Induction of a systemic lupus erythematosus-like disease in mice by a common human anti-DNA idotype

(autoimmune disease/idiotypic network)

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ABSTRACT Systemic lupus erythematosus (SLE) is considered to be the quintessential autoimmune disease. It has not been possible to induce SLE in animal models by DNA immunization or by challenge with anti-DNA antibodies. We here report a murine model of SLE-like disease induced by immunization of C3H.SW female mice with a common human monoclonal anti-DNA idotype (16/6 idiotope). Following a booster injection with the 16/6 idiotype, high levels of murine anti-16/6 and anti-anti-16/6 antibodies (associated with anti-DNA activity) were detected in the sera of the immunized mice. Elevated titers of autoantibodies reacting with DNA, poly(T), poly(dt), ribonucleoprotein, autoantigens [Sm, SS-A (Ro), and SS-B (La)], and cardiolipin were noted. The serological findings were associated with increased erythrocyte sedimentation rate, leukopenia, proteinuria, immune complex deposition in the glomerular mesangium, and sclerosis of the glomeruli. The immune complexes in the kidneys were shown to contain the 16/6 idiotype. This experimental SLE-like model may be used to elucidate the mechanisms underlying SLE.

Among the criteria defining antibody-mediated autoimmune disease one must precisely characterize the autoantigen, the autoantibody, and the induction of a similar condition following immunization with the autoantigen or after the passive transfer of the autoantibody. Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of multiple autoantibodies that are directed against various nuclear antigens (1). Despite the existence of several spontaneous murine models for SLE (2), this classical autoimmune disease could not thus far be experimentally induced. DNA, to which most of the autoantibodies are directed in SLE, is no longer regarded as the antigen initiating the disease (3), mainly because immunization of mice with DNA could not induce SLE (4). Furthermore, transfer of anti-DNA antibodies into mice did not result in the successful induction of SLE (5, 6). Thus the availability of such a model may shed light on the pathogenesis of SLE.

The human monoclonal IgM antibody termed 16/6 bears a common idiotype (idiotype 16/6), defined by a specific rabbit anti-idiotype serum (7). The idiotype was found to have clinical relevance in SLE patients (8). Its levels parallel the titer of anti-double-stranded DNA (dsDNA) antibody and inversely correlate with complement component concentrations in patients with SLE (8). The presence of this idiotype was clearly demonstrated in the glomeruli and epidermal–dermal junction of patients with SLE (9, 10).

In the present study we utilized the idiotype 16/6 to induce a SLE-like disease in a murine strain that does not develop any spontaneous immune disorder. We report here that following immunization of C3H.SW mice with the human anti-DNA monoclonal antibody 16/6, they develop a SLE-like disease characterized by the major hallmarks of SLE.

MATERIALS AND METHODS

Mice. C3H.SW female mice were obtained from The Jackson Laboratory and were used at the age of 2–3 months of age.

Antibodies with the 16/6 Idiotype. The hybridoma secreting antibodies with the idiotype 16/6 was grown in culture. The antibodies with the idiotype 16/6 were precipitated from the culture with 50% (wt/vol) ammonium sulfate, and the affinity-purified material that was eluted from a goat anti-human IgM-Sepharose column was used.

Monoclonal Anti-Idiotype 16/6 Antibodies. Monoclonal anti-idiotype 16/6 antibodies (anti-16/6) were produced by the fusion of splenic lymphocytes of C3H.SW idiotype 16/6–immunized mice with the X63.653 plasmacytoma cells by using 4% (wt/vol) polyethylene glycol. Hybrid cells that secreted antibodies specific to the idiotype 16/6 were cloned by limiting dilution in 96-well microtiter plates. Clone 1A3-2 that secreted anti-16/6 idiotype of the IgM class was used after purification on a goat-anti-mouse immunoglobulin-Sepharose column.

Antigens. The synthetic branched polypeptide antigen poly(Tyr,Glu)-poly(dT-Ala)--poly(Lys) [T,G]-A--L was synthesized and characterized as described (11). Human IgM obtained from the serum of a patient with macroglobulinemia was purified on goat anti-human IgM-Sepharose column.

Immunizations. Several groups of 5–10 mice were immunized with 1 µg of antibodies with the idiotype 16/6 in complete Freund’s adjuvant (CFA; Difco) intradermally into the hind footpads. Three weeks later booster injections were administered with the same amount of antibodies with the idiotype 16/6 in isotonic phosphate-buffered saline (PBS) in the hind footpads.

Radioimmunoassay. Flexible plastic microtiter plates were coated with 50 µl of antigen at 50 µg/ml dissolved in PBS. After a 2-hr incubation the plates were washed with PBS containing bovine serum albumin at 0.5 g/dl. The sera of the mice (diluted from 1:10 to 1:10,000) were then added and incubated for 4 hr. 125I-labeled goat anti-mouse immunoglobulin (105 cpm per well) was added to detect bound antibodies.

ELISA. Single-stranded DNA (ssDNA) and dsDNA were prepared as described (12). Antibodies against the autoantigen Sm and ribonucleoprotein and against autoantigens SS-A and SS-B were determined according to Konikoff et al.

Abbreviations: CFA, complete Freund’s adjuvant; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; SLE, systemic lupus erythematosus; (T,G)-A--L, poly(Tyr,Glu)-poly(dT-Ala)--poly(Lys).

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(13) and Yamagata et al. (14), respectively. Briefly, polystyrene plates with 96 flat-bottom wells (Dynatech, Alexandria, VA) were coated first with poly(L-lysine) (50 μl of 50 μg/ml), then with the antigen (50 μl of 2.5 μg/ml). Polystyrene plates coated with SS-A and SS-B were purchased from BioHytech (Ramat-Gan, Israel). One hundred and fifty microliters of mouse serum (diluted 1:200 in PBS) was added to each well. After incubation and washing, 150 μl of alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (IgG and IgM) was added. Phosphatase conjugate was detected by addition of 150 μl of p-nitrophenyl phosphate (14).

Detection of SLE-Associated Pathological Manifestations. The erythrocyte sedimentation rate was determined by diluting the hemipared 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 by a semiquantitative way by using Combistix kit (Ames, Elkhart, IN).

Immunohistochemistry. Kidneys were removed and frozen immediately in liquid nitrogen. Frozen cryostat sections (6–8 μm thick) were dried and fixed in acetone (Merck) for 10 min. For the detection of immunoglobulin deposits, biotinylated anti-total immunoglobulin antibodies were applied, and avidin–biotinylated peroxidase complex (Vector Laboratories, Burlingame, CA) was used as a second incubation step. Specific staining for the idiotype 16/6 was carried out after blocking of immunoglobulin deposits on the tissue sections with goat anti-mouse immunoglobulin antibodies. Sections were then incubated with the specific monoclonal anti-16/6 (1A3-2) for 30 min, followed by incubation with biotinylated goat anti-mouse IgM antibodies. Finally, the sections were incubated with avidin–biotinylated peroxidase complex. After each incubation, sections were extensively washed with PBS. Specific staining was visualized with diaminobenzidine as a substrate.

Electron Microscopy. Small pieces of renal tissues were fixed in 1% osmic acid and embedded in epoxy resin. Ultra-sections were double stained with 25 g/dl uranyl acetate and 2.8 g/dl lead citrate and were examined with Philips 300 electron microscope.

RESULTS

Several groups of C3H.SW female mice were immunized with 1 μg of the affinity-purified human 16/6 antibodies in CFA in the hind footpads and were given booster injections with the same amount in aqueous solution 3 weeks later. Twenty-one days after the booster injection, the titer of the anti-idiotypic antibodies against the idiotype 16/6 (anti-16/6) reached a maximum level, which remained stable for at least 13 months. Furthermore, high levels of antibodies bearing the idiotype 16/6 (anti-anti-idiotypic 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 dsDNA and ssDNA. Table 1 represents the antibody titers in serum from three of the immunized C3H.SW mice 4 months after booster injection. These antibody titers were detected only in mice immunized with antibodies with the idiotype 16/6 in CFA, but not in aqueous solution. Injection of control mice with human IgM in CFA as a control antigen did not result in significant antibody responses to the above antigens (Table 1). In addition to high antibody levels against dsDNA and ssDNA, we could also detect in the serum of all immunized mice binding activities against other antigens toward which antibodies exist in SLE patients. Table 2 shows the antibody levels against ssDNA, dsDNA, poly(I), Sm, ribonucleoprotein, SS-A, and SS-B in the serum of two representative mice immunized with the idiotype 16/6. Elevated antibody levels against poly(DT) and cardiolipin were also detected (data not shown). Serum of control mice that was immunized

<table>
<thead>
<tr>
<th>Mouse serum</th>
<th>Antibody present in serum, OD₄₀₅ units × 10³</th>
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<tbody>
<tr>
<td>dsDNA</td>
<td>ssDNA</td>
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<tr>
<td>S1</td>
<td>1782</td>
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<tr>
<td>S2</td>
<td>1872</td>
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<tr>
<td>C1</td>
<td>93</td>
</tr>
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<td>C2</td>
<td>63</td>
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<td>NMS</td>
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Antibody binding of serum from mice immunized with the human monoclonal idiotype 16/6 (mice S1 and S2) and from mice immunized with (T,G)-A–L (mice C1 and C2) was examined 4 months after booster injection. The sera were used with a dilution of 1:200. Results are expressed as OD₄₀₅ × 10³. NT, not tested; NMS, normal mouse serum.
idiotype 16/6, confirming the deposition of idiotype 16/6 in the glomerular basement membrane. Similar results were obtained with a polyclonal antiserum against the idiotype 16/6 (data not shown). Kidneys of mice immunized with (T,G)-A--L stained with the monoclonal anti-16/6 antibody 1A3-2 (Fig. 3B) and kidneys of mice immunized with idiotype 16/6 or with (T,G)-A--L stained with an irrelevant control antibody showed no pathological features in the glomeruli. It is noteworthy that in all groups of C3H.SW mice that were independently immunized with the idiotype 16/6 the same pathological features were observed.

**DISCUSSION**

In the present study we have induced an SLE-like disease in a mouse strain that is not susceptible to spontaneous autoimmune diseases. The injection of the common human anti-DNA idiotype (idiotype 16/6) was followed by the appearance of several hallmarks of SLE disease in human and in the spontaneous murine models (NZB/W F1, MRL/1pr/1pr, and BXSB) for this disease. The manifestations included elevated titers of antinuclear antibodies, such as anti-ssDNA, anti-dsDNA, anti-SS-A, anti-SS-B, anti-ribonucleoprotein, and anti-Sm antibodies, as well as antibodies to the synthetic polynucleotides poly(I) and poly(dT). Anti-Sm antibodies were reported to be highly specific for SLE (15) and rarely appear in other autoimmune diseases. The idiotype 16/6 that was found in the immunized mice was also reported to appear in 56% of active SLE patients and to parallel disease activity (8). Since it has been shown (7) that most of the anti-idiotypic antibodies that recognize the idiotype 16/6 bind to its antigen-binding site, it is possible that anti-anti-16/6 antibodies will mimic the 16/6 activity and thus will bind DNA. 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 idiotype 16/6-injected mice, are considered to be pathogenic in patients with SLE. Furthermore, the existence of the idiotype 16/6 has been reported in affected human skin and kidney of patients with SLE (9, 10). It was also found to exist in the renal immune complexes of mice injected with antibodies with the idiotype 16/6 described in this report. The reason for the accumulation of fat, and especially of the brown fat in the suprascapular gland, is obscure. Autoimmune disorders of the thyroid were negated by histology of the thyroid gland, examination of 3,5,3'-triiodothyronine (T3) and free 3,5,3', 5'-tetraiodothyronine (T4) levels and by the absence of antithyroglobulin and anti-microsomal antibodies.

The mechanism by which the injection of the human common idiotype 16/6 induced an SLE-like disease is not yet clear. The facts that the mice themselves produced

**Fig. 1.** Histological examination of kidney sections of mice injected with antibodies with the idiotype 16/6. Paraffin sections (5 μm thick) were fixed and stained with hematoxylin eosin and light green. Kidney sections of mice 4 months after a booster injection with antibodies with the idiotype 16/6 show hypervascularization and hypercellularity as a first stage of pathological changes in the glomerulus (A). (×460.) Twelve months after the same treatment sclerosis and rudimentary necrotic glomerular tissue can be observed in several glomeruli (B). (×460.)
antibodies with the idiootype 16/6 and that this idiootype was found in their kidneys suggest that 16/6--anti-16/6 antibody complexes play a crucial role in the pathogenesis of the disease. No control antigen injected with CFA, including human IgM, could induce the disease. Since 16/6 is an anti-DNA antibody with a common idiootype, 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. Indeed, we have shown (unpublished results) that anti-idiotypic antibodies against the idiootype 16/6 are very potent in the induction of SLE. It is also possible that the anti-idiotypic antibodies against the idiootype 16/6 affect the idiotypic network so that it produces different autoantibodies rather than affect it by crossreacting with the autoantigen. The induction of anti-anti-idiotypic antibodies was associated with the appearance of other antibodies to nuclear antigens, such as anti-DNA and antiribonucleoprotein. Interestingly, the production of antibodies with the idiootype 16/6 was associated with the generation of anti-Sm antibodies, which are highly specific to SLE (15). The reverse chain of events has been reported (16); following a series of injections with a monoclonal anti-Sm antibody, an early appearance of anti-dsDNA antibodies was noted in MRL/1pr/1pr mice.

Several studies have failed to demonstrate SLE induction in naive mice following the injection of either DNA or anti-DNA antibodies. DNA itself is a nonimmunogenic molecule (4), thus the question was raised whether it is the immunizing antigen or the preferred target antigen of anti-DNA antibodies in SLE (3). Several monoclonal anti-DNA antibodies were used for the immunization of naive mice (5). Some of these had high affinity to DNA, were of the IgG isotype, and had alkaline isoelectric points, thus suggesting that they were a nephrogenic subpopulation of the anti-DNA antibodies (17). Yet, none of these antibodies induced SLE-like disease in mice. Furthermore, injection of NZB/W F1 mice with a syngeneic monoclonal anti-DNA antibody with an alkaline isoelectric point was followed by a transient clinical improvement (18). Some of the differences between the system in the present report and that used in previous studies may explain the efficient induction of the SLE-like disease. The antibody with the idiootype 16/6, in contrast to other monoclonal anti-DNA antibodies used, is a human IgM and thus is more potent to the murine immune system. In addition, it was administered intradermally with CFA in contrast to other studies, in which the anti-DNA antibodies were injected either i.v. (6) or i.p. (5). Thus, whereas in the latter studies the clearance of the anti-DNA antibodies is relatively rapid, in our system a continuous triggering was achieved. Finally, the genetic makeup of the mice may be of importance. In the present study, C3H. SW mice were used. These mice possess an efficient immune system but do not spontaneously develop any immune disorder. Indeed, our preliminary data indicate different strain susceptibility to the induction of this SLE-like disease with the antibodies with idiootype 16/6 (unpublished data).

Fig. 2. Electron micrograph of a glomerulus from a mouse injected with antibodies with the idiootype 16/6. Kidneys were removed 4 months after a booster injection. The micrograph shows paramesangial electron-dense deposits (arrows). (×19,500.)
This model of experimental SLE-like disease in mice demonstrates the involvement of the idiotype-anti-idiotype network in SLE and may be further employed for the better understanding of the mechanisms involved in the induction and development of this disease.

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