Transgenic Expression of Interleukin 7 Restores T Cell Populations in Nude Mice
By Benjamin E. Rich and Philip Leder

From the Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts 02115

Summary

The thymic lesion of the nude mouse causes a profound block in T cell development. The failure of most T cells to mature in nude mice is likely to reflect a requirement for signals elaborated in the normal thymus. Interleukin 7 (IL-7), a lymphokine that is normally expressed in the thymus and has been implicated in T cell maturation, might be central to this process. To test this possibility, we introduced a transgene directing lymphoid expression of IL-7 into nude mice and found that it substantially alleviates the block in T cell maturation caused by the thymic defect. IL-7 transgenic nude mice have increased numbers of peripheral cells expressing the T cell marker Thy-1, the T cell antigen receptor complex, and the co-receptors CD4 and CD8. The IL-7 transgene also restores T cell-specific proliferation and activation responses to the peripheral cells of transgene-rescued nude mice. Such findings point toward a fundamental role for IL-7 in the thymic maturation of T cells.

Nude mice are widely used as a model of immunodeficiency. The autosomal recessive nude mutation interferes with hair formation and prevents the normal development of the thymus. The thymic defect caused by the nude mutation results in a nearly complete block in T cell development (1). T cell maturation in nude mice can be restored by thymic epithelial tissue grafts (2). Thus, the failure of most T cell precursors to mature in nude mice is due to their lack of exposure to the normal thymic microenvironment (3).

In passing through the normal thymus, the population of T cell progenitors undergoes a highly selective process of refinement of the large array of TCR generated by somatic rearrangement of their genes. This process involves the progression through three distinct developmental stages that are characterized by the expression of the co-receptors CD4 and CD8. Bone marrow-derived T cell progenitors enter the thymus with germline configuration TCR genes and lack expression of TCR, CD3, CD4, or CD8 (CD4-CD8-). As the cells rearrange and express their TCR genes, they also begin to express both CD4 and CD8 (CD4+CD8+). Cells expressing TCR that interact with self MHC in conjunction with CD4 or CD8, are stimulated to proliferate, turn off either CD4 or CD8, and migrate from the cortex to the medulla and then to the periphery as mature CD4+CD8- or CD4-CD8+ T cells (positive selection). Cells expressing TCR that react with self peptides complexed with MHC molecules are stimulated to die (negative selection). Less than 5% of TCR pass these two tests and persist in the refined repertoire of TCR expressed by the T cells that emerge to the periphery (4, 5).

The signals that normally support this developmental progression in the thymus are not thoroughly characterized. However, in the absence of normal thymic tissue the progression is almost entirely blocked. Lacking normal thymic tissue, nude mice fail to develop mature T cells and are profoundly immunodeficient. Only a very small population of T cells that increases with age is detected in older nude mice (6). These may be a subset of T cells that do not require exposure to thymic tissue to mature (7) or, conversely, nude mice may have some residual function in their defective thymic vestige or in nonthymic tissue that mimics the normal thymic anlage and allows some T cells to mature. Some of these few T cells in nude mice may arise via positive selection (8), however, they appear to escape negative selection (9, 10).

IL-7 conveys proliferative signals to B and T cell progenitors (11-16) and activating signals to mature T cells (17, 18). Several observations suggest a role for this lymphokine in T cell maturation in the thymus. IL-7 is expressed in murine adult (11) and fetal (19, 20) thymus as well as in cultured thymic epithelial cells (14, 21, 22). The treatment of immature CD4-CD8- thymocytes with IL-7 in vitro stimulates their proliferation and preserves their ability to reconstitute lymphocyte-depleted thymic organ culture (23). IL-7 also supports rearrangement and expression of TCR genes in cultured thymocytes (24-27) and administration of IL-7 to normal or lymphopenic mice results in increased numbers of peripheral B and T cells (28, 29). On the other hand, anti-IL-7 antibodies inhibit growth of fetal thymocytes in organ culture (19) and anti-IL-7 or anti-IL-7-receptor antibodies deplete thymocytes in adults (30, 31). Moreover, T cell maturation in IL-7 receptor-deficient mice is sharply curtailed and these mice have very few thymocytes or mature T cells (32).
A

WT

IL-7 Transgenic

nude

nude IL-7 Transgenic

CD3

B

WT

IL-7 Transgenic

nude

nude IL-7 Transgenic

Thy-1

C

WT

IL-7 Transgenic

nude

nude IL-7 Transgenic

CD4

D

WT

IL-7 Transgenic

nude

nude IL-7 Transgenic

CD4 or CD8

CD8

CD3

1224 T Cells in IL-7 Transgenic Nude Mice
Nude mice with intrasplenic transplants of thymic epithelial cell lines that express IL-7 (21), or subcutaneous implants of encapsulated fetal thymic tissue (33), exhibit enhanced T cell maturation. In the latter case, this maturation is partially inhibited by anti-IL-7 antibodies.

In light of these observations we investigated the role of IL-7 in T cell maturation in vivo by examining the effects of transgenic expression of IL-7 in lymphocytes of nude mice. If exposure to IL-7 in the thymus is a critical event in T cell maturation, then lymphocytes that express IL-7 might be able to mature without encountering normal thymic epithelia. To test this hypothesis, we made use of a transgenic mouse strain (TG.UP) that carries an IL-7 transgene expressed under the control of immunoglobulin heavy chain gene promoter and enhancer sequences (34). Lymphoid expression of IL-7 by this transgene, or an engineered retrovirus (35) reduces the control of immunoglobulin heavy chain gene promoter activity in a Cytofluorograf II flow cytometry machine (Becton Dickinson & Co., Mountain View, CA). Dead cells were eliminated from the analyses by gating with forward and perpendicular scatter.

Materials and Methods

Animals. FVB/N mice carrying the TG.UP IL-7 transgene (34) were bred with Swiss or BALB/c nude mice (Taconic Farms Inc., Germantown, NY) and F2 animals were analyzed. All animals were housed under specific pathogen-free conditions. Homozygous nude animals were identified by phenotype and heterozygous transgenic animals were identified by hybridization with DNA prepared from tail biopsy.

Flow Cytometry. Spleen cells were prepared from age matched animals (60-100 d) of each of the four genotypes of F2 animals (wild type, IL-7 transgenic, nude, and nude IL-7 transgenic), stained with fluorescent-labeled antibodies and subjected to flow cytometry. Prominent populations of CD4+CD8- and CD4-CD8+ T cells that is almost completely absent in nude mice and restored in the nude IL-7 transgenic mice (Fig. 1 A). This population is smaller than the CD3+ population of wild-type mice, but it is comparable to that of IL-7 transgenic mice. Similarly, a comparison of the B cell-specific B220 (CD45R) and T cell-specific Thy-1 antigens reveals a population of Thy-1+B220- T cells that is almost completely absent in nude mice and restored in the nude IL-7 transgenic mice (Fig. 1 B). In addition to the CD3/TCR complex and Thy-1, these cells also express the co-receptors CD4 or CD8 (Fig. 1 C).

Results and Discussion

The IL-7 transgene was introduced into homozygous nude mice by conventional mating. On analysis, it was indeed found that of 20 nude mice carrying the IL-7 transgene examined, 18 exhibited partially restored T cell populations. For example, roughly half of the spleen cells derived from wild-type mice and somewhat fewer of the cells derived from euthymic IL-7 transgenic mice express CD3, whereas spleen cells from age-matched nude mice are largely devoid of CD3 (Fig. 1 A). In contrast, the nude IL-7 transgenic spleen cells contain a prominent population of CD3+ cells (Fig. 1 A). This population is smaller than the CD3+ population of wild-type mice, but it is comparable to that of IL-7 transgenic mice.

Figure 1. Immunofluorescent flow cytometry of T cell surface markers. (A) CD3 expression of spleen cells. Cells were stained with anti-CD3 29B and FITC-anti-rat Ig and analyzed. The relative fluorescence intensity (x axis) of dissociated spleen cells from mice of the indicated genotypes is plotted against their frequency (y axis). The entire x axis range represents 3.2 log10 units. (B) Expression of B220 and Thy-1 on spleen cells. Cells stained with FITC-anti-Thy-1, FITC-anti-Thy-1.2 and PE-anti-B220 (CD45R), PE-anti-CD4, PE-anti-CD8 and FITC-anti-CD3 (PharMingen, San Diego, CA), FITC-anti-CD8 and PE-anti-CD4 (Becton Dickinson & Co.).
is normally present only in the thymus. To determine to what extent the CD4+CD8- or CD4-CD8+ cells also express CD3, we utilized a mixture of anti-CD4 and anti-CD8 mAbs and anti-CD3 mAb to define this population (Fig. 1D). While the nude mice had very few cells expressing CD3 and almost none of these express CD4 or CD8, most CD3+ cells derived from the wild type, IL-7 transgenic, and nude IL-7 transgenic mice do express CD4 or CD8. No significant populations of CD4+ or CD8+ cells lacking CD3 were detected.

Since we observed cells expressing T cell surface markers in nude IL-7 transgenic mice, we sought to determine whether they were able to function as T cells. We tested this by measuring their abilities to respond to activation of the TCR complex in vitro (Fig. 2). Spleen cells from mice of each of the four genotypes were cultured in the presence or absence of immobilized anti-CD3 mAb or Con A. The cultures were evaluated for proliferation and for production of IL-2. While cells from euthymic mice responded vigorously to TCR stimulation by proliferating and secreting IL-2 regardless of the presence of the IL-7 transgene, cells from nontransgenic nude mice had minimal responses. Spleen cells from IL-7 transgenic nude mice, however, responded at nearly wild-type levels.

Thus nude mice expressing IL-7 under the control of immunoglobulin regulatory sequences develop significant numbers of peripheral T cells. These T cells are similar in phenotype to those of wild-type mice in that they express Thy-1, CD4 or CD8 and the TCR complex. Moreover, when the TCR complex is engaged, these T cells respond by proliferating and secreting IL-2 in a similar fashion to wild-type T cells.

While it appears that the T cells found in IL-7 transgenic nude mice arise from increased maturation of T cell precursors that are otherwise blocked in nontransgenic nude mice, the data in this study do not rule out the possibility that these T cells represent an expansion of the few T cells that are present in nontransgenic nude mice. Since IL-7 has been shown to stimulate both maturation and proliferation of T lineage cells in various assays, both of these phenomena may contribute to the formation of T cell populations in nude IL-7 transgenic mice.

Transgenic expression of IL-7 in these mice may replace the exposure to IL-7 that T cell precursors normally encounter in the thymus and thus facilitate their maturation in the absence of this organ. Indeed, in the recent analysis of IL-7 receptor-deficient mice it was found that the numbers of thymocytes in those mice are sharply reduced and maturation is variably impeded (32). This demonstrates that signals transmitted via the IL-7 receptor are critical for the development of T cells in the thymus.

Previous work of ours and others has described perturbations in the profile of maturing thymocytes induced by deregulated expression of IL-7 in the thymus (34, 35). As noted above, the depletion of the CD4+CD8- population induced by the IL-7 transgene in euthymic mice may be due to accelerated maturation to CD4+CD8- or CD4-CD8+ cells and exit to the periphery. The present findings are consistent with this model and imply that IL-7 signaling may act at both the CD4+CD8- to CD4+CD8+ transitions.
of IL-7 is probably unaffected by the absence of functional \textit{whn} protein. It is more likely that certain cell lineages within the skin and thymus that express IL-7 are dependent on \textit{whn}.

The restoration of significant populations of T cells in nude mice points toward a central role for IL-7 in thymic maturation of T cells. While it is unlikely that the function of the thymus in generating immunity can be entirely replaced by a single molecule, our results suggest that IL-7 might be an agent useful in the treatment of immunodeficient states.

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Address correspondence to Benjamin E. Rich, Department of Genetics, Harvard Medical School and Howard Hughes Medical Institute, 200 Longwood Avenue, Boston, MA 02115.

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