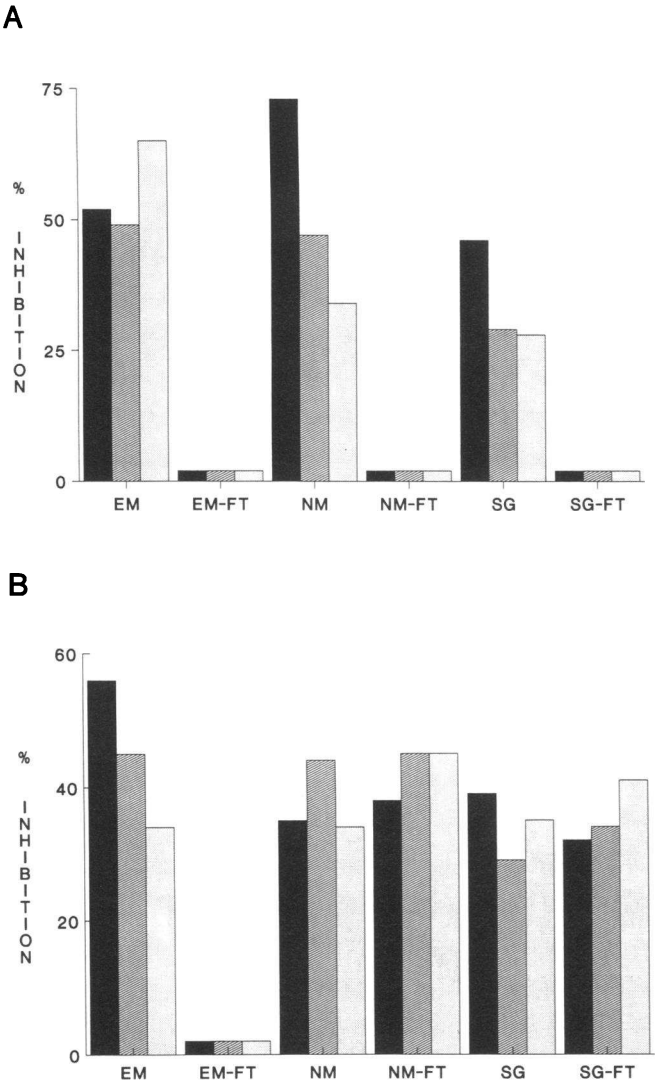

Figure 3. Detection of Ig classes by blocking ELISA. Results are expressed as percentage inhibition (see Methods). ■, IgG; ▨, IgM; □, IgA. EM, purified antitopo-I from scleroderma patient EM-SCL; EM-FT, flow through after absorption of EM-SCL's serum on topo-I; NM, purified antitopo-I from the healthy individual; NM-FT, flow through; SG, purified antitopo-I from scleroderma patient SG-SCL; SG-FT, flow through. (*A*) Ig classes of antitopo-I. Each sample was incubated with 2 μg/ml of topo-I or buffer alone, tested on topo-I-precoated plates and developed with rabbit anti-human gamma, mu, or alpha chain-specific antibodies coupled to HRP. Percentage inhibition was calculated as above (see Methods). (*B*) Ig classes of Ids. Samples preincubated with homologous anti-Id were tested on plates precoated with anti-Id. 2 μg/ml of EM, EM-FT, SG, or SG-FT was incubated with 10–15 μg/ml of homologous anti-Id or the respective preimmune rabbit's purified Ig, or 2.5 μg/ml of NM or NM-FT with 20–25 μg/ml of anti-Id or preimmune rabbit's purified Ig.

Blocking studies using varying amounts of topo-I. EM-SCL, SG-SCL, NM, or the respective FT was incubated with 10 serial dilutions of topo-I starting with 1 μg/ml and added to plates coated with homologous anti-Id as described. Specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha were added and plates were developed as above. Percentage inhibition was calculated and graphs plotted as above.

Detection of Id-NM in control sera. NHS, sera from N.M.'s 3 daughters, and from 18 nonrelated controls all lacking antitopo-I antibody were each incubated as above at 1:600 dilution with anti-Id-NM or preimmune rabbit's Ig. NM whole serum was used as the positive control. The samples were added to plates precoated with anti-Id NM. Specific HRP-conjugated rabbit antibodies to human gamma, mu, or alpha were added and plates were developed as above. Percentage inhibition was calculated as mentioned.

Detection of Id in antitopo-I from other patients. Immunoblots using HeLa chromosome extracts were incubated with 1:1,000 of 10 antitopo-I-containing sera from scleroderma patients. Blots were developed using the electrochemiluminesence system after incubating with either anti-Id SG-SCL or anti-Id NM. SG-SCL- and NM-purified antitopo-I were used a positive controls and their respective FTs as negative controls.

EBV-transformation of S.G.'s B cells. Ficoll/Hypaque fractionated peripheral blood cells from patient S.G. were transformed in the presence of 50% EBV-rich supernatant in 96-well plates, as described (19). Approximately 50 d later culture supernatants were tested on direct binding ELISA using plates precoated with purified topo-I and developed using HRP-conjugated rabbit antibodies to human gamma, mu, alpha, kappa, and lambda chains. The positive cell lines were subcloned to 10 cells per well. Four positive subclones from one cell line were identified and further cloned to one cell per well. After > 100 d in culture, 25 positive antitopo-I clones were successfully established.

mRNA sequencing of antitopo-I heavy chain variable region genes. RNA was extracted from 10^6 to 10^7 cells from each of the 25 clones as described (20). Synthesis of cDNA was carried out using the primer for mu: TAT-TTC-CAG-GAG-AAA-GTG-AT (21), and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). Amplification of 12 cDNA (3 from each of the 4 subclones) was performed by polymerase chain reaction (PCR) (22) using 300–500 ng of 5′ primers for each of the 6 V_H leader sequences: L1, CCA-TGG-ACT-GGA-CCT-GGA-GG; L2, ATG-GAC-ATA-CTT-TGT-TCC-AC; L3, CCA-TGG-AGT-TTG-GGC-TGA-GC; L4, ATG-AAA-CAC-CTG-TGG-TTC-TT; L5, ATG-GGG-TCA-ACC-GCC-ATC-CT; and L6, ATG-TCT-GTC-TCC-TTC-CTC-AT, and the same 3′ mu primer (21). On each PCR reaction a control tube with no DNA was always performed for each of the six V_H leader primers (22).

Eight PCR products (2 of each subclone) were purified using centricon-100 (Amicon, Beverly, MA), ligated into an EcoRV-cut p-bluescript plasmid, and used to transform competent B-SJ72 *Escherichia coli.* The positive colonies were detected by hybridization of the replica filter with α-[^{35}S]-ATP (Dupont NEN, Boston, MA)-labeled mu oligonucleotide: GGA-ATT-CTC-ACA-GGA-GAC-GA. Two to three colonies per cell clone were selected for single-stranded DNA (ssDNA) synthesis (23). A total of 14 ssDNA were sequenced using T7 DNA polymerase (U. S. Biochemical, Cleveland, OH) (24).

Results

Antitopo-I activity in affinity-purified preparations. The amount of IgG, IgM, and IgA in the affinity-purified samples EM-SCL, SG-SCL, and NM is shown in Fig. 1 *A*. Functionally active antitopo-I was detected in EM-SCL, SG-SCL, and NM. When FTs were used 10 times more concentrated than the respective purified antitopo-I, no inhibition of topo-I activity was found (results not shown). Fig. 1 *B* shows the dose–response curve of direct binding to topo-I for the three purified antitopo-Igs. Igs in the respective FTs did not bind to topo-I.

Ig classes of antitopo-I antibody. IgG, IgM, and IgA antitopo-I in EM-SCL, SG-SCL, and NM were detected using immunoblots. Fig. 2 is an example of the immunoblots on HeLa chromosome extracts incubated with SG-SCL or SG-FT and developed with HRP-conjugated rabbit antibodies to human gamma, mu, or alpha chains on HeLa chromosomes blots. All purified antitopo-I were IgG, IgM, and IgA. Igs in the FTs failed to react. ELISA topo-I blocking assay confirmed that

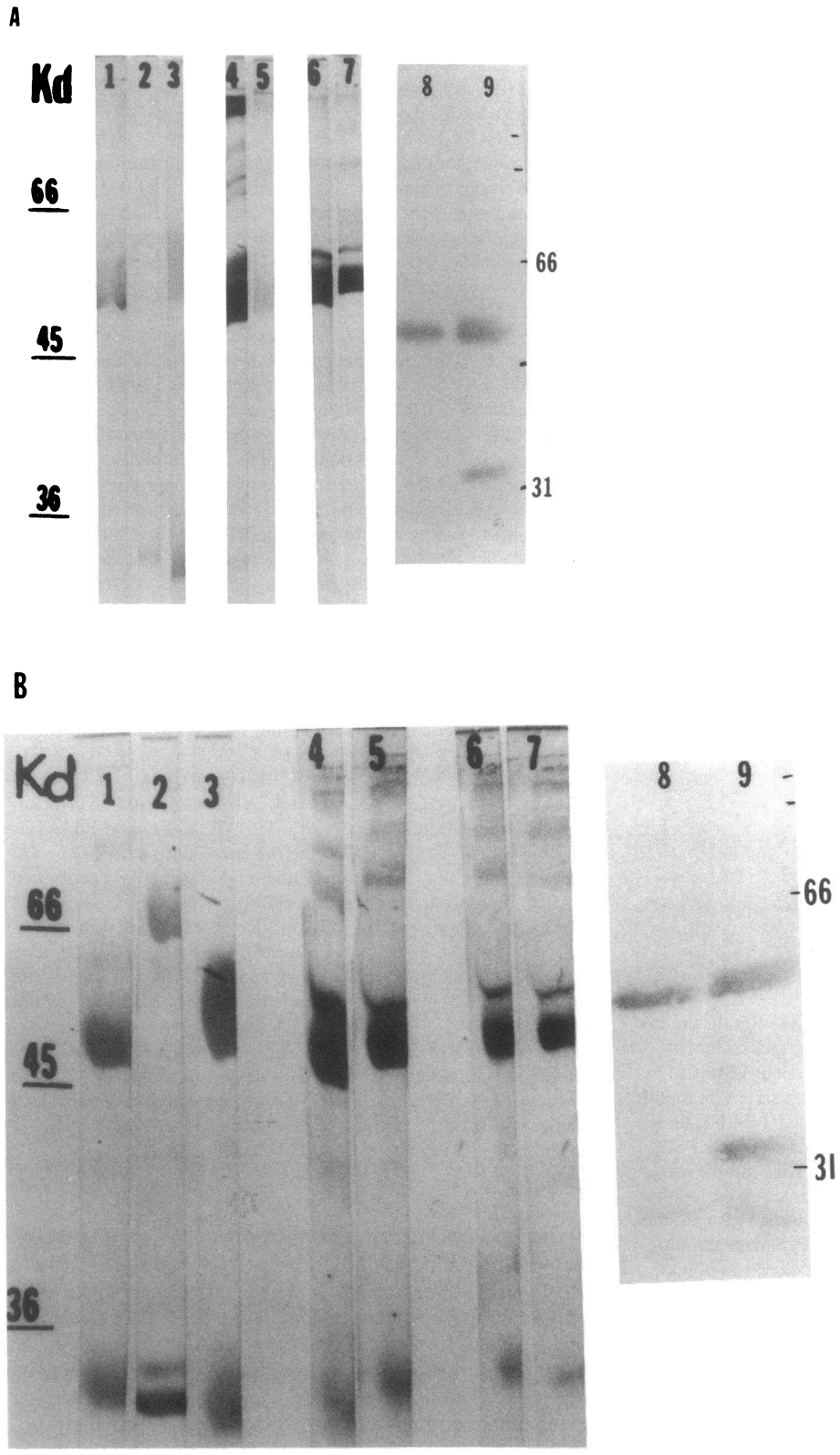

Figure 4. (*A*) Immunoblots using 5–15 μg of each purified sample incubated with 2–5 μg/ml of homologous anti-Id and developed with swine anti-rabbit antibodies coupled to HRP and the TMB membrane system (see Methods). (*B*) Incubation with polyvalent anti-human HRP-conjugate also developed with the TMB membrane system. Lane *1*, control IgG; lane *2*, control IgM; lane *3*, control IgA; lane *4*, EM-SCL; lane *5*, EM-SCL-FT; lane *6*, NM; lane *7*, NM-FT; lane *8*, SG-SCL-FT; and lane *9*, SG-SCL. As shown in *A*, all three Ids are expressed in the heavy chains. Id-EM-SCL is not present in the non-antitopo-I antibodies (lane *5*) whereas Ids SG-SCL and NM are found in both purified antitopo-I and non-antitopo-I antibodies (lanes *6–9*) (see Results).

antitopo-I is present in IgG, IgM, and IgA of EM-SCL, SG-SCL, and NM as shown in Fig. 3 *A*.

Ig classes of Id. Id from each patient's antitopo-I was expressed in all Ig classes of antitopo-I. Fig. 3 *B* shows the blocking ELISA results for all isotypes in EM-SCL, SG-SCL, and NM. Surprisingly, Id-SG-SCL and Id-NM were also expressed in Igs from the corresponding FTs.

These results were confirmed by immunoblots on each of the purified samples of Id. When the blots were incubated with homologous anti-Id, bands corresponding to gamma, alpha, and mu chains were visualized for each of the purified antitopo-I samples (Fig. 4 *A*). The IgG in SG-SCL was at 52 kD but also had a breakdown product of 33 kD (confirmed by immunoblot with specific anti-human gamma chain, not shown).

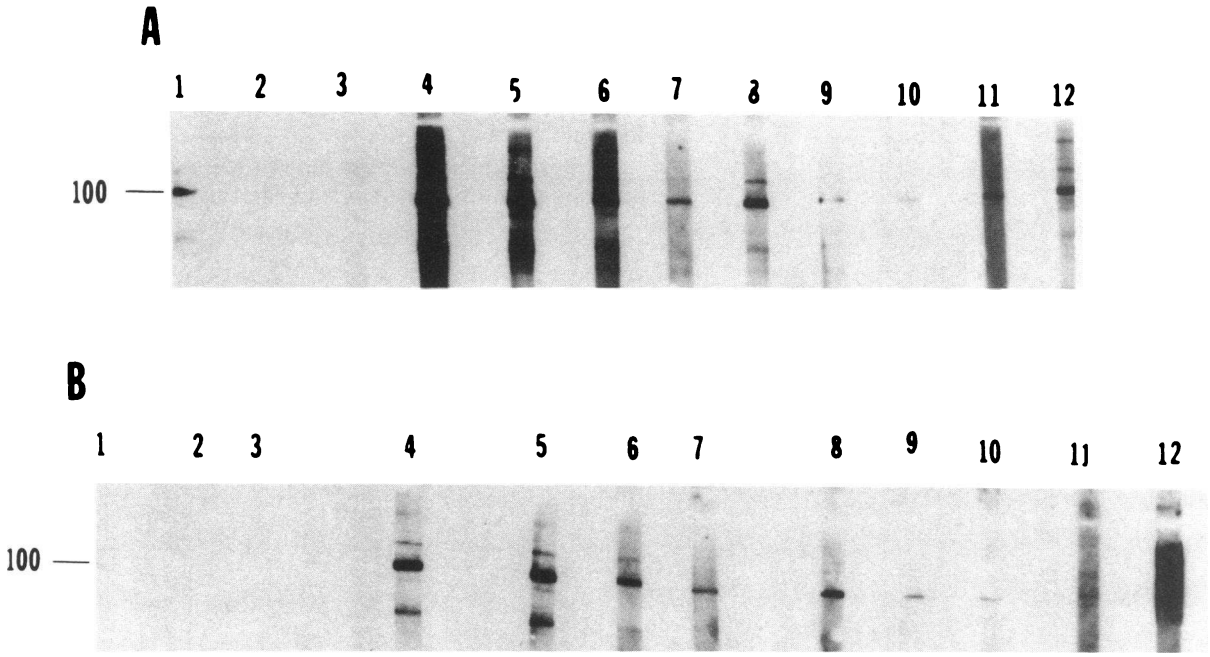

Figure 5. Immunoblots on HeLa chromosome extracts incubated with 1 μg/ml of purified antitopo-I or the respective flow through as positive and negative controls or 1:1,000 dilution of whole sera from 10 nonrelated positive antitopo-I scleroderma patients. After incubation with anti-Id SG-SCL (*A*) or anti-Id NM (*B*) blots were developed with swine anti-rabbit HRP and the ECL system (see Methods). Topo-I runs at a 100-kD band. Lane *1*, purified antitopo-I from SG-SCL (*A*) or NM (*B*); lane *2*, respective FT; lanes *3–12*, 1–10 positive antitopo-I sera. Serum 1 in lane *3* did not express any Id (*A* and *B*). In *B*, serum 9 (lane *11*) when exposed a longer time did not show a clear band at 100 kD and serum 10 (lane *12*) showed a strong reaction even when exposed for 1 s. The expression of Id-NM in these two samples is not as clear as the expression of Id-SG (*A*). All other express both Id-SG-SCL and Id-NM.

Both proteins reacting with the specific anti-human gamma chain also react with the anti-Id. Anti-Ids to SG-SCL and NM were expressed by the non-antitopo-I Ig of their respective FTs and in commercial IgG kappa (Sigma). None of the three anti-Ids bound to the light chain of antitopo-I (Fig. 4 *B*). The blots were negative when incubated with preimmune rabbits' purified Igs (results not shown).

To assess whether Ids to SG-SCL and NM were expressed in other antitopo-I antibodies, immunoblots on HeLa chromosomes using whole sera from 10 nonrelated positive antitopo-I scleroderma patients were performed. As shown in Fig. 5, 9 of 10 expressed Id-SG and some of these coexpressed Id-NM (lane *12* is too dark to detect a specific band at 100 kD). The more intense bands using Id-SG may reflect the higher titers, which were obtained in a direct binding ELISA (results not shown).

The presence of NM-Id in the IgG, IgM, and IgA from N.M.'s 3 daughters' sera and sera from 18 healthy nonrelated controls was studied using blocking ELISA. As shown in Fig. 6 *A*, all three daughters' Ig classes caused meaningful inhibition (25–73%) of Id/anti-Id binding. When 18 unrelated normal controls were tested, 7 inhibited ≥ 35% and 9 ≥ 20% (Fig. 6 *B*).

Thus Id-SG-SCL is expressed in antitopo-I (+) sera and Id-NM is expressed both in antitopo-I (+) sera as well as in all three classes of Ig populations devoid of antitopo-I activity in genetically unrelated and in related individuals.

Relationship between Id and antigen binding site. Fig. 7 *A* shows the blocking percentage of antitopo-I binding to topo-I by homologous anti-Id for all classes of antitopo-I in EM-SCL, SG-SCL, and NM. Anti-Ids also blocked the inhibition of topo-I uncoiling of DNA by antitopo-I in the functional assay (Fig. 8). We have previously assayed every anti-Id on DNA alone, and none of the rabbit's anti-Ids was enzymatically active in uncoiling DNA (25) (anti-Id's NM results shown in Fig. 8, lane *4*); thus, the uncoiled DNA in lane *7* is the result of topo-I activity that is not inhibited by Id-NM after incubation with anti-Id-NM.

Fig. 7 *B* shows that topo-I blocked the binding of all three classes of antitopo-I EM-SCL and SG-SCL to homologous anti-Id. In contrast, topo-I did not block NM's binding to homologous anti-Id.

To investigate whether there is a higher affinity for the anti-Id-NM than for topo-I or whether Id-NM is independent of the antigen binding site, blocking studies were carried out using varying amounts of homologous anti-Id as inhibitor. Fig. 9 *A* shows the dose-dependent inhibition of all classes of antitopo-I EM-SCL, SG-SCL, and NM. In the reverse experiment, topo-I was used to inhibit the binding of Id to homologous anti-Id (Fig. 9 *B*). Interestingly, topo-I inhibited all classes of EM-SCL and SG-SCL from binding to anti-Id in a dose-dependent manner. In contrast, it caused no inhibition of the binding of NM's IgG, IgM, or IgA, suggesting that Id-NM unlike Id-SG-SCL (CRI) or Id-EM-SCL (private) is independent of the antigen binding site.

Expression of Id-SG in monoclonal antitopo-I. Supernatants from the EBV-transformed B cell clones from patient SG-SCL were tested for Id-SG-SCL expression on direct binding ELISA. All positive antitopo-I clones were IgM kappa producers. Id-SG-SCL was expressed in all IgM supernatants (Table I).

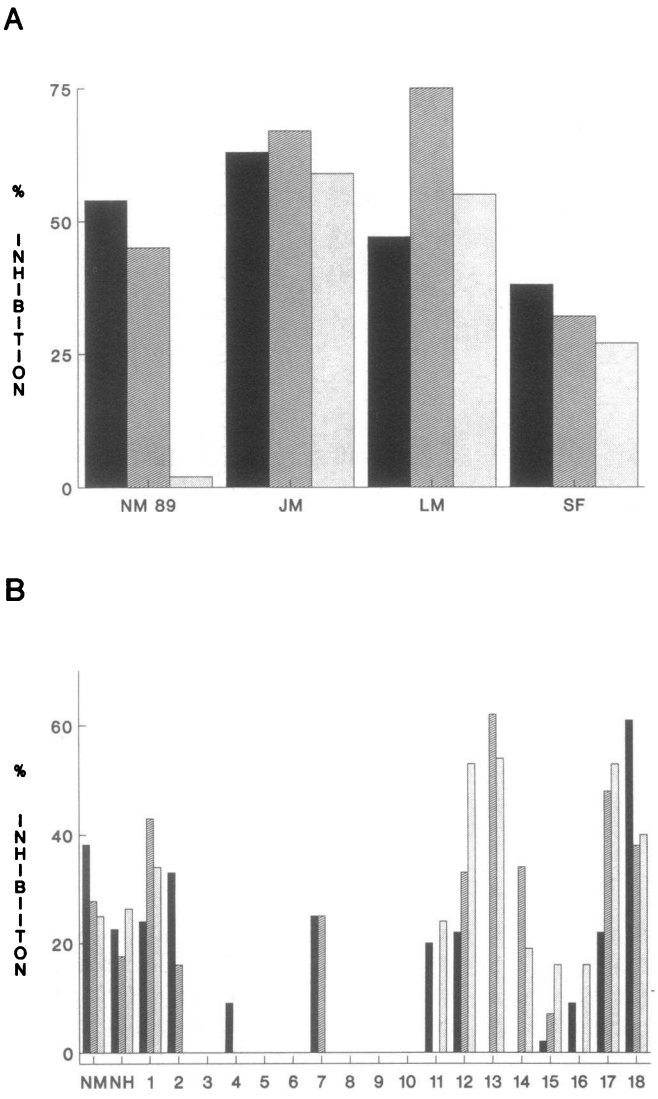

Figure 6. Blocking ELISA for detection of Id-NM in negative anti-topo-I sera. Samples were incubated at a 1:1,000 dilution with 20–25 μg/ml of anti-Id-NM or preimmune rabbit's purified Ig, tested on anti-Id-precoated plates and developed with rabbit anti–human heavy chain–specific antibody coupled to HRP (see Methods). ■, IgG; ▨, IgM; □, IgA. (*A*) NM 89, serum from NM obtained in 1989; JM, LM, and SF, sera from three daughters of NM. (*B*) NM, NM's serum; NH, normal human serum; *1–18,* sera from 18 healthy control individuals.

V H gene use by SG monoclonal antibodies. All 12 clones studied amplified V_H4 family genes (Fig. 10). After transformation of competent *E. coli* and ligation into bluescript plasmid, a total of 14 ssDNA were sequenced from eight clones. All sequences were identical (Fig. 11) and corresponded to the germline genes $V_H4.2$–1, DXP1, and J_H4. When the amino acid sequence was analyzed, the only substitutions were in the boundaries of the D segment, where one amino acid had been added from the N sequences in the rearranged gene (Fig. 11).

Discussion

Antitopo-I is a highly specific autoantibody originally described in patients with scleroderma (4–7, 13–15). Although found in approximately one-third of these patients, its presence represents the only detectable B cell autospecificity in the patient's sera. On the other hand, the frequency of precursors among healthy individuals is extremely low, and only one natural antitopo-I antibody, our NM antitopo-I, has been reported to date (5, 15).

In this paper we analyzed antigen reactivity and idiotype expression of three antitopo-I antibodies and provide evidence

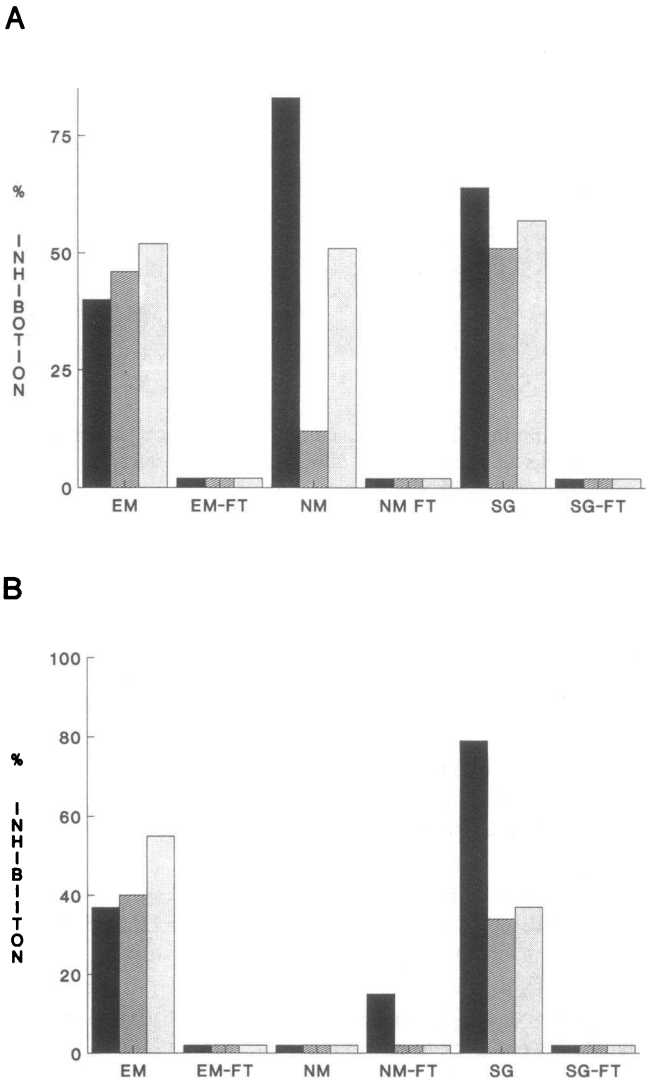

Figure 7. Relationship between Id and antigen binding site. (*A*) Blocking ELISAs performed after incubation of the purified samples with homologous anti-Ids or preimmune rabbits' purified Igs; 10–15 μg/ml of anti-Ids to EM-SCL and SG-SCL, and 20–25 μg/ml of anti-Id-NM. These samples were tested on topo-I-precoated plates. Results are expressed as percentage inhibition (see Methods). (*B*) Blocking ELISAs performed after incubation of the purified samples with 2 μg/ml of topo-I or buffer alone tested on homologous anti-Ids-precoated plates (see Methods). ■, IgG; ▨, IgM, □, IgA. EM, purified antitopo-I from scleroderma patient EM-SCL; EM-FT, flow through after absorption of EM-SCL's serum on topo-I; NM, purified antitopo-I from the healthy individual; NM-FT, flow through; SG, purified antitopo-I from scleroderma patient SG-SCL; SG-FT, flow through. (*A*) Inhibition of binding to homologous anti-Id for all classes of purified antitopo-I. None of the non-antitopo-I purified Igs was inhibited to bind homologous anti-Id. (*B*) Inhibition of binding to homologous anti-Id by topo-I only in the purified antitopo-I from scleroderma patients EM-SCL and SG-SCL (see Results).

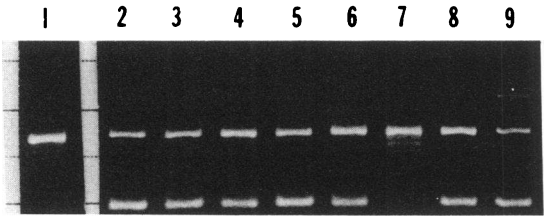

Figure 8. Topo-I functional assay on an DNA-agarose gel (see Methods). Lane *1*, migration of uncoiled DNA by topo-I; lane *2*, supercoiled DNA alone; lane *3*, DNA with preimmune rabbit's purified Ig; lane *4*, DNA with NM and preimmune rabbit's Ig incubated with topo-I; lane *5*, DNA with NM and preimmune rabbit's Ig; lane *6*, DNA with NM and anti-Id-NM; lane *7*, DNA with NM and anti-Id NM incubated with topo-I; lane *8*, DNA with anti-Id-NM; lane *9*, DNA with NM incubated with topo-I. Lane *7* shows that topo-I remains enzymatically active when Id-NM is incubated with homologous anti-Id (see Results).

for the existence of two types of autoantibody idiotypes: one private idiotype uniquely associated with antitopo-I antibodies in patients with scleroderma, and one CRI present on antitopo-I of other scleroderma patients, as well as on non-antitopo-I Ig from these patients and unrelated healthy individuals.

We detected the expression of the private scleroderma-associated Id-EM-SCL using polyclonal Id-EM-SCL (+) antibodies as they occur in the patient's serum and rabbit polyclonal anti-Ids. Id-EM-SCL is expressed mainly in IgG and IgA and to a lesser extent in IgM. The fact that polyclonal anti-Ids do not react with any other Id present in non-antitopo-I Igs from the same donor or in antitopo-I from nonrelated patients (15) strongly suggests the presence in scleroderma EM-SCL antitopo-I of a true private Id. By Western blot Id-EM-SCL is expressed on the heavy chains. Since it is stable after class switch, we can rule out the identification of an allotypic marker or an Id close to the J/constant (C) region boundary. Thus Id-EM-SCL is most likely near one or several CDRs. Moreover, antigen and anti-Id competition experiments suggest that Id-EM-SCL may be close to but not in the antigen binding site.

Unlike Id-EM-SCL, natural NM antitopo-I antibodies express a CRI that is not specific for antitopo-I since it is expressed in homologous non-antitopo-I Igs as well as in non-antitopo-I Igs from related and unrelated individuals. Contrary to that found with the scleroderma-associated Ids, topo-I did not interfere with the natural CRI-NM expression, suggesting that the structural determinants of NM idiotype expression are outside the antigen binding site. The findings in NM are similar to those reported for murine antitopo-Ids from a mouse model of scleroderma (26, 27). As in our natural idiotype NM, topo-I did not block the binding of murine antitopo-I antibodies to murine anti-Id (26).

Both scleroderma-associated Ids (the private Id-EM-SCL and the CRI-SG-SCL) differ from the natural CRI-NM in their association with the antigen binding site. In both cases, the binding to autoantigen altered the capacity of the idiotype to interact with the homologous anti-Ids and vice versa.

We have previously reported that neither NM or EM-SCL express Id-SG-SCL nor SG-SCL expresses the CRI-NM or Id-EM-SCL (15). We here carried out additional experiments and now are able to demonstrate that Id-SG-SCL is expressed in her non-antitopo-I Igs and that other antitopo-I molecules from unrelated scleroderma patients may express this CRI-SG-SCL with or without CRI-NM.

The presence of private and public Ids as well as the independent expression of different CRIs has been reported to occur in several autoantibody responses like rheumatoid factors (RF) and cold agglutinins (CA) (28). Increasing evidence suggests that CRIs may reflect the expression by different antibody

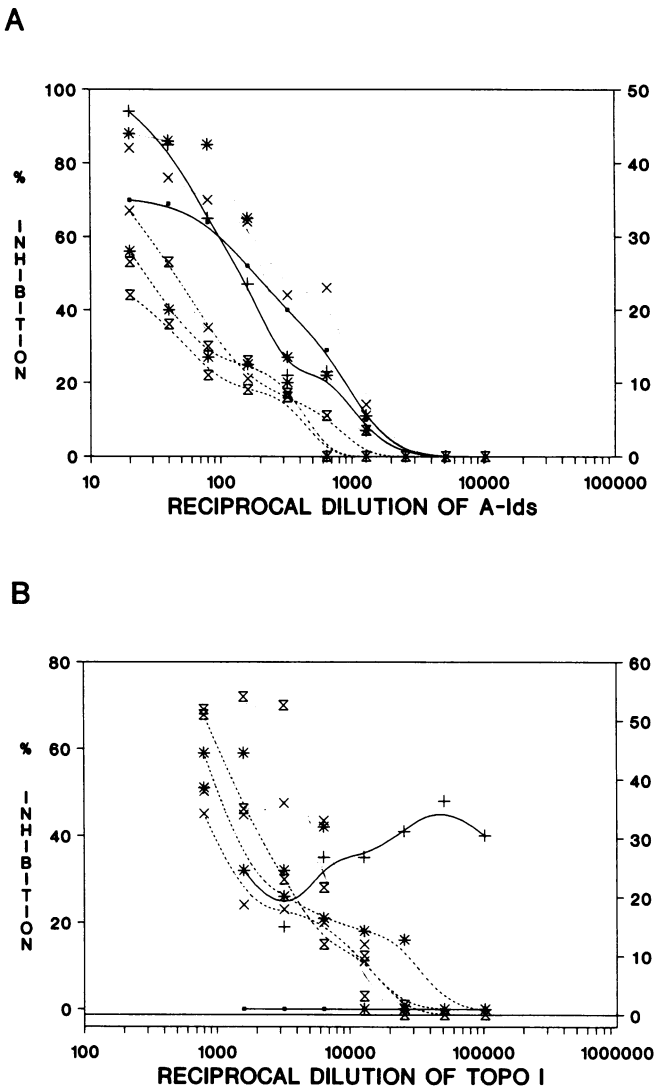

Figure 9. Blocking ELISAs with serial dilutions of inhibitor. EM-SCL IgG (· · ·*· · ·), EM-SCL IgM (· · ·▽· · ·), EM-SCL IgA (· · ·×· · ·), SG-SCL IgG (---*---), SG-SCL-IgM (--- ▽ ---), SG-SCL-IgA (--- × ---), NM IgG (— + —), NM IgM (— ⊡ —), and IgA (both curves overlapped). (*A*) Inhibition percentage of binding to topo-I when the purified antitopo-I have been incubated with serial dilutions of homologous anti-Ids starting at 70–100 μg/ml for each dilution. The IgG and IgA results are plotted in the *y1*-axis, the IgM results are plotted in the *y2*-axis since they required 3 h of incubation at room temperature with the color-substrate in the last step and thus were carried out in different plates. (*B*) Preincubation of purified antitopo-I with serial dilutions of topo-I starting with 1 μg/ml. *A* shows that inhibition of antitopo-I Ids binding to topo-I by anti-Ids follows a dose-response curve for all the Ig classes of antitopo-I. *B* shows how topo-I fails to inhibit Id-NM/anti-Id-NM binding at any concentration used for all NM classes. All classes of EM-SCL and SG-SCL show dose-response inhibition (see Results).

Table I. Direct Binding ELISA on Topo-I and on Anti-ID

	SG-SCL	SG-FT	1	2	3	4	5	6	7	8
Binding on topo-I precoated plates										
IgG	298	160	127	118	126	118	120	123	114	112
IgM	119	118	1803	2025	1655	2245	1130	1434	757	944
IgA	160	117	117	111	121	118	118	115	123	113
Binding on anti-Id to SG-SCL precoated plates										
IgG	1069	1219	146	136	149	133	133	133	131	147
IgM	171	235	573	618	596	854	407	456	413	363
IgA	192	276	128	133	126	131	155	157	170	126

Supernatants from SG-SCL clones corresponding to one to eight sequenced ssDNAs were used in a direct binding ELISA on topo-I precoated plates. Results are given as the mean of the triplicate OD values at 405 nm × 1,000. Positive OD values are IgG > 0.2, IgM > 0.15, and IgA > 0.15.

molecules of particular heavy and/or light chain gene segments in or near germline configuration. The association of these CRIs and autospecificities could then be interpreted as restriction in the usage of the V_H or V_L genes in particular autoimmune responses. Examples of this restriction are the 16/6 Id expressed on IgM antibodies with anti-DNA activity that has been linked to the V_H26 (V_H3 family) germline gene (29). The 17.109 Id related to the 328 kappa IIIb germline gene, expressed on RF and cold agglutinin molecules (30–32), and the 9G4 Id encoded within the V_H4–21 germline gene and is particularly restricted to anti-red blood cell activity (33) and several other CRIs that have been linked to heavy chains (28, 34–48).

CRIs may result, on the other hand, from somatic mutations or specific rearrangements that conform a particular CDR structure within the V_H or V_L regions (49–53). One report clearly defined an interspecies CRI located in the CDR2 of the V_H expressed in nonrelated V_H genes (54).

Idiotypes may be expressed in the light chain (LC), heavy chain (HC), or require the association of both LC and HC. An interesting aspect of our findings is the localization of idiotype to V_H only. We show that the binding of all anti-Ids required only the heavy chains of the antibodies. Idiotypes only at the HC have been documented in the past (28–30, 33, 36–38, 43–45, 49, 50, 53–56) and they may not require the association with the LC (28, 42, 43, 53–56).

The molecular analysis of the V_H gene segment expressing the scleroderma-associated CRI-SG-SCL disclosed the usage of the germline genes V_H4–2.1, DXP1, and J_H4. The use of a V_H4 gene is interesting and adds antitopo-I to the list of other autoantibodies encoded by this small V_H family. Also interesting is the use of germline copies of the DXP1 and J_H4 genes that have also been described in other human autoantibodies like monoclonal RF and CAs (28, 29, 45).

The origin of autoantibodies is still a controversial issue. The Ig molecule results from V_H and V_L gene rearrangements occurring early in B cell maturation and from somatic changes that take place later in B cell development (57, 58). The fetal and neonatal B cell repertoires include genes that have been shown to encode self-reactive antibodies (34, 46–48, 57–59) and some of these genes have been proposed to play an important role in immune regulation and development of normal immune repertoire (34–36, 46, 48, 57–59). Autoreactivity could be a result of immune disregulation leading to the overexpression of particular self-reactive germline Ig genes (34–37, 46, 47, 57, 59–63). On the other hand it has been suggested that autoantibodies result from an antigen-driven process, i.e., the autoantigen selects those somatically mutated B cell clones that produce an antibody with highest affinity for the antigen. This second view is supported by similarities of disease-associated autoantibodies to the antibodies found in the secondary immune response (38, 49, 50, 63–69).

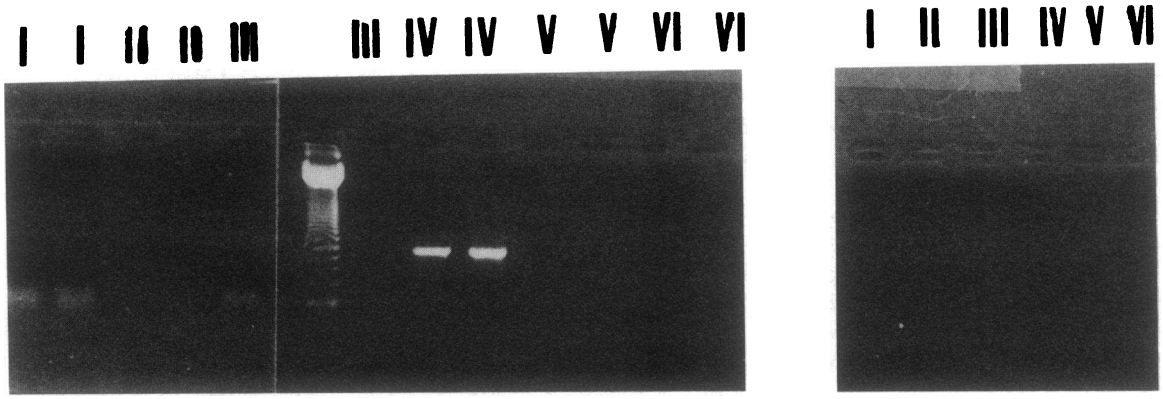

Figure 10. Agarose gel of the DNA-PCR amplified fractions (see Methods). DNA from all clones amplified the V_H4 family gene (clone 3.31 is shown here). Each amplification was carried out by duplicate, using primers for the VH families I, II, III, IV, V, and VI. On the right the PCR control reaction with no DNA is shown (see Methods).

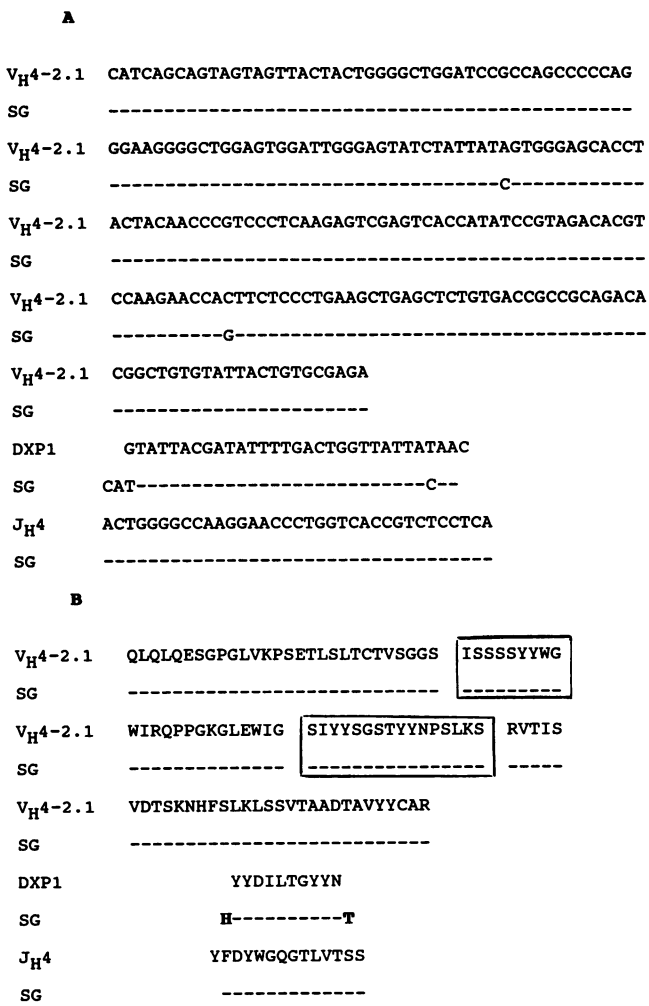

Figure 11. Comparison of the nucleotide (*A*) and amino acid (*B*) sequences of the SG heavy chain variable region and the V_H4.2-1, DXP1, and J_H4 germline genes. Identity of the sequences is denoted by dashes. CDRs are denoted by boxes in the amino acid sequence.

Our data show that all the IgM kappa antitopo-I from SG-SCL clones expressed the same CRI-SG-SCL found in the serum. Furthermore, we showed that all classes of CRI-SG-SCL (+) serum antitopo-I displayed similar dose–response curves on inhibition studies most likely with similar avidities and affinities for the autoantigen as well as for the anti-Ids. Thus the V_H-associated CRI-SG-SCL is likely encoded by the same or very similar germline gene in the B cells secreting different classes of antitopo-I.

Studies of CAs from different individuals has suggested that anti–red blood cells responses are restricted in the usage of the V_H4–21-encoded CRI. Since the anti-Id blocks both the binding and cold agglutination, this CRI may be close to the CDRs (33, 55). The high prevalence of V_H4–21-encoded autoreactive antibodies (70) and the binding of protein A to the V_H3 region of Igs (18) have raised the novel concept of a nonconventional antigen binding site for autoantigens (70). The specificity for antitopo-I could thus be within this V_H4 gene. The future molecular analysis of other scleroderma antitopo-I producers from nonrelated individuals, including NM, will define if there is indeed a preferential use of this or other V_H4 family member and if there is an association between the expression of CRI-SG and disease. It has been suggested that disease-associated IgG autoantibodies may originate from IgM precursors that belong to the pool of natural autoantibodies (71, 72). Some of these disease-associated autoantibodies have undergone class switch and presumably accumulate somatic mutations that increase the affinity for the autoantigen (64–67) and at the same time modify the expressed Id rendering it private (39, 51, 52, 61, 62, 64, 65, 68, 69).

It will be important to identify the gene used by CRI-NM, which may be a non-self–reacting germline gene. It will also be necessary to determine whether other anti-topo-I antibodies that share the CRI-SG use the V_H4–2.1 germline gene. Such antibodies would then be different from CRI-NM. In this case, CRI-SG may be encoded by an autoreactive V_H germline gene combination that has been either autoantigen-selected (60) or expressed after a deletion of a closer, non-self–reactive gene (36), by other immune disregulation at the idiotype network, or at the T cell level (67, 70–72).

In summary, the idiotypic studies presented herein are consistent with the notion that autoantibodies sharing the same specificity can be encoded by either germline genes or genes that have undergone a process of somatic mutation. It is likely that antigen-driven selection of certain somatically mutated B cells may be responsible for the emergence of private idiotypes and possible escape from idiotype network regulation.

The fact that both types of autoantibodies were found in the limited number of patients studied also suggests that it may not be unrealistic to imagine that both private idiotypes and CRIs may coexist in the same patient and that the expression of germline genes plays a protective role against the emergence of somatically mutated autoreactive clones (72).

Acknowledgements

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References

1. Maricq, H. R., M. C. Weinreich, J. E. Keil, E. A. Smith, F. E. Harper, A. L. Nussbaum, F. C. LeRoy, An R. McGregor, F. Diat, and E. J. Roal. 1989. Prevalence of scleroderma spectrum disorders in the general population of South Carolina. *Arthritis Rheum.* 32:998–1006.

2. Douvas, A. S., M. Achten, and E. M. Tan. 1979. Identification of nuclear protein (Scl 70) as a unique target of human antinuclear antibodies in scleroderma. *J. Biol. Chem.* 254:10514–10522.

3. Shero, J. H., B. Bordwell, N. F. Rothfield, and W. C. Earnshaw. 1986. High titers of autoantibodies to topoisomerase I (scl-70) in sera from scleroderma patients. *Science (Wash. DC).* 231:737–740.

4. Shero, J. H., B. Bordwell, N. F. Rothfield, and W. C. Earnshaw. 1987. Antibodies to topoisomerase I in sera from patient with scleroderma. *J. Rheumatol.* 14(Suppl 13):138–140.

5. Weiner, E. S., W. C. Earnshaw, J.-L. Senecal, B. Bordwell, P. Johnson, and N. R. Rothfield. 1988. Clinical association of anticentromere antibodies and antibodies to topoisomerase I. *Arthritis Rheum.* 31:378–385.

6. Weiner, E., S. Hildebrandt, J.-L. Senecal, L. Daniels, G. S. Noell, W. C. Earnshaw, and N. F. Rothfield. 1991. Prognostic significance of anticentromere antibodies and antitopoisomerase I antibodies in Raynaud's Disease. *Arthritis Rheum.* 34:68–77.

7. Hildebrandt, S., E. S. Weiner, J.-L. Senecal, G. S. Noell, W. C. Earnshaw, and N. F. Rothfield. 1990. Autoantibodies to topoisomerase-I (Scl-70): analysis by gel diffusion, immunoblot and enzyme-linked immunosorbent assay. *Clin. Immunol. Immunopathol.* 57:399–410.

8. D'Arpa P., P. S. Machlin, H. Ratrie, N. F. Rothfield, D. W. Cleveland, and W. C. Earnshaw. 1988. cDNA cloning of human DNA topoisomerase-I: catalytic activity of a 67.7-kDa carboxyl-terminal fragment. *Proc. Natl. Acad. Sci. USA.* 85:2543–2547.

9. Heck, M. M., W. N. Hittleman, and W. C. Earnshaw. 1988. Differential

expression of DNA topoisomerases I and II during the eurkaryotic cell cycle. *Proc. Natl. Acad. Sci. USA.* 85:1086–1090.

10. Hoffman, A., M. M. Heck, B. Bordwell, N. F. Rothfield, and W. C. Earnshaw. 1989. Human autoantibody to topoisomerase II. *Exp. Cell Res.* 180:409–418.

11. Oddou, P., U. T. Schmidt, R. Knippers, and A. Richter. 1988. Monoclonal antibodies neutralizing mammalian DNA topoisomerase I activity. *Eur. J. Biochem.* 177:523–529.

12. Juarez C., J. L. Vila, C. Gelpi, M. Agusti, M. J. Amengual, M. A. Martinez, and J. L. Rodriguez. 1988. Characterization of the antigen reactive with anti-Scl-70 antibodies and its application in an enzyme-linked immunosorbent assay. *Arthritis Rheum.* 31:108–115.

13. D'Arpa P., H. Cooper-White, D. W. Cleveland, N. F. Rothfield, and W. C. Earnshaw. 1990. Use of molecular cloning methods to map the distribution of epitopes on topoisomerase-I (Scl-70) recognized by sera of scleroderma patients. *Arthritis Rheum.* 33:1501–1511.

14. Hildebrandt S., E. Weiner, J.-L. Senecal, G. S. Noell, L. Daniels, W. C. Earnshaw, and N. F. Rothfield. 1990. The IgG, IgM, and IgA isotypes of anti-topisomerase I and anticentromere antibodies. *Arthritis Rheum.* 33:724–727.

15. Hildebrandt S., G. S. Noell, D. Vazquez-Abad, W. C. Earnshaw, M. Zanetti, and N. R. Rothfield. 1991. Idiotypic analysis of human anti-topoisomerase I autoantibodies. *Autoimmunity.* 10:41–48.

16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 227:680–685.

17. Gallager, S. R., and J. A. Smith. 1991. Electrophoretic separation of proteins. *In* Current Protocols in Immunology. J. E. Coligan, A. M. Kruisbeek, D. M. Margolies, E. M. Shevach, and W. Strober, editors. Wiley Interscience, Greene Publishing Associates, New York. 841–842.

18. Sasso, E. H., G. J. Silverman, and M. Mannik. 1991. Human IgA and IgG $F(ab)_2$ that bind to staphylococcal protein A belong to the V_H III subgroup. *J. Immunol.* 147:1877–1883.

19. Steinitz, M., G. Kleine, S. Koskimies, and O. Makel. 1977. EB virus-induced B lymphocyte cell lines producing specific antibodies. *Nature (Lond.).* 269:420–422.

20. Chomczynski, P., and N. Sacci. 1987. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

21. Marks, J. D., M. Tristem, A. Karpas, and G. Winter. 1991. Oligonucleotide primers for polymerase chain reaction amplification of human immunoglobulin variable genes and design of family-specific oligonucleotide probes. *Eur. J. Immunol.* 21:985–991.

22. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higushi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science (Wash. DC).* 239:487.

23. Sanger, F. A., R. Carlson, B. G. Barrel, A. J. H. Smith, and B. Roe. 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. *J. Mol. Biol.* 143:161–178.

24. Tabor, S., and C. C. Richardson. 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Nat. Acad. Sci. USA.* 84:4767–4471.

25. Shuster, A. M., G. V. Gololobov, O. A. Kvashuk, A. E. Bogomolova, I. V. Smirnov, and A. G. Gabibov. 1992. DNA hydrolyzing autoantibodies. *Science (Wash. DC).* 256:665–667.

26. Muryoi, T., K. N. Kasturi, M. J. Kafina, Y. Saitoh, O. Usuba, J. S. Perlish, R. Fleischmajer, and C. A. Bona. 1991. Self reactive repertoire of tight skin mouse: immunochemical and molecular characterization of anti-topoisomerase I autoantibodies. *Autoimmunity.* 9:109–117.

27. Muryoi, T., K. N. Kasturi, M. J. Kafina, D. S. Cram, L. C. Harrison, T. Sasaki, and C. A. Bona. 1992. Antitopoisomerase-I monoclonal antibodies from scleroderma patients and tight skin mouse interact with similar epitopes. *J. Exp. Med.* 175:1103–1109.

28. Gorevic, P. D., and B. Frangione. 1991. Mixed cryoglobulinemia cross-reactive idiotypes: implications for the relationship of MC to rheumatic and lymphoproliferative diseases. *Semin. Hematol.* 28:79–94.

29. Dersimonian, H., R. S. Schwartz, K. L. Barrett, and B. D. Stollar. 1987. Relationship of human variable region heavy chain germ-line genes to genes encoding anti-DNA autoantibodies. *J. Immunol.* 139:2496–2501.

30. Crowley, J. J., R. D. Goldfien, R. E. Schrohenloher, H. L. Spiegelberg, G. J. Silverman, R. A. Mageed, R. Jefferis, W. J. Koopman, D. A. Carson, and S. Fong. 1988. Incidence of three cross-reactive idiotypes on human rheumatoid factor paraproteins. *J. Immunol.* 140:3411–3418.

31. Mageed, R. A., M. Dearlove, D. M. Goodall, and R. Jefferis. 1986. Immunogenic and antigenic epitopes of immunoglobulins XVII-monoclonal antibodies reactive with common and restricted epitopes to the heavy chain of human rheumatoid factors. *Rheumatol. Int.* 6:179–183.

32. Mageed, R. A., M. R. Walker, and R. Jefferis. 1986. Restricted light chain subgroup expression on human rheumatoid factor paraproteins determined by monoclonal antibodies. *Immunology.* 59:473–478.

33. Stevenson, F. K., M. Wrightham, M. J. Glennie, D. B. Jones, A. R. Cattan, T. Feizi, T. J. Hamilton, and G. T. Stevenson. 1986. Antibodies to shared idiotypes as agents for analysis and therapy for human B-cell tumors. *Blood* 68:430.

34. Chen, P. P., M.-F. Liu, C. A. Glass, S. Sinha, T. J. Kipps, and D. A. Carson. 1989. Characterization of two immunoglobulin V_H genes that are homologous to human rheumatoid factors. *Arthritis Rheum.* 32:72–76.

35. Chen, P. P., M.-F. Liu, S. Sinha, and D. A. Carson. 1988. A 16/6 idiotype-positive anti-DNA antibody is encoded by a conserved V_H gene with no somatic mutation. *Arthritis Rheum.* 31:1429–1431.

36. Olee, T., P. M. Yang, K. A. Simminovitch, N. J. Olsen, J. Hillson, J. Wu, F. Kozin, D. A. Carson, and P. P. Chen. 1991. Molecular basis of an autoantibody-associated restriction fragment length polymorphism that confers susceptibility to autoimmune diseases. *J. Clin. Invest.* 88:193–203.

37. Mageed, R. A., L. E. MacKenzie, F. K. Stevenson, B. Yuksel, F. Shokri, B. R. Maziak, R. Jefferis, and P. M. Lydyard. 1991. Selective expression of a V_HIV subfamily of immunoglobulin genes in human $CD5^+$ B lymphocytes from cord blood. *J. Exp. Med.* 174:109–113.

38. Martin, T., S. F. Duffy, D. A. Carson, and T. J. Kipps. 1992. Evidence for somatic selection of natural autoantibodies. *J. Exp. Med.* 175:983–991.

39. Kipps, T. J., E. Tomhave, P. P. Chen, and R. I. Fox. 1989. Molecular characterization of a major autoantibody-associated cross-reactive idiotype in Sjogren's syndrome. *J. Immunol.* 142:4261–4268.

40. Silverman, G. J., R. D. Goldfien, P. Chen, R. A. Mageed, R. Jefferis, F. Goni, B. Frangione, S. Fong, and D. A. Carson. 1988. Idiotypic and subgroup analysis of human monoclonal rheumatoid factors. Implications for structural and genetic basis of autoantibodies in humans. *J. Clin. Invest.* 82:469–475.

41. Silverman, G. J., F. Goni, J. Fernandez, P. P. Chen, B. Frangione, and D. A. Carson. 1988. Distinct patterns of heavy chain variable region subgroup use by human monoclonal autoantibodies of different specificity. *J. Exp. Med.* 168:2361–2366.

42. Stevenson, F. K., M. B. Spellerberg, L. C. Jefferies, J. Goldman, T. J. Hamblin, V. Pascual, J. D. Capra, and L. E. Silberstein. 1991. Monoclonal gammopathies and autoimmunity. *In* Monoclonal Gammopathies III. Clinical Significance and Basic Mechanisms. J. Radi and B. van Camp, editors. 1991 Eurage, Brussels. 33–39.

43. Pascual, V., K. Victor, D. Lelsz, M. B. Spellerberg, T. J. Hamblin, K. M. Thompson, I. Randen, J. Natvig, J. D. Capra, and F. K. Stevenson. 1991. Nucleotide sequence analysis of the V regions of two IgM cold agglutinins. *J. Immunol.* 146:4385–4391.

44. Jefferies, L. C., G. J. Silverman, C. M. Carchidi, and L. E. Silberstein. 1992. Idiotypic heterogeneity of V_kIII autoantibodies to red blood cell antigens. *Clin. Immunol. Immunopathol.* 65:119–128.

45. Pascual, V., K. Victor, M. Spellerberg, T. J. Hamblin, F. K. Stevenson, and J. D. Capra. 1992. V_H restriction among human cold agglutinins. *J. Immunol.* 149:2337–2344.

46. Sanz, I., H. Dang, M. Takei, N. Talal, and J. D. Capra. 1989. V_H sequence of a human anti-Sm autoantibody. Evidence that autoantibodies can be unmutated copies of germline genes. *J. Immunol.* 142:883–887.

47. Capra, J. D. 1989. Immunoglobulin V_H genes in systemic lupus erythematosus. *Curr. Opin. Rheumatol.* 1:343–346.

48. Pascual, V., and J. D. Capra. 1992. V_H4–21, A human V_H gene segment overrepresented in the autoimmune repertoire. *Arthritis Rheum.* 35:11–18.

49. Reidl, L. S., D. F. Friedman, J. Goldman, R. R. Hardy, L. C. Jefferies, and L. E. Silberstein. 1991. Structural basis of a conserved idiotope expressed by an autoreactive human B-cell lymphoma. 147:3623–3631.

50. Chen, C., M. P. Stenzel-Poore, and M. B. Rittenberg. 1991. Natural auto- and polyreactive antibodies differing from antigen-induced antibodies in the H chain CDR3. *J. Immunol.* 147:2359–2367.

51. Davidson, A., A. Smith, J. Katz, J.-L. Preud'Homme, A. Solomon, and B. Diamond. 1989. A cross-reactive idiotype on anti-DNA antibodies defines a H chain determinant present almost exclusively on IgG antibodies. *J. Immunol.* 143:174–180.

52. Davidson, A., J.-L. Preud'Homme, A. Solomon, M.-D. Y. Chang, S. Beede, and B. Diamond. 1987. Idiotypic analysis of myeloma proteins: anti-DNA activity of monoclonal immunoglobulins bearing an SLE idiotype is more common in IgG than IgM antibodies. *J. Immunol.* 138:1515–1518.

53. Kang, C.-Y., T. K. Brunk, T. Kieber-Emmons, J. E. Blalok, and H. Kohler. 1988. Inhibition of self-binding antibodies (autobodies) by a V_H-derived peptide. *Science (Wash. DC).* 240:1034–1036.

54. Meek, K., M. Takei, H. Dang, I. Sanz, M. J. Dauphinee, J. D. Capra, and N. Talal. 1990. Anti-peptide antibodies detect a lupus-related interspecies idiotype that maps to H chain CDR2. *J. Immunol.* 144:1375–1381.

55. Thompson, K. M., J. Sutherland, G. Barden, M. D. Melamed, I. Randen, J. B. Natvig, V. Pascual, J. D. Capra, and F. K. Stevenson. 1991. Human monoclonal antibodies against blood group antigens preferentially express a V_H4–21 variable region gene-associated epitope. *Scand. J. Immunol.* 34:509–518.

56. Sollazzo, M., D. Castiglia, R. Billetta, A. Tramontano, and M. Zanetti. 1990. Structural definition by antibody engineering of an idiotypic determinant. *Protein Eng.* 3:531–539.

57. Honjo, T. 1983. Immunoglobulin genes. *Annu. Rev. Immunol.* 1:499–528.

58. Milstein, C. 1985. From the structure of antibodies to the identification of the immune response. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:1083–1092.

59. Cairns, E., P. C. Kwong, V. Misener, P. Ip, D. A. Bell, and K. A. Siminovitch. 1989. Analysis of variable region genes encoding a human anti-DNA antibody of normal origin. *J. Immunol.* 143:685–691.

60. Pascual, V., K. Victor, I. Randen, K. Thompson, J. B. Natvig, and J. D. Capra. 1992. IgM rheumatoid factor in patients with rheumatoid arthritis derive from a diverse array of germline genes and display little evidence of somatic mutation. *J. Rheumatol.* 19:50–53.

61. Siminovitch, K. A., V. Misener, P. C. Kwong, Q.-L. Song, and P. P. Chen. 1989. A natural autoantibody is encoded by germline heavy and lambda light chain variable region genes without somatic mutation. *J. Clin. Invest.* 84:1675–1678.

62. Marion, T. N., A. L. M. Bothwell, D. E. Briles, and C. A. Janeway, Jr. 1989. IgG anti-DNA autoantibodies within an individual autoimmune mouse are the products of clonal selection. 142:4269–4274.

63. Gupta, S., and N. Talal. 1985. Immunology of the Rheumatic Diseases. Plenum Medical Book Co., New York. 818 pp.

64. Schlomchik, M. J., A. Marshak-Rothstein, C. B. Woflowicz, T. L. Rothstein, and M. G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.).* 328:805–811.

65. Berek, C. 1989. Molecular dissection of an antigen-specific immune response. *J. Autoimmun.* 2:195–201.

66. French, D. L., R. Laskov, and M. D. Scharff. 1989. The role of somatic hypermutation in the generation of antibody diversity. *Science (Wash. DC).* 244:1152–1157.

67. Spitzer, R. E., A. E. Stitzel, and G. C. Tsokos. 1992. Autoantibody to the alternative pathway C3/C5 convertase and its anti-idiotypic response. *J. Immunol.* 148:137–141.

68. Behar, S. M., and M. D. Scharff. 1988. Somatic diversification of the S107 (T15) V_H11 germ-line gene that encodes the heavy-chain variable region of antibodies to double-stranded DNA in (NZB × NZW)F_1 mice. *Proc. Natl. Acad. Sci. USA.* 85:3970–3974.

69. Shlomchik, M., M. Mascelli, H. Shan, M. Z. Radic, D. Pisetsky, A. Marshak-Rothstein, and M. Weigert. 1990. Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* 171:265–297.

70. Pascual, V., and J. D. Capra. 1991. B-cell superantigens? *Curr. Biol.* 1:315–317.

71. Gilbert, D., C. Margaritte, B. Payelle-Brogart, and F. Tron. 1992. Development of the B-cell anti-DNA repertoire in (NZB × NZW)F_1 mice. *J. Immunol.* 149:1795–1801.

72. Iwai, K., T. Tsubata, Y. Katsura, S. Kumagai, and H. Imura. 1991. Qualitative difference of anti-DNA antibody-producing cell precursors in the pre-immune B-cell repertoire between normal and lupus-prone mice. *Clin. Exp. Immunol.* 86:106–111.