Brief Definitive Report

AN EXPANDED POPULATION OF NATURAL KILLER CELLS IN MICE WITH SEVERE COMBINED IMMUNODEFICIENCY (SCID) LACK REARRANGEMENT AND EXPRESSION OF T CELL RECEPTOR GENES

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We have recently reported (1) that cloned lines exhibiting NK-like activity rearrange and express the β-chain of the T cell receptor for antigen. Thy-1^+ NK cells from mouse spleen were also shown (1) to express an authentic 1.3 kb transcript. These observations suggested that a population of NK cells belonged to the T cell lineage. To further analyze the origin and differentiation of NK cells in vivo, we have made use of the mouse model exhibiting the severe combined immunodeficiency syndrome (SCID) (2). Mice homozygous for the SCID mutation are selectively deficient in lymphopoiesis but not in myelopoiesis (2, 3). T cell and B cell functions are severely impaired, while NK activity remains normal and increases in response to poly(IC) (4). In this report, we have examined the molecular status of genes encoding the T cell antigen receptor and a third T cell rearranging gene, γ (5) in enriched and flow-sorted NK cells from SCID mice.

Materials and Methods

Mice. C.B-17 SCID male or female mice at 8–10 wk of age, and their C.B-17 isogenic control littermates were bred at the Ontario Cancer Institute (Toronto, Canada) under barrier breeding conditions. For convenience, we will refer to C.B-17 SCID mice simply as SCID mice.

Isolation of Cells and NK Cytotoxicity Assays. SCID or C.B-17 control mice were boosted i.p. with 100 μg of poly(IC) 24 h before each phenotypic or functional assay. Splenocytes were depleted of red blood cells and subsequently added directly to ^{51}Cr-labelled YAC-1.2 tumor cells in a 5-h radioisotope-release assay. To enrich for cells with NK activity, SCID spleens were treated with two cycles of anti-Thy-1.2, -Ly-1.2, -Lyt-2.2, and complement. NK depletions were performed by two cycles of treatment with anti–asialo GM_1 and complement. Lytic units were calculated at 20% lysis.

Antibodies Used in This Study. Anti–mouse T cell mAbs Thy-1.2 (IgG2b), Ly-1.2

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TABLE I

Flow Cytofluorometry Analysis of SCID and C.B-17 Spleen Cells

Marker	Percent positive cells in spleen	
	C.B-17 control	C.B-17 SCID
Ig	63.7 ± 5.3	<1
Thy-1.2	29.5 ± 2.5	16.5 ± 6.9
Ly-1.2	30.7 ± 4.2	2.1 ± 1.8
Lyt-2.2	9.9 ± 0.2	2.5 ± 2.2
NK-2	5.1 ± 3.2	70.3 ± 11.0
Asialo GM_1	13.5 ± 2.1	81.7 ± 6.1
Mac-1	2.0 ± 1.4	22.2 ± 3.2

Mean ± SD from five different experiments using poly(IC)-boosted mice. Each experimental group consisted of 3–10 C.B-17 or SCID mice at 8–10 wk of age.

(IgG2b), and Lyt-2.2 (IgM) were obtained from Cedarlane Laboratories (Hornby, Canada). Anti-asialo GM_1 antiserum (Wako Chemicals, Dallas, TX) detects murine NK cells and pre-T cells. The NK-2 alloantisera were generously donated by Dr. R. Burton (Newcastle, Australia), while the rat mAb Mac-1 was provided by Dr. T. Springer (Harvard Medical School, Boston, MA) and detects the C3bi receptor on activated macrophages and mouse NK cells.

Southern Blot Analysis. DNA was digested with Eco RI, blotted, and hybridized to probes for the T β gene ($J_{\beta 2}$, $C_{\beta 2}$) or the T γ gene (2×10^8 cpm/μgm). After washing under high stringency (60°C), audioradiograms were exposed overnight.

Northern Blot Analysis. Total RNA was extracted, electrophoresed on a 1% formaldehyde/agarose gel, blotted and hybridized to a ^{32}P-labelled T α probe (M. Steimetz, Basel Institute, Basel, Switzerland), $C_{\beta 2}$ probe (T. Mak, Ontario Cancer Institute, Toronto, Canada) or β_2 microglobulin (β_2m) housekeeping gene probe (J. Parnes, Stanford University, Stanford, CA). The membranes were then washed under high stringency and autoradiographed overnight.

Results and Discussion

Functional and Phenotypic Expansion of Cells Bearing the NK-2 and Asialo-GM_1 Markers in SCID Mice. As shown in Table I, spleens from poly(IC)-boosted SCID mice were devoid of surface Ig-positive cells and deficient in mature T cells bearing Ly-1 and Lyt-2 markers compared to normal littermate controls, as previously reported (2). Significant levels of Thy-1.2$^+$ cells were found in SCID mice (11–27%), and the percentage of splenocytes bearing NK-associated markers was high; 53–82.5% for NK-2, 82% for asialo GM_1, and 22% for MAC-1. Identical results were obtained using nonboosted SCID and C.B.-17 spleens. This enrichment of NK cells was confirmed in ^{51}Cr-release assays, in which poly(IC)-boosted SCID spleen cells were functionally enriched three- to four-fold in cytolytic activity against YAC-1.2 over the normal C.B-17 littermates (30.9 ± 7.8 vs. 8.45 ± 1.03 LU per 10^6 cells). The NK cells measured in SCID mice were Thy-1.2$^-$, Ly-1.2$^-$, and Lyt-2.2$^-$, since cytotoxic activity against YAC-1.2 target cells was slightly enhanced (33.5 ± 4.7 LU per 10^6 cells) after treatment with two cycles of anti-T mAb plus complement, whereas NK function was eliminated with asialo GM_1 and complement (0 LU per 10^6 cells). This data is comparable with earlier studies (4) showing that NK-2.1 plus complement treatment depleted NK functional activity in SCID mice. The data suggests that the NK pool has greatly expanded in this mutant strain.

Lack of T Cell Receptor Rearrangement and Expression in Enriched and Flow-

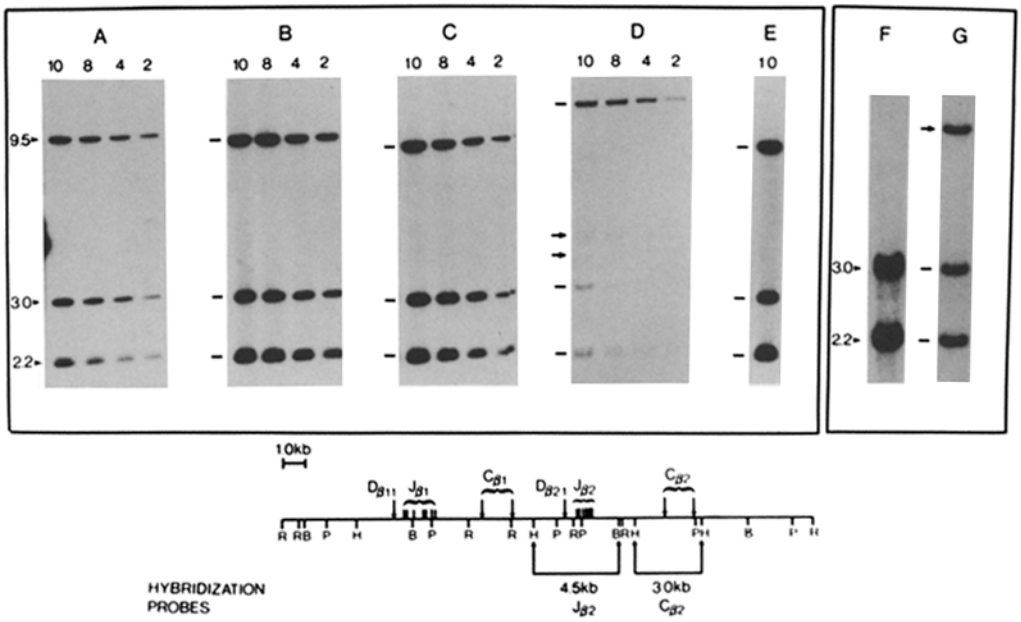

FIGURE 1. Southern blot analysis of T cell receptor β chain rearrangement in NK-enriched and NK-2 flow-sorted NK cells from SCID mice. Varying concentrations of genomic DNA (2–10 μg) were digested with Eco RI, blotted, and hybridized concomitantly to a ^{32}P-labeled 3.0 kb Hind III $C_{\beta 2}$ fragment and a 4.5 kb Hind III–Bam H1 $J_{\beta 2}$ fragment (2×10^6 cpm/ml/probe). *A–E* represent DNA samples from (*A*) SCID liver, (*B*) SCID NK-enriched spleen, (*C*) SCID NK-depleted spleen, (*D*) C.B-17 thymus, (*E*) flow-sorted NK-2$^+$ SCID splenocytes. *F* and *G* were probed with ^{32}P-labeled $J_{\beta 2}$ alone, and represent (*F*) C57BL/6J liver and (*G*) III-F6 NK clone. Bars are indicative of a germline configuration, whereas arrows represent rearrangements.

sorted SCID NK Effector Cells. To study rearrangement of the β locus in NK cells from SCID mice, southern blots were hybridized concomitantly with ^{32}P-labeled $J_{\beta 2}$ and ^{32}P-labeled $C_{\beta 2}$ probes of the same specific activity. Rearrangements can be visualized in a polyclonal cell population by comparing the ratio of the intensity of the germline 9.5 kb $C_{\beta 2}$ band to that of the 3.0 and 2.2 kb $J_{\beta 2}$ Eco RI germline bands (6). Since there is an Eco RI site in the $J_{\beta 2}$–$C_{\beta 2}$ intron, $C_{\beta 2}$ remains as a 9.5 kb germline fragment regardless of the recombination events occurring around the $J_{\beta 2}$ region. On the other hand, rearrangements would lead to a loss of both germline $J_{\beta 2}$ bands (Fig. 1). In the NK-enriched fraction, consisting of 80% NK-2$^+$ cells, and in the NK-depleted spleen cell fraction, the intensity of both $J_{\beta 2}$ germline bands was identical to that of the 9.5 kb $C_{\beta 2}$ band with decreasing concentrations of DNA (Fig. 1 *A–C*). Similar results were obtained with flow-sorted NK-2$^+$ cells (Fig. 1 *E*). However, in the C.B-17 thymus panel, both 3.0 and 2.2 kb germline bands gradually disappear with decreasing concentrations of DNA (Fig. 1 *D*). Distinct rearrangements were also apparent in the thymocyte population and in III F6, an NK clone of C57BL/6 origin in which one $J_{\beta 2}$ allele has rearranged. Identical results obtained at the $J_{\beta 1}$ locus (data not shown) lead us to conclude that the majority of NK cells from SCID spleen do not rearrange the β chain of the T cell receptor for antigen.

We have extended our analysis to the third T cell rearranging gene, γ (7). In

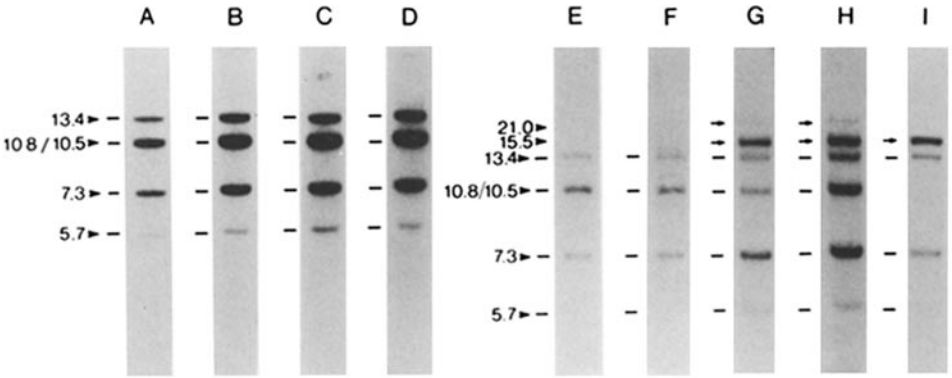

FIGURE 2. Southern blot analysis of T cell γ gene rearrangement in NK cells from SCID mice. Genomic DNA (10 μg) was digested with Eco RI, blotted, and hybridized with a ^{32}P-labelled 1.3 kb cDNA fragment of the T cell γ gene, and autoradiographed. The lanes represent DNA from (*A*, *E*) SCID liver, (*B*) NK-enriched SCID spleen, (*C*) NK-depleted C.B-17 spleen, (*D*) flow-sorted cytotoxic NK-2$^+$ SCID spleen, (*F*) flow-sorted asialo GM$_1^+$ SCID spleen, (*G*) C.B-17 thymocytes, (*H*) nylon wool–passed C.B.-17 spleen, and (*I*) the NK clone III-F6.

control C.B-17 mice, both the 10.8 kb Eco RI variable region segment and the 10.5 kb J-C fragment undergo rearrangements in thymocytes and splenic T cell populations, giving rise to two additional bands of 15.5 and 21 kb. The cross-hybridizing JC 13.4 kb fragment, the 7.3 kb constant region pseudogene, and the 5.7 kb variable gene segment retain a germline configuration (Fig. 2). Southern blot analysis of flow-sorted NK-2$^+$ and asialo GM$_1^+$ SCID NK cells showed a germline pattern (Fig. 2*D* and *F*). On the other hand, the 15.5 kb rearrangement was apparent on both alleles of the III-F6 NK clone (Fig. 2*I*), whereas the 21 kb fragment was found only at low levels in normal C.B-17 thymocytes and nylon wool–passed splenocytes (86% Ly-1.2$^+$ cells) (Fig. 2, *G* and *H*), suggesting that it may either be a rare type of rearrangement or that it may occur only in selected T cell subpopulations. These findings suggest that NK cells from SCID mice do not rearrange T cell γ genes.

Rearrangements of the T cell α gene family are difficult to detect by Southern blot analysis, owing to the large distance between the joining and constant region genes (8). Therefore, we looked for expression of the α gene using Northern blot analysis on NK-enriched populations from SCID spleen. As shown in Fig. 3, all RNAs contained multiple β_2m transcripts, whereas SCID NK-enriched populations lacked expression of the 1.6 kb α transcript found in the cloned NK line III-F6 and T lymphoma EL-4. In addition to the lack of T α expression, full-length (1.3 kb) β chain transcripts were not detected in SCID NK cells.

The findings presented here provide strong evidence that NK cells from SCID mice do not rearrange or express T cell receptor genes. These observations are compatible with the finding of Lanier and colleagues (9), who recently showed that flow-sorted, CD16$^+$ human NK cells retained a germline pattern at the β chain locus. Reynolds et al. (10) reported similar observations in the rat using the transplantable Fisher rat large granular lymphocyte (LGL) leukemia line. In addition, only 1.0 kb truncated β chain transcripts have been found in both the rat LGL (10) and human LGL systems (11).

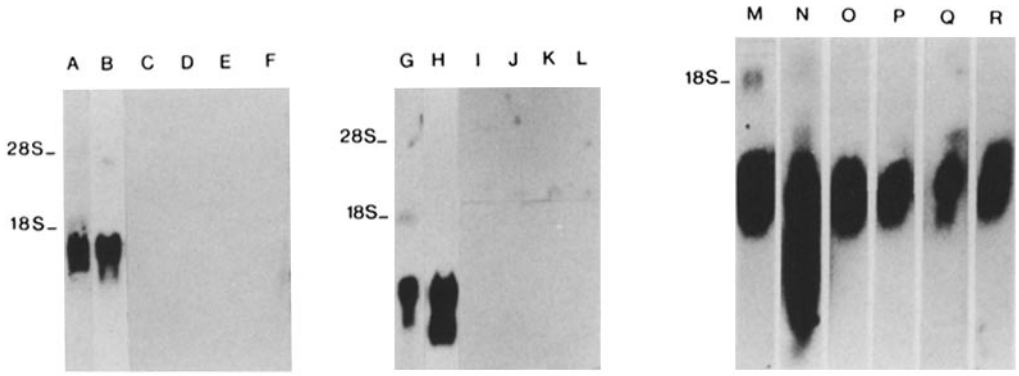

FIGURE 3. Northern blot analysis of T cell receptor α and β chain gene expression in enriched NK cell populations from SCID mice. 10 μg of total RNA was blotted and hybridized either with a (*left*) 1.0 kb cDNA fragment of the murine T cell α gene (V-J-C), (*middle*) 3.0 kb Hind III fragment of the mouse $C_{\beta 2}$ gene, or (*right*) with 0.4 and 0.6 kb Pst 1 fragments of the mouse β_2M gene. *A–M* represent RNA samples from (*A, G, R*) EL-4 thymoma, (*B, H, M*) III-F6 NK clone, (*C, I, N*) SCID liver, (*D, J, O*) NK-enriched SCID spleen, (*E, K, P*) NK-depleted SCID spleen, and (*F, L, Q*) Sp_2/O mouse myeloma.

Our findings on the lack of rearrangement and transcription of T cell receptor genes in NK cells of SCID mice support the contention that a population of NK cells do not use T cell receptor genes in recognition and cytotoxic function. The NK cell described in this study may represent a pre-T stage prior to T cell receptor gene rearrangement, or may belong to an independent lineage altogether, as suggested by Hackett and his colleagues (12). Since the lymphoid component of SCID spleen is extremely low (2), SCID NK cells may also lie on a myeloid pathway. Preliminary morphological studies of cytospins from flow-sorted NK-2 and asialo GM_1 SCID splenocytes revealed both lymphoid and myeloid components.

Summary

Mice with severe combined immunodeficiency syndrome (SCID) exhibit an impairment in both T and B cell maturation, whereas myelopoiesis remains unaffected. We report here that spleens from SCID mice have undergone phenotypic expansion of cells bearing the NK-2 and asialo GM_1 markers (70–80%) characteristic of NK cells and this expansion is accompanied by a 3–4–fold enrichment in NK cytolytic activity over their normal C.B-17 littermates. Furthermore, the NK cells from SCID mice do not rearrange or express T cell receptor α or β genes, or a third T cell rearranging gene, γ. These findings suggest that (*a*) T cell receptors are not necessary for NK-mediated cytolysis, and (*b*) either NK cells constitute an entirely distinct lineage or NK cell function is acquired in pre-T cells prior to the expression of T cell receptor genes.

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