

## A SHARED IDIOTYPE AMONG HUMAN ANTI-Ro/SSA AUTOANTIBODIES

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An idiotypic determinant is unique (private) to a given antibody molecule and is associated with the antigen combining site of the antibody. Crossreactive idiotypes are shared antigenic determinants between antibodies of different antigenic specificities in one individual or among antibodies against the same antigen in different individuals.

Monoclonal IgM rheumatoid factors were the first autoantibodies known to share idiotypic reactivity (1). Shared idiotype has also been demonstrated for autoantibodies found in autoimmune disorders, including human (2) and murine lupus (3).

In systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS)<sup>1</sup> shared idiotypes have been described for antibodies to DNA (2), Sm (4), and IgG (rheumatoid factor) (5). Anti-DNA idiotypes have been related to Ig deposition in the kidneys of SLE patients and mice (6, 7). Idiotypic specificities are consequently thought to reflect the pathogenic potential of autoantibodies in this disease.

Autoantibodies to the RNA protein antigens Ro/SSA and La/SSB are also very common in SLE and SS, often present in serum concentrations >1 mg/ml. These autoantibodies are associated with differences in disease expression (8). To date, there has been little success in defining idiotypic characteristics of these antibodies. Only private idiotypes have been reported for anti-La/SSB in humans (9) and none have been characterized for anti-Ro/SSA. To examine anti-Ro/SSA idiotypes, we prepared a heterologous rabbit antiidiotypic to anti-Ro/SSA F(ab')<sub>2</sub> affinity purified from the serum of a precipitin positive individual and evaluated the possibility of crossreactive idiotypic between anti-Ro/SSA<sup>+</sup> individuals.

### Materials and Methods

*Serum Samples.* Sera from 13 female donors with precipitating levels of anti-Ro/SSA were collected and stored at -20°C. Anti-Ro/SSA levels were quantitated by ELISA as previously described (10-12).

*Anti-Ro/SSA Purification.* Anti-Ro/SSA was prepared from serum by affinity chromatography as previously described (11). The protein yield was ~1 mg of antibody per mg of Ro/SSA

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<sup>1</sup> Abbreviation used in this paper: SS, Sjögren's syndrome.

coupled. Anti-Ro/SSA previously purified and characterized from a normal male donor (11) was also included in this study.

**Antiidiotype Production.** Immune serum against anti-Ro/SSA (antiidiotype) was made by injecting a New Zealand white female rabbit with affinity-purified F(ab')<sub>2</sub> fragments of anti-Ro/SSA from a patient. F(ab')<sub>2</sub> fragments of serum anti-Ro/SSA were prepared as previously described (12), and 300 µg in 1 ml CFA was injected intramuscularly and subcutaneously into a rabbit. The animal was boosted at 25 d with 300 µg anti-Ro/SSA F(ab')<sub>2</sub> in 1 ml IFA and again after 50 d with 300 µg in saline. The rabbit was bled after 7 wk and subsequent sera were collected over a 4-mo period.

**Antiidiotype Purification.** Antiidiotype was purified from the immune rabbit serum by affinity chromatography. Affinity-purified anti-Ro/SSA IgG (~7 mg) was coupled to Sepharose 4B. The immune rabbit serum was dialyzed into Tris buffer and passed over the anti-Ro/SSA column twice. The bound antibody was washed, eluted, and dialyzed in Tris buffer. The purified rabbit Ig was absorbed extensively with Cohn Fraction II IgG (Sigma Chemical Co., St. Louis, MO) coupled to Sepharose to remove reactivity against public framework epitopes.

**Enzyme-linked Immunosorbent Assay (ELISA).** The antiidiotypic activity of the rabbit antibody was detected by a solid-phase enzyme immunoassay modified from methods previously described (12). Briefly, microtiter plates were coated with purified anti-Ro/SSA at 1–2 µg/ml. Absorbed rabbit antiidiotype (0.1–10 µg/ml) or normal rabbit serum (10<sup>-3</sup>–10<sup>-6</sup> dilutions) were added to the anti-Ro/SSA-coated plates. Antiidiotype binding was detected with goat anti-rabbit IgG-alkaline phosphatase conjugate crossabsorbed with human serum proteins to decrease nonspecific binding (Sigma Chemical Co.). Wells were developed with *p*-nitrophenyl phosphate and optical densities determined. The specificity of the rabbit antiidiotype was confirmed by blocking assays where antiidiotype was preincubated with increasing concentrations (0.01–100 µg/ml) of anti-Ro/SSA IgG and F(ab')<sub>2</sub> or Cohn Fraction II IgG. The incubation mixtures were then added to anti-Ro/SSA IgG-coated plates and developed as described above.

The reverse assay was used to further characterize the anti-Ro/SSA idiotype (Id-RoI). Absorbed rabbit antiidiotype (5 µg/ml) was coated followed by 3 µg/ml anti-Ro/SSA and goat anti-human IgG-alkaline phosphatase conjugate (Sigma Chemical Co.). The conjugate was mixed with a 1:100 dilution of normal rabbit serum to decrease nonspecific binding. Additional conjugates used included goat anti-human µ, α, κ, and λ chain-specific reagents. Blocking assays were performed where 3 µg/ml anti-Ro/SSA IgG was preincubated with 1–30 µg/ml purified Ro/SSA for 2 h at room temperature before being added to the rabbit antiidiotype coated plate.

**Western Blot.** SDS-PAGE was performed under nonreducing conditions using the Phast System (Pharmacia Fine Chemicals, Uppsala, Sweden). 100 ng per lane of Cohn Fraction II IgG or affinity-purified anti-Ro/SSA in 2% SDS and 10% glycerol were each electrophoresed in an 8–20% gradient gel (Pharmacia Fine Chemicals) for 30 min at 15°C. The gel was then overlaid with nitrocellulose paper and the proteins were heat transferred for 20 min at 70°C. The immunoblot was blocked overnight in 0.02 M Tris buffer, 0.15 M NaCl, 0.02% azide, pH 8.0, with 0.05% Tween-20 and 2% nonfat dry milk. Each incubation was at room temperature with shaking. The nitrocellulose strips were then incubated with 10 µg/ml absorbed rabbit antiidiotype for 1 h. After several 5-min washes of Tris buffer with Tween-20, the blots were incubated with anti-rabbit IgG-alkaline phosphatase conjugate for 1 h. The protein bands were developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium according to the Protoblot Immunoblotting System (Promega Biotec, Madison, WI). Positive control lanes were blotted with anti-human IgG-alkaline phosphatase and conjugate control lanes were developed only with anti-rabbit IgG-alkaline phosphatase followed by substrate.

## Results and Discussion

To study anti-Ro/SSA idiotypes, a rabbit was immunized with affinity-purified anti-Ro/SSA F(ab')<sub>2</sub>. The immunogen had no Fc reactivity by solid-phase ELISA. The resulting antiidiotype was purified from rabbit serum by affinity chromatog-

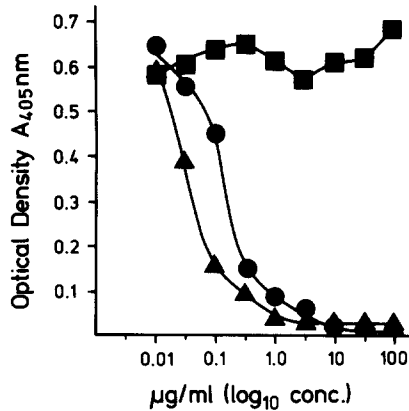


FIGURE 1. Binding specificity of the absorbed rabbit antiidiotype. Rabbit anti-Id-Rol was incubated with increasing amounts of Cohn Fraction II IgG (■), affinity-purified anti-Ro/SSA IgG (●), and affinity-purified anti-Ro/SSA F(ab)<sub>2</sub> (▲). Both IgG and F(ab)<sub>2</sub> anti-Ro/SSA completely inhibited binding of anti-Id-Rol to anti-Ro/SSA IgG in the solid-phase ELISA. Cohn Fraction II IgG was unable to inhibit anti-Id-Rol binding to anti-Ro/SSA.

raphy and absorbed extensively with Cohn fraction II IgG. The absorbed rabbit antiidiotype anti-Id-Rol was specific for the anti-Ro/SSA F(ab)<sub>2</sub> immunogen and did not bind normal human IgG as shown by inhibition ELISA in Fig. 1. The antiidiotype was blocked by anti-Ro/SSA IgG and F(ab)<sub>2</sub> but not by normal human IgG. Conversely, purified anti-Ro/SSA did not bind normal rabbit serum when tested by ELISA (data not shown).

When assayed by ELISA for isotype, the idiotype (Id-Rol) was present on  $\gamma$  heavy chains with either  $\kappa$  or  $\lambda$  light chains. This suggests a heavy chain location for Id-Rol, as idiotype-positive IgG consisted of both light chain types (data not shown).

A competition ELISA was used to determine whether the anti-Ro/SSA idiotype was associated with the Ro/SSA binding site. When anti-Ro/SSA was incubated with Ro/SSA, the binding of rabbit antiidiotype and Id-Rol was completely blocked (Fig. 2). This indicates that the location of Id-Rol on anti-Ro/SSA is in or close to the antigen binding site of anti-Ro/SSA.

The anti-Ro/SSA specificity of the antiidiotype was additionally confirmed by Western blotting. The anti-Id-Rol bound anti-Ro/SSA IgG but did not bind an equal amount of normal human IgG under nonreducing conditions (Fig. 3). Anti-Id-Rol activity was dependent upon the conformational Ig structure for binding, as anti-Ro/SSA was not bound under reducing conditions.

Anti-Ro/SSA was affinity purified from the serum of 12 additional anti-Ro/SSA precipitin-positive donors. Each anti-Ro/SSA preparation was then tested by ELISA

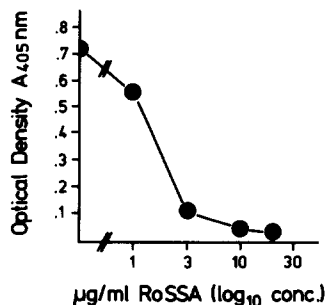


FIGURE 2. Inhibition of idiotype-antiidiotype interaction with Ro/SSA antigen. Affinity-purified anti-Ro/SSA IgG was incubated with increasing amounts of purified Ro/SSA before being added to the anti-Id-Rol-coated microtiter plate. Ro/SSA completely blocked the anti-Ro/SSA/anti-Id-Rol binding.

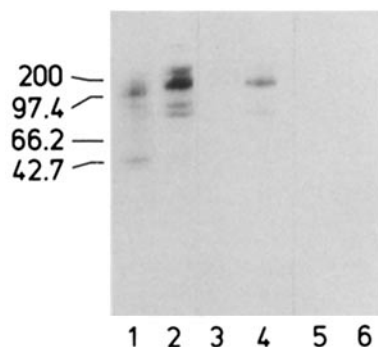


FIGURE 3. Western blot of Cohn Fraction II IgG and anti-Ro/SSA IgG with anti-Id-Rol. Lanes 1, 3, and 5 contain Cohn Fraction II IgG and lanes 2, 4, and 6 contain affinity-purified anti-Ro/SSA IgG. Lanes 1 and 2 were blotted with anti-human IgG to detect total IgG. Lanes 3 and 4 were developed with rabbit anti-Id-Rol followed by anti-rabbit IgG conjugate, while lanes 5 and 6 were developed with rabbit conjugate alone as a control. The anti-Id-Rol bound anti-Ro/SSA IgG (lane 4) but not Cohn fraction II IgG (lane 3).

for reactivity with the rabbit anti-Id-Rol. 3 of the 12 additional anti-Ro/SSA<sup>+</sup> women shared varying degrees of reactivity with the antiidiotype as shown by ELISA in Table I.

In a previous report (11), we affinity purified anti-Ro/SSA from the serum of a normal donor with low levels of anti-Ro/SSA. This antibody was tested and also crossreacted with the prepared rabbit antiidiotype (Table I).

This study is the first report of a shared anti-Ro/SSA idiotype. Crossreactive idiotypes have been described for anti-Sm, anti-DNA, and rheumatoid factor, but none

TABLE I  
Reactivity of Anti-Id-Rol with Affinity-purified Anti-Ro/SSA  
from 12 Additional Patients and a Normal Serum

Anti-Ro/SSA <sup>+</sup> patients	Anti-Ro/SSA serum units	Id-Rol activity of anti-Ro/SSA IgG	
		OD	Score*
DC <sup>†</sup>	$3.01 \times 10^7$	0.675	++
MC	$6.51 \times 10^7$	0.830	+++
JL	$8.08 \times 10^7$	0.055	-
MM	$4.50 \times 10^6$	0.051	-
BN	$4.13 \times 10^7$	0.336	+
WS	$4.47 \times 10^7$	0.051	-
CB	$5.88 \times 10^7$	0.120	-
CC	$1.50 \times 10^7$	0.177	-
SC	$4.21 \times 10^5$	0.213	-
SD	$1.12 \times 10^7$	1.031	++++
FM	$1.02 \times 10^7$	0.247	-
GS	$1.42 \times 10^7$	0.133	-
NK	ND	0.163	-
RC <sup>§</sup>	$2.63 \times 10^4$	1.116	++++
NH IgG <sup>  </sup>	$2.80 \times 10^3$	0.018	-

Id-Rol activity of anti-Ro/SSA IgG was measured by ELISA, and expressed as the average OD of two separate determinations. Units of anti-Ro/SSA binding were also determined by ELISA.

\* Id-Rol activity scale: -, <0.25; +, 0.26-0.50; ++, 0.51-0.75; + + +, 0.76-1.00; + + + +, >1.00.

<sup>†</sup> Immunizing anti-Ro/SSA.

<sup>§</sup> Normal donor with elevated anti-Ro/SSA binding units by ELISA.

<sup>||</sup> Normal human serum or Cohn fraction II IgG.

have previously been reported for anti-Ro/SSA in SLE and SS. A growing body of literature focusing on anti-DNA and rheumatoid factor idiotypes examines their prevalence in patient populations (13), their role in pathogenesis (5, 6), and their genetic basis (14). The development of an anti-Ro/SSA idio type and the demonstration of shared reactivity is the first step towards evaluating differences between these individuals on a molecular level. Idiotypic differences may explain variations between the clinical anti-Ro/SSA association in patient groups.

### Summary

Idiotypes and antiidiotypes are thought to be important immune regulators and have provided clues for the origin and pathogenicity of autoantibodies. Many lupus and Sjögren's syndrome patients, as well as most neonatal lupus infants with congenital heart block or dermatitis, have antibodies to the ribonucleoprotein Ro/SSA, which is one of a group of RNA-protein autoantigens commonly found in human lupus sera. To characterize the fine specificity of anti-Ro/SSA antibodies, a rabbit antiidiotypic serum was prepared against polyclonal affinity purified anti-Ro/SSA F(ab')<sub>2</sub>. The resulting antiidiotype, anti-Id-Rol, is specific for the F(ab')<sub>2</sub> fraction of the anti-Ro/SSA immunogen and its binding to anti-Ro/SSA is inhibited by purified Ro/SSA. These data indicate that the Id-Rol epitope on anti-Ro/SSA is associated with the antigen binding site of these same antibodies. The Id-Rol idio type was present by ELISA in 3 of 12 additional anti-Ro/SSA preparations from precipitin-positive donor sera and in anti-Ro/SSA from one normal donor with low level antibody. This is the first shared idio type to be found in the human autoantibodies binding to this RNA-protein antigen. Idiotypic differences between anti-Ro/SSA autoantibodies have the potential to explain the variation in pathologic associations found in individuals who develop this autoantibody specificity.

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### References

1. Kunkel, H. G., V. Agnello, F. G. Joslin, R. J. Winchester, and J. D. Capra. 1973. Cross idio typic specificity among monoclonal IgM proteins with anti-gammaglobulin activity. *J. Exp. Med.* 137:331.
2. Solomon, G., J. Schiffenbauer, H. D. Keiser, and B. Diamond. 1983. Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with SLE. *Proc. Natl. Acad. Sci. USA.* 80:850.
3. Rauch, J., E. Murphy, J. B. Roths, B. D. Stollar, and R. S. Schwartz. 1982. A high frequency idio typic marker of anti-DNA autoantibodies in MRL-lpr/lpr mice. *J. Immunol.* 129:236.
4. Takei, M., H. Dang, and N. Talal. 1987. A common idio type expressed on a murine anti-Sm monoclonal antibody, and antibodies in SLE sera. *Clin. Exp. Immunol.* 70:546.
5. Fox, R. I., P. Chen, D. A. Carson, and S. Fong. 1986. Expression of a cross-reactive idio type on rheumatoid factor in patients with Sjögren's syndrome. *J. Immunol.* 136:477.
6. Livneh, A., A. Halpern, D. Perkins, A. Lazo, R. Halpern, and B. Diamond. 1987. A monoclonal antibody to a cross-reacting idio type on cationic human anti-DNA antibodies

- expressing  $\lambda$  light chains: a new reagent to identify a potentially differential pathogenic subset. *J. Immunol.* 138:123.
7. Isenberg, D. A., and C. Collins. 1985. Detection of cross-reactive anti-DNA antibody idiotypes on renal tissue bound immunoglobulins from lupus patients. *J. Clin. Invest.* 76:287.
  8. Harley, J. B., and K. K. Gaither. 1988. Autoantibodies. *In* Rheumatic Disease Clinics of North America: Systemic Lupus Erythematosus. J. H. Klippel, editor. W. B. Saunders Company, Philadelphia, PA. 43-56.
  9. Horsfall, A. C., P. J. W. Venables, P. A. Mumford, and R. N. Maini. 1986. Idiotypes on antibodies to the La(SS-B) antigen are restricted and associated with the antigen binding site. *Clin. Exp. Immunol.* 63:395.
  10. Yamagata, H., J. B. Harley, and M. Reichlin. 1984. Molecular properties of the Ro/SSA antigen and enzyme-linked immunosorbent assay for quantitation of antibody. *J. Clin. Invest.* 74:625.
  11. Gaither, K. K., O. F. Fox, H. Yamagata, M. J. Mamula, M. Reichlin, and J. B. Harley. 1987. Implications of anti-Ro/Sjögren's syndrome A antigen autoantibody in normal sera for autoimmunity. *J. Clin. Invest.* 79:841-846.
  12. Gaither, K. K., and J. B. Harley. 1985. Affinity purification and immunoassay of anti-Ro/SSA. *Protides Biol. Fluids Proc. Colloq.* 33:413.
  13. Isenberg, D. A., Y. Shoenfeld, M. P. Madaio, J. Rauch, M. Reichlin, B. D. Stollar, and R. S. Schwartz. 1984. Anti-DNA antibody idiotypes in SLE. *Lancet.* ii:417.
  14. Chen, P. P., K. Albrandt, N. K. Orida, V. Radoux, E. Y. Chen, R. Schrantz, F. T. Liu, and D. A. Carson. 1986. Genetic basis for the cross-reactive idiotypes on the light chains of human IgM-anti-IgG autoantibodies. *Proc. Natl. Acad. Sci. USA.* 83:8318.