Resistance of MHC Class I–Deficient Mice to Experimental Systemic Lupus Erythematosus

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Experimental systemic lupus erythematosus (SLE) can be induced in mice by immunization with a human monoclonal antibody to DNA that bears a common idiotype (16/6Id). These mice generate antibodies to 16/6Id, antibodies to DNA, and antibodies directed against nuclear antigens. Subsequently, manifestations of SLE develop, including leukopenia, proteinuria, and immune complex deposits in the kidney. In contrast, after immunization with 16/6Id, mice lacking major histocompatibility complex (MHC) class I molecules generated antibodies to 16/6Id but did not generate antibodies to DNA or to nuclear antigen. Furthermore, they did not develop any of the above clinical manifestations. These results reveal an unexpected function of MHC class I in the induction of autoimmune SLE.

Systemic lupus erythematosus is a human autoimmune disease characterized by the presence of an array of autoantibodies, including antibodies to DNA (anti-DNA), antibodies to nuclear antigen (anti–nuclear antigen), and antibodies to ribonucleoprotein (anti-RNP) (*1*). Progression of the disease is associated with leukopenia, proteinuria, and immune complex deposits in the kidney. An experimental model of SLE can be induced in mice by immunization with a human monoclonal anti-DNA derived from an SLE patient and that expresses a common idiotype, designated 16/6Id (*2*). After immunization and a single boost with 16/6Id, mice produce antibodies to 16/6Id (anti-16/6Id), to DNA, and to nuclear antigens. After a period of 4 to 6 months, the immunized mice develop leukopenia and proteinuria, and immune complexes are observed in their kidneys (*3*). This experimental model closely parallels the human disease with respect to the production of autoantibodies and to clinical manifestations. The immunological basis for disease induction in 16/6Id-immunized mice is not known. However, administration of a monoclonal anti-16/6Id has been shown to be even more effective in eliciting experimental SLE than 16/6Id itself (*4*). Furthermore, T cell lines specific for 16/6Id can also induce disease when injected into mice (*5*). All 16/6Id-specific T cell lines are of the $CD4^+CD8^-$ phenotype and are MHC restricted (*5*).

MHC class I molecules, which present intracellularly derived peptides to $CD8^+$ T cells, are expressed in nearly all somatic tissues, including the kidney (*6*). Mice that lack cell surface MHC class I molecules have been generated by inactivation of the gene for β_2-microglobulin, which is required for the proper assembly and cell surface expression of the MHC class I molecule (*7*). The $CD4^-CD8^+$ T cell subset does not develop in these MHC class I–deficient mice. Mice that lack MHC class I (MHC class I^-) generally are healthy and are capable of mounting antibody responses and surviving various viral infections; however, they are more sensitive to intracellular parasites than their normal (MHC class I^+) littermates (*8*). To determine whether MHC class I molecules are involved in the induction or propagation of experimental SLE, we tested class I^- mice for their ability to develop this disease.

MHC class I^- mice and control 129 mice were immunized with 16/6Id, followed by a single boost 3 weeks later (*9*). In MHC class I^+ control strain 129 mice, we detected anti-16/6Id and anti-DNA in the sera within 10 days after the boost. These responses persisted for at least 6 months (Fig. 1, A and B). MHC class I^- mice immunized with 16/6Id developed anti-16/6Id with the same kinetics and with similar titers as control strain 129 mice (Fig. 1A). In contrast, the sera of MHC class I^- mice did not contain significant amounts of anti-DNA (Fig. 1B). Anti-DNA titers in the sera of 16/6Id-immunized MHC class I^- mice remained at background levels for up to 6 months, although anti-16/6Id titers remained high. Furthermore, anti–nuclear antigen titers were not detected in sera of MHC class I^- animals, but were found in sera of 16/6Id-immunized strain 129 animals (Fig. 1C) (*10*). The MHC class I^- mice were not generally poor responders to antigen, because immunization with ovalbumin elicited an antibody response not markedly different from that of normal mice (Fig. 1D).

The development of anti-DNA in normal mice immunized with 16/6Id is associated with the generation of antibodies to anti-16/6Id (*3*); immunization with anti-16/6Id triggers antibodies to DNA and nuclear extract antigen, and experimental SLE (*4*). The failure of class I^- mice to develop anti-DNA in response to immunization with 16/6Id raised the possibility that they do not respond to anti-16/6Id. We assessed this possibility by immunizing class I^- mice with mouse monoclonal anti-16/6Id (*11*). Although the control strain 129 mice all responded to the anti-16/6 idiotype, the class I^- mice did not respond at all (Fig.

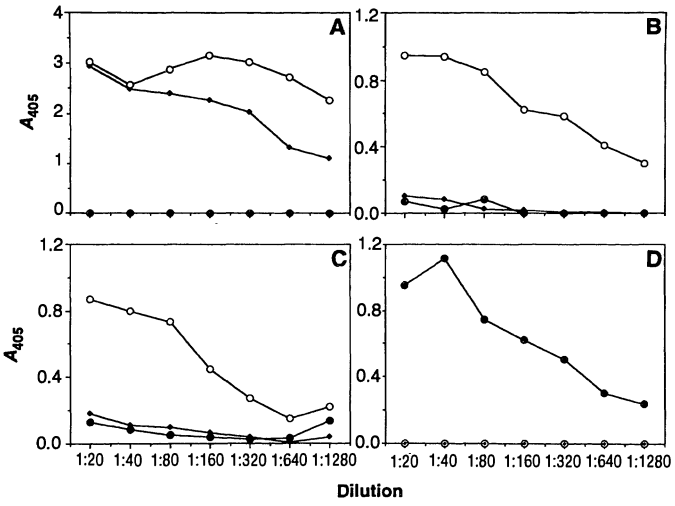

Fig. 1. Generation of antibodies in mice injected with 16/6Id. Serial twofold dilutions of sera were assayed by enzyme-linked immunosorbent assay (ELISA) 10 weeks after the boost (*5*). Results are the average of measurements from five animals and are expressed as absorbance at 405 nm × 10^3, as a function of serial serum dilutions (*5*). Standard deviations did not exceed 10% of the mean. (○), sera of 16/6Id-immunized control 129 mice; (◆), sera of 16/6Id-immunized class I^- mice; and (●), sera of ovalbumin-immunized class I^- mice. [Sera from 16/6Id-immunized animals did not contain significant amounts of antibodies to human immunoglobulin (*13*)]. (**A**) Titration of 16/6Id binding in the sera of immunized mice; purified 16/6Id was immobilized on plates. (**B**) Titration of single-stranded DNA binding in the sera of immunized mice; single-stranded DNA was immobilized on plates. (**C**) Titration of nuclear antigen binding in the sera of immunized mice; nuclear extract was immobilized on plates. (**D**) Titration of ovalbumin binding in the sera of immunized mice; ovalbumin was immobilized on plates.

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2). Thus, MHC class I–deficient mice could respond to ovalbumin and 16/6Id, but they were defective in their response to anti-16/6Id.

Immunization of control 129 mice with 16/6Id not only elicited a prolonged antibody response, it also induced leukopenia, proteinuria, and immune complex disease in the kidney (Table 1 and Fig. 3). Because MHC class I^- mice did not mount the full range of antibody responses after 16/6Id immunization, we monitored their susceptibility to these clinical manifestations of disease. None of the class I^- mice showed any evidence of either leukopenia or proteinuria (Table 1). Furthermore, whereas immune complex deposits were readily detected in the kidneys of 16/6Id-immunized control mice, no such deposits were found in the kidneys of MHC class I^- animals (Fig. 3). Taken together, these data indicate that MHC class I^- mice do not develop experimental SLE.

Our results demonstrate that MHC class I plays a key role in the induction of autoimmune SLE. The mechanism by which 16/6Id induces experimental SLE, and the involvement of MHC class I in this induction, are not known. The role of MHC class I could be either direct, through its role in peptide presentation, or indirect, through its role in generating $CD8^+$ T cells. It is unlikely that the role of MHC class I is indirect, because mice depleted of $CD8^+$ T cells remained susceptible to experimental SLE (*12*). We favor a model in which experimental SLE depends on the induction of an idiotype network in response to 16/6Id and in which this induction requires peptide presentation by MHC class I. Consistent with this model is the observation that MHC class I^- mice are nonresponders to anti-16/6Id, suggesting that failure to generate an idiotype network may be protective for experimental SLE. Understanding the function of MHC class I in inducing disease may be important not only in experimental SLE, but in other antibody-mediated autoimmune diseases as well.

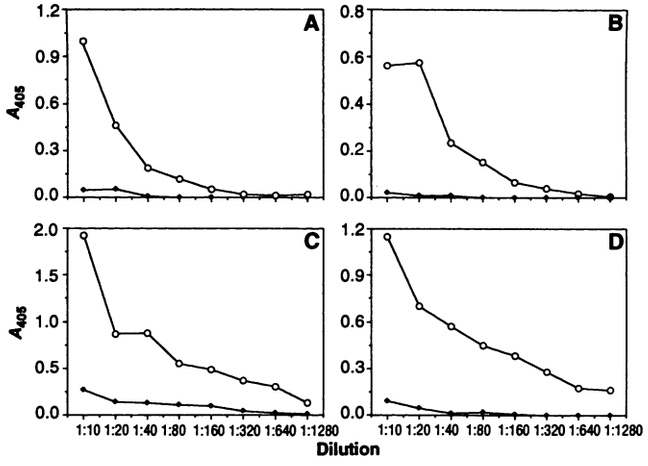

Fig. 2. Response to immunization with monoclonal anti-16/6Id. Serial twofold dilutions of sera were assayed by ELISA 7 weeks after immunization (*5*). Results are the average of measurements from six animals. Standard deviations did not exceed 10% of the mean. (○) Sera of anti-16/6Id–immunized control 129 mice; and (◆) sera of anti-16/6Id–immunized class I^- mice. (**A**) Titration of anti-16/6Id binding in the sera of immunized mice; purified rabbit polyclonal anti-16/6Id was immobilized on plates. (**B**) Titration of single-stranded DNA binding in the sera of immunized mice; single-stranded DNA was immobilized on plates. (**C**) Titration of 16/6Id binding in the sera of immunized mice; 16/6Id was immobilized on plates. (**D**) Titration of nuclear antigens binding in the sera of immunized mice; nuclear extract was immobilized on plates.

Table 1. Clinical manifestations of SLE in mice immunized with 16/6Id (*9*).

Animals	Treatment	Mean (SEM) leukocyte counts* (cells/mm^3)	Proteinuria (mg/dL)
		Experiment 1	
129	16/6Id	3150 (50)	100
MHC class I^-	16/6Id	5530 (250)	Trace
MHC class I^-	Ovalbumin	5130 (155)	Not determined
		Experiment 2	
129	16/6Id	2680 (135)	30–100
MHC class I^-	16/6Id	4480 (193)	Negative–trace
MHC class I^-	Ovalbumin	4033 (88)	Negative

*The leukocyte counts of the 16/6Id-immunized MHC class I^- and control 129 animals are significantly different ($P < 0.002$); those of the MHC class I^- mice immunized with 16/6Id or ovalbumin are not significantly different ($P < 0.2$) and fall within the normal range.

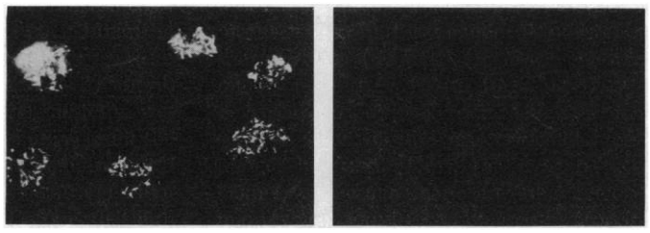

Fig. 3. Immunohistological staining of kidney sections of MHC class I^- (right) and control 129 (left) mice injected with 16/6Id. Frozen kidney sections (5 μm thick) were fixed and stained with fluorescein isothiocyanate–conjugated goat antibodies to mouse immunoglobulin G (γ chain–specific; Sigma). Magnification, ×200. The kidney sections shown are from one animal in each group and are representative of that group.

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9. Mice (groups of four to six) were immunized intradermally in the hind footpads with 1 μg of affinity-purified human monoclonal 16/6Id in complete Freund's adjuvant (CFA; Difco, Detroit, MI) and were boosted 3 weeks later with 1 μg of 16/6Id in phosphate-buffered saline (PBS) (*3*). Immunization of control C3H.SW mice with human immunoglobulin M in CFA does not elicit significant autoantibody responses (*3*). Immunization of mice with ovalbumin (20 μg) followed the same procedures as with 16/6Id. Five or six months after immunization, blood was drawn from MHC class I^- and control 129 mice. Leukocyte counts were done on each animal. Protein in the urine was measured with an Ames 2855 uristix (Miles, Inc.); normal mice are negative to trace.
10. Previously it has been reported that C57BL/6 mice are nonresponders to 16/6Id [S. Mendlovic *et al.*, *Immunology* **69**, 228 (1990)]. Because the C57BL/6 mice did not generate anti-16/6Id, this nonresponse is distinct from that of the MHC class I^- mice, which make anti-16/6Id but not anti-DNA or anti–nuclear antigen. Furthermore, C57BL/6 × MHC class I^- F1 mice responded normally to 16/6Id (*13*).
11. Mice (groups of six) were immunized intradermally in the hind footpads with 20 μg of monoclonal anti-16/6Id 1A3-2 (*4*) in CFA and were boosted 3 weeks later with the same amount of monoclonal antibody in PBS. Mice injected with a control anti-Id do not develop an anti-16/6Id response (*4*).
12. P. Ruiz and E. Mozes, unpublished observations.
13. E. Mozes, L. D. Kohn, F. Hakim, D. S. Singer, unpublished data.

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Interleukin-7: A Cofactor for V(D)J Rearrangement of the T Cell Receptor β Gene

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The diversity of the T cell receptor repertoire is generated by rearrangement of gene elements in immature thymocytes. To identify a thymic signal that induces this rearrangement, a variety of agents were tested for their ability to induce rearrangement of the T cell receptor β gene in suspensions of thymocytes from mouse embryos at day 14 of gestation. Of 16 agents tested, only interleukin-7 (IL-7) induced V(D)J gene rearrangement and sustained expression of the *RAG-1* and *RAG-2* genes, which are known to control rearrangement. These data implicate IL-7, a cytokine that is abundantly expressed in embryonic thymus, in driving gene rearrangement during early T cell development.

The antigen receptors of lymphocytes are encoded by genes that are assembled from separate gene elements (*1*). During rearrangement of the T cell receptor β (TCRβ) gene (*2*, *3*), variable (V), diversity (D), and joining (J) elements are cut, recombined, and joined together (*4*, *5*), creating the enormous diversity of T cell receptors. Similar phenomena occur in the TCRα, γ, and δ genes in T cells and in immunoglobulin (Ig) genes in B lymphocytes. This gene rearrangement appears to be strictly regulated: only precursor lymphocytes rearrange and the process occurs in a certain sequence. Thymocytes for example, first rearrange the D to J elements, then the V to D elements in the TCRβ locus, and later the TCRα locus is rearranged. We investigated the signals from the thymic microenvironment that induce rearrangement of the TCRβ locus.

Because the precursor T cells (pre-T cells) in which the rearrangement occurs are few in number, we used a polymerase chain reaction (PCR) for detection of V_β rearrangement (Fig. 1A). V and J regions are separated by 200 to 600 kb in the germline configuration (too long a span to generate a PCR product). If these regions are joined by the rearrangement process in a T cell, the PCR amplification product is 330 bp; the specificity of this product was verified by hybridization with a probe located between the primers. We used this technique to detect rearrangement of $V_\beta 8$ (Figs. 1 and 2), $V_\beta 3$, $V_\beta 6$, and $V_\beta 11$ (*6*).

To find an unrearranged T cell precursor population, we analyzed thymic subsets from adult mice. Triple negative cells ($CD4^-CD8^-CD3^-$), which represent 2% of the adult thymus, do not yet express TCR proteins. Nonetheless, most had already rearranged the TCRβ locus and were therefore not useful for these studies (*7*). We next examined fetal hematopoietic tissue to define the onset of V_β rearrangement (Fig. 1B). T cell precursors are generated in the fetal liver; they then migrate to the thymus where we detected V(D)J rearrangement at day 15 of gestation (longer autoradiographic exposure revealed presumably postthymic cells in the liver at day 19). A similar time frame for V_β rearrangement had previously been shown with the use of hybridomas generated from thymocytes at different time points during fetal development (*8*). At day 14, we detected less than one rearranged cell per thymus lobe (containing approximately 30,000 cells).

We therefore used these pre-T cells to study signals for rearrangement. The intact thymus was cultured, and the timing of V(D)J rearrangement was assayed by PCR (Fig. 2). V(D)J rearrangement occurred between 24 and 48 hours ($V_\beta 8$ is shown in Fig. 2, but the result was also reproduced for $V_\beta 3$ and $V_\beta 6$). For quantitation of the rearranged DNA, titration (*9*) indicated at least a 500-fold increase during 48 hours of organ culture, far more than could be a result of cell proliferation. The time course of rearrangement in organ culture resembled that seen in vivo (Figs. 1B and 2A). Thus, the isolated thymus provided a sufficient environment for rearrangement. Fetal thymic organ culture has required floating the organ on medium to achieve growth and differentiation processes (*10*); however, we observed no difference in the rates of V(D)J rearrangement within the first 48 hours in floating versus immersion cultures of the organ (*6*).

Intact organ structure was required for V(D)J rearrangement in vitro (Fig. 2B) because no rearrangement was detectable if the thymocytes were cultured as suspensions. Even when thymocyte suspensions were cultured in U-bottom plates (in order to provide more cell contact among the

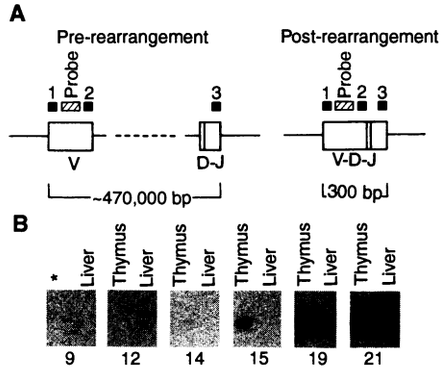

Fig. 1. V(D)J rearrangement of the TCRβ gene during fetal development. (**A**) Diagram of the murine TCRβ gene before (left) and after (right) rearrangement. Locations are indicated for PCR primers 1, 2, and 3 (below), as well as for the probe used for detecting the PCR product by hybridization (Probe). (**B**) PCR with DNA from C57Bl/6 embryonic mice. Organs were removed on the indicated days, and DNA was extracted (*26*) and quantitated. A constant amount of DNA (1 μg) from each treatment group was analyzed by PCR with the $V_\beta 8.1,2,3$ primer (primer 1, GAGGCTGCAGTCACCCAAAGTCCAA) and with opposite strand primers for $V_\beta 8.1,2,3$ (primer 2, ACAGAAATATACAGCTGTCTGAGAA) or $J_\beta 2.1$ (primer 3, TGAGTCGTGTTCCTGGTCCGAAGAA). The reaction mixture contained 35 mM tris (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, bovine serum albumin (100 μg/ml), nucleoside triphosphates (NTPs) (2 μM each), the primers (0.5 μM each), and 1 U of Taq polymerase. The amplification was performed for 30 cycles (1 min at 94°C, 2.5 min at 54°C, and 1.5 min at 70°C, with a 7-min extension period). The PCR product was then separated on a 1.2% agarose gel and blotted. The specific product of 330 bp was detected by hybridization with a ^{32}P-labeled oligonucleotide probe (100 mer) from the $V_\beta 8.1$ sequence between primers 1 and 2. Prehybridization and hybridization were performed at 54°C overnight in 6× sodium saline citrate (SSC), 5× Denhardt's solution, and denatured salmon sperm DNA (50 μg/ml). Membranes were washed 20 min at room temperature and then for 20 min at 54°C in 6× SSC. The membranes were then exposed for autoradiography overnight. This PCR method is relatively quantitative, in that the amount of product is related to the amount of rearranged DNA, and increasing the number of cycles gives more product (*9*). This experiment, showing the onset of rearrangement in the thymus at day 15, is representative of three separate experiments. Asterisk indicates embryo without liver.

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