

## Cutaneous Lymphoproliferation and Lymphomas in Interleukin 7 Transgenic Mice

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### Summary

To investigate the role of interleukin 7 (IL-7) in the development of the lymphoid system, we have generated two lines of transgenic mice carrying an IL-7 cDNA fused to an immunoglobulin heavy chain promoter and enhancer. This transgene is expressed in the bone marrow, lymph nodes, spleen, thymus, and skin provoking a perturbation of T cell development characterized by a marked reduction of CD4<sup>+</sup>CD8<sup>+</sup> (double-positive) thymocytes. Quite unexpectedly, however, both lines also develop a progressive cutaneous disorder involving a dermal lymphoid infiltrate that results in progressive alopecia, hyperkeratosis, and exfoliation. Although the infiltrate is primarily composed of T lineage cells, its development is not impeded in the athymic *nu/nu* background. Furthermore, the phenotype can be transmitted horizontally by transplanting lymphoid tissues or skin to syngeneic wild-type mice. Thus, the phenotype is conveyed by skin-homing, mobile cells (presumably the infiltrating lymphocytes) in a cell-autonomous fashion. In addition to the skin phenotype, this transgene also provokes the development of a lymphoproliferative disorder that induces B and T cell lymphomas within the first 4 mo of life. These findings suggest potential physiologic actions of IL-7 in T cell development and in cutaneous immunity. They also demonstrate that IL-7 can act as an oncogene in the living organism.

The mammalian immune system comprises a complex network of interacting cell types and functions. Much of the communication between cells that regulates their growth and differentiation appears to be accomplished by the elaboration of soluble factors that interact with specific receptors found on responsive cells. Several of these factors, known as cytokines, elicit very different responses from multiple cell types. IL-7 is a cytokine that was discovered initially as an active component of murine bone marrow B lymphocyte cultures (1, 2). It is produced by cultured adherent bone marrow cells and stimulates DNA synthesis in feeder-dependent lymphocytes. However, mRNA studies indicate that the primary organs in which IL-7 synthesis takes place are the spleen, kidney, and thymus (3).

The cellular response to IL-7 is complex and varied, involving both B and T cell lineages. Early B cell progenitors, but not more mature B cells, respond to IL-7 in proliferation and colony formation assays (2, 4–6). Treatment of fetal thymocytes in dissociated culture with IL-7 results in only a brief mitogenic response and enhanced viability, whereas the same cells cultured in intact tissue exhibit a dramatic and prolonged proliferative response to exogenous IL-7 (7). The IL-7-induced population includes an increased number of cells that can exhibit cytolytic function in response to stimulation by IL-2 (8). Adult thymocytes also proliferate in response to IL-7,

largely resulting in increased numbers of CD4<sup>-</sup>CD8<sup>-</sup> (double-negative) and CD4<sup>+</sup>CD8<sup>-</sup> or CD4<sup>-</sup>CD8<sup>+</sup> (single-positive) cells (9, 10). Additionally, IL-7, acting in concert with TCR engagement, phorbol ester, or Con A treatment activates and stimulates the cytolytic function of mature peripheral T cells (11–14).

The *in vivo* response to systemic administration of highly purified recombinant IL-7 is equally complex, resulting in the reversible increase in the number of early B cell progenitors in murine bone marrow, spleen, and LN, and in single-positive (CD4<sup>+</sup> or CD8<sup>+</sup>) T cells in spleen and LN (15, 16). Similarly, transgenic mice expressing an IL-7 cDNA under the control of an immunoglobulin  $\kappa$  L chain promoter and a H chain enhancer develop expanded populations of mature and immature bone marrow and splenic B cells and increased numbers of thymic and lymphoid T cells (17). In light of the many *in vitro* activities of IL-7, this lymphokine appears to have roles both in the normal development of the lymphoid system and in the reactive phase of the immune response.

As with several cytokine systems, the cellular response to a particular factor can differ depending upon the microenvironment of the responding cell. For example, cultured bone marrow pre-B cells divide vigorously when they encounter IL-7 in the presence of stromal cells, whereas in the absence of stromal cells, DNA synthesis is only briefly stimulated

(2). As mentioned above, IL-7 evokes very different responses from fetal thymocytes depending upon whether they are dissociated or in thymic tissue (7). Thus, the context in which the cell encounters IL-7 can make a critical difference in its response. With this in mind, we sought to investigate the effects of IL-7 expression *in vivo* using transgenic mice. More specifically, we directed expression of IL-7 to cells of the B and T cell lineages by utilizing the Ig H chain promoter and enhancer (18). The data we present below show that IL-7 perturbs thymic T cell development and induces a novel population of lymphocytes that migrates to and substantially disrupts the cytoarchitecture of the skin. In addition, we find that IL-7 expression promotes the malignant transformation of B and T lineage cells.

## Materials and Methods

**Construction of the  $E\mu P\mu$ -IL7 Transgene.** cDNA was prepared from Swiss mouse spleen RNA with reverse transcriptase and oligo-dT. An IL-7 cDNA molecule containing the whole coding sequence was amplified from this preparation by PCR using oligonucleotide primers derived from the published sequence of an IL-7 cDNA (3). The primers corresponded to nucleotides 525-551 in the positive sense and 1158-1184 in the negative sense with four nucleotides (GGTC) added on to the 5' end of each of the primers to create a Sall restriction site. This IL-7 cDNA molecule was inserted into the XhoI site of pIgTE/N (19) to create pIG17. pIgTE/N contains a murine H chain Ig enhancer ( $E\mu$ ) and a human H chain Ig promoter ( $P\mu$ ) 5' of the XhoI site and SV40 sequences containing an intron and polyadenylation signal 3' of the XhoI site. A 3.1-kb fragment of DNA containing these sequences was excised from the plasmid with Sall and BamHI and purified by agarose gel electrophoresis.

**Transgenic Animals.** All animals used in this study were obtained from Taconic Farms, Inc. (Germantown, NY) or raised in our facility. Animals were maintained under Specific Pathogen Free (SPF) conditions in microisolator cages and were handled using sterile technique in laminar flow hoods. The 3.1-kb  $E\mu P\mu$ -IL7-SVPA fragment described above was introduced into the pronuclei of 0.5 d postcoitum fertilized FVB/N eggs by microinjection (20). The injected oocytes were implanted into oviducts of pseudopregnant Swiss-Webster mice. Transgenic animals were identified by hybridization to DNA prepared from tail biopsies. A fragment derived from the same SV40 sequences as the transgene was radiolabeled and used as a hybridization probe. Two positive founders were identified, TG.UP and TG.QD, and propagated by crossing back into FVB/N. TG.UP/TG.UP animals were confirmed by breeding with wild-type animals and scoring offspring. To cross TG.UP into the *nu/nu* background, TG.UP/+ FVB/N males were bred with *nu/nu* Swiss-Webster females. Progeny from this cross were scored for the presence of the TG.UP transgene. TG.UP/+, *nu/nu* males were bred with +/+, *nu/nu* females and the resulting offspring were scored for nude phenotype and TG.UP genotype.

**Northern Blots.** RNA was prepared from tissue or cultured cells by disruption in guanidinium isothiocyanate (GIT) followed by pelleting through CsCl gradients (21). 5  $\mu$ g of RNA per lane was loaded onto formaldehyde/agarose gels for electrophoresis (21). RNA was then blotted from the gel onto Genescreen (Dupont, Wilmington, DE) membrane and cross-linked with UV light. Filters were hybridized to the same SV40 DNA probe described above, washed, and exposed to film.

**Skin Cell Suspensions.** Mice were killed and shaved if necessary (wild-type only). Pelts were removed in a single piece and scraped free of subcutaneous tissue with the back of a no. 12 scalpel. Particular attention was paid to removing all LN. Cells were dissociated from skin by an adaptation of a method used to obtain dendritic cells (22). Briefly, the skin was cut into  $\sim$ 0.5 cm pieces and incubated in 1 mg/ml trypsin in culture media without serum for 5-6 h at room temperature. Dermis and epidermis of each piece were then gently teased apart with forceps and incubated in 0.7 mg/ml trypsin, 1.4 mg/ml collagenase, 0.14 mg/ml DNase I for 40 min at 37° with frequent agitation. The suspensions were then filtered through gauze and the dissociated cells were rinsed and cultured overnight in complete medium supplemented with IL-2 and IL-7. Cells were harvested by vigorous pipetting, but strongly adherent cells were not removed from the culture dishes.

**Flow Cytometry.** Cell preparations described above, dissociated noncutaneous tissues, or cultured cells were suspended in normal saline, reacted with antibodies, and analyzed with a Cytofluorograf II (Ortho Diagnostic Systems Inc., Westwood, MA). mAb used were: Thy-1 (clone M5/49) (23); B220/CD45R (clone 6B2) (24); Mac-1 (25); and TCR- $\alpha/\beta$  (clone H57) (26). FITC goat anti-rat IgG or FITC goat anti-hamster IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) were used as secondary reagents. Commercially prepared directly conjugated mAb used were: FITC-anti-Thy1.1 (New England Nuclear, Boston, MA); PE-anti-CD4, FITC-anti-CD5, FITC-anti-CD8 (Becton Dickinson & Co., Mountain View, CA); FITC-anti-CD3, PE-anti-CD3, PE-anti-TCR- $\alpha/\beta$ , PE-anti-TCR- $\gamma/\delta$ , FITC-anti-B220/CD45R (Pharmingen, San Diego, CA); FITC-anti-CD4 (Caltag Laboratories, South San Francisco, CA); and FITC goat anti-mouse IgM (Southern Biotechnology Associates, Inc., Birmingham, AL).

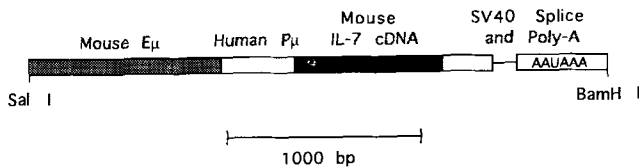
**Histology.** Tissue samples were preserved in Optimal<sup>+</sup> Fix (American Histology Reagent Co., Stockton, CA). Standard paraffin embedding, sectioning, and staining with hematoxylin and eosin was performed by the Transgenic Pathology Laboratory (University of California, Davis, CA).

**Southern Blots.** DNA was prepared from the supernatant of the GIT/CsCl gradients described above for RNA preparation, or directly from tissues and cell pellets by proteinase K digestion, phenol extraction, and ethanol precipitation. Southern blots were performed using standard techniques (21). To detect Ig H chain  $\mu$  locus rearrangements (see Fig. 8 A), DNA samples were digested with EcoRI and BamHI and hybridized to a radioactively labeled 1.5-kb PstI-PstI fragment containing  $J_H$  sequences (27). To detect TCR- $\beta$  locus rearrangements (see Fig. 8 B), samples were digested with HindIII and probed with a 2-kb EcoRI-EcoRI fragment containing  $J_2\beta$  (28). TCR- $\gamma$  rearrangements (see Fig. 8 C) were detected by digesting DNA with EcoRI and probing the filters with a 0.5-kb HindIII-HindIII fragment containing exon I of  $C\gamma 3$  (28).

**Tissue Transplants.** Portions of tumor or other noncutaneous tissue (0.1-0.3 g) were suspended in 1-2 ml PBS by repeated extrusion through a 1-ml syringe. Skin samples were dissociated by incubation in trypsin. 0.05-0.2 ml of cell suspension was injected subcutaneously or intravenously into naive syngeneic or *nu/nu* animals.

**Skin Grafts.** Pelts were prepared as for cell suspensions and cut into 1.5-2.0 cm. squares. Recipient mice were anesthetized with avertin, immobilized, and shaved. A square of flank skin was carefully removed and replaced with a piece of donor skin which was held in place with flexible collodion (Eastman Kodak Co., Rochester, NY). Vaseline gauze and plaster dressings were applied. After 8 d, the dressings and collodion were removed.

**Tissue Culture.** Tumor tissue was dissociated with forceps and



**Figure 1.** Structure of the  $E\mu P\mu$ -IL7-SVpA transgene. Map of the 3.1-kb SalI-BamHI fragment used to generate the TG.UP and TG.QD strains is shown. Construction is detailed in the text.

cultured in RPMI 1640 medium supplemented with 10% bovine calf serum, 2 mM glutamine, 50 U/ml penicillin, 50  $\mu$ g/ml streptomycin, and 50  $\mu$ M 2-ME at 37° in 7% CO<sub>2</sub>. Some cultures were supplemented with mitomycin-C-treated NAIL-7 cells or culture supernatants from NAIL-7 cells. NAIL-7 is a NIH3T3 cell that has been engineered to secrete IL-7 (Rich, B. E., and P. Leder, manuscript in preparation). IL-7 assays were performed as described (2).

## Results

**Generation of Transgenic Mice and Expression of the IL7 Transgene.** Two lines of transgenic mice were generated carrying an engineered transgene comprising (5'-3') a mouse Ig H chain enhancer, a human Ig H chain promoter, a murine IL-7 cDNA, and sequences from SV40 virus which contain an intron and a polyadenylation signal (Fig. 1). One of these lines, TG.UP, carries the transgene on an autosome, whereas in the other, TG.QD, the transgene is assumed to have integrated into the Y-chromosome as it is only inherited by males. Significant variability in the transgene's genomic Southern blot pattern and the severity of the associated phenotype were observed in the Y integrant line (TG.QD), suggesting that the locus undergoes inactivating rearrangements in this line (data not shown). In contrast, the phenotype (described below) of the autosome-borne TG.UP line is quite uniform and the transgene appears to be integrated in a stable fashion. For this reason, most of the analyses were carried out on the TG.UP line, though the TG.QD line displayed the same phenotype when the transgene was inherited intact.

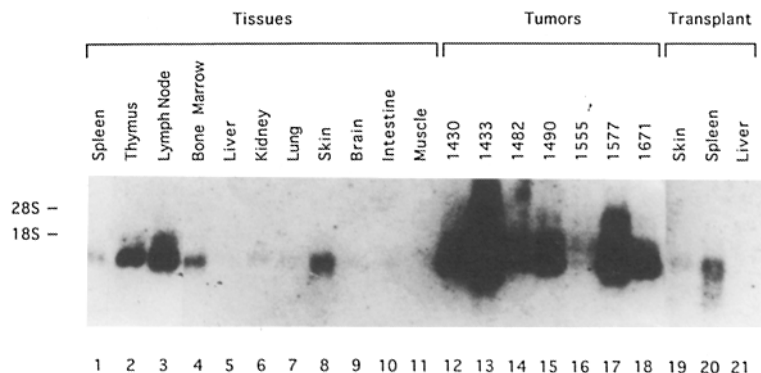
The levels of expression of the IL-7 transgene in various tissues were assessed by Northern blot analysis and the results for a heterozygous autosomal transgenic (TG.UP/+) mouse are shown in Fig. 2 (lanes 1-11). Significant levels of RNA derived from the transgene were found in the thymus, LN, bone marrow, spleen and, interestingly, the skin. Lower levels

were also detected in kidney, lung, and intestine. No transgene RNA was detected in liver, brain, or skeletal muscle.

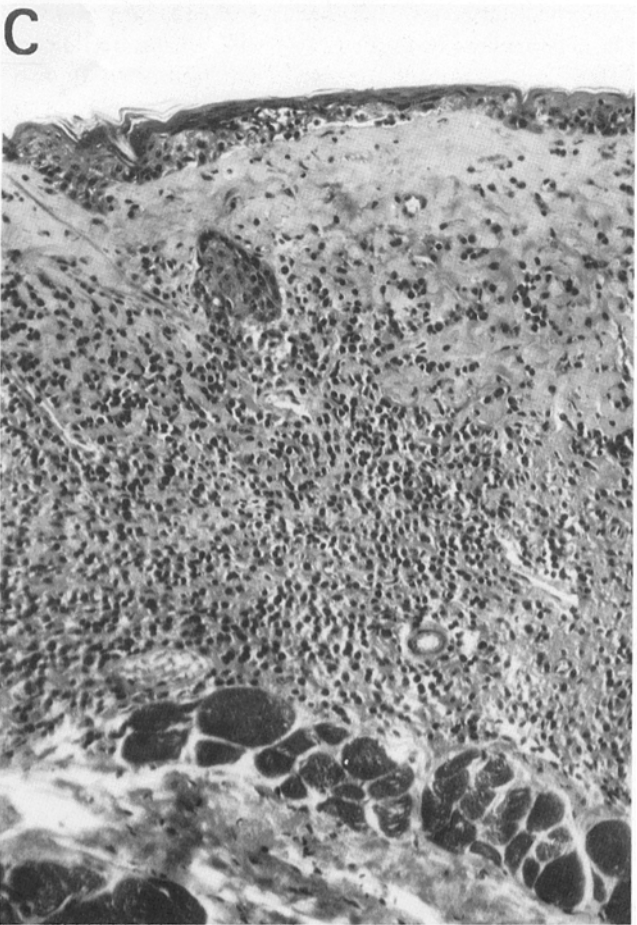
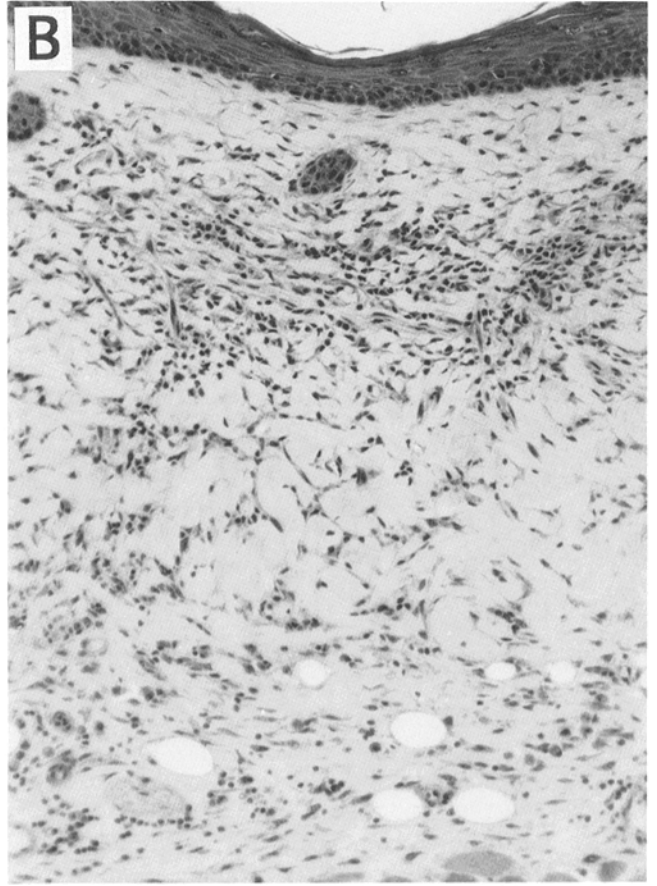
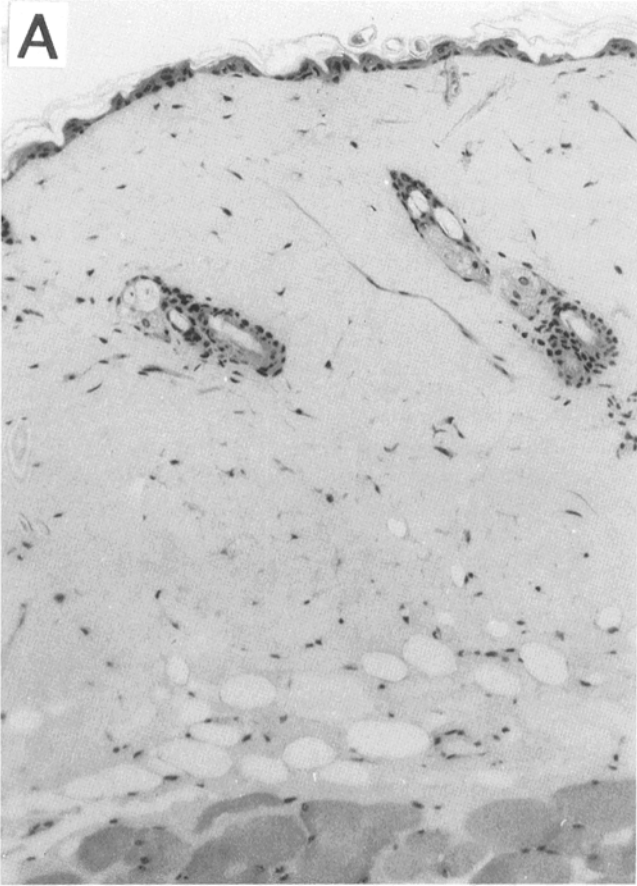
**IL7 Transgenic Mice Develop Alopecia and Abnormal Lymphoproliferation.** The most dramatic result of the transgenic expression of IL-7 is the progressive alopecia that both of these lines develop in a characteristic pattern. This phenotype is observed in all mice carrying the autosomal transgene and (as indicated above) in almost all of the mice carrying the Y chromosome-borne transgene. Heterozygous animals begin to develop the skin phenotype at about 12 wk of age. Alopecia initially develops in the inguinal area and subsequently spreads to the flank, eventually affecting most of the body. As the skin becomes increasingly affected it loses suppleness and elasticity. Homozygous (TG.UP/TG.UP) animals develop a more rapidly progressing alopecia which is first noticeable on the flank and haunches at 6-8 wk of age. Because of the accelerated development of their phenotype, it is not practical to maintain the line in the homozygous state. For the purposes of this study heterozygous TG.UP/+ mice were used unless specified otherwise.

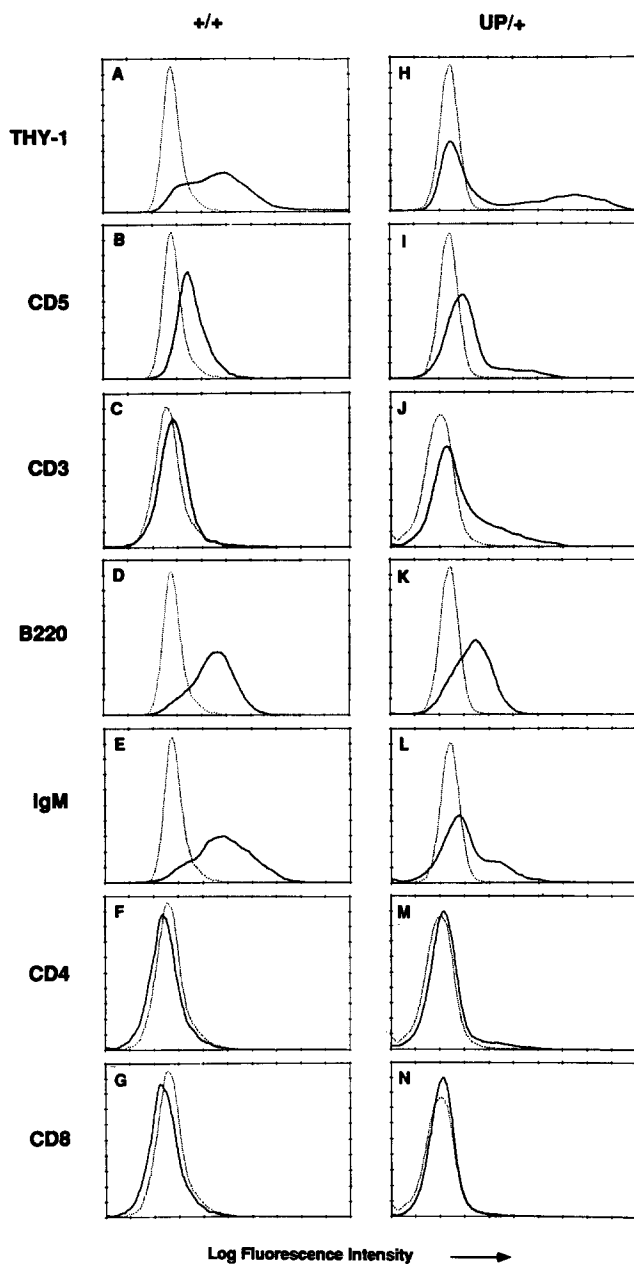
Before the skin phenotype is evident, autopsy examination of younger (6-8-wk-old) heterozygous IL-7 transgenic mice reveals splenomegaly and lymphadenopathy with a marked increase in subcutaneous vascularity. The lymphadenopathy becomes more pronounced and more evident as the alopecia becomes more extensive. In later stages, the lymphadenopathy progresses to frank lymphomatosis, and large asymmetric masses develop in some animals. In addition, the life span of the transgenic mice is shorter than that of their wild-type litter mates. Half the heterozygous carriers of the transgene are dead by about 220 d of age, whereas, wild-type FVB/N mice live roughly 2 yr in our facility.

**Skin of the Affected Transgenics Is Hyperkeratotic and Is Infiltrated with Lymphoid Cells Bearing the Markers Thy-1, CD5, and CD3.** Histologic examination of affected skin reveals atrophy and loss of hair follicles, a thickening of the epidermis, and the appearance of lymphoid infiltrates in the dermis. Figs. 3, A and B compare typical sections of skin from about 14-wk-old normal and transgenic animals. Note that the transgenic skin (Fig. 3 B) has fewer hair follicles than wild-type (Fig. 3 A) and that the remaining follicles are atrophied. Note also that the epidermis is hyperplastic and hyperkeratotic and that a prominent infiltrate of lymphoid cells is seen throughout the dermis of the transgenic skin.



**Figure 2.** Expression of the transgene. Unfractionated RNA samples isolated from tissues from transgenic (lanes 1-11) or skin graft recipient (lanes 19-21) mice, or cultured tumor cells (lanes 12-18) as indicated were separated by gel electrophoresis, blotted to a membrane, and hybridized to radiolabeled SVpA fragment. The mobilities of 18S and 28S rRNAs are indicated.





**Figure 4.** Immunofluorescent flow cytometry of dissociated dermal cells. Trypsin/collagenase-dissociated cells were prepared from dermis of wild-type (A–G) and transgenic (H–N) mice, stained with the indicated antibody and analyzed as described in the text. (Smoothed plots) Number of cells (y axis) vs log fluorescence intensity (x axis). Three divisions on the x axis represent a 10-fold increase in the intensity of fluorescence.

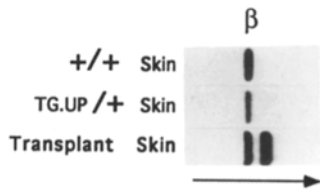
To determine the identity of the infiltrating lymphoid cells, flow analyses of cells dissociated from the infiltrated dermis of transgenic mice and from their wild-type litter mates were compared. Skin from transgenic animals was found to con-

tain increased numbers of cells expressing high levels of the Thy-1 antigen with a concomitant decrease in cells expressing low levels of Thy-1 (Fig. 4, A and H). Similar increases were also detected in the numbers of cells expressing CD5 in transgenic skin (Fig. 4, B and I) and CD3 (Fig. 4, C and J). Little reactivity with either anti-CD4 or anti-CD8 was found on any of these cells (Fig. 4, F, M and G, N, respectively). TCR- $\alpha/\beta$  molecules were detected on most of the CD3<sup>+</sup> skin-derived transgenic cells, however, a significant fraction of the cells in these preparations expressed TCR- $\gamma/\delta$  (data not shown). Note that the frequency of transgenic cells expressing surface IgM was reduced (Fig. 4, E and L), as was the level of B220 expression (Fig. 4, D and K). Thus, the predominant infiltrating transgenic cell type seems to be of the T lineage.

*The Transgenic Skin Phenotype Can Be Transmitted by Transplantation in a Cell-autonomous Fashion.* We postulated that the infiltrative skin phenotype in these transgenic mice might be brought about by circulating, IL-7-producing lymphocytes that “home” to the dermis. To test this notion, we transplanted intact transgenic and wild-type skin from littermates to syngeneic wild-type hosts. Within 8 wk, the infiltration and alopecia spread from the initially affected transgenic graft to involve all of the wild-type host skin. At autopsy, splenomegaly, lymphadenopathy, and pulmonary infiltrates were also observed. As would be expected from the gross morphology, the skin of the recipient animals was invariably found to have an inflammatory, cellular infiltrate, and the thickened, hyperkeratotic epidermis characteristic of the affected transgenic mice (Fig. 3 C). The skin of recipients of wild-type grafts remained normal (data not shown). Recipients of dissociated transgenic spleen, LN, thymus, or skin cells developed similar lesions, but more slowly. In addition, RNA derived from the transgene was detected in infiltrated skin distal to the graft site and in spleens (but not in the livers) of wild-type recipients of transgenic grafts (Fig. 2, lanes 19–21). This indicates that organ-specific migration of transgenic cells occurred and that the phenotype is conveyed in a cell-autonomous fashion.

*Infiltrated Skin from Serially Transplanted Animals Contains Clonal T Cells.* To determine the clonality of the infiltrating cells, DNA samples were extracted from the infiltrated skin of transgenic and transplanted animals and examined for rearrangements of the TCR- $\beta$  and - $\gamma$  and Ig  $\mu$  genes. No obviously unique TCR- $\beta$  rearrangement was detected in DNA extracted from the infiltrated skin of newly affected transgenic mice (Fig. 5). However, when DNA samples from the infiltrated skin of wild-type recipients of transgenic transplants were examined, unique alterations of TCR- $\beta$  genes were detected (Fig. 5). Rearrangements were not detected at the TCR- $\gamma$  or H chain Ig  $\mu$  loci, (data not shown). Indeed, two separate lines have been carried for more than eight

**Figure 3.** Skin histology. Photomicrographs of typical histological preparations of skin samples from a 15-wk-old wild-type mouse (A); a 15-wk-old TG.UP/+ transgenic mouse (B); a distal site from a syngeneic transplant recipient (C); and a *nu/nu* TG.UP/+ mouse (D).



**Figure 5.** Rearrangement of the TCR- $\beta$  gene detected in infiltrated graft recipient skin. Southern blots were performed using DNA prepared from wild-type (+/+), transgenic (TG.UP/+), or graft recipient skin as indicated. DNA samples were digested with HindIII and the filter was hybridized to a  $\beta$ -specific probe as described in the text. (→) Gel migration.

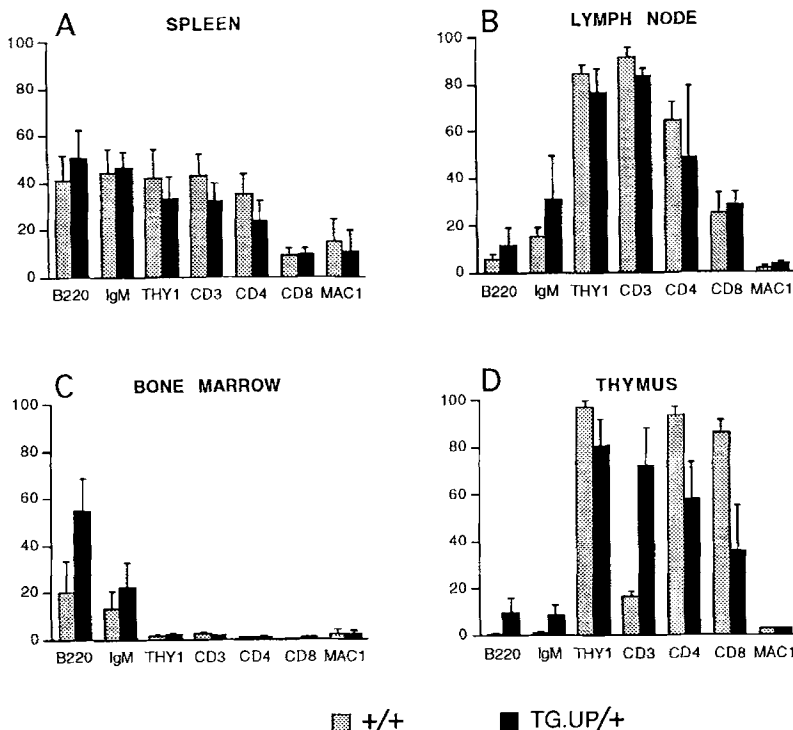
sequential transplants, and both retain rearranged TCR- $\beta$  genes. Thus the dermal infiltrate in the transgenic mice appears to arise as a polyclonal T cell response, and transplantation results in the selection of a clonal subset of these skin-homing lymphocytes.

**Dermis-infiltrating Cells Develop in the *nu/nu* Background.** Since the infiltrating cells bear T cell markers, it was important to determine what role, if any, the thymus played in their development. To do this, a mating strategy was used to introduce the IL-7 transgene into an athymic *nu/nu* background. As shown in Fig. 3 D, the characteristic dermal infiltrate readily developed in these animals, indicating that this infiltrating T cell population did not require the thymus for its development.

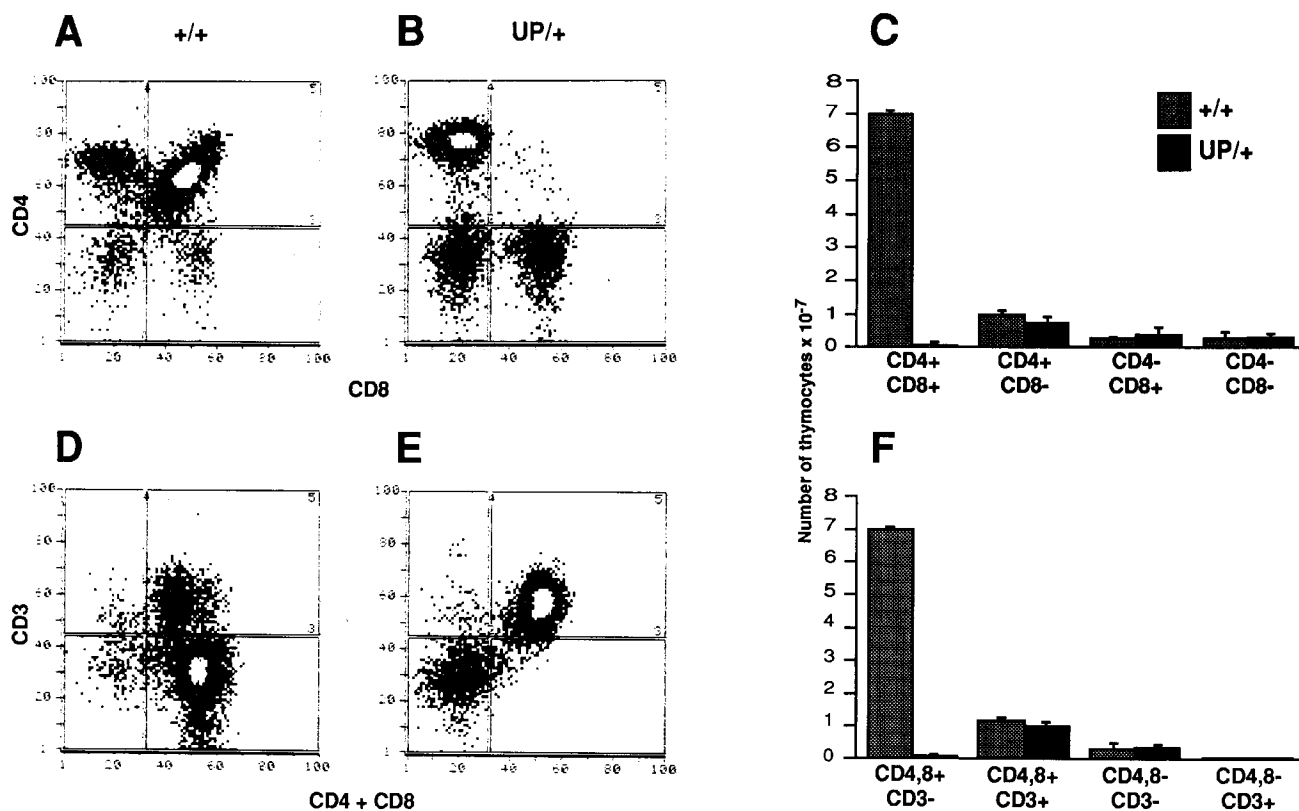
**The IL-7 Transgene Perturbs Lymphoid Cell Development.** Since IL-7 is a pleiotropic effector of lymphocytic development, it was also important to assess the effect of its aberrant expression. As shown in Fig. 6, A-C, expression of the IL-7 transgene results in increased frequencies of B220<sup>+</sup>sIgM<sup>+</sup>

cells in the bone marrow, LN, and, to a lesser extent, in the spleens of the transgenic animals. It is more remarkable that a similar population of B220<sup>+</sup>sIgM<sup>+</sup> cells are found in the thymuses of transgenic mice (Fig. 6 D), whereas B220<sup>+</sup>sIgM<sup>+</sup> cells are entirely absent in the thymuses of their wild-type litter mates. In addition, there is a concomitant reduction of the fraction of Thy-1<sup>+</sup> thymocytes in transgenic animals that can be roughly accounted for by the presence of these B220<sup>+</sup>sIgM<sup>+</sup> cells. Marked reductions are also observed in the frequencies of cells expressing CD4 and CD8 surface molecules, whereas there is a sharp increase in the percentage of thymocytes expressing the TCR-associated antigen, CD3.

To clarify this perturbation, we performed further analyses of the thymocytes of the transgenic mice using two-color flow immunofluorescence (Fig. 7). A comparison of the CD4 versus CD8 plots of wild-type and transgenic thymocytes reveals a dramatic difference and pinpoints the abnormality as an absence of double-positive (CD4<sup>+</sup>CD8<sup>+</sup>) cells. Although most of the wild-type thymocytes carry both CD4 and CD8 on their surface ( $82 \pm 1.5\%$  double-positive), very few of the transgenic thymocytes express both antigens. The largest population of the transgenic thymocytes are single-positive CD4<sup>+</sup>CD8<sup>-</sup> cells ( $47 \pm 15\%$ ), with smaller populations of single-positive CD4<sup>-</sup>CD8<sup>+</sup> cells ( $27 \pm 13\%$ ) and double-negative CD4<sup>-</sup>CD8<sup>-</sup> cells ( $21 \pm 8\%$ ) (Fig. 7, A and B). Note, however, that fewer thymocytes were recovered from the transgenic (mean of  $1.5 \times 10^7$ ) than from wild-type animals (mean of  $8.5 \times 10^7$ ). When this reduction is taken into account and the total number of cells of each population is calculated, the difference in the total number of cells is roughly accounted for by the missing



**Figure 6.** Flow cytometry of lymphoid tissues. Fractions of lymphoid cells expressing the indicated cell surface markers are represented as a percentage of the whole population of lymphoid cells dissociated from spleen (A), LN (B), bone marrow (C), and thymus (D). (Hatched columns) Percentages for wild-type tissues. (Solid columns) Percentages for transgenic TG.UP/+ tissues. Error bars, SD.



**Figure 7.** Two-color flow cytometry of thymocytes. (A) Wild-type and (B) TG.UP/+ thymocytes were stained with PE-anti-CD4 and FITC-anti-CD8 antibodies, analyzed as described, and presented in log-log scatter plots. Scales of both axes are identical to the x axis of Fig. 4. (C) Ratios determined from two-color analyses shown in A and B were used to calculate number of each thymocyte population which plotted as wild-type (hatched) or TG.UP/+ (solid) columns. (D) Wild-type and (E) TG.UP/+ thymocytes were stained with PE-anti-CD3, FITC-anti-CD4, and FITC-anti-CD8 antibodies, analyzed and presented as in A and B. (F) Number of thymocyte populations were calculated using ratios from D and E and plotted as in C.

CD4<sup>+</sup>CD8<sup>+</sup> (double-positive) cells (Fig. 7 C). Therefore, the total numbers of the three other populations of cells are not dramatically altered.

To determine whether TCR appear on the surface of the thymocytes, we stained them with a PE-labeled anti-CD3 mAb and a mixture of FITC-labeled anti-CD4 and anti-CD8 mAb and analyzed. Representative scatter plots are shown in Fig. 7, D and E. Whereas the great majority of wild-type thymocytes stain for either CD4 or CD8 or both (96% CD4, 8<sup>+</sup>), most do not express CD3 (82% of total). Indeed, only a small fraction of these CD4<sup>-</sup> and/or CD8-expressing cells also express CD3 (14% of total). The transgenic thymocytes, however, consist predominantly of a population displaying TCR as well as either CD4 or CD8 (66% CD3<sup>+</sup>CD4, 8<sup>+</sup>), accompanied by a smaller fraction of cells lacking all three antigens (25% CD3<sup>-</sup>CD4, 8<sup>-</sup>). Thus, whereas most of the wild-type thymocytes express CD4 and CD8, they do not express CD3. In contrast, most of the transgenic thymocytes express CD3 as well as either CD4 or CD8. By comparing the total number of thymocytes as described above, we see that the IL-7 transgenic mice appear to be specifically missing CD3<sup>-</sup>CD4<sup>+</sup>CD8<sup>+</sup> thymocytes, whereas the other populations of thymocytes are minimally altered (Fig. 7 F). This is consistent with the histological observation that the thymic

cortex, the primary repository of CD3<sup>-</sup>CD4<sup>+</sup>CD8<sup>+</sup> T cells in normal mice, is greatly reduced in size in these animals (data not shown).

**Tumors in IL-7 Transgenic Mice.** As a biologic effector molecule, IL-7 might be expected to perturb not only the development of lymphoid cells, but their growth as well. Thus, we noted that over time, both lines of IL-7 transgenic mice began to develop B and T cell lymphomas. Since lymphomas can be outwardly difficult to distinguish from benign lymphadenopathy, we have relied on histology and tumor transplantation to diagnose malignancy in these animals. It is remarkable that every examined transgenic animal older than 130 d had histological evidence of lymphoma ( $n = 42$ ). In some of these animals, there was evidence of more than one type of tumor (see below). Five of eight animals autopsied before they reached 130 d of age (87–126 d) had histologically abnormal LN architecture, but appeared free of neoplastic disease (data not shown). Two obviously ill younger mice (nos. 1433 and 1707, see below) had greatly enlarged (0.57 and 1.15 g) thymus glands that proved to be neoplastic. In each case, the thymus was enlarged to a point at which it appeared to impair pulmonary function. This phenomenon and other tumor-associated processes must contribute to the markedly decreased life span of these mice.

In addition to histologic examination, six tumors were transplanted to syngeneic wild-type mice and grew rapidly as tumor masses at the site of transplant. As mentioned above, cells from enlarged LN from a transgenic mouse that was subsequently judged histologically to be nonmalignant were also transplanted. These failed to form localized tumors, but months later did develop the infiltrative skin phenotype associated with the survival of transplanted, transgenic skin-homing T cells.

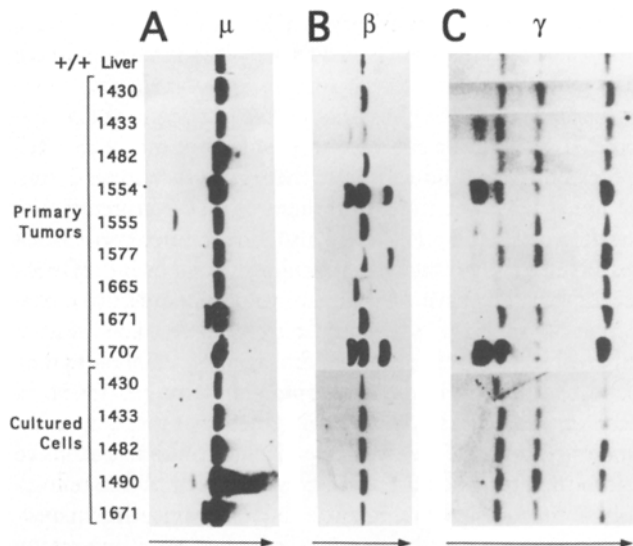
Samples of the primary tumors and cell lines derived from them were subjected to more detailed analyses to determine their cell types and clonality. Immunofluorescent flow cytometry of dissociated primary tumor tissue revealed cells bearing a series of B and T cell-associated markers. Several of the samples were essentially monoclonal with respect to these surface markers (see nos. 1430, 1490, 1555, and 1577 below and in Fig. 8), whereas others obviously contained mixtures of lymphocytes (nos. 1433, 1671, and 1707). Although much of this heterogeneity may be due to reactive cells, it is likely that some of these tumors were oligoclonal.

Seven of the tumors were successfully adapted for growth in culture and further analyzed. Each of these lines grew as a solid mass at the site of injection into FVB/N or nude mice. Immunofluorescent flow analyses of five of these cultured cell lines (nos. 1430, 1433, 1482, 1490, and 1671) revealed that they carried B lineage markers (B220 and/or IgM), whereas only one expressed T cell markers (no. 1577, Thy-1<sup>+</sup>, CD3<sup>+</sup>, TCR- $\gamma/\delta$ <sup>+</sup>, CD4<sup>-</sup>, and CD8<sup>-</sup>). This last line was also unique in that it grew in culture only in the presence of exogenous IL-7 and then, only slowly. It is interesting that early cultures of this line exhibited cytotoxic activity against

adherent feeder cell layers that were used in coculture (data not shown).

To establish the presence of clonal populations of genetically committed cells, we also assessed the primary tumors and cultured tumor cells for rearrangements of Ig and TCR genes. Southern blots of these samples are shown in Fig. 8. Rearrangements at the  $\mu$  locus (indicating commitment to the B lineage) were detected in DNA prepared from tumors or cell lines from three of the mice, nos. 1490, 1555, and 1671 (Fig. 8A). Of these three cell lines, nos. 1490 and 1671 expressed IgM; no. 1555 was not tested. Rearrangements of TCR- $\beta$  and - $\gamma$  genes were detected in samples from five of the tumors (Fig. 8, nos. 1433, 1554, 1577, 1665, and 1707). One of these grew in culture and expressed TCR (no. 1577), whereas three failed to grow. A tumorigenic cell line grew out of the fifth tumor (no. 1433), but only after prolonged culture in the presence of feeder cells and exogenous IL-7. This cell line, and two others (nos. 1430 and 1482) expressed B220, but did not appear to contain rearranged Ig or TCR genes. Thus, the cell line derived from tumor no. 1433 probably did not originate from the same clone detected in the primary tumor by virtue of its TCR- $\beta$  and TCR- $\gamma$  rearrangements (Fig. 8, B and C). In summary, the solid tumors that develop in these mice appear to be derived from both committed B and T lineage cells, as well as from lymphoid cells that may be less mature.

High levels of RNA derived from the transgene were detected in six of the seven tumor cell lines analyzed (Fig. 2, lanes 12–18) and a lower level of transgene-derived RNA was detected in the seventh (no. 1555). IL-7 activity (10–100 U/ml) was also detected in the culture supernatants of all of the lines except nos. 1555 and 1577 (data not shown). As noted above, no. 1577, which is the only T lineage tumor cell line we have been able to culture, is also the only line that requires exogenous IL-7 for growth in vitro. Consistent with the notion that IL-7 plays an autocrine role in the growth of these transformed cells, all six of the tumor lines grow poorly at low cell concentrations (<10<sup>4</sup>/ml) unless the culture medium is supplemented with exogenous IL-7. Furthermore, they are responsive to IL-7 when tested for [<sup>3</sup>H]thymidine incorporation or enhanced cloning efficiency (data not shown).



**Figure 8.** Ig and TCR gene rearrangements in tumors. Southern blots were performed using DNA prepared from the indicated tissues or cells as described in the text. (A) DNA samples were digested with EcoRI and BamHI and the filter was hybridized to a  $\mu$ -specific probe. (B) DNA samples were digested with HindIII and the filter was hybridized to a  $\beta$ -specific probe as in Fig. 5. (C) DNA samples were digested with HindIII and the filter was hybridized to a  $\gamma$ -specific probe. (→) Gel migration.

## Discussion

The phenotype associated with the expression of this transgene comprises three distinct, but interrelated phenomena. First, severe dermatological perturbations appear as a consequence of thymus-independent, T lymphoid cell infiltration of the dermis. Second, this is accompanied by distinct abnormalities of thymocyte development primarily involving an absence of double-positive thymocytes. Finally, there is a generalized lymphoproliferation that progresses to malignancy. Each of these manifestations represents an abnormality of the lymphoid system that may reflect an exaggerated, but otherwise physiologic function of IL-7.

**The Dermal Infiltrative Disorder.** In addition to being a mechanical barrier, skin is a complex immunologically active organ (29). An emerging model suggests that antigenic



or traumatic stimuli induce skin cells to release arrays of cytokines that recruit and activate effector cells of the immune response (30). Among a number of other cytokines (29, 31), IL-7 is produced by keratinocytes (32) and dendritic epidermal T cells (33).

Flow cytometry of cells dissociated from the affected skin of these IL-7 transgenic mice reveals a novel population of T cells that express Thy-1, CD3, and CD5, but lack CD4 and CD8. We postulate that the skin disorder is caused by the IL-7-induced autocrine growth and activation of these cells which migrate to the dermis, secreting IL-7, and possibly other factors. The expression of these cytokines may mimic, in part, the cutaneous immune response and cause a chronically reactive state to develop. We have tested the hypothesis that mobile IL-7-producing lymphocytes are responsible for this syndrome by transplanting affected skin or lymphoid tissue from transgenic donors to syngeneic wild-type animals. The skin response in both types of graft recipients was generalized and not confined to the site of the transplant, indicating that the disorder is conveyed by nonessile cells in a cell-autonomous fashion.

It is interesting that a similar skin phenotype has been observed in transgenic mice broadly expressing human IL-2 (34). Histological examination of the affected tissues also reveals lymphocytic infiltrates, but in contrast to our results, transplantation experiments indicate that this skin phenotype is induced by transgene expression in sessile and not mobile cells (14). Since IL-2 and IL-7 are both mitogens and T cell activators, and since IL-7 stimulates expression of IL-2 and IL-2 receptors in T cells (14), it is possible that the skin phenotypes of these two strains of mice are related.

The occurrence of the dermal infiltrative syndrome in transgenic mice bearing the *nu/nu* genotype is especially important and indicates that, unlike most T cells (35), the skin-infiltrating cells we observe mature without a strict requirement for the thymus. These transgenic lymphocytes may be related to the small numbers of CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup>TCR- $\alpha/\beta$ <sup>+</sup> cells that are normally found in murine thymus (36–38), LN (39), or human skin (40) and can have cytotoxic activity (41). The small population of normal CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup> thymocytes is unique in that such cells appear to be terminally differentiated by virtue of their singular inability to reconstitute thymuses of irradiated mice (37, 38). A fraction of these thymocytes may be derived from CD4<sup>+</sup>CD8<sup>+</sup> precursors because their TCR repertoire is modified by clonal deletion (42, 43), but some of these CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup> cells may escape this process and progress directly to maturity without ever expressing CD4 or CD8 (38, 42, 43). Thus, as with the infiltrating cells we observe in the transgenic mice, some CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup>TCR- $\alpha/\beta$ <sup>+</sup>, wild-type cells may not be entirely dependent on the thymus for their maturation. Indeed, IL-7 is normally expressed at significant levels in the murine thymus (3) and CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup> thymocytes are highly sensitive to IL-7 (44). It is plausible that the transgenic IL-7 expression in CD4<sup>-</sup>CD8<sup>-</sup>CD3<sup>+</sup> cells has allowed them to mature in the absence of a thymus.

It is also interesting to consider mechanisms that might

be responsible for the homing of the infiltrating lymphocytes to the skin. Certain surface molecules of hematopoietic cells mediate infiltration into specific tissues via binding to adhesion molecules in those tissues (45, 46). It is possible that the dermal infiltrate we observe is a result of the autocrine expression of the transgene in an IL-7-responsive cell that normally migrates to skin. Alternatively, the expression of the transgene might induce a lymphocyte not normally found in skin to migrate there by autocrine induction of skin-specific adhesion molecules or by the paracrine induction of lymphocyte-specific adhesion molecules on resident dermal cells.

*The Transgenic Syndrome and Human Disease.* The transgenic syndrome we observe shares features with certain human diseases. Whereas there are no precise human counterparts to the disorder seen in these mice, the infiltrating cells seen in certain human cutaneous T cell lymphomas appear histologically similar to those seen in these IL-7 transgenic mice. Human cutaneous lymphomas most often express the T cell markers TCR- $\alpha/\beta$ , CD3, and CD5 that are also expressed by the infiltrating cells seen in the transgenic syndrome. However, in the majority of human cases, the transformed T cells also express CD4 or CD8, although some malignancies are seen that lack both these markers (47, 48). The transgenic infiltrates are also distinct from those seen in many human cutaneous T cell lymphomas in that they do not significantly invade the epidermis. In addition to similarities to cutaneous T cell lymphoma, the polyclonal expansion of cutaneous lymphocytes is similar to certain benign human disorders (such as lymphomatoid papulosis) that are thought to be precursors of malignant disease (49, 50). Indeed, CD4<sup>-</sup>CD8<sup>-</sup>TCR- $\alpha/\beta$ <sup>+</sup> cells have been described in normal human skin (40) and in cutaneous and lymphoid infiltrates of Omenn's syndrome, a heritable, recessive, combined immunodeficiency and lymphoproliferative disease (51, 52).

*Abnormalities of T Cell Maturation.* We have found that CD4<sup>+</sup>CD8<sup>+</sup> (double-positive) thymocytes are largely missing in these adult transgenic mice. A depletion of double-positive thymocytes, similar to that which occurs in the IL-7 transgenic mice, is also seen when corticosteroid is administered in vivo or when dissociated thymocytes are cultured in the presence of IL-7 and IL-2 (10). In the case of corticosteroid treatment in vivo, this effect is due to triggering of programmed cell death in the susceptible double-positive population. Thymocytes cultured in vitro exhibit a proliferative response to IL-7, and as noted above, CD4<sup>-</sup>CD8<sup>-</sup> thymocytes are the most responsive, single-positive cells respond more modestly, and double-positive cells have no detectable response (10, 53). Thus the disappearance of double-positive thymocytes cultured in the presence of IL-7 and IL-2 reflects the enhanced survival and proliferation of the three other populations of cells (double-negative and single-positive) rather than an increased rate of cell death among double-positive cells.

It is noteworthy that the normally predominant population of double-positive thymocytes is absent in IL-7 transgenic mice whereas double-negative and single-positive cells (representing stages that immediately precede and follow, respectively, double-positives in thymocyte development) are

minimally affected. Any of several mechanisms might account for this specific loss. For example, it is possible that this loss represents accelerated death and clearance of double-positive cells that fail to mature. Alternatively, the loss of double-positive cells could represent an increased and possibly indiscriminate rate of maturation to single-positive cells and their subsequent exit to the periphery. A third possibility is that the reduction of double-positive cells could be due to a decreased rate of progression of thymocytes from the double-negative to double-positive stage. In light of the *in vitro* results described above, the first of these explanations, that is, accelerated death and clearance of double-positive cells, seems most likely, although none of the other possibilities can be ruled out. It also remains to be determined whether this effect is caused by expression of IL-7 in T cell precursors or in the small percentage of B220<sup>+</sup>sIg<sup>+</sup> cells that are found in the thymus. B lineage thymocytes are not normally detected in significant levels, although they are found in some autoimmune mice and have been seen previously in transgenic mice with hyperproliferative B cells (54).

*IL-7 As Autocrine Tumor-provoking Cytokine.* In that IL-7 is a potent growth factor, it is reasonable to expect that it might act as a factor in lymphoid tumorigenesis, conceivably in an autocrine manner. We have seen that the expression

of IL-7 in the lymphoid compartment of these transgenic mice leads to a prolonged preneoplastic polyclonal lymphoproliferative state followed by the development of lymphomas. These lymphomas and thymomas are readily distinguished from the pathogenic skin-infiltrating cells by their pattern of growth in transplant experiments. Moreover, these tumors are of both B and T lineages. Although IL-7 expression by itself is not sufficient to transform pre-B cells *in vitro* (55), our experiments indicate that prolonged expression *in vivo* does promote tumor development. The resulting transgenic tumors are in many cases readily adapted to culture, and the fact that the tumors both secrete and respond to IL-7 suggests that IL-7 might be acting as an autocrine growth factor. We have not unambiguously demonstrated that continuous IL-7 expression in these cells is essential for transformation, however, the reduced ability of these cells to grow at low density without exogenous IL-7 suggests that this might be the case. Since these tumors are largely monoclonal, we infer that secondary events are necessary to cause their progression to tumorigenesis. These putative secondary events might be rendered more likely by IL-7 by inducing a proliferative state in target cells and/or by expanding the population of cells at risk for tumor formation.

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We thank T. Vasicek for assistance with intravenous injections; M. Harty for instruction in skin graft technique; C. Daugherty for assistance with cell culture; and W. Havran for helpful discussions about skin lymphocyte preparations. We thank R. Cardiff and the Transgenic Pathology facility of The University of California at Davis for assistance with histological analysis, and D. Taber for careful maintenance of the mice.

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*Received for publication 15 September 1992 and in revised form 16 October 1992.*

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