Brief Definitive Report

Homozygous *scid/scid;beige/beige* Mice Have Low Levels of Spontaneous or Neonatal T Cell–induced B Cell Generation

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Summary

The autosomal recessive *scid* mutation results in defective immunoglobulin and T cell receptor gene rearrangement. The *scid* mutation occurred in the allotype congenic C.B-17 line, and up to 25% of C.B-17 *scid* mice spontaneously produce both T cells and immunoglobulin, a phenotype known as "leaky." Moreover, introduction of neonatal T cells into C.B-17 *scid* mice leads to immunoglobulin production by 100% of animals. We have produced mice homozygous for both the *scid* and *beige* mutations. By contrast with C.B-17 *scid* mice, BALB/c *scid.beige* mice have a <2% incidence of "leakiness." This percentage does not increase with age, and introduction of neonatal T cells fails to rescue immunoglobulin production. This suggests that a gene (or genes) closely linked to the *beige* locus regulates B and/or T cell development.

Mice with the *scid* mutation (1) are deficient in T and B lymphocytes because of extremely inefficient production of coding joins during rearrangement of Ig and TCR gene segments (2). However, C.B-17 SCID mice show an age-dependent increase in the percentage of animals spontaneously producing limited numbers of T lymphocytes and oligoclonal patterns of Ig (3, 4). This would appear to reflect a low rate of successful gene rearrangement, with subsequent rescue of lymphocytes by antigen exposure. If one transfers neonatal (but not adult) thymocytes to C.B-17 SCID mice, all animals produce Ig bearing the IgM allotype of the SCID recipient (5, 6). This phenomenon may reflect a more efficient rescue of small numbers of B cells than occurs during spontaneous T cell– and antigen-dependent stimulation in unreconstituted SCID mice.

We have produced BALB/c mice homozygous for both the *scid* and *beige* mutations (7) (hereafter, SCID.BG) with the goal of depressing NK activity, which is spared by the *scid* mutation (8). Production colonies of SCID.BG mice were screened for IgM levels at 8 wk of age to determine the incidence of "leakiness," which was designated as IgM levels >5 μg/ml. Only 1.9% of SCID.BG mice were "leaky" by this criterion, whereas 23.9% of SCID mice reared in the same environment had IgM levels <5 μg/ml. The incidence of IgM production increased to nearly 100% in 8-mo-old SCID mice, whereas the incidence of leaky SCID.BG mice was 3% at 8 mo of age. Injection of neonatal thymocytes rescued IgM production in all SCID mice, but in only 1 of 10 SCID.BG mice. The addition of this second mutation onto the BALB/c background has clearly reduced the basal rate of successful B cell production, and suggests that B cell differentiation may be influenced by the *beige* locus or a closely linked gene.

Materials and Methods

Mice. C.B-17/NIcr *scid/scid* mice were obtained from Dr. Ken Dorschkind (University of California, Riverside) and bred at Medical Biology Institute, with annual caesarean-rederivation to limit transmission of environmental pathogens. BALB.*beige* congenic mice were obtained from Dr. Carl Hansen (National Institutes of Health, Bethesda, MD) with the *beige* gene introduced onto the BALB/cAnN background. C.B-17 SCID mice were crossed with BALB.*beige* mice and the F_2 progeny tested for IgM levels (to detect homozygous *scid/scid* mice) and for large myeloperoxidase-positive granules (9) in peripheral blood granulocytes (to detect homozygous *beige/beige* mice). Mice homozygous for both mutations were inbred for four generations. No attempt has been made to make the mice homozygous at the IgH locus. The mice have been typed using PCR primers and Southern blotting to detect polymorphisms between the IgH^b allele from C.B-17 and the IgH^a allele from BALB/c. Most of the SCID.BG mice express only the IgH^a allele, but some mice also express the IgH^b allele. The few leaky SCID.BG mice analyzed had the IgM^a allotype, but given the frequency of the IgH^a allele in the population, this result is to be expected.

IgM Detection. ELISA techniques were used to detect both total IgM and IgM^a and IgM^b. These techniques have been described in detail elsewhere (10). The assay standard was an IgM mAb purified by affinity chromatography. The assay gave linear results with IgM concentrations between 2 and 400 μg/ml; sera with higher values were diluted and reassayed.

T Cell Transfer. Thymocytes were prepared from 2–4-d-old

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BALB.xid mice and 5 × 10^6 cells injected into the lateral tail vein of 8-wk-old SCID and SCID.BG recipients. IgM levels were determined at weekly intervals after T cell transfer. The use of BALB.xid donors ensures that contaminating donor B cells cannot expand in SCID or SCID.BG recipients (5).

Results and Discussion

Distribution of IgM Levels in Young SCID and SCID.BG Mice. 8-wk-old SCID and SCID-BG mice housed under identical pathogen-free conditions were bled and total IgM levels determined by ELISA. Mice with >5 μg/ml of IgM have been designated as leaky, although this value is somewhat arbitrary and differs between laboratories. We found that only 5 of 260 SCID.BG mice were leaky by this criterion, whereas 137 of 574 SCID mice had higher IgM levels. The distribution of IgM levels for the 137 leaky SCID mice and the 5 leaky SCID.BG mice is shown in Fig. 1 *A*. Most of the leaky mice had IgM levels <20 μg/ml, but two SCID.BG mice and eight SCID mice had >100 μg/ml of IgM. The SCID.BG mouse has a much lower incidence of leakiness than the SCID mouse, but the addition of the *beige* mutation does not preclude the generation of functional B cells in rare mice.

Distribution of IgM Levels of Old SCID and SCID.BG Mice. It is known that the incidence of leaky SCID mice and the levels of Ig produced increase dramatically in older animals. We compared cohorts of 8-mo-old SCID and SCID.BG mice born and reared in the same isolation room. The IgM levels of 35 mice from each group are shown in Fig. 1 *B*. As expected, 33 of 35 old SCID mice had IgM levels >5 μg/ml, and many were substantially higher. By contrast, only 1 of 35 old SCID.BG mice had >5 μg/ml IgM, and that mouse had <20 μg/ml. More important, 90% of both old and young SCID.BG mice had IgM levels below the 2 μg/ml limit of detection, indicating that no increase in the incidence of leakiness was occurring with increasing age. This was confirmed by comparing IgM levels at 8 wk and 8 mo of age on the same SCID.BG mice; the same mice had IgM levels >2 μg/ml at both time points (data not shown; n = 16). This result suggests that the rare successful generation of a B cell is limited to early life in SCID.BG mice, while it appears to be an ongoing process in SCID mice.

Induction of IgM Production by Neonatal T Cell Transfer. We have reported previously (5, 6) that injection of neonatal thymocytes or CD4 T cells induces the production of high levels of IgM in every SCID recipient. Both BALB.xid and C.B-17 neonatal thymocytes were capable of rescuing B cells in C.B-17 SCID recipients, so there was no evidence to suggest that recognition of IgH incompatibility was required. To determine if SCID.BG mice harbored latent B cells (or their precursors), we repeated this experiment using both IgH^b C.B-17 SCID and IgH^a SCID.BG recipients of neonatal BALB.xid thymocytes. By 3 wk after T cell transfer, all 10 SCID recipients had >100 μg/ml of IgM, while only one SCID.BG recipient had IgM levels in this range (Fig. 2). Interestingly, that animal had 9 μg/ml of IgM before T cell transfer, while the other SCID.BG recipients had <5 μg/ml. It appears that most SCID.BG mice have few if any cryptic B cells that can be revealed by neonatal T cell transfer.

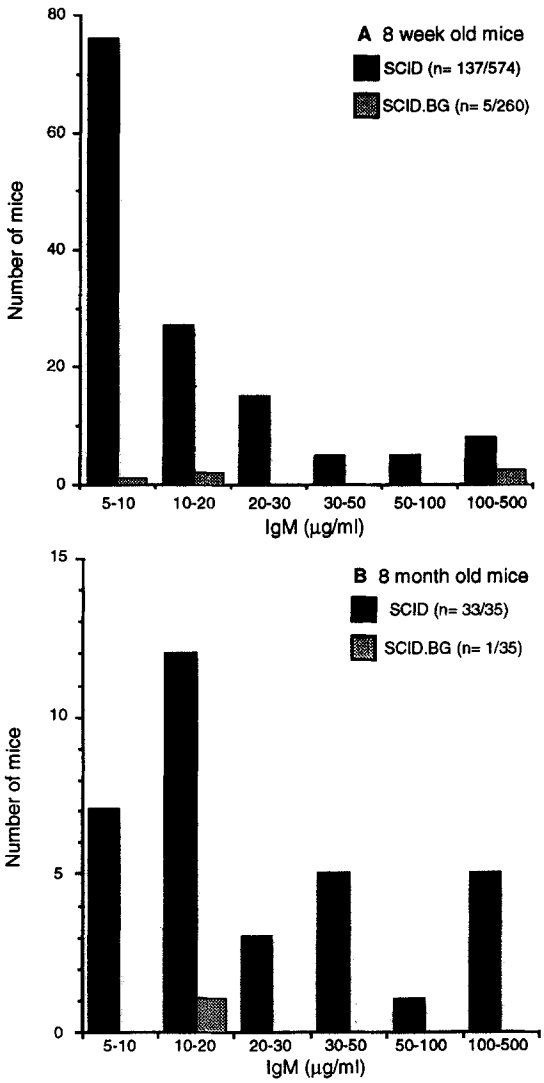

Figure 1. (*A*) IgM levels in 8-wk-old SCID and SCID.BG mice. Only IgM levels >5 μg/ml are shown, with the number of mice within each indicated range of IgM levels shown. 137 of 574 SCID and 5 of 260 SCID.BG mice analyzed had IgM levels >5 μg/ml. For comparison, IgM levels in normal BALB/c mice are in the 400–800 μg/ml range, so few "leaky" SCID mice achieve this normal range. (*B*) IgM levels in 8-mo-old SCID and SCID.BG mice. The number of mice with IgM levels >5 μg/ml are shown. 33 of 35 SCID mice were in this category, whereas only 1 of 35 SCID.BG mice had elevated IgM.

To confirm that neonatal T cells, including the critical CD4 T cell subset, survived as well in SCID.BG recipients as in SCID mice, we analyzed the splenic T cells in the same mice depicted in Fig. 2 at 10 wk after thymocyte injection. The results (Fig. 3) indicate that both CD4 and CD8 BALB.xid T cells survive as well in IgH^a BALB.SCID.BG as IgH^b C.B-17 SCID recipients. The failure of SCID.BG mice to produce IgM thus cannot be attributed to poor survival of neonatal T cells.

The gene product altered by the *scid* mutation has yet to be identified. The mutation on chromosome 16 affects not only joining of Ig and TCR gene segments but radiation

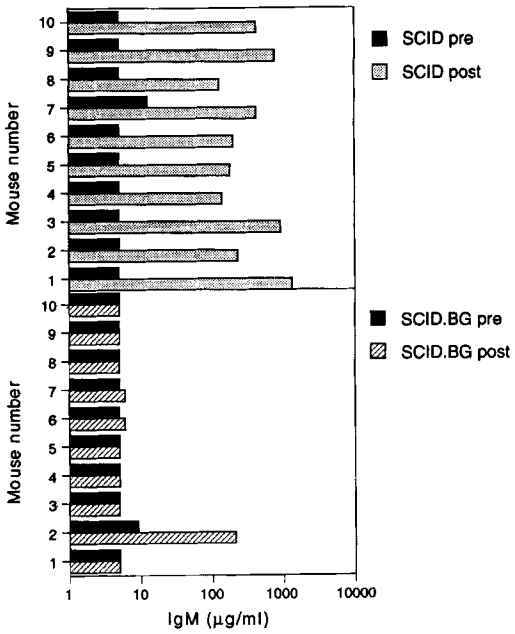

Figure 2. IgM levels in 8-wk-old SCID (*A*) and SCID.BG (*B*) mice before injection of thymocytes and IgM levels of the same individual mice 3 wk after injection of neonatal BALB.*xid* thymocytes. Note that the one SCID.BG mice showing a substantially elevated IgM level had 9 μg/ml of IgM before T cell transfer.

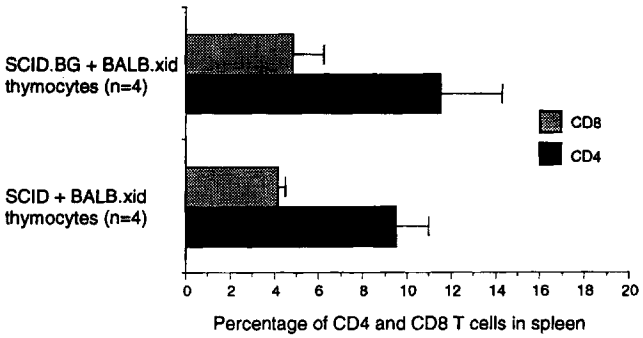

Figure 3. Percentages of CD4 and CD8 T cells in the spleens of SCID and SCID.BG recipients of neonatal BALB.*xid* thymocytes at 10 wk after thymocyte injection. Numbers were determined by FACS® analysis, and represent the mean ± SE of four individual animals in each group. SCID or SCID.BG mice not injected with T cells had no detectable reactivity with CD4 or CD8, despite the presence of some cells expressing low-density Thy-1 (data not shown).

damage repair as well (11). The failure to produce coding joins in recombination substrate experiments and the increased sensitivity to radiation damage suggest that the *scid* gene may encode a ligase activity. The observed lower rate of spontaneous or T cell–induced B cell generation in SCID.BG mice could be due to a second locus reducing the already low rate of recombination, or to a reduction in the rescue rate that allows the survival of rare B cells. While the *beige* mutation on chromosome 13 is known to reduce NK cell activity, these experiments do not necessarily imply that NK cells regulate (directly or indirectly) B cell generation. NK cells are present in SCID.BG mice and can be activated to produce cytokines such as IFN-γ (our unpublished observations). Cytolytic activity against YAC cells is reduced in SCID.BG mice. It is thus equally likely that a locus closely linked to *beige* rather than a defect in NK cells is responsible for the phenotype of SCID.BG double mutants. The only locus closely linked to *beige* that is known to influence lymphocyte development is the TCR-γ locus, which is 3 cM telomeric with respect to *beige*. Since rearrangement of the TCR-γ gene should be inhibited by the *scid* mutation, it is not obvious how this locus could contribute to the phenotype we have observed. It is also possible that genes linked to the IgH locus could influence the rate of B cell generation, since most of the SCID.BG mice analyzed had the IgH^a allele, and little information is thus available on the rate of leakiness in IgH^b SCID.BG mice. The rare leaky SCID.BG mouse (e.g., mouse no. 2 in Fig. 2 *B*) expressed IgM^a. The results presented here thus suggest that other genetic loci can modify the effect of the *scid* mutation, and localize one (or more) such gene(s) to the region of the *beige* locus on chromosome 13.

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