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The genetic regulation of the induction of experimental SLE

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SUMMARY

We have recently reported the induction of systemic lupus erythematosus (SLE) in C3H.SW female mice by their immunization with a human monoclonal anti-DNA antibody that bears a common idiotype termed 16/6 Id. In the present study, the ability to induce experimental SLE in seven inbred mouse strains by immunization with the 16/6 Id was examined. Two out of the seven strains failed to develop the disease. These two strains did not produce antibodies specific to the 16/6 Id, while the other five strains produced high titres of anti-16/6 Id antibodies. The anti-16/6 Id antibody response, followed by the induction of the disease, was not found to be MHC or Ig heavy chain allotype linked. F_1 hybrids between a resistant strain and two of the susceptible strains were found to be resistant to the induction of the disease, indicating that susceptibility is inherited as a recessive trait. In the autoimmune NZB/W F_1 female mice, immunization with the 16/6 Id resulted in an early onset of the SLE-like disease. The results of the present study indicate the role of the anti-16/6 Id antibodies in the induction of experimental SLE, and provide direct evidence for the importance of the genetic background in determining susceptibility to SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease of unknown etiology and cure. This disease is also considered as the prototype immune complex disease, as the involvement of the immune system entails the formation of immune complexes that are subsequently deposited and injure various tissues (Morrow & Isenberg, 1987).

The notion that the genetic background, and mainly the major histocompatibility complex (MHC), is important in immune responses has promoted the examination of a possible association between the MHC and autoimmune diseases. The linkage between different HLA types and SLE is not well established. Thus, based on statistical analysis, it was reported that HLA-B8 and HLA-DR3 are more frequent in patients with SLE, but these two alleles were also found to be more frequent in some other autoimmune diseases (Svejgaard, Platz & Ryder, 1983). A further association was suggested between different HLA haplotyes and the severity of SLE. Hence, HLA-DR5 was found frequently in patients with mild SLE, as opposed to HLA-DR7, which was observed in a higher percentage of patients with severe SLE (Shalev *et al.*, 1985).

The study of many of the pathological and immunological aspects of SLE was facilitated by the availability of murine autoimmune strains. Mice of these strains, including the NZB/W F_1, BXSB males and MRL/lpr/lpr develop spontaneously an autoimmune disease that resembles SLE (Steinberg, 1984). The close examination of the autoimmune murine strains shed light on many of the factors that are of importance in the development of SLE (e.g. sex hormones, dietary factors, etc.; Roubinian, Papalian & Talal, 1977; Morrow *et al.*, 1986). However, these strains could not contribute to our understanding of the combined effect of exogenous and genetic factors in SLE, as in all cases the disease develops spontaneously.

We have recently reported the successful induction of experimental SLE in a murine strain that does not develop any immune disorders (Mendlovic *et al.*, 1988). The induction was carried out by the immunization of C3H.SW female mice with 1 μg of the human monoclonal anti-DNA antibody termed 16/6. The 16/6 idiotype (16/6 Id) was shown to have clinical relevance in human SLE (Shoenfeld & Isenberg, 1987; Shoenfeld *et al.*, 1983a). Priming and boosting the mice with this antibody resulted in the appearance of antibodies against the 16/6 Id, but also of murine antibodies that bear the 16/6 Id. In addition, binding activities against various nuclear antigens (such as ssDNA, dsDNA, RNP, Sm, Ro, La and others) were detected in the sera of all immunized mice. Four months after the booster injection, the mice exhibited significant proteinuria, leukopenia and elevated erythrocyte sedimentation rates. In the kidneys,

Abbreviations: dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbent assay; HIgM, human IgM; 16/6 Id, idiotype of the anti-DNA monoclonal antibody 16/6; PBS, phosphate-buffered saline; poly(I), polyinosinic acid; poly(G), polyguanosilic acid; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA.

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immune complexes could be detected. The 16/6 Id was found to be present in the renal complexes. One year following immunization, focal sclerosis of the glomeruli was apparent. None of the above abnormalities could be found in control C3H.SW female mice that were injected with either another human IgM or with an unrelated antigen, namely the synthetic polypeptide poly(Tyr,Glu)-poly(*DL*Ala)--(poly(Lys), designated (T,G)-A--L. We have recently shown that another human monoclonal antibody (SA-1) that was derived from a polymyositis patient and bears the 16/6 Id is capable of inducing experimental SLE in mice (Blank *et al.*, 1989). An additional monoclonal antibody (SA-2) that was derived from the same patient but did not bear the 16/6 Id could not induce the disease. The availability of an inducible model allows the examination of the pathogenesis of SLE and of the role of genetic factors determining the susceptibility to this disease.

We hereby report that the successful induction of SLE by the 16/6 Id in mice is strain dependent and is in direct correlation to their ability to produce anti-16/6 Id-specific antibodies. The susceptibility to the induction of the disease is inherited as a recessive trait. This is, therefore, the first clear evidence for the important role played by the genetic makeup and external stimuli in determining susceptibility to the induction of SLE.

MATERIALS AND METHODS

Mice

Mice of the different strains were obtained from either The Jackson Laboratory, Bar Harbor, ME, or Olac, Blackthorn, Bicester, Oxon, U.K. Four to 10 mice were used in each of the studied strains. The NZB/W F_1 females and (BALB/c × C57BL/6)F_1 were obtained from The Jackson Laboratory. (SJL × C57BL/6)F_1 mice were bred in the animal facilities at the Weizman Institute of Science. 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 with 50% ammonium sulphate, and the affinity-purified material that was eluted from a goat anti-human IgM–Sepharose 4B column was employed in this study.

Human IgM

Human IgM (HIgM, kappa$^+$) was obtained from the serum of a patient with macroglobulinemia following purification on a goat anti-human IgM–Sepharose 4B column.

Immunizations

Several groups of four to 10 mice were immunized with 1 μg of the 16/6 Id 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 16/6 Id in PBS in the hind footpads.

Radioimmunoassay

Flexible plastic microtitre plates were coated with 50 μl of 50 μg/ml of either 16/6 Id, HIgM or ssDNA 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 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 c.p.m./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) was prepared as described previously (Shoenfeld *et al.*, 1983b). Antibodies against Sm and RNP and against SS-A and SS-B (Tan, 1982) were determined according to Konikoff *et al.* (1987) and Yamagata, Harley & Reichlin (1984), 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 SS-A and SS-B were purchased from BioHytech Ltd, Ramat-Gan. One-hundred and fifty microlitres of mouse serum (diluted 1:200 in PBS) were 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°. Optical densities were read at 405 nm in a Dynatech model MR 580 Micro ELISA reader (Denkendorf, FRG).

Detection of SLE-associated pathological manifestations

The erythrocyte sedimentation rate 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 semi-quantitatively, using Combistix kit (Ames-Miles, Stoke Poges, Surrey, U.K.).

Immunohistochemistry

Kidneys were removed and were frozen immediately in liquid nitrogen. Six micrometer frozen cryostat sections were air dried for at least 2 hr, and fixed in acetone for 10 min. 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

Antibody response to the 16/6 Id and formation of murine antibodies with the 16/6 Id

Four to 10 mice of the 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 PBS. Animals were bled every 2 weeks following the boost injection with the 16/6 Id, and their sera were tested for the presence of antibodies against 16/6 Id and for the presence of murine antibodies that bear the 16/6 Id. The murine antibodies with the 16/6 Id were identified by a rabbit antiserum specific to the 16/6 Id. As can be seen in Fig. 1, which represents results determined with sera withdrawn 4 months after the boost, all the strains except C57BL/6 and

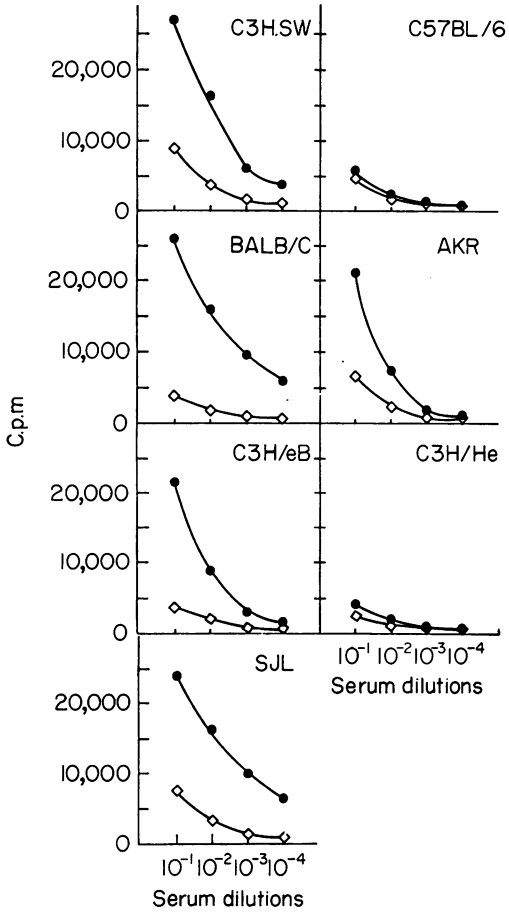

Figure 1. Antibody responses of different mouse strains following immunization with the 16/6 Id. Flexible plastic microtitre plates were coated with human monoclonal antibody 16/6 (50 μg/ml). The pooled sera of four to 10 mice of the different strains, obtained 4 months after the boost, were added at different dilutions, and bound Ig were detected by ^{125}I-labelled goat anti-mouse. (●) Sera of mice immunized with the 16/6 Id; (◇) preimmune sera.

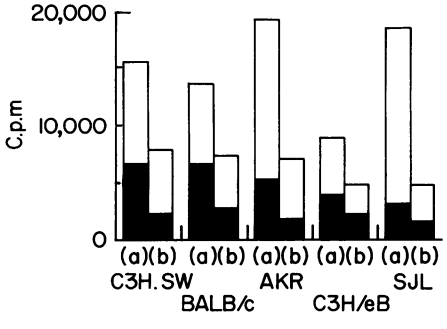

Figure 2. Specificity of antisera from 16/6 Id-immunized mice. Flexible plastic mictotitre plates were coated with either the human monoclonal antibody 16/6 or with HIgM (50 μg/ml). The pooled sera of different mouse strains obtained 4 months after the boost were added at 1:10 (a) and 1:100 (b) dilutions and bound Ig were detected by ^{125}I-labelled goat anti-mouse. Open bars represent binding to the 16/6 Id and closed bars represent binding to the HIgM.

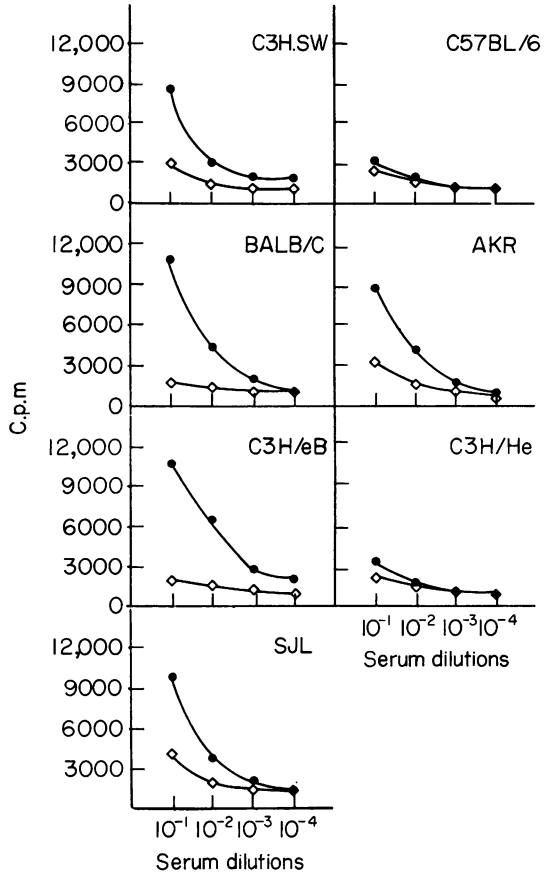

Figure 3. Binding of antibodies of different mouse strains immunized with the 16/6 Id to anti-16/6 Id. Flexible plastic microtitre plates were coated with a rabbit antiserum specific to the 16/6 Id at the dilution of 1:100. The pooled sera of four to 10 mice of the different strains, obtained 4 months after the boost, were added at different dilutions and bound Ig were detected by ^{125}I-labelled goat anti-mouse. (●) Sera of mice immunized with the 16/6 Id; (◇) Preimmune sera.

C3H/He reacted to the 16/6 Id by producing anti-16/6 Id antibodies. Figure 2 demonstrates the specificity of the immune response to the 16/6 Id by comparing the binding of the immune sera to an irrelevant human IgM. As can be seen, in sera of all responding strains the binding to the HIgM was significantly lower than that to the 16/6 Id. Figure 3 shows that in all the mouse strains that produced antibodies directed to the 16/6 Id, antibodies expressing the 16/6 Id could be detected. C57BL/6 and C3H/He mice did not produce 16/6 Id-bearing antibodies either. As previously reported (Mendlovic *et al.*, 1988; Blank *et al.*, 1989), sera of mice immunized with monoclonal human IgM that does not bear the 16/6 Id did not bind the 16/6 Id and anti-16/6 Id above the levels shown with preimmune sera.

Anti-nuclear antibody profile 4 months after immunization

Four months after immunization the sera of the different mice were tested for the presence of antibodies against various nuclear antigens. Figure 4 presents the antibody levels of representative responder (BALB/c) and non-responder (C3H/He) mouse strains to various nuclear antigens at three sera dilutions. Table 1 presents the antibody levels to ssDNA,

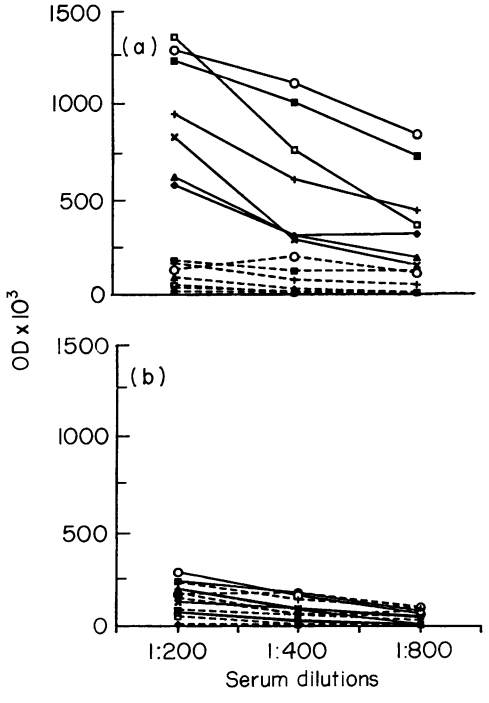

Figure 4. Anti-nuclear antibodies in BALB/c and C3H/He mice immunized with the 16/6 Id. Anti-nuclear antibodies were determined by ELISA in pooled sera of BALB/c (a) and C3H/He (b) mice 3 months after the booster injection. Solid lines represent sera of immunized mice, dashed lines represent sera of pre immune mice. (□) Anti-ssDNA; (■) anti-poly (I); (○) anti-poly (G); (▲) anti-Sm; (+) anti-RNP; (×) anti-Ro; (◆) anti-La antibodies.

poly(G), poly(I), Ro (SS-A), La (SS-B), RNP and Sm in all the strains at a 1:200 dilution. As can be seen in Fig. 4 and Table 1, the BALB/c mice produced antibodies against all the nuclear antigens examined. In the C3H.SW, AKR, C3H/eB and SJL mice, elevated levels of antibodies against most of the antigens were detected. The C57BL/6 mice had antibodies against poly(I), Ro and La. The sera of the C3H/He mice did not react against any of the nuclear antigens.

Clinical status of the mice 4 months following booster injection

Three parameters were examined in order to evaluate the clinical status of the mice of the different strains: proteinuria, erythrocyte sedimentation rate and leukopenia. As indicated in Table 2, all strains, besidese C3H/He and C57BL/6, showed a considerable proteinuria. The BALB/c mice were found to develop the most severe proteinuria compared with all other strains. The erythrocyte sedimentation rate was found to be elevated in the C3H.SW, C3H/eB, BALB/c and SJL mice, but not in the C3H/He, C57BL/6 and AKR mice (Table 2). Significant leukopenia was also determined, and was found to exist in all strains apart from C3H/He and C57BL/6 (Table 2). As mentioned for the proteinuria, the BALB/c mice were also found to suffer from the most severe leukopenia. The white blood cell count in control mice of the different strains was found to be similar to that shown for control C3H.SW mice (Table 2). It should be noted that during the course of the experiment one BALB/c mouse (out of four), one SJL mouse (out of four), and four (out of 10) AKR mice died. The reason of death in the last two strains was due to malignancy. The cause of death of the BALB/c mouse was unclear, but just prior to death severe proteinuria was observed.

Table 1. Anti-nuclear antibodies in the different mouse strains immunized with the 16/6 Id

Strain		Antibodies to: ssDNA	Poly(G)	Poly(I)	LA	Ro	RNP	Sm
C3H.SW	Immunized	280	359	605	424	400	356	288
	(Preimmune)	(155)	(87)	(242)	(70)	(73)	(275)	(101)
C57BL/6	Immunized	74	80	136	210	233	338	172
	(Preimmune)	(52)	(86)	(58)	(58)	(48)	(204)	(84)
BALB/c	Immunized	535	582	826	355	271	578	202
	(Preimmune)	(147)	(72)	(274)	(85)	(77)	(231)	(85)
AKR	Immunized	295	164	273	117	159	312	123
	(Preimmune)	(62)	(80)	(120)	(62)	(62)	(161)	(82)
C3H/eB	Immunized	359	229	443	324	286	651	292
	(Preimmune)	(144)	(156)	(334)	(124)	(101)	(286)	(100)
C3H/He	Immunized	83	92	312	82	85	336	94
	(Preimmune)	(207)	(201)	(283)	(102)	(157)	(279)	(127)
SJL	Immunized	167	243	279	147	184	282	369
	(Preimmune)	(117)	(129)	(171)	(95)	(136)	(231)	(130)

Different anti-nuclear antibodies were assessed in the pooled, 1:200 diluted sera, of the different strains about 3 months after the boost with the 16/6 Id. The numbers represent the OD values read at 405 nm, $\times 10^3$. The numbers in parentheses are the OD values obtained with pooled normal mouse sera of the different strains

Immunized, 16/6 Id immunized.

Preimmune, pooled preimmune sera.

Table 2. Clinical features of the different strains following immunization with the 16/6 Id

Strain	Immunogen	WBC counting	ESR*	Proteinuria
C3H.SW	16/6 Id	4·1+0·4†	++	++
C57BL/6	16/6 Id	5·3+0·5	−	−
BALB/c	16/6 Id	2·4+0·7†	++	+++
AKR	16/6 Id	4·2+0·6†	−	++
C3H/eB	16/6 Id	4·2+0·2†	+	++
C3H/He	16/6 Id	4·8+0·7	−	−
SJL	16/6 Id	4·6+0·3†	+	++
C3H.SW	HIgM	5·3+0·1	−	−

White blood cell (WBC) counting, erythrocyte sedimentation rates and proteinuria were assessed in each of the 16/6 Id-immunized strains, as well as in C3H.SW mice immunized with a control HIgM. Results express the readings of four to 10 mice, and were recorded 4 months after the booster injection.

* Erythrocyte sedimentation rates: −, < 1 mm/6 hr; +, 1–10 mm/6 hr; ++, > 10 mm /6 hr. Proteinuria: −, <0·3 g/l; +, 0·3–1 g/l; ++, 1–3 g/l; +++, >3 g/l.

† $P<0·01$ (Student's t-test compared to the WBC counting in the C3H.SW immunized with the control HIgM.

Immunohistology of kidney sections of different mouse strains immunized with the 16/6 Id

Immunohistological examination of the kidneys of the 16/6 Id immunized mice 6 months following boost revealed immunoglobulin deposits of both the IgG and IgM isotypes in all the mice that developed experimental SLE except for C3H/eB mice. Although the latter were found to develop experimental SLE according to all other criteria tested, no clear deposition of immunoglobulin could be detected in their examined kidneys. In the C57BL/6 and C3H/He mice that did not develop the disease, immune complexes could not be detected. Figure 5 demonstrates the immunoglobulin deposits in the 16/6 Id-immunized C3H.SW (a) and BALB/c (c) mice. As shown in Fig. 5, no immune complexes could be detected in the 16/6 immunized C3H/He mice (d). Control C3H.SW mice immunized with an irrelevant HIgM did not show any immunoglobulin deposits (b).

Immune responses and susceptibility to experimental SLE of F_1 hybrids between resistant and susceptible mice

F_1 hybrid mice between C57BL/6 mice that are resistant to the induction of the disease and either BALB/c or SJL mice that are susceptible to experimental SLE were immunized with the 16/6 Id. Table 3 demonstrates the antibody responses of the F_1 mice to the 16/6 Id as compared to those of the parental strains. As can be seen, (BALB/c × C57BL/6) F_1 are low responders to the 16/6 Id and produce low titres of antibodies expressing the 16/6 Id. In the (SJL × C57BL/6) F_1 mice immunized with the 16/6 Id, elevated anti-16/6 Id antibody titres could be detected, which appear to be due to an anti-HIgM response (Table 3). No significant antibody titres characteristic of SLE could be detected in the groups of immunized F_1 hybrid mice. The mice did not exhibit any of the clinical manifestations or kidney damage demonstrated in the susceptible mice, as tested during a period of over 8 months. Thus, the immune response potential to the 16/6 Id and susceptibility to the disease induction are inherited as a recessive trait.

Effects of 16/6 Id immunization on SLE-prone mice

Ten NZB/W F_1 mice were divided into two groups. Five mice were immunized with the 16/6 Id as the other strains, and five were left as a control group. The mice were analysed for the appearance of SLE by the assessment of serology, proteinuria, and eyrthrocyte sedimentation rate. Six months after the immunization, the titre of antibodies against 16/6 Id, ssDNA, dsDNA and murine antibodies that bear the 16/6 Id was examined. As can be seen in Table 4, the antibody levels detected in the 16/6 Id-immunized mice were comparable to these recorded in the control group that was not immunized. The only difference was a higher titre of anti-ssDNA antibodies in the 16/6 Id-immunized mice. On the other hand, it was found that the 16/6 Id-immunized mice developed the disease much earlier compared to non-treated NZB/W F_1 mice. Thus, the clinical parameters were evident 3 months after the boost with the 16/6 Id (at the age of 5 months) in all the mice (5/5). This is in contrast to the control group, where only a single mouse (1/5) exhibited any clinical abnormalities at that time. Figure 6 presents microscopical examination of the kidney sections taken from a representative 16/6 Id-injected NZB/W F_1 mouse 3 months following boost, and from a control mouse at the age of 5 months. Figure 6 indicates the massive renal pathology in the 16/6 Id-immunized mouse compared with the mild changes seen in the representative control mouse. The life span of the 16/6 Id-immunized mice was significantly shortened. Seven months after the immunization, at the age of 9 months, all of the 16/6 Id-treated mice died (5/5) compared to one out of five in the control group.

DISCUSSION

Natural and experimental evidence support a genetic predisposition to many autoimmune diseases, including SLE. Thus, family studies have shown an increased incidence of SLE in first degree relatives of SLE patients. Later studies, based on HLA typing, have demonstrated the higher frequency of HLA-B8 and HLA-DR3 in SLE patients (Svejgaard *et al.*, 1983). The statistical association between different HLA types and SLE suggests a role for the genetic background in determining susceptibility to SLE.

The study of murine autoimmune strains, although contributing information on the pathology of SLE, could not be used to investigate sensitivity or resistance to the disease. In all three autoimmune strains, NZB/W F_1, BXSB males and MRL/lpr/lpr, the development of the autoimmune disease is spontaneous, without any apparent or known exogenous inducer (Theofilopoulos & Dixon, 1985). The genetic background in these strains is therefore of immense importance. Indeed, the genes of the different autoimmune phenoma were partially located in the NZB strain, and were found to directly control the appearance of their matched clinical features (Shirai, 1982). In the MRL/lpr/lpr strain the lpr gene was found to be the one responsible for the SLE-like disease (Theofilopoulos & Dixon, 1981), while in BXSB strain genes linked to the Y chromosome were found to be important for the disease (Theofilopoulos & Dixon, 1981). In human, however, the role of the genetic background in SLE is

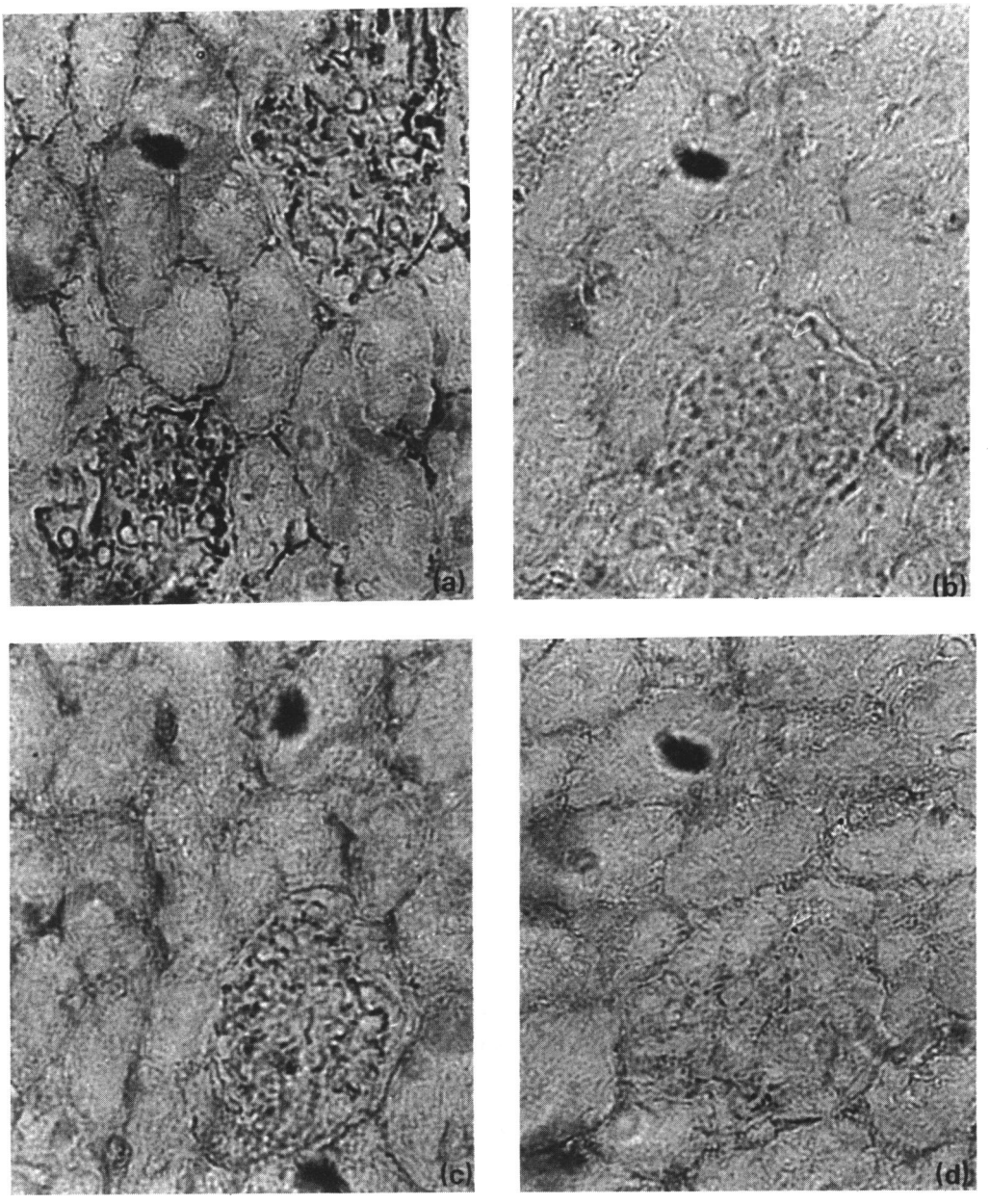

Figure 5. Immunohistology of kidney sections of different immunized mouse strains. Immunohistochemical staining of 5-μm frozen cryostat sections of 16/6 Id-immunized (a, c and d) and control HIgM-immunized (b) mice of different strains: (a, b) C3H.SW; (c) BALB/c; (d) C3H/He. Sections show peroxidase staining of IgM deposits on the glomerular basement membrane in the kidneys of the 16/6 Id-immunized C3H.SW (a) and BALB/c (c) mice. In the kidneys of HIgM-immunized C3H.SW (b) and of the 16/6 Id immunized C3H/He mice (d) no such deposits are detectable (magnification $\times$ 270).

not clear, and environmental factors are known to contribute to its induction (Shoenfeld & Cohen, 1987). Therefore, the generation of a model of experimental SLE, in which the disease is externally induced, enables the analysis of factors determining susceptibility to this disease.

In the present study we report that the induction of experimental SLE in mice is strain dependent. Out of the seven strains that normally do not develop an SLE-like syndrome, five were found to be sensitive to the induction following immunization with the 16/6 Id, whereas two strains (C3H/He and C57BL/6) were found to be resistant (Tables 1, 2, Figs 1, 3, 4 and 5). All the strains that developed experimental SLE responded to the 16/6 Id by the production of high titres of anti-16/6 Id-specific antibodies, while in the resistant strains (C3H/He and C57BL/6) such antibodies could not be detected (Fig. 1). The C57BL/6 strain developed considerable amounts of antibodies against Sm, Ro and La. However, all the other clinical parameters were found to be negative. In addition, this strain did not exhibit proteinuria, elevated erythrocyte sedimentation rate or leukopenia. The disparity between the anti-Sm, Ro and La antibodies and the other clinical parameters tested is not clearly understood. The production of these antibodies was

Table 3. Immune responses of F_1 hybrid mice following immunization with the 16/6 Id

Strain		Antibodies to: 16/6 Id 1:100*	16/6 Id 1:1000*	HIgM 1:100*	HIgM 1:1000*	Anti-16/6 Id 1:100*	Anti-16/6 Id 1:1000*
BALB/c	Immunized	26,540	22,493	10,818	5206	15,878	6412
	Preimmune	2857	1499	3425	2648	3403	2393
C57BL/6	Immunized	4507	1217	3130	1680	3429	2628
	Preimmune	2533	913	2714	1509	3496	2447
(BALB/c × C57BL/6) F_1	Immunized	8357	2455	5180	3244	4398	2767
	Preimmune	3923	1754	3821	2506	2765	2220
SJL	Immunized	24,044	16,556	6566	3470	15,226	6870
	Preimmune	7806	3304	1855	1458	4845	3727
(SJL × C57BL/6) F_1	Immunized	17,025	6456	21,443	10,388	6774	3387
	Preimmune	2740	1421	4352	4619	3918	2612

Flexible plastic microtitre plates were coated with either 16/6 Id, HIgM (50 μg/ml) or a rabbit antiserum specific to the 16/6 Id at a dilution of 1:100. The pooled sera of five mice in the different groups bled 2 months after the booster injection were added and bound Ig were detected by ^{125}I-labelled goat anti-mouse Ig (10^5 c.p.m./well).

* Sera dilutions.

Table 4. Antibody profile in 16/6 Id-immunized and non-immunized NZB/W F_1 female mice

Treatment	Sera dilution	Antibodies to: 16/6 Id	R-anti-16/6 Id	ssDNA	dsDNA
16/6 Id	1:10	13,302	11,544	25,072	13,135
16/6 Id	1:1000	5326	7363	19,354	6739
—	1:10	10,768	13,243	17,315	9546
—	1:1000	5782	6495	9363	5494

The antibodies against 16/6 Id, ssDNA, dsDNA and murine antibodies that bear the 16/6 Id (anti-rabbit anti-16/6 Id) were measured in 16/6 Id immunized and non-immunized NZB/W F_1 mice at the age of 6 months (3 months after the boost). Bound Ig were detected by ^{125}I-labelled goat anti-mouse Ig (10^5 c.p.m./well). The results express mean c.p.m. of pooled sera of five mice.

observed only in the 16/6 Id-immunized C57BL/6 mice, but not in mice immunized with an irrelevant antigen (data not shown). It seems, therefore, that the production of these antibodies is associated with a certain, specific determinant on the 16/6 Id antibody. However, as these 16/6 Id immunized mice did not develop SLE it is likely that they could not recognize the critical determinant on the 16/6 Id which is of importance in the induction of SLE. Indeed, specific antibodies to the 16/6 Id could not be detected in these mice. The disparity between different autoantibodies and SLE has been already reported (Munfrod *et al.*, 1985).

It can be therefore concluded that the production of anti-16/6 Id antibodies is a crucial step in the pathogenesis of experimental SLE, and the production of these antibodies is genetically controlled.

The segregation of the tested mice into sensitive and resistant strains does not appear to be linked to a single MHC gene. Thus the C3H.SW and C57BL/6 strains are both H-2^b, but the first one was found to be susceptible and the second resistant to the induction of SLE. The same is true also to the AKR and C3H/He strains, both having the H-2^k haplotyes. No correlation could be found between the Ig heavy chain allotypes and the sensitivity to the disease as well. It seems, therefore, that the susceptibility of the murine strains to experimental SLE is not under direct control of a single gene linked to either MHC or heavy chain allotypes.

The study of F_1 hybrid mice between the resistant strain C57BL/6 and two susceptible strains, namely BALB/c and SJL, indicated that the ability to respond to the 16/6 Id by the production of anti-idiotypic antibodies and subsequent development of clinical manifestations is under the control of functionally recessive genes or gene complexes. This observation is in agreement with previous reports dealing with autoimmunity in mice and man (Wicker *et al.*, 1987; Sasazuki *et al.*, 1983). Thus, it has been shown that diabetes in NOD mice is controlled by at least three functionally recessive diabetogenic

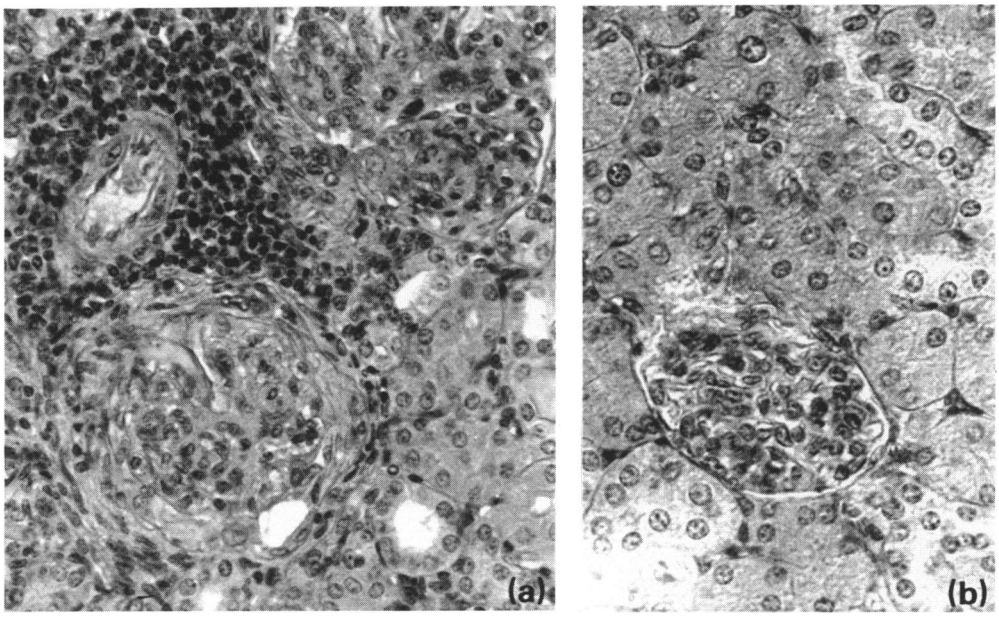

Figure 6. Histological sections of the 16/6 Id-immunized (a) (approximately 3 months after the boost) and non-treated (b) NZB/W F_1 mice at the age of 5 months. In the 16/6 Id-immunized mouse the kidney is extensively damaged, and some of the glomeruli are sclerotic, while in the non-treated mouse only mild changes could be observed (magnification × 300).

gene complexes, one of which is linked to the MHC of the NOD (Wicker *et al.*, 1987). In humans it was suggested that susceptibility to autoimmune diseases and allergy is transmitted recessively, due to the existence of HLA-linked immune suppressor genes (Sasazuki *et al.*, 1983).

In the present study we have also investigated the effects of immunization of the autoimmune strain NZB/W F_1 with the monoclonal anti-DNA antibody 16/6. It was found that the immunization resulted in a significant facilitation of the disease (Fig. 6). The injected mice exhibited proteinuria and eventually died earlier, compared with the non-immunized control group. A previous report has shown delayed onset of the spontaneous disease by the injection of murine monoclonal anti-DNA antibody (Hahn & Ebling, 1982). Our results, therefore, support the role played by the 16/6 Id–anti-16/6 Id antibodies in the induction of SLE.

This is the first direct report to show the importance of the genetic background in determining susceptibility to SLE. The results of this study should lead to the characterization of the gene(s) that are responsible for the differential sensitivity of the murine strains to experimental SLE.

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REFERENCES

Blank M., Mendlovic S., Mozes E. & Shoenfeld Y. (1988) Induction of SLE like disease in naive mice with a monoclonal anti-DNA antibody derived from a patient with a polymyositis carrying the 16/6 ID. *J. Autoimmunity*, **1**, 683.

Hahn B.H. & Ebling F.M. (1982) Suppression of NZB/NZW murine nephritis by administration of a synegeneic monoclonal antibody to DNA. *J. clin. Invest.* **71**, 1728.

Konikoff F., Shoenfeld Y., Isenberg D.A., Barisson I., Sobe T., Theodor E. & Slor H. (1987) anti-RNP antibodies in chronic liver disease. *Clin. exp. Rheumatol.* **5**, 359.

Mendlovic S., Brocke S., Shoenfeld Y., Ben-Bassat M., Meshorer A., Bakimer R. & Mozes E. (1988) Induction of a systemic lupus erythematosus like disease in mice by a common anti-DNA idiotype. *Proc. natl. Acad. Sci. U.S.A* **85**, 2260.

Morrow J. & Isenberg D. (1987) *Autoimmune Rheumatic Disease*, pp. 48–147. Blackwell Scientific Publication, London.

Morrow W.J.W., Homsy J., Swanson C.A., Ohashi Y., Estes J. & Levy J.A. (1986) Dietary fat influences the expression of autoimmune disease in MRL/lpr/lpr mice. *Immunology*, **59**, 439.

Munfrod T.A., Room G.R.W., Venables P.J.W. & Maini R.N. (1985) IgG antibodies to SS-B, RNP/Sm and DNA are produced by normal lymphocytes in culture. *Rheumatol. Int.* **5**, 109.

Roubinian J.R., Papalian R. & Talal N. (1977) Adrogenic hormones moderate autoantibody responses and improve survival of murine lupus. *J. clin. Invest.* **59**, 1066.

Sasazuki T., Nishimura Y., Muto M. & Ohta N. (1983) HLA-linked genes controlling immune response and disease susceptibility. *Immunol. Rev.* **70**, 51.

Shalev Y., Bentwich Z., Katz D., Brautbar C. & Mozes E. (1985) (T,G)-A--L specific immune response potential and HLA typing of Israeli patients with systemic lupus erythematosus (SLE). *Clin. exp. Immunol.* **60**, 355.

Shirai T. (1982) The genetic basis of autoimmunity in murine lupus. *Immunol. Today*, **3**, 187.

Shoenfeld Y. & Cohen I.R. (1987) Infections and autoimmunity. In: *The Antigens* (ed. M. Sela), Vol. VII, p. 307. Academic Press, New York.

Shoenfeld Y. & Isenberg D. (1987) DNA antibody idiotypes: a review of their genetics, clinical and immunological features. *Seminars Arth. Rheum.* **16**, 245.

Shoenfeld Y., Isenberg D.A., Rauch J., Madaio M.P., Stollar B.D. & Schwartz R.S. (1983a) Idiotypic cross reactions of human lupus autoantibodies. *J. exp. Med.* **158**, 718.

Shoenfeld Y., Rauch J., Massicotte H., Datta S.K., Andre-Schwartz J., Stollar B.D. & Shwartz R.S. (1983b) Polyspecificity

of monoclonal autoantibodies produced by human–human hybridomas. *N. Engl. J. Med.* **308,** 414.

STEINBERG A.D. (1984) Systemic lupus erythematosus: Insights from animal models. *Annal. Internal Med.* **100,** 714.

SVEJGAARD A., PLATZ P. & RYDER L.P. (1983) HLA and disease 1982—A survey. *Immunol. Rev.* **70,** 193.

TAN E.M. (1982) Autoantibodies to nuclear antigens. *Adv. Immunol.* **33,** 167.

THEOFILOPOULOS A.N. & DIXON F.J. (1981) Ethiopathogenesis of murine SLE. *Adv. Immunol.* **37,** 269.

THEOFILOPOULOS A.N. & DIXON F.J. (1985) Murine models for systemic lupus erythematosus. *Adv. Immunol.* **37,** 269.

WICKER L.S., MILLER B.J., COKER L.Z., MCNALLY S.E., SCOTT S., MULLEN Y. & APPER M.C. (1987) Genetic control of diabetes and insulitis in the non-obese diabetic (NOD) mouse. *J. exp. Med.* **165,** 1639.

YAMAGATA H., HARLEY J.B. & REICHLIN M. (1984) Molecular properties of the Ro/SS-A antigen and enzyme-linked immunosorbent assay for quantitation of antibody. *J. clin. Invest.* **74,** 625.