Brief Definitive Report

Adoptive Transfer of Neonatal T Lymphocytes Rescues Immunoglobulin Production in Mice with Severe Combined Immune Deficiency

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Summary

Mice with the autosomal recessive severe combined immune deficiency (*scid*) mutation lack mature lymphocytes because of defective joining of T cell receptor and immunoglobulin (Ig) gene segments. Penetrance of this mutation is incomplete since 10–25% of SCID mice produce some T or B lymphocytes. This "leaky" phenotype could be due to a reversion of the mutation in some mice or to a constant, low frequency of functional lymphocytes generated in all SCID mice with variable survival of such cells. We report here that all SCID mice can be stimulated to produce functional B cells by the transfer of normal neonatal, but not adult, T cells. T cell–induced rescue of C.B-17*scid* B cells results in high levels of Ig expressing the Igh^b allotype of the SCID recipient. These results show that all SCID mice generate some functional B cells, the majority of which do not survive in the absence of a subset of T cells present in high frequency in the neonate.

The autosomal recessive *scid* mutation results in defective joining of TCR and Ig gene segments (1, 2). However, 10–25% of SCID mice spontaneously produce serum Ig (designated as "leaky" SCID mice; 3, 4), and normal TCR and Ig junctions have been demonstrated in these SCID mice (5, 6). Two explanations have been proposed to explain the "leaky" phenotype. The first postulates reversion of the *scid* mutation to generate functional recombinase activity (5). The second is that functional TCR or Ig gene rearrangements occur at a low frequency in all SCID mice (6), but the rare lymphocytes generated are unlikely to persist in the absence of antigenic stimulation or other undefined regulatory interactions. The fact that rearrangement of both Ig and TCR genes appears to be required for Ig production in leaky SCID mice (4, 7) suggests that functional T cells may be required to rescue any B cells that are generated. If provision of normal T cells were to rescue B cells in all SCID mice, it would argue for a constant, low level production of functional Ig joins rather than a sporadic genetic reversion event. We have accordingly adoptively transferred T cells from allotype-congenic BALB/c (Igh^a) donors to C.B1-7*scid* (Igh^b) recipients. Our results demonstrate that neonatal, but not adult, T cells induce IgM expressing the Igh^b allotype in all SCID recipients. These studies show that productive Ig gene rearrangements occur in every SCID mouse, but that B cell survival is ordinarily infrequent.

Materials and Methods

Mice. C.B-17*scid* (SCID; H-2^d, Igh^b), BALB/c (H-2^d, Igh^a), BALB.*xid* (XID; H-2^d, Igh^a), CBA/J (H-2^k, Igh^e), and CBA/NCAHN-*xid*/J (XID; H-2^k, Igh^e) mice were bred and maintained in accord with National Institutes of Health guidelines at the Medical Biology Institute. Mice were used between the ages of 3 d and 35 wk. All SCID mice were bled at 8 wk of age, and only those mice (84%) with <5 μg/ml of serum IgM were used in these studies. This same criterion was applied for younger and older (4 and 35 wk) SCID recipients. This screening reduced the frequency of subsequent spontaneous "leakiness" to <5%; no IgM production was observed in control SCID mice, which did not receive T cells.

Cell Preparations and Adoptive Transfer. Spleen and thymus cell suspensions were prepared, and T cell enrichment, or T or B cell depletion, was conducted as described (8, 9). 10^7 cells in a volume of 0.2 ml of HBSS were injected intravenously into the lateral tail vein of SCID recipients.

Isotype- and Allotype-specific ELISA. Serum Ig isotype levels were determined by ELISA using polyvinylchloride plates (Dynatech Laboratories, Alexandria, VA) coated with affinity-purified rabbit anti–mouse IgM or goat anti–mouse IgG3, or IgG1 (Fisher Scientific Co., Pittsburgh, PA) antibodies. Serial twofold dilutions of test sera and the purified isotype standards, HPCM2 (IgM, κ), 5-1E4.2 (IgG_3, κ), and 137.5G6 (IgG1, κ), generously provided by Dr. Ann Feeney (MBI), were applied in duplicate to the plates. Rabbit anti–mouse Ig $F(ab')_2$-specific horseradish peroxidase (HRPO) conjugates (Jackson ImmunoResearch Laboratories, Inc., Avondale, PA) or goat anti–mouse κ HRPO conjugates (Fisher Scientific Co.) were used for detection. Allotype-specific ELISAs were conducted as described previously (8). Calculation of Ig concentrations was done by comparison to standard curves using a minimum of three data points with correlation coefficients >0.95.

Results and Discussion

We tested the hypothesis that B cell maturation in SCID mice requires T cells by providing normal BALB/c (Igh^a)

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thymocytes or splenic T cells to C.B-17*scid* (Igh^b) recipients. Production of Igh^b Ig was used as a measure of the rescue of SCID B cells with functional Ig rearrangements. Transfer of adult BALB/c thymocytes resulted in minimal production of IgM^b in SCID recipients (Fig. 1). However, the injection of neonatal (3–5 d of age) BALB/c thymocytes did rescue IgM^b production in SCID recipients, but donor IgM^a was also detected. This result implies that neonatal BALB/c thymocytes contain a population of cells capable of rescuing SCID B cell Ig production, and in addition, contain some B cells capable of initiating IgM secretion upon transfer to SCID recipients. Two experimental approaches were taken to resolve these activities.

We have shown that cotransfer of Igh allotype disparate B lymphocyte populations to SCID recipients can result in dominance by one population (8). This dominance is not seen with B cells from mice with the *xid* mutation and appears to be associated with the $CD5^{+/-}$/Mac-1^+ subset of B cells (10), which accounts for the majority of B cells in adult thymus (11). We have therefore used thymocytes or T cells from BALB.*xid* donors to eliminate the possibility that growth of a small number of donor B cells will obscure emergence of B cells of SCID origin. In addition, we have rigorously depleted the B cells present in neonatal BALB/c thymus to confirm that normal (as well as XID; 12) T cells are sufficient for rescue of SCID B cells.

Neonatal, but not adult, BALB.*xid* thymocytes stimulated SCID IgM production, which was entirely of host origin (Fig. 1). This effect was not unique to XID thymocytes since transfer of BALB/c neonatal thymocytes depleted of B cells by anti-IgM panning also rescued IgM^b secretion. Similar findings were obtained after transfer or neonatal BALB.*xid* spleen cells (not shown). These results lead to three conclusions: (*a*) all SCID mice contain a small number of B cells (or their immediate precursors) that can be rescued by normal, neonatal T cells; (*b*) neonatal T cell sources mediate this rescue, but adult T cell sources do not; this could be because the T cells responsible for this result are present only in the neonate or because the activity of these cells is blocked in the adult; and (*c*) transfer of even a small number of normal, donor

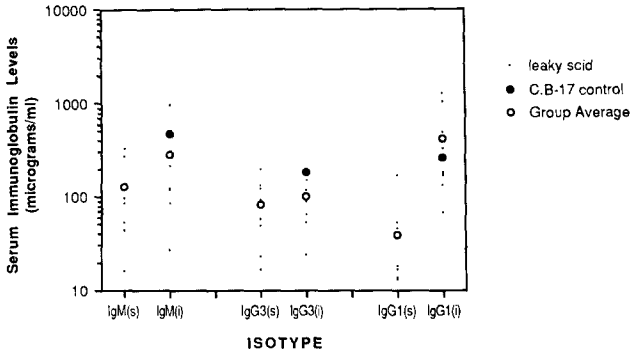

Figure 2. Ig subclass levels in spontaneous (s) and induced (i) leaky SCID mice. Data for spontaneous Ig production are representative (85 mice tested) of the MBI C.B-17*scid* colony. Serum Ig levels for spontaneous leaky SCID mice 12–40 wk of age (n = *9* for each group) were determined by ELISA. Sera from SCID recipients assessed 3–5 wk post-transfer of 10^7 neonatal (3–5 d of age) BALB.*xid* thymocytes. Donor contribution to IgG1 reconstitution is negligible at these timepoints as determined in previous experiments comparing IgG1 levels in irradiated vs. unirradiated SCID recipients. Unreconstituted cagemates of SCID recipients fail to produce detectable levels of serum Igs. C.B-17 control values represent averages generated for 20 mice.

B cells results in the detection of their Ig rather than that of the SCID recipient, reaffirming the potential for "feedback competition" among B cell subpopulations (8, 13).

Normal T-B cell interaction is MHC restricted. We transferred CBA/N neonatal thymocytes (XID, H-2^k, Igh^e) to SCID recipients to determine if T cell–mediated rescue of IgM^b production was likewise MHC restricted. The results (Fig. 1, *line* 7) indicate that rescue was not strictly dependent upon MHC identity, although CBA/N thymocytes were less effective than BALB.*xid* thymocytes. It appears that MHC-restricted T-B cell interaction is not essential for rescue of SCID B cells.

The majority of spontaneous leaky SCID mice produce IgM and IgG3, but IgG1 production is observed less frequently (3). IgG1 is more dependent upon helper T cells (14), whereas IgM and IgG3 production can be stimulated by T-independent

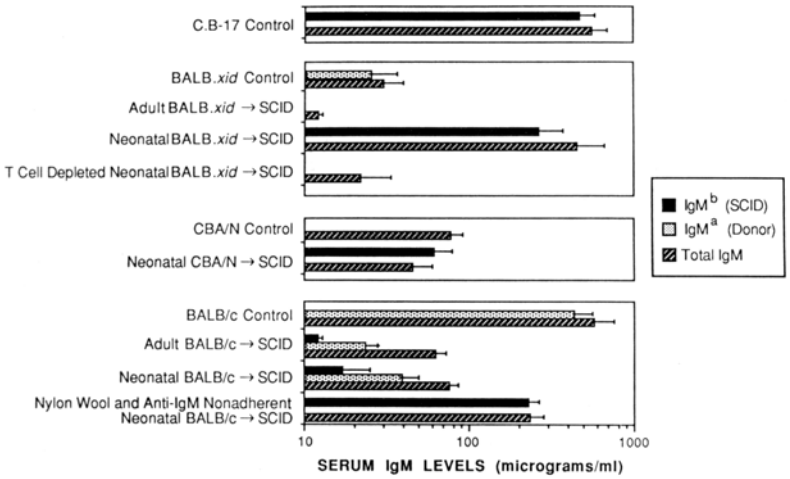

Figure 1. Serum IgM levels in C.B-17*scid* recipients of various thymocyte preparations. Adult thymocyte donors are 8 wk of age; neonate donors are 3–5 d of age. Control sera obtained day of assay from 8–12-wk-old mice. Serum IgM levels determined 3 wk post-transfer by ELISA (three to six mice/group).

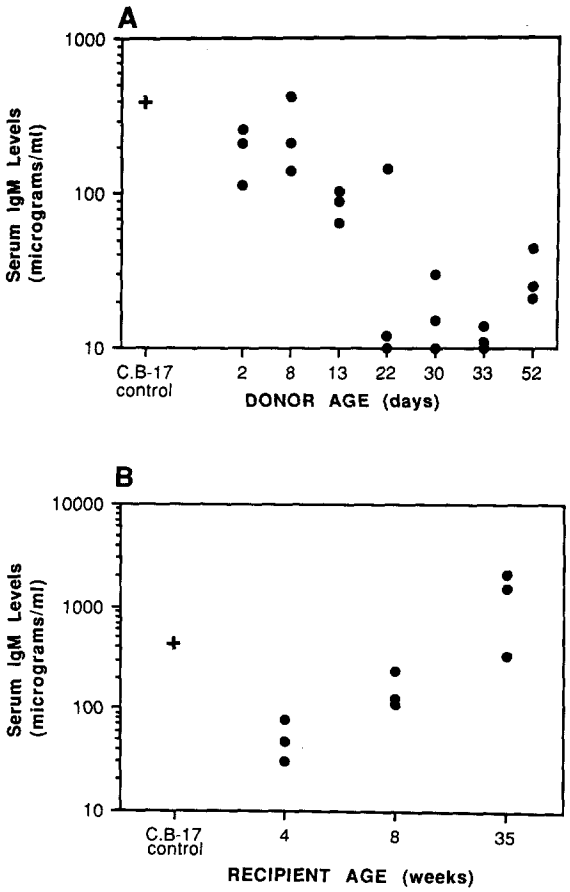

Figure 3. Influence of age of donor and recipient age on SCID IgM production. (*A*) Influence of donor age on SCID IgM production. 10^7 neonatal BALB.*xid* thymocytes from donors of the ages listed were transferred to SCID recipients. (*B*) Influence of recipient age on SCID IgM production. 10^7 neonatal BALB.*xid* spleen cells were transferred to SCID recipients of the ages listed. All recipients had <5 μg/ml serum IgM before transfer. Serum IgM levels were determined 3 wk post-transfer.

(TI) antigens (15). SCID recipients of neonatal thymocytes might be expected to produce both TI and thymus-dependent (TD) isotypes. We compared IgM, IgG3, and IgG1 levels in spontaneous vs. thymocyte-induced leaky SCID mice. Fig. 2 shows relatively similar levels of IgM and IgG3 in induced vs. spontaneous leaky SCID mice; in contrast, IgG1 levels were significantly greater in induced leaky SCID mice and approached those seen in normal mice. These data show that, in addition to increasing the frequency of SCID mice producing serum Ig, neonatal T cells increase Ig levels in individual leaky SCID mice. The variation in isotype levels between individual leaky SCID mice suggests that B cell rescue involves relatively few clones, as has been demonstrated for spontaneous leaky SCID mice (3).

Having found that neonatal, but not adult, T cells induce SCID Ig production, we wished to determine the age at which the donor thymus loses this function. Equal number of thymocytes from BALB.*xid* mice between the ages of 2 and 52 d were transferred to SCID recipients. Fig. 3 *A* illustrates that only thymocytes obtained from donors 13 d of age or younger rescued SCID IgM production. The ability of T cells to mediate rescue thus declines rapidly with increasing donor age. The age of SCID recipients was also shown to influence T cell–induced leakiness in the following experiment.

We rescreened old SCID recipients (35 wk of age) to confirm no spontaneous IgM production (older SCID mice are known to have a higher incidence of spontaneous Ig production [3]) and transferred neonatal BALB.*xid* spleen cells to them as well as to 4- and 8-wk-old SCID recipients. Fig. 3 *B* illustrates that older SCID recipients produced substantially higher levels of IgM than young SCID recipients. This result suggests that T cell transfer rescues B cells that either accumulate during the lifetime of the SCID mouse or are generated with higher frequency in older mice.

Only small numbers of B cells are detectable in spontaneous leaky SCID mice (3, 4). Functional B cells (Igh^{b+}) are detectable in low numbers in the spleen, bone marrow, and peritoneal cavity of SCID recipients of neonatal BALB.*xid* thymocytes (manuscript in preparation). These preliminary data and the results cited above suggest that neonatal T cell transfer is increasing the frequency (or success rate) of relatively rare events in SCID mice, rather than dramatically altering the process of B cell differentiation. That these events occur in every SCID mouse, not just the 10–25% of spontaneous leaky animals, argues in favor of a higher success rate of functional Ig gene recombination than previously suspected.

Why are neonatal, but not adult, T cells efficient at rescuing SCID Ig production? Several possibilities remain to be tested. The increased representation of self-reactive clones in the neonatal T cell pool (16) may facilitate SCID B cell differentiation, particularly due to the T cell deficiency of these mice (17). Neonatal T cells may have different patterns of cytokine production that favor rescue of SCID B cells. T cells bearing the TCR-γ/δ, which are prominent in the neonate (18), could regulate B cell differentiation. Whether donor T cells are directly involved in this process, or function via sequential rescue of SCID T cells (19), which then induce SCID B cell differentiation, remains to be determined. As noted above, adult T cells may contain a functionally blocked subset of cells capable of SCID B cell rescue, so it is not formally demonstrated that rescue activity is restricted to neonatal T cells. In summary, we conclude that all SCID mice generate functional B cells, the majority of which do not survive in the absence of a T cell subpopulation present at high frequency during the neonatal period.

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References

1. Bosma, G.C., R.P. Custer, and M.J. Bosma. 1983. A severe combined immunodeficiency in the mouse. *Nature (Lond.).* 301:527.
2. Schuler, W., I.J. Weiler, A. Schuler, R.A. Philips, N. Rosenberg, T.W. Mak, J.F. Kearney, R.P. Perry, and M.J. Bosma. 1986. Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. *Cell.* 46:963.
3. Bosma, G.C., M. Fried, R.P. Custer, A. Carroll, D.M. Gibson, and M.J. Bosma. 1988. Evidence of functional lymphocytes in some (leaky) scid mice. *J. Exp. Med.* 167:1016.
4. Carroll, A.M., R.R. Hardy, and M.J. Bosma. 1989. Occurrence of mature B (IgM^+, $B220^+$) and T ($CD3^+$) lymphocytes in SCID mice. *J. Immunol.* 143:1087.
5. Petrini, J.H.-J., A.M. Carroll, and M.J. Bosma. 1990. T-cell receptor gene rearrangements in functional T-cell clones from severe combined immune deficient (scid) mice: reversion of the scid phenotype in individual lymphocyte progenitors. *Proc. Natl. Acad. Sci. USA.* 87:3450.
6. Ferrier, P., L.R. Covey, S.C. Li, H. Suh, B.A. Malynn, T.K. Blackwell, M.A. Morrow, and F.W. Alt. 1990. Normal recombination substrate V_H to DJ_H rearrangements in pre-B cell lines from SCID mice. *J. Exp. Med.* 177:1909.
7. Habu, S., M. Kimura, M. Katsuki, K. Hioki, and T. Nomura. 1987. Correlation of T cell receptor gene rearrangements to T cell surface antigen expression and to serum immunoglobulin level in scid mice. *Eur. J. Immunol.* 17:1467.
8. Riggs, J.E., R.S. Stowers, and D.E. Mosier. 1990. The immunoglobulin allotype contributed by peritoneal cavity B cells dominates in SCID mice reconstituted with allotype-disparate mixtures of splenic and peritoneal cavity B cells. *J. Exp. Med.* 172:475.
9. Wysocki, L.J., and V.L. Sato. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA.* 75:2844.
10. Herzenberg, L.A., A.M. Stall, P.A. Lalor, C. Sidman, W. Moore, D.R. Parks, and L.A. Herzenberg. 1986. The Ly-1 B cell lineage. *Immunol. Rev.* 93:53.
11. Miyama-Inaba, M., S-I. Kuma, K. Inaba, H. Ogata, H. Iwai, R. Yasumizu, S. Muramatsu, R.M. Steinman, and S. Ikehara. 1988. Unusual phenotype of B cell in the thymus of normal mice. *J. Exp. Med.* 168:811.
12. Teale, J.M. 1983. Abnormalities in isotype expression in CBA/N mice due to stimulatory environment rather than a B cell defect. *J. Immunol.* 130:72.
13. Lalor, P.A., L.A. Herzenberg, S. Adams, and A.M. Stall. 1989. Feedback regulation of murine Ly-1 B cell development. *Eur. J. Immunol.* 19:507.
14. Snapper, C.M., and W.E. Paul. 1987. B cell stimulatory factor (BSF-1) activates resting murine B cells to secrete IgG_1 upon subsequent stimulation with bacterial lipopolysaccharide. *J. Immunol.* 139:10.
15. Slack, J., G.P. Der-Balian, M. Nahm, and J.M. Davie. 1980. Subclass restriction of murine antibodies. II. The IgG plaque-forming cell response to thymus-independent type 1 and type 2 antigens in normal mice and mice expressing an X-linked immunodeficiency. *J. Exp. Med.* 151:853.
16. Smith, H., I.-M. Chen, R. Kubo, and K.S.K. Tung. 1989. Neonatal thymectomy results in a repertoire enriched in T cells deleted in adult thymus. *Science (Wash. DC).* 245:749.
17. Sakaguchi, S., and N. Sakaguchi. 1990. Thymus and autoimmunity: capacity of the normal thymus to produce pathogenic self-reactive T cells and conditions required for their induction of autoimmune disease. *J. Exp. Med.* 172:537.
18. Havran, W.L., and J.P. Allison. 1988. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. *Nature (Lond.).* 335:443.
19. Shores, E.W., S.O. Sharrow, I. Uppenkamp, and A. Singer. 1990. T cell receptor-negative thymocytes from SCID mice can be induced to enter the CD4/CD8 differentiation pathway. *Eur. J. Immunol.* 20:69.