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SCID-bg mice as xenograft recipients

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Summary

SCID-bg (scid/scid, beige/beige) is a strain of double-mutant mice with impaired lymphoid development and reduced natural killer (NK) cell activity. The present study was undertaken to evaluate the usefulness of SCID-bg mice as xenograft recipients. Fetal guineapig tissues (liver, thymus, spleen) were transplanted under the kidney capsule of the mice and their serum guineapig IgG levels were measured weekly thereafter. C.B.-17-scid and anti-asialo GM₁ antiserum-treated (NK-depleted) C.B.-17-scid (C.B.-17-scid-AGM₁) mice that received the identical transplants were used as controls. Throughout the experimental period (1, 2, and 3 weeks after transplantation), the average serum guineapig IgG concentrations was highest in C.B.-17-scid-AGM₁ mice followed by SCID-bg mice and lowest in C.B.-17-scid mice without antiserum treatment, though we could not find any statistical significance among these groups. However, SCID-bg mice always showed the smallest within-group variance (individual difference) in the serum guineapig IgG concentrations [P<0.05, versus C.B.-17-scid-AGM₁ mice at 1,2, and 3 weeks and versus C.B.-17-scid mice at 2 weeks]. The graft size was not significantly different among these three groups, but the spleen grafts in C.B.-17-scid mice contained fewer nucleate cells than the other two groups. These results indicate that the reduced NK cell activity by beige mutation is not crucial for the success of xenogenic transplantation, though SCID-bg mice may be useful as xenograft recipients with a consistent potential to retain the viability and function of engrafted tissues.

Keywords SCID mice; beige; NK cells; anti-asialo GM₁; xenograft

Mice homozygous for scid mutation (SCID mice) lack mature T and B lymphocytes and are unable to reject xenogenic cells (Bosma & Carroll 1991). Therefore, human haematopoietic cells or peripheral blood mononuclear cells (PBMC) can be successfully transplanted into SCID mice. They are generally called SCID-hu mice and have been used for the research in human immunology and virology, especially for the study of HIV-infection [McCune et al. 1991]. However, the extent of growth or maintenance of transplanted cells in SCID mice is very variable [Shiptz et al. 1994], because of phenotypical differences in the recipient SCID mice such as leaky phenotype, as well as the difference in donors. Several investigators have tried to improve the transplantation of human cells in SCID mice by γ-irradiation, administration of anti-NK cell antibody, immunosuppressive drugs, and growth hormone; and, some success has resulted [Shiptz et al. 1994, Somasundaram et al. 1995, Murphy et al., 1992a, 1992b]. In this connection, the beige...
mutation is known to reduce NK cell activity (Roder & Duwe 1979), and is therefore expected to enhance xenogenic cell-growth if introduced into SCID mice. In this study, we have attempted to evaluate the usefulness of SCID-bg mice, which are homozygous for both scid and beige mutations, as xenograft recipients. For this purpose, we engrafted guineapig fetal liver, thymus and spleen into three groups of SCID mice; namely, SCID-bg mice, C.B.-17-scid mice and anti-asialo GM₁ antiserum-treated [NK-depleted] C.B.-17-scid (C.B.-17-scid-AGM₁) mice. The levels of serum guineapig IgG concentrations were measured weekly thereafter and compared to assess the survival and growth of donor-derived B lymphocytes. Histological examination in transplanted tissues was also carried out to see the growth of donor-derived T lymphocytes and cells other than B lymphocytes.

Materials and methods

Mice

C.B.-17-scid (scid/scid) mice and SCID-bg mice [scid/scid, beige/beige] were obtained from the breeding colony in the Department of Veterinary Science, National Institute of Health [Tokyo, Japan]. SCID-bg mice, which were originally generated at the Institute for Experimental Animals, Faculty of Medicine, Kanazawa University [Kanazawa, Japan] by mating C.B.-17-scid mice with KSN-bg mice, have been maintained in the National Institute of Health. The SCID-bg mice used were the 15th or 16th generation of sister-brother mating. All the mice used here were 6 to 10 weeks old. Sixteen male C.B.-17-scid mice, and two male, five female SCID-bg mice were used. They were maintained in SPF condition, and the room temperature and humidity were at 23 ± 2°C and 55 ± 5%, respectively. Lighting was provided in a cycle of 14 h light, 10 h dark. A standard commercial diet (CMF: Oriental Yeast Co., Ltd., Tokyo, Japan) and acidic water added 3 ppm sodium hypochlorite solution [Wako Pure Chemical Industries Co., Ltd., Osaka, Japan] were provided ad libitum.

Antibody treatment

To deplete NK cells, some C.B.-17-scid mice were intravenously injected with 20 μl of anti-asialo GM₁ antiserum [Wako Pure Chemical Industries Co., Ltd., Osaka, Japan] [Kasai et al. 1980] one day before the transplantation as previously described [Murphy et al. 1992a].

Transplantation of fetal guineapig tissues

Two pregnant Hartley guineapigs were purchased from Japan SLC, Inc. [Hamamatu, Japan] and used at 40 to 50 days of gestation. A guineapig fetus from each pregnant animal was aseptically removed and samples of the liver, thymus, and spleen of about 1 cubic mm were transplanted under the kidney capsules of the mice.

Determination of guineapig IgG

Mice were bled weekly from the retro-orbital vein and sera were stored at −40°C until use. The level of serum guineapig IgG was determined by a sandwich enzyme-linked immunosorbent assay [ELISA] using affinity-purified goat anti-guineapig IgG [Cappel, Durham, NC] as the capture agent and affinity-purified alkaline phosphatase-conjugated goat anti-guineapig IgG [American Qualex, La Mirada, CA] as the detection agent. The absorbance at 405 nm was quantified on an ELISA reader [Multiskan Bichromatic, Labsystems, Helsinki, Finland].

Measurement of graft size

Three weeks after transplantation, the mice were necropsied and the graft size [length, width and depth at point of incision] was measured for each thymus and spleen graft. The graft growth index was calculated as follows.

\[
\text{Graft growth index (mm)} = \sqrt{\text{length} \times \text{width} \times \text{depth (thymus)}} + \sqrt{\text{length} \times \text{width} \times \text{depth (spleen)}}
\]

Histological analysis

Tissues were fixed in 10% neutral buffered formalin, and paraffin sections at 4 μm thickness were stained with haematoxylin and eosin [H&E].
Fig 1  Levels of serum guinea pig IgG in three groups of mice transplanted with fetal guinea pig tissues. Horizontal bars show average values of serum guinea pig IgG in each group. □: SCID-bg mice (n=6). △: Anti-asialo GM1 antiserum-treated C.B.-17-scid mice (n=6). ○: C.B.-17-scid mice without antibody treatment (n=10). One anti-asialo GM1-treated C.B.-17-scid mouse had died at 2 weeks during the experimental period.

Statistics

Statistical significance in the difference of variances in serum guinea pig IgG concentrations between each experimental group was analysed by an F-test of the ratio of the two variances. These data was also examined by Kruskal–Wallis analysis of variance of ranks to test if there was any difference in the average serum guinea pig IgG concentrations among the three experimental groups.

Results

Acceptance of transplanted tissues

Out of 16 C.B.-17-scid mice and 7 SCID-bg mice, 15 and 6 had been successfully engrafted with fetal guinea pig tissues, respectively, as confirmed by viable thymus and spleen tissues at the time of necropsy.

Levels of serum guinea pig IgG

The levels of serum guinea pig IgG are shown in Fig. 1. Throughout the experimental period, the average serum guinea pig IgG concentration was always highest in C.B.-17-scid-AGM1 mice, followed by SCID-bg mice, though there was no statistically significant difference in the average serum guinea pig IgG concentrations among the three experimental groups by Kruskal–Wallis analysis. On the other hand, the variance of serum guinea pig IgG concentrations within each experimental group was calculated and analysed by an F-test of the ratio of the two variances (Table 1). The difference of the variances of serum guinea pig IgG concentrations between C.B.-17-scid-AGM1 mice and SCID-bg mice was always significant throughout the experimental period, and that of between C.B.-17-scid mice without antiserum treatment and

Table 1 An F-test of the ratio of the variances in serum guinea pig IgG concentrations between each experimental group

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To test if there are any statistical significance between the standard deviations (or variances) of each experimental group, the variances of serum guinea pig IgG concentrations in each experimental group (V) were calculated, and the variance ratio distributions (F) were then determined. These F values were examined by the F-test of the ratio of the two variances.
Fig 2  Graft growth index in three groups of mice. Error bars show standard deviations in each group

SCID-bg mice was also statistically significant at 2 weeks post-transplantation. The difference of variance between C.B.-17-scid mice with and without anti-asialo GM₁ antiserum treatment was not significant throughout the experimental period. These results may suggest that SCID-bg mice show less within-group variance on the growth of transplanted xenogenic cells when compared to C.B.-17-scid mice with or without anti-asialo GM₁ antiserum treatment.

Graft size

Well-grown grafted thymus and spleen were detected in the majority of mice examined, but there was no significant difference in their sizes among the three mouse groups [Fig. 2]. The grafted thymus and spleen were easily distinguished from recipients' renal tissues [Fig. 3]. On the other hand, liver grafts could not be seen in any mice examined.

Histological findings

As mentioned above, graft components and recipients' renal tissues were well separated [Fig. 4]. Although there was no significant difference in the histology of the grafted thymus, which showed well-developed structures in all three mouse groups; grafted spleen in C.B.-17-scid mice without antiserum treatment contained fewer nucleate cells and more abundant erythrocytes. Moreover, in C.B.-17-scid mice without antiserum treatment, eosinophil infiltration was commonly found in the grafted spleen, which was almost absent in the other two groups [Figs 5, 6].

Discussion

NK cells, which still exist in SCID mice [Dorshkind et al. 1985], are considered to be the major effector cells that prevent survival and growth of foreign haemopoietic cells in recipient SCID mice [Murphy et al. 1987]. In this study, we attempted to evaluate the...
usefulness of SCID-bg mice as xenogenic cell recipients, because it is well known that beige mutation affects only some subsets of NK cells (MacDougall et al. 1990). But our present results also showed smaller within-group variance of the serum guineapig IgG concentrations in SCID-bg mice than the other two groups, and that was always statistically significant when compared to C.B.-17-scid-AGM1 mice, and to C.B.-17-scid mice at 2 weeks post-transplantation. This smaller variation in SCID-bg mice may be due to beige mutation, because NK cells have been shown to have a close relation with the maintenance of human PBMC in SCID-hu system (Murphy et al. 1992a). However, we cannot deny the possibility that other background genes are also factors. To elucidate that question, experiments using SCID mice and SCID-beige mice with the same genetic background will be required.

We found dominant eosinophil infiltration only in the grafted spleen in the C.B.-17-scid mice without antibody treatment, and it was absent in both SCID-bg and C.B.-17-scid-AGM1 mice. This suggests the possibility that the recipient's NK cells introduce granulocytes to the transplanted tissue, and take part in the rejection of foreign cells by an indirect way such as cytokine production, as well as by lysing foreign cells directly. The proliferation of nucleate cells in the grafted spleen was also more dominant in SCID-bg mice and C.B.-17-scid-AGM1 mice than C.B.-17-scid mice without antiserum treatment, suggesting that NK cells affected by beige mutation may influence the reconstruction of the grafted tissue, even if they have only minor roles. To clarify the detailed effects of host's NK cells against transplanted tissues, it would be necessary to examine the tissue distribution of recipient-derived cells in the graft, and monitor the development of donor-derived haematopoietic cells. In conclusion, our present results indicate that NK cells affected by beige mutation do not play a critical role in the rejection of xenograft, for beige mutation affects only some subsets of NK cells (MacDougall et al. 1990). But our present results also showed smaller within-group variance of the serum guineapig IgG concentrations in SCID-bg mice than the other two groups, and that was always statistically significant when compared to C.B.-17-scid-AGM1 mice, and to C.B.-17-scid mice at 2 weeks post-transplantation. This smaller variation in SCID-bg mice may be due to beige mutation, because NK cells have been shown to have a close relation with the maintenance of human PBMC in SCID-hu system (Murphy et al. 1992a). However, we cannot deny the possibility that other background genes are also factors. To elucidate that question, experiments using SCID mice and SCID-beige mice with the same genetic background will be required.
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