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Induction of experimental systemic lupus erythematosus (SLE) in mice with severe combined immunodeficiency (SCID)

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SUMMARY

A model in which experimental SLE is induced in normal mice by the injection of a human anti-DNA MoAb expressing a common idiotype 16/6 Id has been established in our laboratory. In the present study we have attempted the induction of experimental SLE in mice with SCID by the transfer of lymphocytes obtained from mice with experimental SLE. Disease could not be induced by direct immunization of SCID mice with the 16/6 Id nor by transfusion of normal splenocytes and immunization with the 16/6 Id thereafter. In contrast, disease was induced in SCID mice which were transplanted with splenic lymphocytes obtained from SLE afflicted BALB/c mice. The disease was expressed by the presence of high titres of antibodies and glomerular immune complex deposits were present in the kidney sections of these mice. Mice that received spleen cells from donors with experimental SLE together with the 16/6 Id developed higher titres of autoantibodies and had, in addition to the immune complex deposits, glomerular histological pathology. The model of experimental SLE induction in SCID mice should help in the elucidation of the role of different cell types in the pathogenesis of SLE.

Keywords Experimental SLE SCID mice

INTRODUCTION

The autoimmune disease SLE is causally related to the appearance of autoantibodies, yet the antigen triggering this phenomenon and the mechanisms leading to the various clinical manifestations of the disease are not identified. The establishment of an experimental SLE model in mice [1] has made possible a direct approach to induce the disease and to analyse the mechanisms underlying development of the autoantibodies and their systemic involvement in SLE. Experimental SLE is induced in normal mice by injection of human anti-DNA MoAbs expressing the common idiotype 16/6 Id [2]. The disease induced in this manner is expressed by the production of a variety of autoantibodies (e.g. anti-ss, ds DNA, Sm, RNP, Ro, La, and cardiolipin antibodies) and by clinical manifestations characteristic of human SLE; proteinuria, elevated erythrocyte sedimentation rates and leukopenia. Kidney sections obtained from these mice revealed glomerular immune complex deposits. 16/6 Id was shown to be present in these complexes. MoAbs produced against the idiotype 16/6 Id (anti-16/6 Id) are also potent in the induction of pathological manifestations of the disease [3]. In addition, monoclonal T cells specific to the 16/6 Id were found capable of inducing efficiently experimental SLE in mice, indicating the important role of T cells in the induction and development of the disease [4]. For the determination of the role of the different components of the immune system in the development of experimental SLE, the transfer of immunocytes from SLE afflicted mice to immunodeficient recipients appeared to be an appropriate tool.

SCID mice are known as ideal recipients since they are deficient in active B and T lymphocytes [5]. They have, however, normal natural killer (NK) cells, myeloid cells, antigen-presenting cells and splenic colony stem cells [6–8]. The basis of the SCID defect has been ascribed to abnormality in the process of gene rearrangement for immunoglobulin and T cell receptor [9,10]. Consequently, these mice can be reconstituted with different allogeneic as well as xenogeneic haematopoietic and lymphoid cells [11,12]. Indeed, studies using SCID mice as recipients of peripheral blood lymphocytes (PBL) from SLE patients were reported [13]. The transplanted cells showed long term presence in the SCID mice and prolonged antibody synthesis, including IgG, IgM, and anti-nuclear antibodies (ANA). Immune complex deposits were also observed in the kidneys of these mice [13]. It thus seemed that an attempt to induce experimental SLE in SCID mice by adoptively transferring cells from SLE-afflicted donors will make possible the dissociation of the processes leading to the development of the disease. We report here that whereas SCID mice do not respond to the 16/6 Id either by production of antibodies or by renal pathology, transfer of splenic lymphocytes from the SLE-

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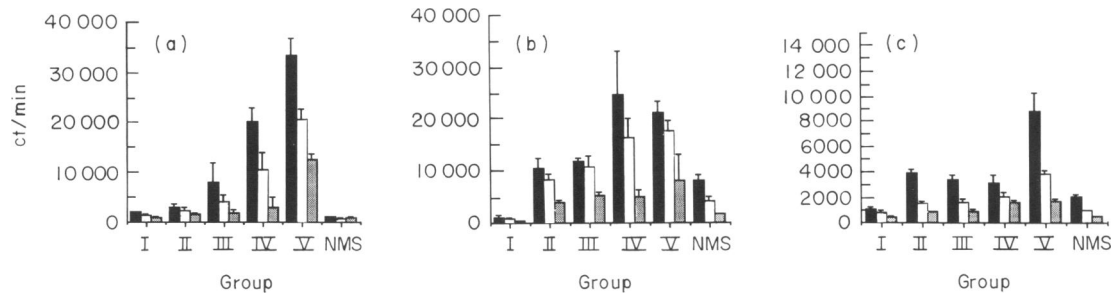

Fig. 1. Levels of autoantibodies in SCID mice 2 months following transplantation of spleen cells obtained from normal or SLE afflicted BALB/c mice. (a) Anti-16/6 Id antibodies. (b) Levels of anti-ss DNA antibodies. (c) Levels of anti-anti-16/6 Id antibodies (16/6 Id^+). I, Group 1, immunized with 16/6 Id; II, group 2, transplanted with 10^7 normal BALB/c spleen cells; III, group 3, same as group 2, and immunized with the 16/6 Id; IV, group 4, transplanted with 10^7 splenocytes of BALB/c with experimental SLE; V, group 5, same as group 4, together with the 16/6 Id; NMS, normal BALB/c mouse serum. Results represent mean ± s.d. of ct/min values obtained from radioimmunoassay (RIA) of different dilutions of serum samples from four mice per group. In group 5 all antibody levels at all serum dilutions were significantly elevated as compared with group 2 ($P < 0{\cdot}001$, two samples *t*-test). In group 4 only antibodies to 16/6 Id and ssDNA were significantly elevated as compared with group 2, at serum dilutions of 1:10 and 1:100 ($P < 0{\cdot}001$ and 0·005, respectively). Serum dilutions: ■, 1:10; □, 1:100; ▨, 1:1000.

afflicted mice led to the appearance of high titres of autoantibodies, as well as to massive glomerular immune complex deposits in the kidneys of the recipient SCID mice.

MATERIALS AND METHODS

Mice

BALB/c female mice were obtained from the Jackson Laboratory, Bar Harbor, ME. SCID C.B-17 homozygous mice described by Bosma [5] are grown in our laboratories under virus-free filters at constant air conditioned environment, and food and water *ad libitum*. Routine surveillance includes test for gross pathology, microbiology, parasitology and serology. Each mouse is routinely checked for production of immunoglobulins.

Antibodies and antigen

Human anti-DNA MoAbs bearing the 16/6 Id were isolated from the culture supernatants of a hybridoma secreting these antibodies on a goat anti-human IgM-Sepharose 4B column [1]. Rabbit anti-16/6 Id serum was produced by immunization of rabbits with the 16/6 Id and was used to assay levels of 16/6 Id^+ (anti-anti-16/6 Id) antibodies. Calf thymus DNA was purchased from Sigma (St Louis, MO). Single-stranded DNA (ssDNA) was obtained by heating the commercially available DNA for 10 min and immediately cooling on ice.

Induction of experimental SLE and cell transfer

Female BALB/c mice 2 months old were immunized by injection of 1 μg of the 16/6 Id in Freund's complete adjuvant (FCA) intradermally into the hind foot-pads. Three weeks later, booster injections with the same amount of antibody in PBS were administered in a similar manner. The BALB/c mice were killed 6 months post-immunization when high titres of anti-16/6 Id, anti-anti-16/6 Id (16/6 Id^+) and anti-DNA antibodies were determined. Single cell suspensions were prepared from their spleens, and 10^7 splenocytes were administered intravenously to each SCID mouse.

Radioimmunoassay

Flexible plastic microtitre plates were coated with 50 μl of 10 μg/ml of the 16/6 Id, or ssDNA or dsDNA and with rabbit anti-16/6 Id antibodies at a dilution of 1:200 in PBS. After a 2 h incubation period the plates were washed and serial dilutions (1:10 to 1:1000) of sera were added and incubated for an additional 3 h. Subsequently, ^{125}I-labelled goat anti-mouse immunoglobulin (100 000 ct/min per well) was added to the washed plates and incubated for 18 h. After extensive washing of the radioactive reagent, plates were dried, wells were cut out and counted in a gamma counter.

ELISA

Levels of autoantibodies against calf thymus dsDNA (Sigma) were determined as previously described [1]. Antibodies to Sm/RNP and SS-B (La) were determined according to Konikoff [14] and Yamagata [15], respectively.

Immunohistology

Kidneys were removed and immediately frozen in liquid nitrogen. Cryostat sections (5–7 μm thick) were dried and fixed in acetone for 5 min and then rinsed with PBS. For detection of immunoglobulin deposits, FITC-conjugated goat anti-mouse immunoglobulin antibodies were applied for 60 min. After extensive rinsing in PBS, the specific binding was visualized using fluorescence microscopy.

Histology

Tissues were fixed in Bouin's fixative, paraffin embedded and 4 μm sections were stained with haematoxylin, eosin and light-green.

RESULTS

SCID mice were divided into five groups, four mice in each group. Group 1 was immunized and boosted with the 16/6 Id in the same manner used for induction of the experimental disease in BALB/c mice (see Materials and Methods). Group 2 received 10^7 spleen cells obtained from normal BALB/c mice. Group 3 was injected with 10^7 normal BALB/c splenocytes and then immunized and boosted with the 16/6 Id. Group 4 was inoculated with 10^7 splenocytes from BALB/c mice that developed experimental SLE. Group 5 was injected with 10^7 splenocytes of mice with experimental SLE, together with 1 μg of the

Table 1. Autoantibody levels in SCID and BALB/c mice with experimental SLE

Antibodies to			
16/6 Id	anti-16/6 Id	ssDNA	dsDNA
BALB/c, Exp. SLE			
30170	8770	29000	30500
25200	2900	25800	27000
14300	670	11600	13500
SCID, group 5			
29300	7500	23200	22500
17600	1900	20500	21000
6500	970	13600	13000
BALB/c, normal (NMS)			
3800	2100	12300	13100
3600	950	7400	7200
1200	550	2000	2700

Antibody titres of pooled sera of mice, comparing the recipient SCID mice (group 5) with the donors, BALB/c mice with the experimental SLE. Results represent mean of duplicates obtained from radioimmunoassay of serum at different dilutions (1:10–1:1000). S.D. did not exceed 10%.

NMS, Normal BALB/c mouse serum.

Table 2. Autoantibody levels in SCID mice recipients of spleen cells from normal or SLE-afflicted BALB/c mice

Group	ds DNA	Sm/RNP	La
1	0·005	0·002	0·017
2	0·192	0·021	0·011
3	0·251	0·001	0·002
4	0·383	0·138	0·132
5	0·550	0·404	0·386
NMS	0·170	0·033	0·025

Autoantibody levels of pooled serum (diluted 1:200) 4 months following transplantation were tested by ELISA. Results are expressed as mean of duplicates at OD 405. S.D. did not exceed 10%.

NMS, Normal BALB/c mouse serum.

16/6 Id. All mice were bled periodically and their sera were tested for autoantibody production. Figure 1 represents the antibody levels 2 months following cell transfer. Figure 1a demonstrates 16/6 Id-specific antibody responses. As can be seen in Fig. 1, no antibody activity could be detected in sera of SCID mice that either were immunized with the 16/6 Id (group 1) or received normal spleen cells (group 2). Low levels of anti-16/6 Id antibodies were observed in mice that received normal splenocytes and were immunized thereafter (group 3). The antibodies were detected 4 weeks following booster injection. High antibody titres could be observed in sera of mice belonging to groups 4 and 5. Antibody levels were higher in group 5 and were detected as early as 1 week following inoculation of cells (data not shown). The elevated antibody levels in both groups 4 and 5 were maintained for 9 months, when the mice were killed. As shown in Fig. 1b, the differences between the groups in the titres of anti-ssDNA antibodies parallelled those observed for anti-16/6 Id antibodies. The levels of anti-anti-16/6 Id antibodies (16/6 Id^+) as detected by rabbit anti-16/6 Id serum are shown in Fig. 1c. Significantly elevated 16/6 Id^+-specific antibody levels could be detected only in sera of mice of group 5. Table 1 demonstrates autoantibody levels in the donor SLE afflicted BALB/c mice as compared with those in the recipient SCID mice (group 5). Autoantibodies to dsDNA, Sm/RNP and La were measured by ELISA and are shown in Table 2. Elevated antibody levels could be detected in the SCID mice from groups 4 and 5. In agreement with the results of the radioimmunoassay (RIA) (Fig. 1), the highest antibody levels were observed in group 5.

Since SCID mice are severely leukopenic and suffer from agammaglobulinaemia, the clinical examinations performed routinely to evaluate the experimental disease, e.g. white cell count and erythrocyte sedimentation rate, were not relevant in this study. Proteinuria which is present in the experimental disease was found not only in the experimental mice but also in most 'normal healthy' SCID mice. The reason for the latter is not clear since the kidneys of 'healthy' SCID mice appeared to be normal as could be judged by conventional histology and immunohistology. Urea and creatinine levels were not elevated in any of the experimental groups as compared with the normal control mice (results not shown).

Nine months following inoculation, mice were killed and immunuhistology was performed on their kidney sections. In agreement with the results of serology no immune complex deposits could be detected in the kidneys of mice in groups 1 and 2. A small number of immune complex deposits that were stained weakly were observed in kidney sections of mice in group 3. In contrast, multiple immune complex deposits were detected in the kidney sections of all the mice in groups 4 and 5, and the staining was more dense in those of group 5. Figure 2 demonstrates the immune complex deposits observed in representative kidney sections of a mouse from group 5 in comparison with a kidney with no deposits from a group 2 mouse. Histological examination of the kidney sections revealed pathological changes in all mice from group 5. No pathology was detected in any of the other groups. As can be seen in Fig. 3, mild to moderate thickening of the glomerular basement membrane, moderate mesangial proliferation and mild fibrotic changes are observed in kidney sections of a mouse from group 5 (Fig. 3a). These changes are not seen in kidney sections of mice of group 2 that received normal splenocytes (Fig. 3b).

DISCUSSION

The present study indicates that it is possible to induce experimental SLE in SCID mice by an adoptive transfer of splenocytes from donors with experimental SLE. The disease was expressed by the presence of autoantibodies and glomerular immune complex deposits as well as histological changes in the kidney sections of those mice that received the immunized cells

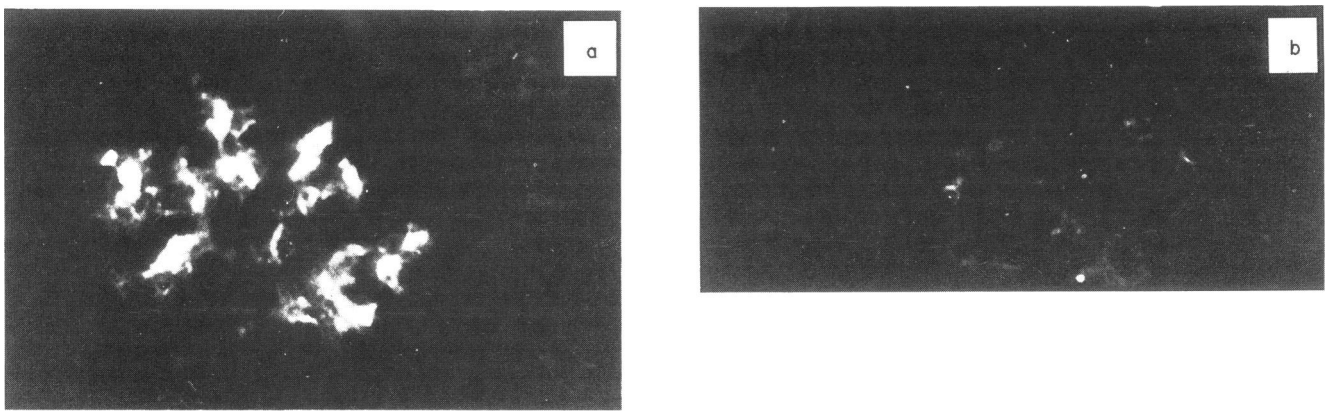

Fig. 2. Immunohistology of kidney sections from SCID mice 9 months following transplantation of spleen cells from SLE-afflicted mice. Immunofluorescent staining of 5 μm frozen cryostat sections of kidneys from (a) mice transplanted with 10^7 splenocytes from donors with experimental SLE together with the 16/6 Id; (b) mice transplanted with 10^7 splenocytes from normal BALB/c donors. Immunoglobulin deposits were detected in (a). The control section (b) is negative.

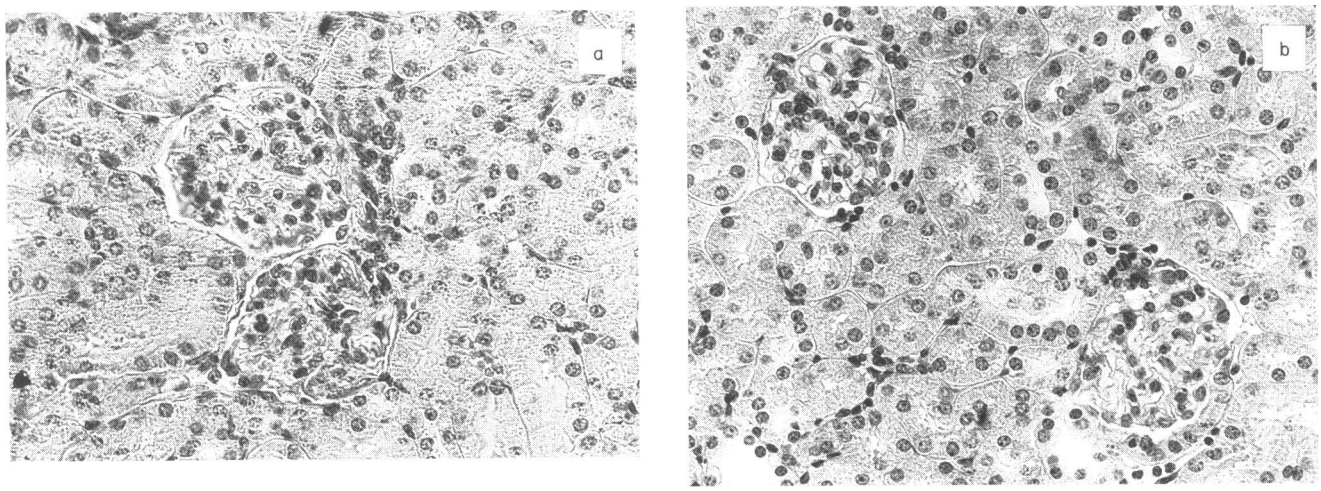

Fig. 3. Histological examination of kidney section of SCID mice, 9 months following transplantation of spleen cells from SLE-afflicted BALB/c mice. Paraffin sections (5 μm thick) were fixed and stained with haematoxylin eosin and light green. Thickening of parietal and visceral glomerular basement membrane, mild mesangial hypercellularity and fibrotic changes are seen in kidney section of mice in group 5 (a) in comparison with a normal section, group 2 (b) ($\times$ 460).

together with the 16/6 Id. The disease could not be induced by immunization of the mice with the 16/6 Id, in agreement with the fact that these mice do not possess an active lymphoid system. The feasibility of employing these mice as recipients is due to their deficiency in functional B and T lymphocytes. The model of the SCID mouse as compared with the use of normal irradiated recipients has several advantages. First, the possible existence of residual radioresistant cells is ruled out. Second, since onset of symptoms of the disease is manifested after relatively long periods, the complexity of radiation recovery and subsequent involvement of recipient cells is eliminated in the SCID mouse. Third, this model avoids damage to non-lymphatic tissues that might be caused by irradiation. The SCID mice may thus provide a unique tool for studies of cellular induction and control of SLE, by limiting T and B lymphocyte responses strictly to the donor cells.

In our experiments we have demonstrated that transfer of experimental SLE to SCID mice was accomplished by their transfusion with splenocytes from SLE-afflicted BALB/c mice. It was of interest to note that transfer of splenocytes from normal untreated donors and then immunization of the recipient SCID mice with the 16/6 Id led to the production of low antibody titres (Fig. 1) and to a mild immune-complex deposition in their kidneys. Several possibilities may be considered to account for these observations: (i) the results might be related to an insufficient number of inoculated lymphocytes in our experimental protocol. Hence, the transfer of 10^7 spleen cells from untreated donors may have had a limited proportion of a crucial cell subset, which is increased in the pre-immunized splenocytes to initiate the process in the host environment; (ii) homing of the unimmunized cells in the SCID mice may be limited as compared with that of immunized cells. It is possible that the more active disease observed in recipients of splenocytes of mice with experimental SLE given together with the 16/6 Id is due to a more efficient homing in the presence of the triggering antibody, namely the 16/6 Id. This possibility is in line with

previous observations that stimulation with antigen or mitogen enabled acceptance of antigen-specific T cell lines in the appropriate recipients ([16], and Apte & Mozes, unpublished).

The other aspect of our study concerns the appearance of renal disease in the SCID mice. The presence of immune-complex deposits in the kidneys of the recipient SCID mice in correlation with the presence of autoantibodies in their sera suggests that essential factors which are necessary for the glomerular pathology in this autoimmune disease (e.g. complement or possibly cytokines) do exist and are functional in the SCID.

The present findings that different manifestations of experimental SLE, including production of autoantibodies and the appearance of glomerular immune-complex deposits, can be initiated in SCID mice by the grafted immunized splenocytes now provide an experimental approach for analysis of the role of different cell types (B or T lymphocytes, as well as their distinct subclasses) in the induction of the disease. In this study it has been shown recently that $CD4^+$ but not $CD8^+$ T cell populations could be engrafted in the spleens of SCID mice [17]. Furthermore, our model may make possible the distinction between the role of immune cells and that of other essential factors, and the interrelationships between them in the pathogenesis of autoimmune diseases.

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