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The Role of the Idiotypic Network in the Induction of Experimental Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) has been induced in C3H.SW mice by their immunization with a human monoclonal anti-DNA antibody that bears a common idiotype-16/6 Id. Following immunization, high levels of murine anti-16/6 and anti-anti-16/6 antibodies were detected in the sera of the immunized mice. Elevated titers of autoantibodies reacting with ssDNA, dsDNA, poly(I), poly(G), RNP, Ro, and La were also observed. The serological findings were associated with significant proteinuria, leukopenia, and elevated erythrocyte sedimentation rate. Immune complex deposition in the glomerular mesangium and sclerosis of the glomeruli were demonstrated. To study whether or not anti-idiotypic antibodies are involved in the induction of the disease, a murine monoclonal antibody against the 16/6 Id was prepared and injected into C3H.SW mice. The anti-16/6 Id antibody induced experimental SLE similarly to the 16/6 Id with an accelerated kidney pathology. A study performed on different mouse strains indicated that the susceptibility to the induction of SLE by the 16/6 Id is strain dependent and directly correlates to their ability to produce anti-16/6 Id specific antibodies.

Key words: experimental SLE, genetically regulated susceptibility

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown origin and cure. This disease, being characterized by the formation of antibodies against various autoantigens, is considered to be the prototype of autoimmunity. Systemic lupus erythematosus, however, could not thus far be induced neither by immunization of mice with DNA, to which the autoantibodies are directed in SLE [1], nor by transfer of anti-DNA antibodies [2,3].

The human monoclonal antibody 16/6 is an anti-DNA antibody that was derived from an SLE patient [4]. This antibody bears a common idiotype, termed 16/6 idiotype (16/6 Id) and was shown to exist in most active SLE patients [5]. The 16/6 Id was also detected in the immune complex deposits in the kidneys [6] and in the dermal-epidermal

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junction [7] of SLE patients. This idiotype was, therefore, suggested to play a role in the pathogenesis of SLE.

Since the idiotypic network was shown to have a role in regulating immune responses [8,9], it has been suggested that idiotypic-anti-idiotypic antibodies may be involved in the induction and progression of autoimmunity [10]. However, despite extensive studies the exact role of the idiotypic network in the pathogenesis of SLE is not yet clear.

Natural and experimental evidence support a genetic predisposition to many autoimmune diseases, including SLE. Thus, based on statistical analysis, it was reported that HLA-B8 and HLA-DR3 are more frequent in patients with SLE [11]. Yet, the role of the genetic background in SLE in humans is not clear, and environmental factors are known to contribute to its induction [12].

In the present study we have used the 16/6 Id for the induction of experimental SLE in a murine strain that does not develop any spontaneous autoimmune disease. We also investigated the role of the idiotypic network in the induction of experimental SLE by using a murine monoclonal antibody specific to the 16/6 Id. We further examined the importance of the genetic background in the induction of experimental SLE in mice.

MATERIALS AND METHODS

Mice

Different mouse strains were obtained from either The Jackson Laboratory, (Bar Harbor, Maine) or Olac, Blackthorn, Bicester (Oxon, UK). Several groups of four to ten mice were used in each experiment. All the mice were used at the age of 2–3 months.

The 16/6 Idiotype

The hybridoma secreting the 16/6 Id was grown in culture. The 16/6 Id was precipitated from the culture supernatant with 50% ammonium sulfate, and the affinity purified material that was eluted from a goat-anti-human IgM-Sepharose column was employed in this study.

Production of Anti-Idiotypic Monoclonal Antibodies

Monoclonal anti-16/6 Id antibodies were produced by fusing 16/6 Id immunized splenocytes of C3H.SW mice with the plasmacytoma X63.653 by the use of 41% polyethylene glycol. Hybrid cells that secreted antibodies specific to the 16/6 Id were cloned by limiting dilution. The subclone secreting the McAb1A3-2 was employed in this study.

Immunizations

Several groups of four to ten mice were immunized with 1 μg of the 16/6 Id or with 20 μg of McAb1A3-2 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 the immunizing antibodies in aqueous solution in the hind footpads.

Radioimmunoassay

Flexible plastic microtiter plates were coated with 50 μl of 50 μg/ml antigen dissolved in phosphate buffered saline (PBS). For the identification of murine antibodies

with the 16/6 Id, plates were coated with rabbit antiserum specific to the 16/6 Id at the dilution of 1:100. After 2 hr of incubation the plates were washed with PBS containing 0.5 g/dL bovine serum albumin. The sera of the mice (diluted 1:10 to 1:10,000) were then added for 4 hr. Thereafter, plates were washed three times and incubated for 18 hr with ^{125}I-labelled goat anti-mouse immunoglobulin (1×10^5 cpm/well). After extensive washing of the radioactive reagent, plates were dried, wells were cut out and counted in a gamma counter.

Enzyme-Linked Immunosorbent Assay (ELISA)

Single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) were prepared as previously described [4]. Antibodies against Sm and RNP and against Ro and La were determined according to Konikoff et al. [13] and Yamagata et al. [14], respectively. Briefly, polystyrene plates with 96 flat-bottomed 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 Ro and La were purchased from BioHytech Ltd. (Ramat-Gan, Israel). One hundred and fifty microliters of mouse serum (diluted 1:200 in PBS) was added to each well, and the plates were incubated for 1 hr at room temperature. After washing with PBS-0.1% Tween 20, 150 μl alkaline-phosphatase-conjugated goat anti-mouse immunoglobulin (IgG + IgM) were added. Plates were then incubated for 18 hr 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.

Detection of SLE-Associated Pathological Manifestations

Erythrocyte sedimentation rate (ESR) was determined by diluting the heparinized blood in PBS at a ratio of 1:1. The diluted blood was then passed to a microsampling pipette, and the sedimentation was measured 6 hr later. Proteinuria was measured in a semiquantitative way by the use of the Combistix kit (Ames-Miles, UK).

Immunohistochemistry

Kidneys were removed and were frozen immediately in liquid nitrogen. Frozen cryostat sections were air dried for at least 2 hr and fixed in acetone. For the detection of Ig deposits, sections were incubated with biotinylated anti-IgG or anti-IgM antibodies, and avidin biotinylated peroxidase complex (Vector Laboratories, Burlingame, CA) was used as a second incubation step. After each incubation, sections were extensively washed with PBS. Specific staining was visualized with 3,3′-diaminobenzidine (Sigma, St. Louis, MO) as a substrate.

RESULTS

Induction of Experimental SLE by the 16/6 Id

Several groups of C3H.SW female mice were primed with 1 μg of the affinity purified human anti-DNA 16/6 antibody in complete Freund's adjuvant (CFA) in the hind footpads, and were boosted with the same amount in aqueous solution 3 weeks later. Twenty-one days after the boost, the titer of the anti-idiotypic antibodies against the 16/6 Id (anti-16/6 Id) reached a maximum level, which has remained stable for the last

TABLE I. Clinical Features of the 16/6 Id Immunized C3H.SW Mice

	16/6 Id injected	HIgM injected
Serology		
Anti-16/6 Id	+[a]	–
Murine 16/6 Id	+	–
Anti-ss/dsDNA	+	–
Anti-poly(I)/poly(G)	+	–
Anti-Sm/RNP	+	–
Anti-Ro/La	+	–
Anti-cardiolipin	+	–
Blood		
WBC counts (cells/mm^3)	3,580 ± 299	4,987 ± 680
ESR (mm/6 hr)	2–5	0.5–0.75
Kidney		
Proteinuria	1–3 g/L	<0.3 g/L
Immunostaining for MIg	+	–
Immunostaining for 16/6 Id	+	–
Electron dense deposits	+	–

[a] +, indicates a positive SLE parameter in all the mice injected with the 16/6 Id (n = 10).

15 months. The antibody response was mainly directed against the 16/6 Id as the sera bound more efficiently the 16/6 antibody than an irrelevant monoclonal human IgM (21,749 cpm vs. 8,367 cpm, respectively, in 1:10 diluted pooled sera of 16/6 Id immunized mice). Furthermore, high levels of antibodies bearing the 16/6 idiotype (anti-anti-Id specific antibodies) were produced in the immunized mice. The elevation in the titer of the latter antibodies occurred concomitantly with the production of antibodies directed against double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA). Injection of mice with human IgM (HIgM) in CFA as a control antigen did not result in significant antibody responses to the above antigens. In addition to high antibody levels against dsDNA and ssDNA, we could detect in the sera of all immunized mice binding activities against poly(I), poly(G), Sm, RNP, Ro, La, and cardiolipin, as indicated in Table I, which summarizes the clinical features observed in a representative group of ten immunized mice. Four months following booster injection, the 16/6 Id immunized mice had moderate leukopenia, elevated ESR, and proteinuria (Table I). The histological examination of kidney sections of the mice sacrificed 4 months after boosting disclosed some mild pathological changes in the glomeruli. Twelve months following the immunization a focal sclerosis of the glomeruli was evident. Immunohistochemical staining 4 months after boosting revealed immune complexes of both IgM and IgG isotypes in the glomerular basal membrane, as shown in Figure 1. The existence of these complexes was confirmed by electron microscopy that demonstrated electron dense deposits in the paramesangial tissue. The complexes were shown to bear the 16/6 Id, as demonstrated by staining of the kidney sections with a specific monoclonal antibody (McAb1A3-2) prepared against the 16/6 Id (Table I). It is noteworthy that in all groups of C3H.SW mice that were independently immunized with the 16/6 Id the same pathological features were observed.

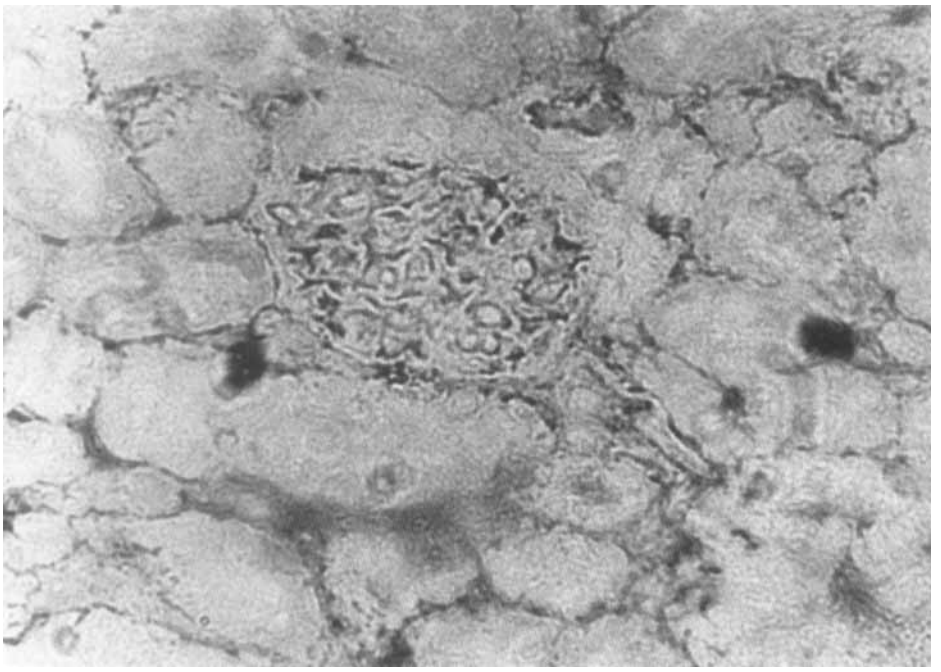

Fig. 1. Immunohistochemical staining of kidney sections of 16/6 Id immunized C3H.SW mice. Sections (5 μm) show peroxidase staining of IgM deposits on the glomerular basement membrane. ×300.

Induction of Experimental SLE by the McAb1A3-2

To assess the role of anti-16/6 Id antibodies in the induction of experimental SLE, C3H.SW female mice were immunized in the hind footpads with 20 μg of the affinity purified McAb1A3-2 in CFA. The mice were boosted with 20 μg of this antibody in PBS 3 weeks later. Following the boost, the mice were bled and their sera were tested for the presence of antibodies with the 16/6 Id (identified by a rabbit antiserum specific to the 16/6 Id) against the 16/6 Id and antibodies against ssDNA. As can be seen in Figure 2, the McAb1A3-2 immunized mice produced all the above antibodies. In addition to the antibodies shown in the Figure, the McAb1A3-2 injected mice produced the characteristic pattern of autoantibodies found in SLE. Thus, a very similar autoantibody pattern to that found in the 16/6 Id immunized mice (Table I) was also detected in the McAb1A3-2 immunized mice.

Three months after the booster injection, all the mice immunized with the monoclonal anti-16/6 Id antibody McAb1A3-2 exhibited proteinuria, leukopenia, and elevated ESR. The histological and immunohistological examination of the kidneys at that time revealed a more advanced injury pattern to that found in the 16/6 Id immunized mice.

The Genetic Control of the Induction of SLE in Mice

Four to ten mice of seven different strains were immunized with 1 μg of the 16/6 Id in CFA. The mice were boosted 3 weeks later with the same amount of the 16/6 Id in

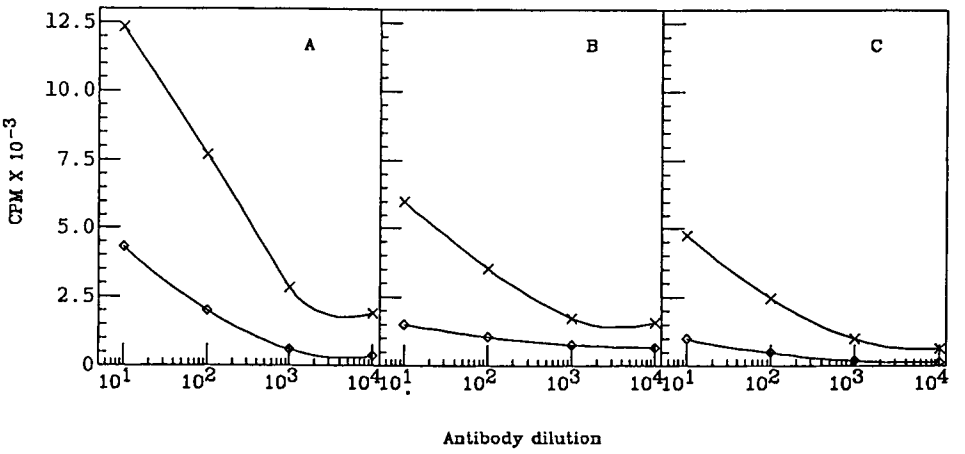

Fig. 2. Antibody responses of C3H.SW mice immunized with McAb1A3-2. Pooled sera of 5 C3H.SW mice were examined for the presence of different antibodies 3 months following immunization with McAb1A3-2: **A:** Anti-16/6 Id antibodies. **B:** Murine antibodies that bear the 16/6 Id. **C:** Anti-ssDNA antibodies. Antibody levels of McAb1A3-2 immunized mice (×) and of normal mouse serum (◇) were detected by a radioimmunoassay.

TABLE II. Clinical Features of the Different Murine Strains Immunized With the 16/6 Id*

Strain	H-2 type	Serology[a]		Leukopenia	Elevated ESR[b]	Proteinuria
		16/6 Id	Anti-16/6 Id			
C3H.SW	b	27,231	8,229	4,100 ± 400[c]	+	(7/7)[d]
C57BL/6	b	5,779	3,150	5,300 ± 500	−	(0/5)
BALB/c	d	25,705	10,745	2,400 ± 700[c]	++	(5/5)
AKR	k	20,700	5,392	2,700 ± 400[c]	+	(10/10)
C3H/eB	k	21,177	10,920	4,200 ± 200[c]	+	(5/5)
C3H/He	k	4,461	3,529	4,800 ± 700	−	(0/5)
SJL	s	24,044	9,812	4,600 ± 300[c]	+	(5/5)

*Leukopenia, elevated ESR, and proteinuria were assessed in each of the 16/6 Id immunized strains. Results express the reading of four to ten mice and were recorded 4 months after the booster injection.
[a]Pooled sera of four to ten mice of the different strains obtained 4 months after the boosters were tested by a radioimmunoassay at a dilution of 1:10.
[b]ESR: −, <1 mm/6 hr; +, 1–15 mm/6 hr; ++, >15 mm/6 hr.
[c]$P < 0.01$ calculated in comparison to control mice of the same strain.
[d]Incidence of proteinuria.

PBS. Four months after the booster injection, animals were bled and their sera were tested for antibody activities. As demonstrated in Table II, all the strains, except C57BL/6 and C3H/He, developed high titers of antibodies against the 16/6 Id and anti-16/6 Id. All the strains responding to the 16/6 Id produced elevated antibody titers to the nuclear antigens listed in Table I. A considerable proteinuria, elevated ESR, and a significant leukopenia were detected in all strains, except for C57BL/6 and C3H/He (Table II). An immunohistology study confirmed the glomerular damage in all the strains, but not in the C57BL/6 and C3H/He strains.

The effect of the 16/6 Id immunization on the development of SLE in the autoimmune strain NZB/W F1 was also examined. It was found that the immunization

of NZB/W F1 mice accelerated the development of the spontaneous disease and resulted in the reduction of the life span of the immunized mice.

DISCUSSION

In the present study we have induced an SLE-like disease in a mouse strain (C3H.SW) that is not susceptible to spontaneous autoimmune diseases. The injection of the common human anti-DNA idiotype (16/6 Id) was followed by the appearance of several hallmarks of SLE in human [15] and in the spontaneous murine models (NZB/W F1, MRL/lpr/lpr, and BXSB [16]) for this disease. The manifestations included elevated titers of antinuclear antibodies. Other findings demonstrated in the immunized mice, such as high erythrocyte sedimentation rate and leukopenia, are also found in SLE patients. Renal immune complexes, as shown in the 16/6 Id injected mice, are considered to be pathogenic in patients with SLE. Furthermore, the existence of the 16/6 Id has been reported in affected human kidneys and skin of patients with SLE [6,7]. It was also found to exist in the renal immune complexes of the mice immunized with the 16/6 Id.

The mechanism by which the injection of the human monoclonal anti-DNA antibody with the common 16/6 idiotype induced an SLE-like disease is not clear yet. No control antigen injected with complete Freund's adjuvant, including monoclonal human IgM, could induce the disease. Since the 16/6 is an anti-DNA antibody with a common idiotype, it is likely to trigger the immune system for the production of anti-idiotypic antibodies that might bear crossreactive epitope(s) with the autoantigen related to SLE. It has been previously demonstrated that idiotypic and anti-idiotypic antibodies can have a similar effect on the expansion of cells bearing a specific idiotype [17,18]. Indeed as we have further shown in this study, we could induce an experimental SLE in mice by the murine monoclonal anti-16/6 Id antibody termed McAb1A3-2. Moreover, the onset of the proteinuria and damage detected in the kidney sections was earlier and more pronounced when the inducer of the disease was the anti-16/6 Id antibody as compared to the 16/6 Id. It thus appears that the anti-idiotypic antibody specific to the 16/6 Id is capable of inducing SLE in mice more efficiently than the 16/6 Id. This substantiates the proposition that the induction of experimental SLE by the 16/6 Id is mediated by the formation of anti-16/6 Id specific antibodies.

Several studies demonstrated either facilitation or inhibition of spontaneous SLE in mice by various anti-idiotypic antibodies [19–21]. It might be that the nature of the idiotype or of the anti-idiotypic antibody used in each of these studies determines the enhancement or suppression of the autoimmune phenomenon. Nevertheless, to our best knowledge, this is the first study to show the induction of experimental SLE by anti-idiotype antibodies in a murine strain that does not develop any autoimmune disorders.

It has been reported that anti-idiotypic antibodies to major idiotypes that are antigen-binding site specific can replace the original antigen against which the idiotype is directed [22–24]. It is possible that through a mimicry between the anti-idiotype and the unknown original antigen of the 16/6 Id, the McAb1A3-2 is capable of stimulating the immune system for the production of the 16/6 Id-bearing antibodies. Murine 16/6 Id^+ antibodies were indeed identified in the sera of the McAb1A3-2 injected mice. These

antibodies, being directed also against self determinants (e.g., DNA), elicit anti-self response, and subsequently autoimmunity.

Although the statistical association between different HLA types and SLE suggests a role for genetic factors in determining susceptibility to SLE [11], the nature of this linkage is not yet clear. The study of the murine autoimmune strains, although shedding light on the pathology of SLE [25,26], could not contribute to our understanding of the combined effect of exogenous factors and the role of the genetic background in SLE, as the disease in all of them is spontaneous in its essence. Therefore, our newly established model of experimental SLE, in which the disease is externally induced, enabled the analysis of factors determining susceptibility to this disease. In this study we demonstrated that the induction of experimental SLE in mice is strain dependent. All the strains that developed experimental SLE responded to the 16/6 Id by the production of high titers of anti-16/6 Id specific antibodies, while in the resistant strains (C3H/He and C57BL/6) such antibodies could not be detected. These results are in agreement with the notion that the development of anti-16/6 Id antibodies is a crucial step in the pathogenesis of experimental SLE and they indicate that their production is genetically controlled.

No correlation could be found between MHC genes or Ig heavy chain allotypes and the sensitivity to the disease. It seems, therefore, that the susceptibility of the murine strains to experimental SLE is controlled by other genes than those linked to either MHC or heavy chain allotypes.

Immunization of the autoimmune mice NZB/W F1 with the 16/6 Id resulted in an advanced onset of the disease and an earlier death of the immunized mice compared with the nonimmunized control group. A previous report has shown delayed onset of the spontaneous disease as a result of the injection of murine monoclonal anti-DNA antibody [21]. Our results, therefore, further support the role played by the 16/6 Id-anti-16/6 Id antibodies in the induction of SLE.

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