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

By James E. Riggs, Ronald S. Stowers, and Donald E. Mosier

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. Penetration 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-17scid B cells results in high levels of Ig expressing the Ig^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.

Materials and Methods

Mice. C.B-17scid (SCID; H-2^d, Ig^b), BALB/c (H-2^d, Ig^b), BALB.xid (XID; H-2^d, Ig^b), CBA/J (H-2^k, Ig^b), and CBA/NCAHN-xid/J (XID; H-2^k, Ig^b) 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 polystyrene plates (Dynatech Laboratories, Alexandria, VA) coated with affinity-purified rabbit anti-mouse IgM or goat anti-mouse IgG, 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 (IgG1, κ), and 137.5G6 (IgG1, λ), 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 (Ig^b)
thymocytes or splenic T cells to C.B-17scid (Ighb) recipients. Production of Ighb 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 Ighb in SCID recipients (Fig. 1). However, the injection of neonatal (3–5 d of age) BALB/c thymocytes did rescue Ighb production in SCID recipients, but donor IgM 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 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 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-2k, Igh) to SCID recipients to determine if T cell-mediated rescue of Ighb 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
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. 3A 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. 3B 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 (IgM) 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-α/β 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.
This work was supported by National Institutes of Health grants RO1 AI-22871 and PO1 AI-2456. J. E. Riggs is supported by NIH training grant T32-AI-07259. This is manuscript no. 202 from the Medical Biology Institute.

Address correspondence to James E. Riggs, Department of Immunology, Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037.

Received for publication 14 September 1990 and in revised form 10 October 1990.

References

1. Bosma, G.C., R.P. Custer, and M.J. Bosma. 1983. A severe combined immunodeficiency in the mouse. Nature (Loud.). 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. Petini, 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_{α} to DJ_{α} 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.I. 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, 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.

268 Neonatal T Cell Transfer Rescues Immunoglobulin Production in SCID Mice