Brief Definitive Report

Early Lymphocyte Expansion Is Severely Impaired in Interleukin 7 Receptor-deficient Mice

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Summary

Interleukin 7 (IL-7) stimulates the proliferation of B cell progenitors, thymocytes, and mature T cells through an interaction with a high affinity receptor (IL-7R) belonging to the hematopoietin receptor superfamily. We have further addressed the role of IL-7 and its receptor during B and T cell development by generating mice genetically deficient in IL-7R. Mutant mice display a profound reduction in thymic and peripheral lymphoid cellularity. Analyses of lymphoid progenitor populations in IL-7R–deficient mice define precisely those developmental stages affected by the mutation and reveal a critical role for IL-7R during early lymphoid development. Significantly, these studies indicate that the phase of thymocyte expansion occurring before the onset of T cell receptor gene rearrangement is critically dependent upon, and mediated by the high affinity receptor for IL-7.

The development of lymphoid cells from multipotential progenitors is dependent upon growth, survival, and differentiation factors produced by various stromal cells. Long-term cultures supporting the growth of murine B lineage cells from uncommitted precursors can be established from adult bone marrow and have been instrumental in defining factors that influence B cell development (1, 2). IL-7 was identified and cloned based upon its ability to induce short-term proliferation of B cell progenitors in the absence of stromal cells (3). The requirement for IL-7 in the proliferation of B cell precursors has been clearly established in that stromal cell variants that no longer express IL-7 are incapable of efficiently supporting B cell progenitor growth, and neutralizing antibodies to IL-7 or IL-7R severely inhibit B cell development in vitro and in vivo (4-6).

Evidence suggests that the IL-7–responsive B lineage population displays an early/late pro-B cell phenotype characterized by the expression of B220, heat stable antigen (HSA), and leukosialin (CD43), the presence of D-J and V-D-J rearrangements and the absence of surface IgM (7). Less mature B lineage cells do not proliferate or differentiate in response to IL-7 in vitro, although they do express IL-7R (8). IL-7R expression is lost after productive light chain rearrangement in pre-B cells, and thus subsequent stages of B cell maturation are likely to be directly IL-7 independent (9).

The role of IL-7 in T cell development is less well defined. IL-7 is expressed in the thymus and has been shown to stimulate the growth of immature $CD4^-/CD8^-$ adult and fetal thymocytes (10, 11) and promote rearrangement of T cell receptor β and γ chains in fetal thymus and fetal liver culture systems (12, 13). Additionally, treatment of mice with neutralizing antibodies to IL-7 or IL-7R results in thymic hypoplasia (5, 6), and overexpression of IL-7 in transgenic mice perturbs thymocyte cellularity (14). However, it is not known to what extent T cell development within the thymus is IL-7 dependent and which of the thymic subpopulations have specific requirements for IL-7–induced proliferation and survival in vivo.

We have taken a genetic approach to further elucidate the role of IL-7 and its high affinity receptor in the proliferation and maturation of B and T lineage cells in vivo by analyzing lymphoid development in mice lacking the high affinity IL-7R. Results obtained from these analyses implicate IL-7R as a critical signaling molecule in the proliferation of early B cell progenitors undergoing heavy chain rearrangements and in early thymocyte expansion prior to the acquisition of rearranged T cell receptor genes.

Materials and Methods

Gene Targeting. An IL-7R gene targeting construct was prepared by inserting a 1-kb MCneo cassette (15) into a HindIII site within the third exon of the IL-7R gene, occurring at approximately amino acid 90 of the 180 amino acid extracellular domain (16). A thymidine kinase cassette (MC-TK) was inserted into the 3′ end of the vector (15). AB1 embryonic stem (ES) cells were maintained, electroporated, and selected in the presence of G418 and ganciclovir as described previously (17). Approximately 1/250 G418 plus ganciclovir resistant colonies carried an IL-7R allele altered

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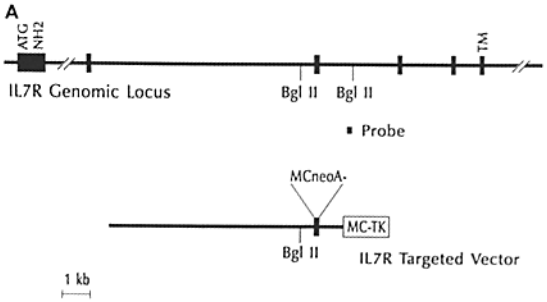

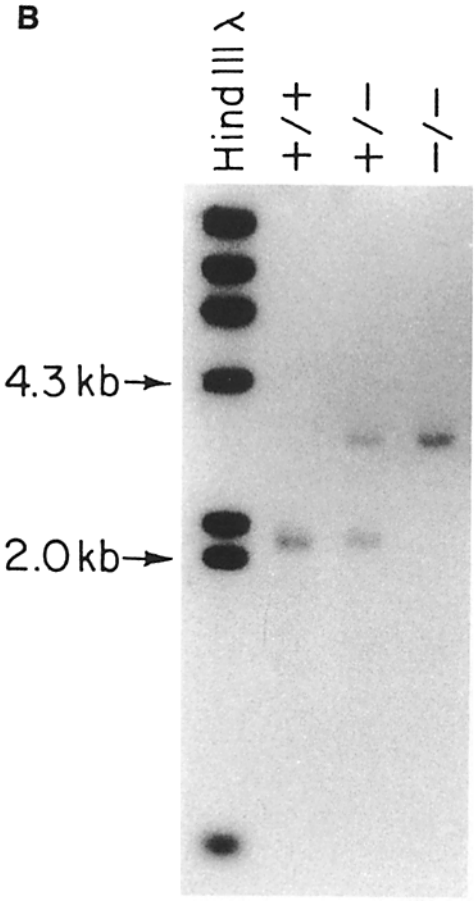

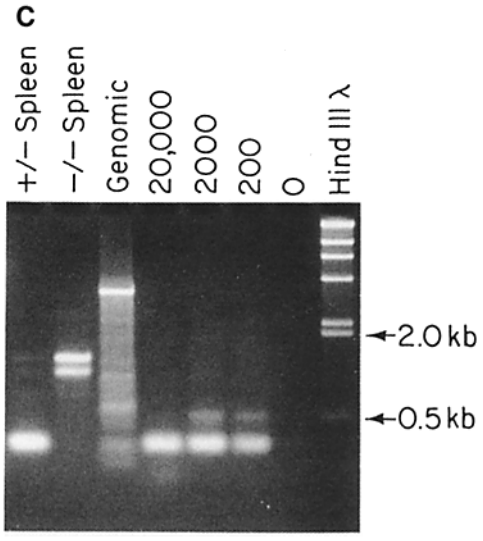

by homologous recombination as determined by both PCR and Southern blot analyses.

Mice. C57Bl/6 were obtained from Charles River Laboratories (Wilmington, MA), 129/J were obtained from The Jackson Laboratory (Bar Harbor, ME), and Swiss Webster were obtained from Taconic Farms, Inc. (Germantown, NY). All mice were maintained under specific pathogen-free conditions. ES cells carrying the mutant IL-7R allele were injected into day 3.5 C57Bl/6 blastocysts and transferred to day 2.5 pseudopregnant Swiss Webster recipients. Resulting male chimeras were bred to 129/J females and offspring analyzed for germline transmission of the altered allele by PCR analysis of ear biopsy DNA. Mice homozygous for the IL-7R mutation (IL-7R−/−) were generated either from intercrosses between heterozygotes (IL-7R+/−) or from IL-7R−/− breeding pairs. When necessary, genotypes were determined by PCR analysis of ear biopsy DNA. Throughout these studies either age-matched or littermate IL-7R+/− and IL-7R+/+ mice were used as controls.

Molecular and Biochemical Analyses. Reverse transcriptase PCR reactions were performed on poly(A)+ RNA according to manufacturer's specifications (Pharmacia LKB, Piscataway, NJ). Scatchard analyses were performed on primary cell suspensions using iodinated murine IL-7 as described (18). Bone marrow–derived macrophages were isolated and cultured in the presence of colony stimulation factor 1 as described (19).

Immunofluorescent Staining and Flow Cytometry. Four-color analysis of bone marrow was performed using reagents and protocols described previously (6, 7). Analyses of thymic and peripheral lymphoid populations were performed as described (20).

Results and Discussion

Generation and Gross Characterization of IL-7R–deficient (IL-7R−/−) Mice. Mice lacking a functional IL-7R gene were created by inserting a neo cassette in the third exon of one IL-7R allele by homologous recombination of a gene targeting vector in 129Sv-derived ES cells (Fig. 1 *A*). The mutant allele encodes less than half of the IL-7R extracellular domain and is thus expected to represent a null mutation. IL-7R−/− mice were generated from matings between IL-7R+/− mice at the expected Mendelian frequency (data not shown) and identified by genomic Southern blot analysis (Fig.

Figure 1. Targeted disruption of the IL-7R locus and molecular characterization of IL-7R−/− mice. (*A*) The first six exons encoding the extracellular domain of murine IL-7R are depicted, highlighting the initiation codon (*ATG*), NH_2 terminus (*NH2*), transmembrane domain (*TM*), and diagnostic BglII sites. The vector constructed to target the IL-7R gene and the probe used to identify an IL-7R locus altered by homologous recombination are also shown. (*B*) DNAs isolated from wild-type mice (+/+), and mice heterozygous (+/−) or homozygous (−/−) for the mutant IL-7R gene were digested with BglII and subject to Southern blot analysis using the indicated probe. The positions of various HindIII λ size markers are shown. (*C*) Reverse transcriptase PCR analysis of splenic cDNA prepared from adult mice heterozygous (+/−) and homozygous (−/−) for the IL-7R mutation using a primer 5′ of the MCneo insertion in exon 3 and a primer derived from exon 4. The PCR products are derived from ~4,000 splenic cell equivalents and are compared with PCR products obtained from 200, 2,000, or 20,000 molecules of control IL-7R cDNA. Reagent only (0) and genomic DNA controls are included and the positions of various HindIII λ size markers are shown.

Table 1. *Analysis of High Affinity IL-7 Binding to Control and IL-7R −/− Cells*

	Control		IL-7R −/−	
Source	Site number	K_a	Site number	K_a
		M^{-1}		M^{-1}
Thymus	166	1.6×10^{10}	ND*	ND
Spleen	420	7.0×10^{9}	ND	ND
BM‡	160	4.3×10^{9}	ND	ND
BMM§	30	3.2×10^{10}	ND	ND

Cells were prepared as described (19, 20) and examined for high affinity binding to radiolabeled murine IL-7 (18). Site numbers and K_a values were determined by Scatchard analyses of complete sets of binding data.
* Not detectable. Site numbers <~10 molecules/cell are below the limit of accurate detection and are considered not detectable.
‡ Bone marrow.
§ Bone marrow–derived macrophages (19).

1 *B*). Reverse transcriptase PCR analysis using primers that flank the neo insertion in exon 3 confirmed the lack of wild-type IL-7R message in IL-7R−/− splenocytes, although expression of the expected mutant IL-7R-MCneo hybrid transcript was readily apparent (Fig. 1 *C*).

Several lines of evidence indicate that IL-7R−/− hematopoietic cells fail to bind IL-7 with high affinity and do not respond to IL-7. As shown in Table 1, Scatchard analyses from control marrow, spleen, and thymus cell suspensions as well as from cultured bone marrow–derived macrophages revealed site numbers and binding affinities comparable to those described previously (18), whereas high affinity IL-7 binding to IL-7R−/− cells was undetectable. These data indicate that the cloned IL-7R is the only molecule on murine hematopoietic cells capable of binding IL-7 with high affinity and that the introduced IL-7R mutation is in fact a null mutation. Additionally, B cell progenitor proliferation was readily apparent in IL-7 supplemented newborn liver cultures established from IL-7R+/− mice but not in cultures derived from IL-7R−/− littermates (data not shown). In contrast, parallel cultures supplemented with steel factor supported myeloid development indistinguishable from that of control cultures (data not shown).

Gross pathological analyses of IL-7R−/− mice did not reveal any nonlymphoid anomalies and both sexes were fertile. However, newborn and young adult IL-7R−/− mice displayed dramatically reduced thymic and splenic lymphoid cellularity relative to either age-matched or littermate controls (Fig. 2, *A* and *B*). This deficit was observed in both B and T cell compartments and affected both $CD4^+$ and $CD8^+$ T cell subsets (Fig. 2 *A*). Peripheral and mesenteric lymph node cellularities were also greatly decreased (data not shown). Total bone marrow cellularity and CFU-GM potential in IL-7R−/− mice were both comparable to those observed in controls, whereas colony formation in response to IL-7 was observed with control but not IL-7R−/− bone marrow (data not shown).

Thymocyte Development in IL-7R−/− Mice Is Blocked at an Early Developmental Stage. During T cell development, prothymocytes migrate from the bone marrow into the thymus and undergo a phenomenal expansion within the cortex that

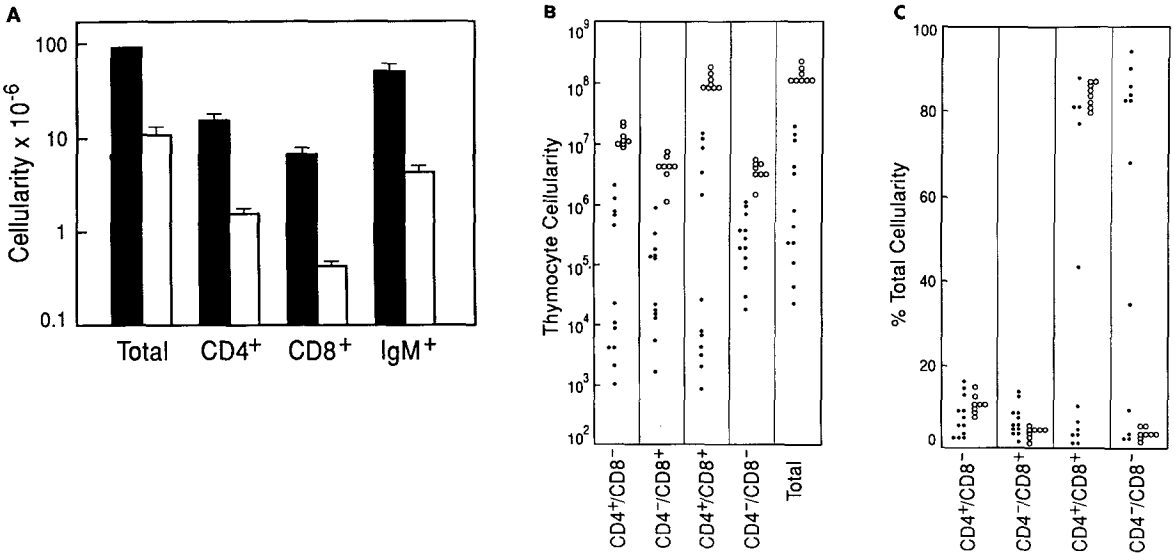

Figure 2. Analysis of lymphoid cellularity in control and IL-7R−/− mice. (*A*) Spleens from 5–12-wk-old IL-7R−/− mice (*open bar*) and age-matched or littermate controls (*solid bar*) were evaluated for total cellularity and cellularity of $CD4^+$, $CD8^+$, and IgM^+ subpopulations. The data represent mean and SEM values of 7–12 IL-7R−/− mice and 5–7 controls. (*B*) Analysis of thymocyte cellularity in control (*open circle*) and IL-7R−/− mice (*solid circle*). Thymuses from 12 IL-7R−/− mice (5–24 wk of age) and 8 age-matched or littermate controls were evaluated for total cellularity and cellularity of the various CD4/CD8 subpopulations. (*C*) Analysis of the relative distribution of thymocyte subsets in IL-7R−/− (*solid circle*) and control mice (*open circle*). Levels of the CD4/CD8 thymocytes subsets determined in *B* are expressed as a fraction of total thymocyte cellularity.

is accompanied by T cell receptor gene rearrangements and a sequential progression through $CD4^-/CD8^-$ and $CD4^+/CD8^+$ stages to ultimately yield mature medullary $CD4^+$ or $CD8^+$ T cells (21). The cytokine(s) required for this pathway have not been defined, although IL-7 is a reasonable candidate (3, 10–12, 22). IL-7R−/− mice displayed a marked reduction in thymocyte cellularity. Whereas splenic T cell levels in IL-7R−/− mice were consistently approximately 10% those observed in control mice (Fig. 2 *A*), total thymic cellularity among these mice varied between 0.01 and 10% of control values (Fig. 2 *B*). The relative proportions of thymic subsets in IL-7R−/− mice were similarly variable (Fig. 2 *C*). $CD4^+/CD8^+$ thymocytes normally comprise approximately 80% of the thymus (21). This distribution was also observed in IL-7R−/− thymuses where total cellularity was at least 1% that of control mice. In marked contrast, those IL-7R−/− thymuses displaying a total cellularity significantly less than this were composed primarily of $CD4^-/CD8^-$ cells. The source of this variability is presently not known, although it cannot be attributed to differences in age or sex and is unlikely to be due to strain variation as the IL-7R−/− mutation has been maintained on a 129 background.

To determine more precisely what stage of thymocyte development is affected in IL-7R−/− mice, $CD4^-/CD8^-$ thymocytes were analyzed for the expression of HSA and CD25 by flow cytometry. The dynamic expression of these markers defines distinct stages of $CD4^-/CD8^-$ thymocyte development (23). The $HSA^+/CD25^+$ fraction present in a control thymus was clearly underrepresented in an IL-7R−/− thymus composed primarily of $CD4^-/CD8^-$ cells (Fig. 3, *A* and *B*) indicating that T cell development is affected before the surface expression of CD4 and CD8 and before the initiation of T cell receptor β chain rearrangement. The acquisition of CD25 expression by $CD4^-/CD8^-$ HSA^+ cells is accompanied by extensive proliferation induced by undefined factors provided by the thymic microenvironment (21). The failure of this proliferative transition to occur in IL-7R−/− mice suggests an obligatory requirement for IL-7 and its high affinity receptor for efficient expansion of $CD4^-/CD8^-$ thymocytes and implicates IL-7R as a key signaling molecule driving the first major phase of early thymocyte expansion. Mutations affecting β chain rearrangement or signal transduction through this chain arrest the second major phase of thymocyte expansion (24, 25), which ultimately yields postmitotic cortical $CD4^+/CD8^+$ thymocytes. That the IL-7R−/− mutation manifests itself prior to this phase precludes a direct evaluation of the role of IL-7R in this process.

B Cell Development in IL-7R−/− Mice Is Affected at the Pro-B Cell Stage. To more closely examine the stage at which development of conventional B cells is affected in IL-7R−/− mice, the $B220^+$ IgM^- B cell precursor population from adult bone marrow was further separated on the basis of CD43 and HSA expression (7). As shown in Fig. 4, the predominant $CD43^+$ HSA^{bright} and $CD43^-$ HSA^{bright} fractions present in gated $B220^+IgM^-$ control marrow were undetectable in IL-7R−/− marrow. These fractions correspond to, respectively, the IL-7 responsive early/late pro-B population and the IL-7 nonresponsive pre B population. The $CD43^+$ HSA^{dull} fraction representing pre–pro-B cells was not diminished in IL-7R−/− mice, suggesting that B cell development up to and including this stage is unaffected by the mutation and that survival of these populations does not depend upon high affinity IL-7R. It is interesting to note that a population of $CD43^+$ cells expressing very low levels of HSA was easily detected in IL-7R−/− marrow but undetectable in control marrow. This fraction may represent yet another discernible fraction of B cell precursors that lies intermediate between uncommitted progenitors and pre–pro-B cells whose presence is unmasked in IL-7R−/− marrow simply due to inefficient proliferation of subsequent developmental stages.

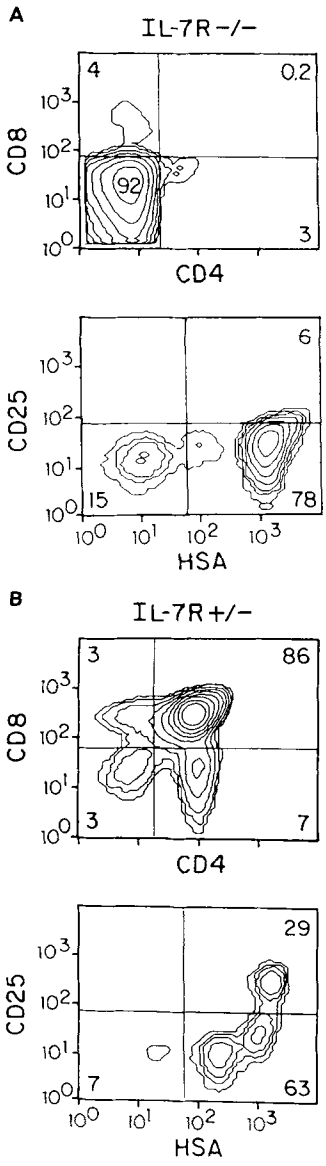

Figure 3. Immunofluorescent profiles of $CD4^-/CD8^-$ thymocytes in control and IL-7R−/− mice. Gated $CD4^-/CD8^-$ thymocytes from a young adult IL-7R−/− mouse (*A*) and an age-matched control (*B*) were analyzed for the expression of HSA and CD25 by flow cytometry. The axis divisions refer to $\log_{10}$ fluorescence units. The data are representative histograms of mutant thymuses composed predominantly of $CD4^-/CD8^-$ cells.

The results described in this report argue that efficient lymphoid development in mice is critically dependent upon IL-7R. However, the observation that mature B and T cells were present in the periphery of IL-7R−/− mice indicates that to a limited extent, lymphoid development can proceed in

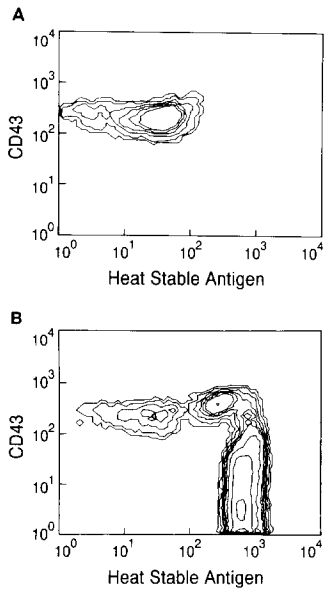

Figure 4. Immunofluorescent profiles of B cell progenitor subpopulations in the bone marrow. Gated $B220^+/IgM^-$ bone marrow cells from a 5-wk-old IL-7R−/− mouse (*A*) and an age-matched control (*B*) were analyzed for the expression of CD43 and HSA by flow cytometry. The axis divisions refer to $\log_{10}$ fluorescence units. The data are representative histograms.

the absence of an IL-7R–mediated phase of expansion. The extent to which impaired expansion during development affects the function of these lymphocytes is currently under investigation.

Several members of the hematopoietin receptor family share common receptor subunits (26) and thus receptor mutations may yield phenotypes more severe than those observed with mutations in their corresponding ligands. For example, mutations in the IL-2Rγ subunit result in human X-linked immunodeficiency disease (XSCID) (27), whereas lymphoid development is normal in mice lacking IL-2 (28). Recently it has been demonstrated that the IL-2Rγ chain is a functional component of both the IL-4 and IL-7 receptors (29–31). Impaired lymphoid development in IL-7R−/− mice formally supports the hypothesis that the severity of XSCID is in part due to a compromised IL-7/IL-7R interaction. However, the observation that peripheral B cell levels are depressed in IL-7R−/− mice and are apparently unaffected in XSCID patients suggests the possibility that IL-7R function in B cell progenitors may not be strictly dependent on IL-2Rγ.

Although IL-7R−/− mice display a qualitatively similar phenotype to mice treated with neutralizing antibodies to IL-7, the propensity for hematopoietin receptors to use shared subunits raises the possibility that the IL-7R mutation has impinged upon additional receptor systems. We have recently cloned a cDNA encoding a novel stromal cell–derived cytokine, thymic stromal-derived lymphopoietin (TSLP), whose activities on a variety of lymphoid cells parallel those of IL-7 (Williams, D. E., manuscript in preparation). Interestingly, IL-7R is a subunit of the TSLP receptor (Park, L. S., manuscript in preparation). Thus the phenotypes displayed by IL-7R−/− might be attributed to a functional inactivation of both TSLP and IL-7.

Analyses of previous cytokine and cytokine receptor gene knock-out mice have been particularly notable in that none have resulted in lymphoid deficiency (28, 32–34). In contrast, lymphoid development in IL-7R−/− mice is severely impaired and reveals a unique, nonredundant role of IL-7R in the formation of B and T cell lineages.

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