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Induction of experimental systemic lupus erythematosus in mice by immunization with a monoclonal anti-La autoantibody

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Abstract

Experimental systemic lupus erythematosus (SLE) in mice can be induced by immunization either with a human monoclonal anti-DNA antibody bearing the 16/6 idiotype (16/6 Id) or with a mouse monoclonal anti-idiotypic antibody specific for the 16/6 Id. In the present report we investigated the pathogenic role of a monoclonal anti-La autoantibody in the induction and mediation of experimental SLE in mice. The monoclonal anti-La antibody was derived from a mouse in which experimental SLE was induced by immunization with the monoclonal anti-16/6 Id antibody. Following immunization with the anti-La antibody the mice produced antibodies to double-stranded DNA, single-stranded DNA, Sm, SS-A/Ro, SS-B/La, and ribonucleoprotein. Furthermore, even though the anti-La antibody does not express nor react with the 16/6 Id, the immunized mice produced high titers of anti-16/6 Id antibodies as well as 16/6 Id bearing antibodies. Four months following immunization the mice exhibited significant proteinuria, and kidney sections revealed immune complex deposits on the basement membrane of the glomeruli. These results suggest that anti-La autoantibodies are involved in the induction and mediation of SLE in mice.

Introduction

Systemic lupus erythematosus (SLE) is regarded as the prototype of systemic autoimmune disease. Among a large number of different autoantibodies occurring in lupus, antibodies against native double-stranded DNA (dsDNA) and the Sm nuclear antigen are highly specific for the disease (1) Other autoantibodies to intracellular antigens also detectable in SLE are directed against ribonucleoprotein (RNP), SS-A/Ro, and SS-B/La (1). The La protein with which anti-La autoantibodies react was found to be associated with precursor forms of RNA polymerase III transcripts (2) and appears to be a regulator of RNA polymerase III transcription (3). Some small viral transcripts were also shown to be complexed with the La protein (4); however, the exact function in RNA metabolism remains uncertain. As mentioned above, the La protein is one of the target antigens of autoantibodies in the sera of patients with SLE and primary Sjögren syndrome. Approximately 10% of patients with SLE and 40–60% with Sjögren syndrome have this antibody (1). Except for an association between a congenital complete heart block and autoantibodies to ribonucleoproteins in maternal connective tissue disease (5), and anti-Ro antibodies and cutaneous lupus erythematosus (6), the pathogenic relevance of these antibodies is not clear. Moreover, attempts to induce a lupus like disease by immunizing mice with DNA or preparations of purified nuclear proteins have failed.

We have recently reported the induction of experimental SLE in mice (7) by immunization with a human monoclonal anti-DNA antibody. This antibody bears a common idiotype (16/6 Id) (8) which was found to have clinical relevance in SLE patients (9). Following immunization and boost with the 16/6 Id the mice developed high titers of anti-16/6 Id and anti-anti-16/6 Id antibodies (murine 16/6 Id bearing antibodies with anti-DNA activity). Concomitantly with the anti-anti-16/6 Id antibodies, autoantibodies directed against various nuclear antigens (Sm, RNP, SS-A/Ro, SS-B/La) could be demonstrated. The induction

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of the disease was found to be strain dependent and to correlate to the production of anti-16/6 Id antibodies (10). Experimental SLE in mice could be induced even more efficiently by immunizing the mice with a murine monoclonal anti-16/6 Id antibody mAb 1A3-2 (11). We have recently established a monoclonal autoantibody (mAb 2D12) specific to the La protein derived from a mouse in which the disease was induced by immunization with the anti-16/6 Id antibody (11). The anti-La antibody was found to react similarly to anti-La antibodies from sera of SLE patients in all parameters tested. In order to evaluate the pathogenic role of this anti-La autoantibody in the induction and mediation of experimental SLE, the antibody was injected into female C3H.SW mice and was found to induce experimental SLE similar to the disease obtained by using the 16/6 Id or the monoclonal anti-16/6 Id antibody as the immunizing agent.

Methods

Mice

C3H SW mice were obtained from the Jackson Laboratory, Bar Harbor (USA) and used for experiments at the age of 8 – 12 weeks.

Human mAbs

The human mAb 16/6 in an anti-DNA antibody that was derived from an SLE patient (8). The hybridoma secreting the antibody is grown in culture. The antibodies were precipitated from the culture with 50% ammonium sulfate, and the affinity purified material eluted from a goat-anti-human IgM – Sepharose 4B column was used

Murine mAb

The monoclonal anti-La autoantibody mAb 2D12 was produced from the fusion of X63.853 plasmacytoma cells with splenocytes of a female C3H.SW mouse, in which experimental SLE was induced by immunization with the monoclonal anti-idiotypic antibody mAb 1A3-2 (11) directed against the 16/6Id. The mAb 2D12 was found to be of IgM isotype, with a κ light chain. It does not bear nor react with the 16/6 Id. In immunoblotting experiments using gel separated nuclear proteins of HeLa cells the mAb 2D12 was shown to bind to the 48 kd La peptide. Using protease digested nuclear extracts we could demonstrate the recognition of identical epitopes on the 48 kd La protein by mAb 2D12 and human anti-La sera from SLE patients. Furthermore, the binding of McAb 2D12 to the 48 kd La protein could be inhibited by human anti-La sera (D.Offen *et al.*, unpublished results).

The mAb A-6 is an IgM anti-idiotypic antibody against monoclonal antibodies specific to the synthetic polypeptide (T,G)-A--L (12) and was used as the control antibody for mAb 2D12. The three hybridomas mAb 2D12, mAb 1A3-2, and mAb A-6 were injected intraperitoneally into $(C57BL/6 \times BALB/c)F_1$ previously injected with 2,6,10,14-tetramethylpentadecane (pristane). Ascitic fluids were harvested usually 20 days after inoculation of the hybridoma cells. The mouse anti-La antibody mAb SW-5 (13) was kindly provided by D.Williams (Kennedy Institute, London, UK).

Purification of murine mAbs

The different ascitic fluids containing the antibodies were affinity purified on a goat anti-mouse Ig Sepharose 4B column. The eluted Ig fraction was used after extensive dialysis against PBS.

Immunizations

Several groups of 5 – 10 mice were immunized with 12.5 μg of the mAb 2D12, mAb SW-5, or mAb A-6 in complete Freund's adjuvant (Difco Laboratories Inc., Detroit, MI) intradermally into the hind footpads. Three weeks later the mice were boosted with the same amount of antibodies in PBS injected into the same sites.

Radioimmunoassay

For the detection of 16/6 Id+ antibodies flexible plastic microtiter plates were coated with mAb 1A3-2; for anti-anti-La antibodies mAb 2D12 and for anti-mouse IgM antibodies mAb A-6 were used The different ascitic fluids containing the antibodies were used at a dilution of 1:150 and 50 μl of the latter were added to each well. After 2 h incubation the plates were washed with PBS containing 0.5% bovine serum albumin. The sera of the mice (diluted 1:10 to 1 1000) were then added for 4 h. Thereafter, plates were washed and incubated for 18 h with ^{125}I-labeled Protein A (1×10^5 cpm/well). The plates were washed, wells were cut out, and counted for bound radioactivity. For the detection of anti-16/6 Id antibodies, wells were coated with the human monoclonal 16/6 Id (50 μl of 10 μg/ml) and ^{125}I-labeled goat anti-mouse Ig (1×10^5 cpm/well) was added.

Enzyme linked immunosorbent assay (ELISA)

Single-stranded DNA (ssDNA) and dsDNA were prepared as previously described (14) Antibodies against Sm and RNP, and against SS-A/Ro and SS-B/La were determined according to Konikoff *et al.* (15) and Yamagata *et al.* (16), respectively Briefly, polystyrene plates with 96 flat-bottom wells were coated first with poly-L-lysine (50 μl of 50 μg/ml), then with the antigen (2.5 μg/ml). Polystyrene coated plates with SS-A/Ro and SS-B/La were purchased from BioHytech Ltd, Ramat-Gan, Israel. Aliquots of 150 μl of mouse serum (diluted 1:200 in PBS) were added to each well, and the plates were incubated for 1 h at room temperature. After washing with PBS – 0.1% Tween 20, 150 μl alkaline-phosphatase-conjugated goat anti-mouse Ig were added. Plates were then incubated for 18 h and washed again. Phosphatase conjugate was detected by addition of 150 μl *p*-nitrophenyl phosphate (1 mg/ml in 50 mM $NaHCO_3$, 2 mM $MgCl_2$, pH 9.5) at 23°C. Optical densities were read at 405 nm in a Dynatech model MR 580 Micro ELISA reader (Dynatech, Denkendorf, FRG).

Production of an extract of nuclear proteins from HeLa cells

Nuclear extracts of HeLa cells were prepared as described by Dignam *et al* (17), and stored frozen at – 20°C in the presence of 0 01 mM phenylmethylsulfonyl fluoride (PMSF). The protein concentration of these nuclear extracts was 5 mg/ml, as determined by the method of Bradford (18).

Immunoblotting

Immunological detection of gel-separated nuclear proteins was done according to Towbin *et al.* (19). Proteins were displayed on 12% polyacrylamide gels in the presence of SDS. The gel-separated proteins were transferred to nitrocellulose sheets by electrophoresis at 250 mA for 8 h at 4°C, in a buffer containing 15 mMTris – base, 115 mM glycine, and 20% methanol. All subsequent steps were carried out at room temperature. The

blots were incubated for 1 h in a blocking solution consisting of 10% low fat milk, 0.02% Triton X-100, and 0.01% NaN_3 in PBS. Hybridoma supernatants, and human and mouse sera were incubated with the blots for 1 – 4 h in heat-sealed plastic bags with gentle shaking The filters were washed three times for 10 min with blocking solution, and probed with ^{125} I-labeled Protein A or goat anti-mouse Ig (10^6 cpm/lane) in PBS containing 0.01% Triton X-100 and 0.01% NaN_3, for 1 h. The filters were washed 3 times for 10 min with blocking solution, blot-dried, and autoradiographed.

Detection of proteinuria

Proteinuria was measured semi-quantitatively using Combistix kit (Ames-Miles, Slough, UK).

Immunohistology

Kidneys were removed from the mice and frozen immediately in liquid nitrogen. Cryostat sections (5 μm) were air dried for 2 h and fixed in acetone for 10 min. For the detection of Ig deposits, the sections were covered for 30 min with FITC-labeled rabbit anti-mouse Ig (Nordic, Tilburg, The Netherlands). After washing in PBS, the specific staining was analyzed by a fluorescent microscope.

Results

Serological and clinical manifestations in mice immunized with mAb 2D12

Three groups of 5 – 10 female C3H.SW mice were immunized and boosted with 12.5 μg of the mAb 2D12 purified either from culture supernatant or from ascitic fluid. Additional groups were immunized with identical amounts of the control antibodies. The mice were bled in 3-week intervals and their sera were tested for antibody activity. No significant differences in the antibody profile were observed in the sera of mice immunized with the mAb 2D12 purified either from the culture supernatant or from the ascitic fluid Antibody levels determined 3 months after the booster injection are presented in Fig. 1. As can be seen in Fig. 1, the mAb 2D12 immunized mice reacted to mAb 2D12 by producing anti-anti-La antibodies (Fig. 1C). These antibodies are specific since the titer of antibodies against mouse IgM is significantly lower (Fig. 1D). Additionally, the mice exhibited high titers of anti-16/6 Id antibodies (Fig. 1A) as well as antibodies expressing the 16/6 Id (Fig. 1B). No such antibodies could be detected in mice that were injected with the anti-La antibody mAb SW-5 or with mAb A-6.

Since the presence of antibodies against a variety of nuclear antigens and oligonucleotides is typical in SLE, the sera of all groups of mice were tested for antibodies against dsDNA, ssDNA, Ro, La, Sm, RNP, cardiolipin, histone, poly(G), and poly(I). As shown in Table 1, the mice immunized with mAb 2D12 produced high antibody titers against all the above-mentioned antigens. The data were confirmed by immunoblotting experiments using gel separated nuclear proteins of HeLa cells. As shown in Fig. 2, antibodies binding to the 68, 48, 34, and 28 kd bands, representing U1RNP, La, RNP, and SM, respectively, were present in the sera of mice immunized with mAb 2D12. No such antibodies were detectable in the sera of mice immunized with mAb SW-5 and mAb A-6.

Anti-anti-La antibodies do not react with a polyclonal rabbit anti-16/6 Id antiserum

The presence of antibodies expressing the 16/6 Id in the sera of mAb 2D12 injected mice prompted us to investigate whether anti-anti-La antibodies share idiotypic determinants that can be recognized by a polyclonal rabbit antiserum specific to the 16/6 Id. However, as shown in Table 2, we could not demonstrate any binding of the rabbit anti-16/6 serum to the anti-anti-La antibodies. As also shown in Table 2, the mAb 2D12 does not bind or express the 16/6Id. It should be mentioned that, as shown in Fig. 1, the sera used in this experiment exhibited high levels of 16/6 Id$^+$ antibodies as well as anti-anti-La antibodies.

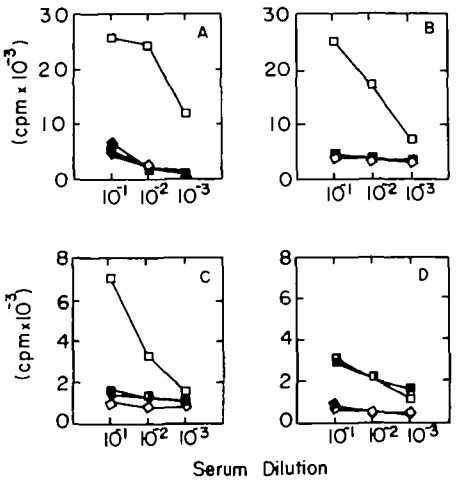

Fig. 1. Antibody responses of C3H.SW mice immunized with mAb 2D12 Groups of C3H SW mice immunized with mAb 2D12 (□), mAb SW-5 (◆), and mAb A-6 (■) were bled 3 months after immunization The pooled sera of mice including preimmune mouse serum (◇) were tested for antibody activity. Flexible microtiter plates were coated with 16/6 Id (A), murine anti-16/6 Id (mAb 1A3-2) (B), anti-La (mAb 2D12) (C), or murine IgM (mAb A-6) (D) Following incubation with the diluted sera either [^{125}I]Protein A (B, C and D) or [^{125}I]goat anti-mouse Ig (A) were added

Table 1. Immune response to nuclear antigens in C3H SW mice immunized with mAb 2D12

Antibodies	Mice immunized with:			
	2D12	SW-5	A-6	NMS[a]
dsDNA	636	422	379	258
ssDNA	513	398	343	180
SS-A/Ro	1360	370	417	360
SS-B/La	1341	431	364	318
Sm	768	90	158	98
Cardiolipin	700	n d.	498	374
Histones	562	n d.	287	204
poly(G)	250	160	179	174
poly(I)	667	n d	235	330

Three months following immunization with either mAb 2D12 or control antibodies mAb SW-5 and mAb A-6 mice were bled and their pooled sera were tested for the presence of different anti-nuclear antibodies by ELISA at a dilution of 1.200. Results are expressed as optical density at 405 nm x 10^3.

[a]Preimmune mouse serum

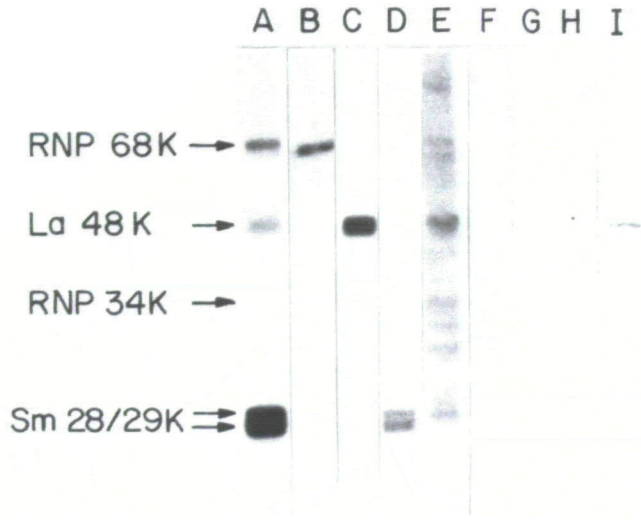

Fig. 2. Nuclear antigens recognized by human and murine immune sera and mAb. Gel separated and electroblotted proteins of HeLa cells nuclear extract were probed with sera from different SLE patients (A, B, and D) specific for RNP, SS-B/La, and Sm, (C) shows the blotting of an anti-La serum from a patient with primary Sjögren Syndrome, sera from mice immunized with mAb 2D12, mAb SW-5, and mAb A-6 are shown in (E), (F), and (G), respectively; (H) represents the blot of preimmune mouse serum; (I) shows the binding of mAb 2D12 to the 48 kd La protein (A) – (D) were probed with [^{125}I]Protein A (E) – (I) were probed with [^{125}I]goat anti-mouse Ig.

Table 2. Lack of expression of the 16/6 Id on anti-anti-La antibodies

	cpm
Experiment 1[a]	
Non-specific binding of ^{125}I-goat anti rabbit immunoglobulin to mAb 2D12 coated plates	4466
Sera of mice immunized with mAb 2D12	5473
Preimmune serum	4847
Experiment 2[b]	
Binding of mAb 2D12 to human 16/6 Id	1270 (860)[c]
Binding of mAb 2D12 to rabbit anti-16/6 Id	1628 (1013)[c]

[a]Plastic microtiter plates were coated with mAb 2D12 and incubated with sera from mice immunized with mAb 2D12 (serum dilution 1:100). The specific binding of a polyclonal rabbit anti-16/6 Id antibody to anti-anti-La antibodies was detected by the addition of [^{125}I]goat anti-rabbit serum. The values of antibodies expressing the 16/6 Id in the sera used in this RIA gave 17,258 cpm for mice immunized with mAb 2D12 versus 3542 cpm for preimmune serum

[b]To examine the binding of mAb 2D12 to the 16/6 Id, the plates were coated with the human 16/6 Id or the polyclonal rabbit anti 16/6 Id antibody. Specific binding of mAb 2D12 (dilution 1 100) was detected by [^{125}I]goat anti-mouse Ig.

[c]The values of the non-specific binding of [^{125}]goat anti-mouse Ig to human 16/6 Id and rabbit anti-16/6 Id are given in brackets.

Kidney disease in the mAb 2D12 injected mice

Four months after the booster injection all the mice immunized with mAb 2D12 developed moderate proteinurea of 0.3 – 3 g/l (as compared to <0.3 g/l in the control groups). As shown in Fig. 3, immunohistology of the kidneys demonstrated a granular pattern of immunecomplex deposits on the basement membranes in the glomeruli of the mouse injected with mAb 2D12 (Fig. 3A). No such deposits could be detected in preimmune mice (Fig. 3D) or in mice immunized with the anti-La antibody mAb SW-5 (Fig. 3B) or the control antibody mAb A-6 (Fig. 3C).

Discussion

In the present study we report the induction of experimental SLE in mice by immunization with a mouse monoclonal anti-La autoantibody (mAb 2D12). Immunization of female C3H.SW mice with mAb 2D12 led to the production of high titers of antibodies against a variety of nuclear antigens (dsDNA, ssDNA, La, Ro, Sm/RNP, cardiolipin, and histones). Additionally high titers of antibodies bearing the 16/6 Id and anti-16/6 Id antibodies were detectable. Four months after the boost, immune deposits in the kidneys, typical to SLE, could be demonstrated in the immunized mice (Fig. 3).

The presence of autoantibodies is a prominent feature in autoimmune diseases and there is no doubt that in the case of autoantibodies to hormone receptors and some other cell surface antigens the causal relationship between the antibody and the disease is clearcut (20). However, for numerous autoantibodies against intracellular antigens there is still a question as to their pathogenic role in mediating disease. Some of the nuclear autoantigens in SLE have functions that are of essential biological importance to eukaryotic cell survival (21,22). Reports have addressed the question as to whether autoantibodies to nuclear antigens are inducible in mice by immunization with purified preparations of the corresponding antigen. These attempts resulted in the production of antibodies reactive with heterologous (23) and autologous (13) protein, whereas it has not been possible to induce SLE-like disease in the immunized animals. In the present study we have shown that experimental SLE is inducible in naive non-autoimmune mice by immunization with the monoclonal anti-La autoantibody mAb 2D12 However, just the antibody specificity for an autoantigen as the La peptide is not sufficient to induce experimental SLE since immunization of mice with mAb SW-5, which is a monoclonal anti-La antibody produced by immunizing mice with purified La protein (13), did not cause any autoimmune disorder (Figs 1 – 3). In experiments carried out for the characterization of mAb 2D12 we could demonstrate that it is a true autoantibody and that identical epitopes on the 48 kd La protein are recognized by human anti-La sera from SLE patients and mAb 2D12 (D.Offen *et al.*, unpublished data). Yet, the additional signals that direct the immune system of mice immunized with mAb 2D12 towards an autoimmune response are not known. We have demonstrated previously that experimental SLE in mice can be induced by immunization with a human monoclonal anti-DNA antibody expressing the 16/6 idiotype (7) or with a murine monoclonal anti-16/6 Id antibody (11).

Additional studies showed clear evidence for the important role of the 16/6 Id in the pathogenesis of SLE in that the suscepti-

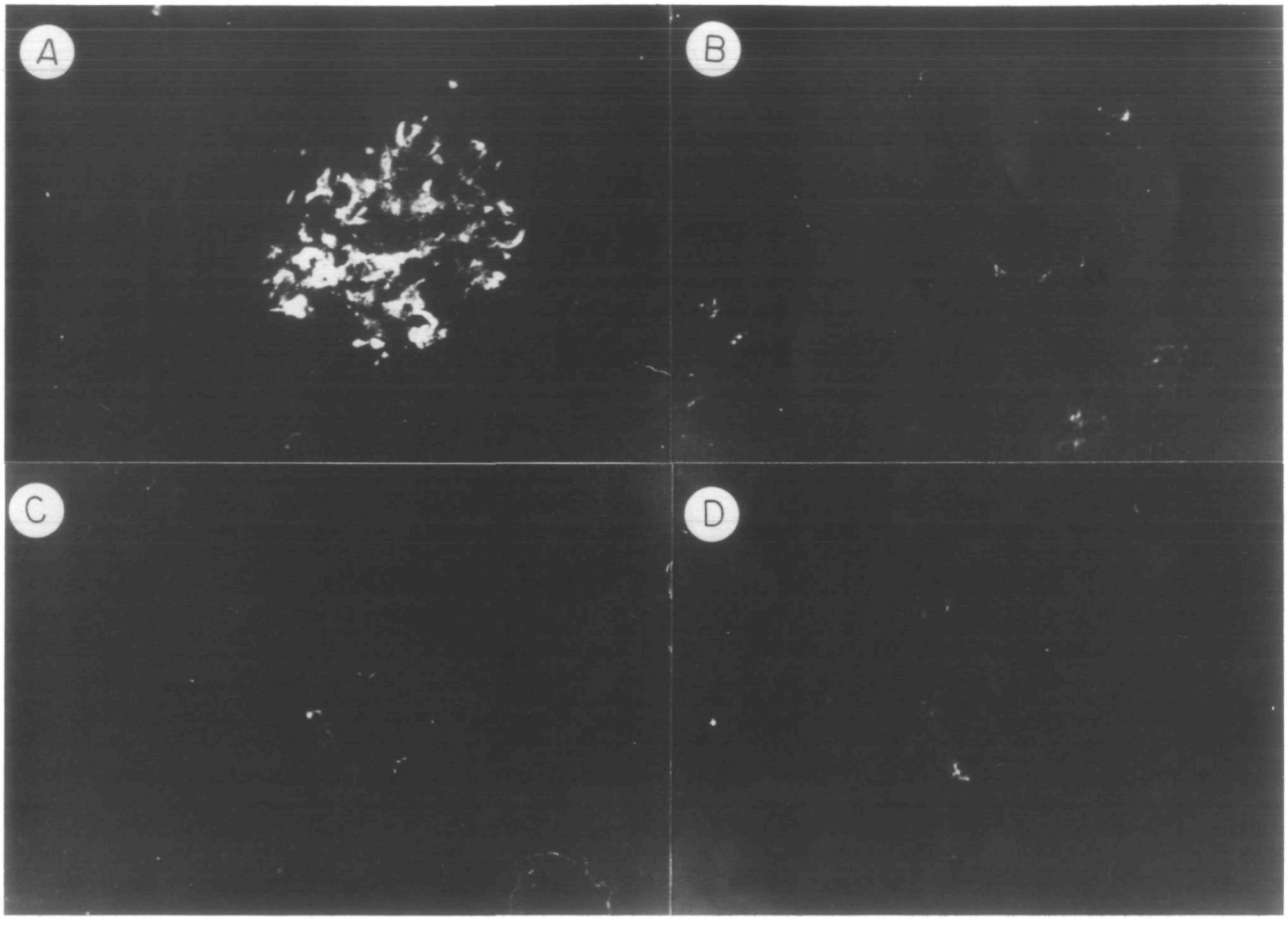

Fig. 3. Immunohistology of kidneys four months following immunization with mAb 2D12. Immunofluorescent staining of 5 μm frozen cryostat sections of kidneys from mice immunized with mAb 2D12 (A), mAb SW-5 (B), and mAb A-6 (C); (D) shows a kidney section of a non-immunized mouse. Ig deposits were detected in (A). The control sections (B) – (D) are negative.

bility to the induction of experimental SLE in mice correlated to their ability to respond to the 16/6 Id by production of anti-16/6 Id antibodies (10). However, the mechanism that causes the production of anti-16/6 Id and 16/6 Id bearing antibodies following immunization with the mAb 2D12 is not clear. As shown in Table 2, antibodies specific to the mAb 2D12 do not carry the 16/6 Id and also the mAb 2D12 neither binds to nor bears the 16/6 Id. However, even though there is no 'cross-reactivity' between mAb 2D12 and anti-16/6 Id or 16/6 Id bearing antibodies on the 'antibody level', these antibodies may share V region sequence homologies encoding related T cell epitopes that can give rise to idiotypically interacting T cell clones and thus establish a connection to the 16/6-idiotypic network.

The mAb 2D12 was established from a fusion carried out for the preparation of murine 16/6 Id bearing anti-DNA antibodies. From this fusion we could establish a complete parallel set of antibodies (16/6 $Id^{+/-}$, anti-$DNA^{+/-}$) (S. Mendlovic *et al.*, unpublished results), suggesting a possible mode of interaction of the 'double-negative' (16/6 Id^{-}, anti-DNA^{-}) mAb 2D12 with the 16/6 idiotypic network via functionally related parallel sets of autoantibodies. A similar mechanism was shown for anti-DNA and anti-RNP antibodies in MRL-lpr/lpr mice (24).

The diversity of antinuclear antibodies detectable in SLE is explained by being either the result of a non-specific polyclonal B cell activation (25 – 27) or an antigen driven autoimmune response to nuclear proteins (28). The question of whether lupus autoantibodies are encoded in the germ line or are generated and expanded in a response originally directed against exogenous antigens is important, since it identifies the developmental stage at which a given B cell clone acquires self specificity and escapes the control of tolerance towards self. It is likely that mAb 2D12—and probably other autoantibodies reactive with nuclear antigens—can arise by mutation of a V gene segment that originally encoded anti-DNA autoantibodies. Nucleotide sequencing of the above-mentioned antibodies, as well as the establishment of other monoclonal autoantibodies reactive with nuclear antigens—now in progess—should reveal the basis of their structural relationship.

The experiments described in the present study provide, to the best of our knowledge for the first time, clearcut evidence for a direct involvement of anti-La autoantibodies in the induction and mediation of SLE.

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Abbreviations

dsDNA	double-stranded DNA
Id	idiotype (idiotypic)
Ig	immunoglobulin
mAb	monoclonal antibody(ies)
PBS	phosphate-buffered saline
poly(I)	polyinosine acid
RNP	ribonucleoprotein
SLE	systemic lupus erythematosus
SN	supernatant
ssDNA	single-stranded DNA
16/6 Id	idiotype of the anti-DNA monoclonal autoantibody 16/6

References

1 Bernstein, R M and Matthews, M B. 1987. Autoantibodies to intracellular antigens with particular reference to transfer RNA and related proteins in myositis *J. Rheumatol* 14 83.
2 Rinke, J. and Steitz, J. A. 1982. Precursor molecules of human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies *Cell* 29.149.
3 Gottlieb, E and Steitz, J. A. 1989. Function of the mammalian La protein· evidence for its action in transcription termination by RNA polymerase III. *EMBO J* 8.851.
4 Kurilla, G. K. and Keene, J D 1983 The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-la lupus antibodies. *Cell* 34 837.
5 Scott, J S., Madison, P. J , Taylor, P. V., Escher, E., Scott, O , and Skinner, R. P. 1983 Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block *N. Engl. J. Med.* 309:209
6 Provost, T T and Reichlin, M. 1988. Immunopathologic studies of cutaneous lupus erythematosus. *J. Clin Immunol.* 4.223.
7 Mendlovic, S., Brocke, S., Shoenfeld, Y., Ben-Bassat, M., Meshorer, A., Bakimer, R , and Mozes, E 1988. Induction of a systemic lupus erythematosus like disease in mice by a common anti-DNA idiotype *Proc. Natl. Acad. Sci. USA* 85·2260.
8 Shoenfeld, Y., Hsu Lin, S. C , Gabriel, J. E , Silberstein, L. E., Furie, B C , Furie, B., Stoller, B. D., and Schwartz, R. S. 1982. Production of autoantibodies by human human hybridomas *J. Clin. Invest.* 70:205
9 Isenberg, D. A., Shoenfeld, Y., Madaio, M. P., Rauch, J , Reichlin, M., Stollar, B. D., and Schwartz, R. S. 1984 Anti-DNA antibody idiotypes in systemic lupus erythematosus. *Lancet* ii:417.
10 Mozes, E , Brocke, S., Shoenfeld, Y., and Mendlovic, S. 1989. The role of the idiotypic network in the induction of experimental Systemic Lupus Erythmatosus. *J. Cell Biochem* 40.173.
11 Mendlovic, S., Fricke, H , Shoenfeld, Y , and Mozes, E 1989. The role of anti-idiotypic antibodies in the induction of experimental lupus erythematosus in mice *Eur. J. Immunol* 19 729.
12 Mozes, E. and Zinger, H. 1987 Characterization and biological activities of an anti-idiotypic specific T-cell line and its derived clones. *J. Immunol.* 139.3564
13 Smith, P R , Williams, D. G., Venables, P J W., and Maini, R. N. 1985. Monoclonal antibodies to the Sjögren's Syndrome associated antigen SS-B/La *J. Immunol. Methods* 77 63
14 Shoenfeld, Y , Rauch, J., Massicotte, H , Datta, S. K., Andre-Schwartz, J., Stollar, B D , and Schwartz, R 1983. Polyspecificity of monoclonal autoantibodies produced by human – human hybridomas *N Engl. J Med.* 308.414.
15 Konikoff, F., Shoenfeld, Y , Isenberg, D A , Barisson, J , Sobe, T , Theodore, E , and Slor, H. 1987. Anti-RNP antibodies in chronic liver disease. *Clin. Exp. Rheumatol* 5·359.
16 Yamagata, H., Harley, J. B., and Reichlin, M 1984 Molecular properties of the SS-A/Ro-antigen and enzyme linked immunosorbent assay for the quantitation of antibody. *J Clin. Invest* 74: 625.
17 Dignam, J D., Lebovitz, R M , and Roeder, R. G. 1983 Accurate transcription initiation by RNA polymerase II in soluble extract from isolated mammalian nuclei *Nucleic Acids Res.* 11.1475.
18 Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dyes binding *Anal Biochem* 71:248
19 Towbin, H , Staehelin, T., and Gordon, J 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. procedure and some applications *Proc. Natl. Acad. Sci USA* 76:4350.
20 Bottazzo, G F , Todd, I , Mirakian, R , Belfiore, A , and Bujol-Borell, R 1986 Organ specific autoimmunity. A 1986 overview. *Immunol Rev* 94.137.
21 Shero, J H , Bordwell, B , Rothfield, N F , and Earnshaw, W C 1986. High titers of autoantibodies to topoisomerase I (Sc170) in sera from scleroderma patients. *Science* 231·737
22 Matthews, M. B., Bernstein, R M , Franza, B R , and Garrels, J I 1984 Identity of the proliferating cell nuclear antigen and cyclin. *Nature* 309 374
23 Chan, E. K. L. and Tan, E M 1987 Human autoantibody-reactive epitopes of SS-B/La are highly conserved in comparison with epitopes recognized by murine monoclonal antibodies. *J Exp. Med.* 166·1627
24 Migliorini, P., Ardman, B , Karburaki, J., and Schwartz, R. S. 1987. Parallel sets of autoantibodies in MRL-1pr/1pr mice *J Exp Med* 165.483.
25 Steinberg, A D , Baron, S , and Talal, N. 1969. The pathogenesis of autoimmunity in New Zealand mice I Induction of anti-nucleic acid antibodies by polyinosinic – polycytidylic acid. *Proc. Natl Acad. Sci. USA* 63 1102.
26 Smith, H. R., Green, D. R., Smathers, P A , Gershon, R K , Raveche, E S , and Steinberg, A. D. 1983. Induction of autoimmunity in normal mice by thymectomy and administration of polyclonal B cell activators: association with contrasuppressor function. *Clin. Exp. Immunol* 51·579
27 LeMing, H., Slack, J H , Amundson, C , Izui, S , Theofilopoulos, A. N., and Dixon, F. J. 1983. Induction of murine autoimmune disease by chronic polyclonal B cell activation *J. Exp Med* 157·874
28 Schlomchik, M. N., Marshak-Rothstein, A , Wolfowicz, C. B., Rothstein, Th L , and Weigert, M G. 1987. The role of clonal selection and somatic mutation in autoimmunity *Nature* 328.805.