Brief Definitive Report

Transfer of Human Systemic Lupus Erythematosus in Severe Combined Immunodeficient (SCID) Mice

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Summary

To study the role of peripheral blood leukocytes (PBL) in the pathogenesis of human systemic lupus erythematosus (SLE), we transferred PBL from 5 SLE patients into 15 severe combined immunodeficiency (SCID) mice. Such reconstituted mice showed long-term presence of autoantibodies characteristic of the donor in their sera, as well as human immunoglobulin deposition, and in some cases mouse C3, in the renal glomeruli. SCID mice repopulated with PBLs from normal donors do not develop serologic abnormalities or immunodeposits. It is concluded that human SLE serology and some associated renal changes can be reproduced solely by PBL transferred from afflicted patients, and that SCID-human-SLE mice may serve as an in vivo laboratory model for the study of human SLE.

Animal models of autoimmunity, particularly mouse models, have been invaluable tools to the study of human SLE (1). Both share serologic and pathologic abnormalities, although human and murine SLE may differ in manner of expression (2). Since the etiology of both human and murine SLE remains unknown, it remains to be determined whether they share common etiologic factors and if the conclusions from the study of murine SLE will be directly applicable to the human counterpart. The recent report of long-term engraftment of human lymphoid cells, particularly functional human PBL into SCID mice (3, 4), prompted us to study the effect of the PBL transfer from SLE patients into SCID mice (SCID-hu-SLE), in an attempt to create a human SLE model that could be manipulated within the context of a laboratory animal.

Materials and Methods

Cell Transfer. PBL from healthy donors or SLE patients were isolated by Ficoll Hypaque separation. Within 6 h of drawing, 15 × 10^6 PBL were injected intraperitoneally into 8-wk-old nonleaky C.B-17 *scid/scid* (SCID) mice. A successful reconstitution was considered if the level of human IgG in the mouse was >1,000 μg/ml in 90 d or less after the cell transfer. We included one SCID-hu-SLE mouse (No. 10, see Results and Discussion) that, although not fulfilling our criteria for a good reconstitution, displayed a high antinuclear antibody (ANA) titer.

Human Ig Level Determination. Human IgG and IgM were quantified as described using a modification of the particle concentration fluorescence immunoassay procedure of Jolley et al. (5). Briefly, polystyrene particles previously adsorbed with a polyvalent, mouse-absorbed, goat anti-human Ig (Caltag, South San Francisco, CA) were incubated with serum samples or respective standards of human IgG, Fx II (Pentex 46), or human IgM (Calbiochem-Behring Corp., San Diego, CA). Bound IgG or IgM were revealed using an Fc-specific goat anti-human IgG, or a goat anti-human IgM conjugated to FITC (Caltag), respectively. These manipulations were performed in special assay plates using an automated fluorescent concentrated analyzer (Pandex Laboratories, Inc., Mundelein, IL).

Human ANA Titer Determination. Serum human ANA titers were determined using HEP-2 cells as a substrate (Bion Enterprises, Ltd., Park Ridge, IL), and specific human ANA were revealed using a Burro anti-human total Ig conjugated to fluorescein (Kallestad Diagnostics, Austin, TX). The human ANA isotypes were determined using the same procedure; the ANA were revealed with the FITC-conjugated antibodies described in the human Ig level determination section. Slides were examined under an Olympus BH2-RFL microscope equipped with a reflected light fluorescence illuminator (Olympus, Tokyo, Japan).

Histology. Tissues were fixed in Bouins fixative, paraffin embedded, and 4-μm sections were stained with periodic acid Schiff (A). Alternatively, unfixed frozen sections were incubated with a Burro anti-human total Ig conjugated to fluorescein (Kallestad Diagnostics) (B), a goat anti-mouse C3 (Cappel Laboratories, Malvern, PA), or an anti-human C3, and examined under reflected light fluorescence.

Results and Discussion

15 SCID mice were injected intraperitoneally with 15 × 10^6 PBL from 5 SLE patients (SCID-hu-SLE), and 9 mice were injected with 15 × 10^6 PBL from 3 normal donors (SCID-hu), and bled periodically thereafter. As depicted in Table 1, such reconstituted mice showed long-term presence

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Table 1. *Quantitation of Human IgG and IgM Levels (μg/ml) in SCID-hu-SLE and SCID-hu Mice*

SCID-hu SLE	D15	D30	D60	D90	D120	D150	D180	D270
No. 1	122/2.4	703/3.6	1,277/14.0	1,320/8.9	2,165/78.0	959/4.0	573/1.2	363/0.3
2	253/2.8	1,398/22.0	1,710/66.0	1,282/47.0	992/27.0	719/13.0	594/9.2	265/2.2
3	185/3.0	760/9.6	865/56.0	1,222/120.0	917/105.0	–	–	–
4	265/1.8	1,065/6.9	2,041/112.0	2,701/249.0	1,689/101.0	–	–	–
5	182/2.8	1,657/32.0	3,002/283.0	2.944/133.0	1,200/7.8	1,211/35.0	982/16.0	484/2.0
6	932/16.0	1,968/11.0	1,359/10.0	1,210/5.8	1,004/2.9	573/2.2	744/2.1	–
7	3,222/41.0	3,191/18.0	2,691/38.0	2,548/42.0	1,662/15.0	1.007/11.0	1,120/8.5	261/2.2
8	3,557/35.0	2,488/22.0	2,714/71.0	–	–	–	–	–
9	3,555/21.0	2,148/8.4	2,419/90.0	855/1,984*	–	–	–	–
10	164/0.7	247/0.3	298/11.0	261/1,004*	136/2,803*	–	–	–
11	2,858/54.0	2,337/12.0	3,512/13.0	1,498/9.7	762/5.4	393/2.6	248/1.2	–
12	2,288/55.0	2427/15.0	2,569/7.6	2,237/7.1	1,476/19.0	2,168/36.0	1,342/24.0	–
13	1,863/36.0	2,045/18.0	2,050/11.0	1,121/6.0	566/2.9	334/3.1	207/9.2	–
14	1,160/14.0	1,375/20.0	1,654/31.0	705/7.8	492/4.2	467/2.2	195/1.1	–
15	1,827/34.0	1,897/7.9	–	–	–	–	–	–
SCID-hu‡	884/3.4	918/13.5	1,643/17.2	1,776/20.6	1,394/22.2	1,037/4.6	775/7.3	118/0.5

Determination of human IgG/IgM levels 15, 30, 60, 90, 120, 150, 180, and 270 d after engraftment of human PBL. –, dead.
* SCID-hu-SLE mice 9 and 10 developed human lymphoma, which caused their death. These two mice have not been included in the histology/immunofluorescent kidney results.
‡ For comparison the mean human IgG/IgM level of nine SCID-hu mice is included.

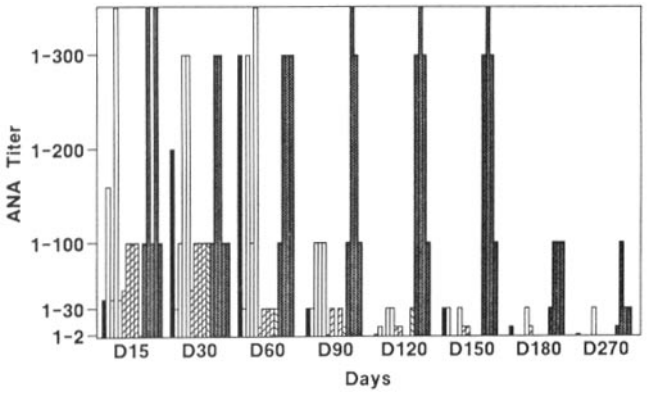

Figure 1. Evolution of human ANA titers in SCID-hu-SLE mice. Mouse 1 (■), donor 1 ANA titer 1:2,240; Mice 2–5 (□), donor 2 ANA titer 1:1,600; Mice 6–9 (▨), donor 3 ANA tier 1:320; Mouse 10 (▧), donor 4 ANA titer 1:4,480; Mice 11–15 (▦) donor 5 ANA titer 1:640. Some mice demonstrated human ANA titers of 1:1,000 and 1:3000 and are represented by histograms with titers higher than titer 1:300. When tested for IgG and IgM isotypes, all SCID-hu-SLE mice and the majority of patient ANA were positive for IgG only. The exceptions were donor 4 and mouse 2, who presented equal reactivity for both IgG and IgM ANA, and donor 2, whose ANA were mainly of the IgG isotype but presented also low IgM ANA titer (1:100). The ANA titers of 29 SCID-hu mice repopulated with PBL from three different donors were 1:10 in 5 mice, 1:2 in 7, and negative at 1:2 in 17.

of human IgG, reaching maximum levels ~2 mo after transfer and then gradually declining over the next 4–7 mo, although great individual variety exists. At maximum values, the human IgG levels in the mice were approximately one-fifth of the normal human levels. The evolution of human IgM in the SCID recipient was more variable than that of human IgG, as illustrated in Table 1.

FACS analysis on PBL from SCID-hu mice demonstrates the presence of human B and T cells (representing up to 15% of the total PBMC population). Among T cells, the ratio of $CD4^+CD8^-/CD4^-CD8^+$ at 1 and 2 mo post-engraftment are 3:1, and 1:2, respectively (data not shown). Human ANA titers reflecting those of the SLE donors were found in the 15 SCID-hu-SLE mice, while little or no such antibody was found in 29 SCID-hu mice, comprising the 9 depicted in Table 1 (Fig. 1, legend). This ANA must have been produced by the transferred cells in the murine host since the cells had been well washed to eliminate contaminating antibody, and since it persisted well beyond the time expected for passive antibody transfer (half-life of human IgG and IgM in the SCID recipient are 184 and 108 h, respectively; data not shown). The SCID-hu-SLE mice had a ratio of ANA titers to human IgG levels of the same order of magnitude as the SLE patient donors, and, as in the donors, the ANA autoantibodies are primarily of the IgG isotype (Fig. 1, legend).

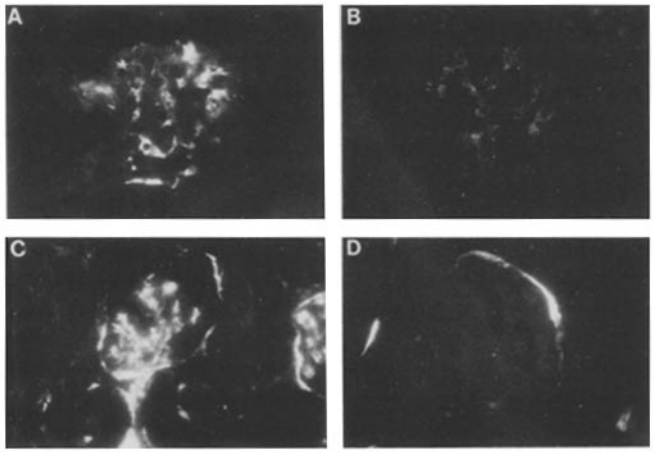

Figure 2. Fluorescence photomicrograph of glomeruli of SCID-hu-SLE mice 224 d after transfer of PBL (*A*,*C*), and age and IgG level-matched SCID-hu mice (*B*,*D*) stained with a Burro anti-human Ig (*A*,*B*), or a goat anti-mouse C3 (*C*,*D*). Ig and mouse C3 deposits are seen in a pattern consistent with capillary wall and mesangial localization.

It appears that the cells transferred from the peripheral blood of afflicted patients have already undergone IgM to IgG switching in their ANA production. Such IgG anti-DNA autoantibodies are believed to be the primary pathogens in mice as well as in humans (6, 7).

The persistence of antibody synthesis by autoreactive clones in the SCID-hu-SLE model might be secondary to the presence of human or murine antigen(s). The additional possibility that this B cell population is polyclonally stimulated in this model cannot be excluded. ANA titers in some SCID-hu-SLE mice were maintained up to 7 mo, although the majority decreased by 3–4 mo post-engraftment. Although there is variability between individual mice, the general ratio of ANA titer vs. human IgG decreased with time. This decreased ANA production may reflect the lifespan of the B cell autoreactive clones in the model, and/or the termination of B cell stimulation in the murine reconstituted human immune system. The progressive loss of $CD4^+CD8^-$ human T cell subset in SCID-hu recipients, strongly implicated in the stimulation of IgG autoantibody-producing B cells in human and mouse (8, 9), might play an important role in the autoantibody decline in this model.

Autoantibodies in SLE patients form immune complexes detectable in the circulation and target organs, such as kidneys (2). We examined seven kidney biopsies from SCID-hu-SLE mice, and four from SCID-hu mice. Conventional histologic examination revealed no alteration in the SCID-hu model, and only minimal mesangial proliferation in the SCID-hu-SLE model (data not shown). Immunofluorescence studies on six of seven SCID-hu-SLE kidney biopsies showed the presence of granular deposits of human Ig in a pattern suggestive of mesangial and capillary loop distribution (Fig. 2 *A*). Half of those kidneys positive for human Ig were also positive for mouse C3 (Fig. 2 *C*). SCID-hu mice do not present such Ig or mouse C3 deposits in their glomeruli (Fig. 2, *B* and *D*). All kidneys examined were negative for human C3 (data not shown). The lack, or minimal activation of, this important effector pathway of the immune response, as well as the relatively short time of autoantibody production, might explain the paucity of lesions seen on conventional histology, as well as the absence of significant proteinuria or elevated BUN level in SCID-hu-SLE mice (data not shown).

In summary, we report the first transfer of human SLE serology and some glomerular pathology into SCID mice. This new in vivo model expands our potential to directly evaluate the influence of cellular and humoral functions in the progression of this disease. In addition, this ability to transfer PBL from one donor into multiple mice allows us to compare various potential therapies simultaneously on the disease-producing cells of a single patient.

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