9. D. D. Taub, unpublished data.
10. Neutrophils may be participating in the induction of IL-8–mediated T cell chemotaxis because T cells do migrate in response to IL-8 in neutrophil-depleted rats (*19*). Our lymphocyte preparations may be free of contaminating neutrophils or of other cells that produce such an indirect mechanism of T cell chemotaxis, and therefore lymphocyte attraction in response to IL-8 was not detected.
11. To distinguish between chemotaxis (active and directional cell migration) and chemokinesis (random cell migration), we performed a T cell migration assay in which MIP-1α, MIP-1β, and RANTES were added at equal concentrations on both sides of the collagen-coated polycarbonate filter. Under these conditions, migration was substantially reduced, demonstrating that a concentration gradient is required for active T cell migration to MIP-1α, MIP-1β, and RANTES. Less than 8% of T cell migration to these chemokines could be attributed to chemokinesis.
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14. Human tetanus toxoid– and diptheria toxoid–reactive CD4+ T cell clones and cell lines were established in our laboratory with standard cloning procedures (*12*). Several clones were isolated and were found to be CD4+ and CD29+ T cells with a T helper cell–1–like lymphokine profile. We maintained these cells on a 15- to 21-day stimulation schedule by using irradiated autologous peripheral blood mononuclear cells and antigen (2 μg/ml) and rhIL-2 (25 U/ml). Before use, T cell clones were passaged over a Ficoll-Hypaque gradient to enrich for viable cells.
15. Several TTx- and diptheria toxoid (DTx)–reactive T cell clones were tested for chemotactic activity. Four CD4+ TTx-reactive clones migrated to rhMIP-1β and RANTES but not to rhMIP-1α. One DTx-reactive CD4+ T cell clone did not respond to rhMIP-1α, rhMIP-1β, and RANTES. Additionally, several long-term nonclonal TTx- and DTx-reactive CD4+ T cell lines demonstrated a preferential migration to MIP-1β and not to MIP-1α. CD8+ alloreactive T cell lines and clones demonstrated variable responses to both MIP-1 compounds.
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Requirement for Tyrosine Kinase p56^{lck} for Thymic Development of Transgenic γδ T Cells

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The Src-related protein tyrosine kinase p56^{lck} is essential for antigen-specific signal transduction and thymic maturation of T cells that have an αβ T cell receptor (TCR), presumably by physical association with CD4 or CD8 molecules. To evaluate the requirement for p56^{lck} in the development of T cells that have γδ TCRs, which generally do not express CD4 or CD8, p56^{lck} mutant mice were bred with TCRγδ transgenic mice. Few peripheral cells that carried the transgenes could be detected in p56$^{lck-/-}$ mice, although 70 percent of thymocytes were transgenic. Development of transgenic γδ$^+$ thymocytes was blocked at an early stage, defined by interleukin-2 receptor α expression. However, extrathymic development of CD8αα$^+$TCRγδ$^+$ intestinal intraepithelial lymphocytes appeared to be normal. Thus, p56^{lck} is crucial for the thymic, but not intestinal, maturation of γδ T cells and may function in thymic development independently of CD4 or CD8.

The molecular requirements for the development of TCRγδ$^+$ lymphocytes are poorly characterized (*1*). Thymic development of TCRαβ cells unfolds in stages defined by expression of CD4 and CD8 (*2*). Early CD4$^-$CD8$^-$ precursors become CD4$^+$CD8$^+$ cells and finally mature into CD4$^+$ or CD8$^+$ single-positive cells. Specific recognition of self major histocompatibility complex (MHC) molecules by the TCRαβ$^+$ thymocytes is the prerequisite for thymic selection events and maturation to single-positive lymphocytes (*2*). Besides the specific antigen receptor, CD4 and CD8 molecules are crucial for thymic selection and maturation of TCRαβ$^+$ lymphocytes (*3*).

Antigen-specific stimulation of T cells results in the activation of protein tyrosine kinases and subsequent tyrosine phosphorylation of several intracellular substrates (*4*). The Src-related protein tyrosine kinase p56^{lck} (*5*) is essential for antigen-induced signal transduction mediated by TCRαβ-CD3 complexes (*6*). Because p56^{lck} is physically associated with the cytoplasmic portions of CD4 or CD8 molecules (*7*) expressed on TCRαβ$^+$ cells, activation of αβ$^+$ T cells is probably modulated by CD4 or CD8 molecules by means of p56^{lck} (*8*); mice rendered deficient for p56^{lck} by homologous recombination have a block in thymic development of TCRαβ$^+$ cells and few TCRαβ$^+$ cells are present in the lymph nodes and spleens of these mice (the number of TCRαβ$^+$ cells in p56$^{lck-/-}$ mice is 5 to 15% of that in p56$^{lck+/-}$ and p56$^{lck+/+}$ controls) (Fig. 1) (*9*). However, the role of p56^{lck} in signal transduction and in the development of TCRγδ$^+$ lymphocytes, which generally do not express CD4 or CD8 accessory molecules, is not known (*1*).

To evaluate the requirement for p56^{lck} in the development of γδ$^+$ T cells, we crossed the p56^{lck} mutation (*9*) into mice that expressed a transgenic (tg) γδ TCR (V_α11.3J_δ1C_δ/V_γ2J_γ1C_γ1) (*10, 11*). In these mice, approximately 70% of peripheral T cells express the tg γδ TCR, as assessed by staining with a fluoresceinated monoclonal antibody (mAb) to V_γ2 (Fig. 1) (*11, 12*). The total number of T lymphocytes in the lymph nodes and spleens of tg$^{+/+}$p56$^{lck+/+}$ mice was equivalent to the numbers found in tg$^{-/-}$p56$^{lck+/+}$ wild-type mice. The total number of T cells in the spleen and lymph nodes of heterozygous tg$^{+/-}$p56$^{lck+/-}$ mice was similar to that in tg$^{+/+}$p56$^{lck+/+}$ mice, although the number of cells that expressed V_γ2 was slightly lower (~60% of the total number of T cells).

In contrast to p56$^{lck+/+}$ and p56$^{lck+/-}$ mice, V_γ2$^+$ T lymphocytes were nearly absent from the spleen, blood, and lymph nodes of tg$^{+/-}$p56$^{lck-/-}$ mice, which thus resembled tg$^-$p56$^{lck-/-}$ animals (Fig. 1). In addition, the total peripheral T cell number in tg$^{+/-}$p56$^{lck-/-}$ and tg$^{-/-}$p56$^{lck-/-}$ mice was reduced as described (Fig. 1) (*9*). Thus, tg V_γ2$^+$ lymphocytes were absent in the periphery of p56^{lck}-deficient mice, and the presence of rearranged TCRγδ transgenes could not restore normal numbers of peripheral T cells.

The V_α11V_γ2 TCR expressed in these tg mice was originally cloned from a BALB/c

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($H\text{-}2^d$) *nu/nu*-derived T cell line with alloreactivity against MHC class I Tla molecules of $H\text{-}2^b$ mice (*10*). Thymocytes that express this γδ TCR undergo positive selection mediated by $H\text{-}2^d$ class I molecules (*11, 12*). Because the most profound defect in $p56^{lck-/-}$ mice is a severe block in thymic maturation (9), we wanted to know whether tg $V_\alpha 11V_\gamma 2$ T cells could mature in the thymus of $tg^{+/-}p56^{lck-/-}$ animals even though cells that expressed the tg γδ TCR were not present in peripheral organs. In $tg^{-/-}p56^{lck+/+}$ mice, fewer than 1% of thymocytes expressed $V_\gamma 2$, whereas ~75% of thymocytes in $tg^{+/+}p56^{lck+/+}$ mice were positive for $V_\gamma 2$ expression (Fig. 2). Most (>90%) tg $V_\gamma 2^+$ thymocytes did not coexpress CD4 or CD8 molecules (Fig. 2) (*11, 12*). The same results were obtained in $tg^{+/-}p56^{lck+/-}$ mice. Because of a maturational defect, 20 to 40% of thymocytes in $tg^{-/-}p56^{lck-/-}$ mice had a $CD4^-CD8^-$ phenotype, whereas 60 to 80% of thymocytes were $CD4^+CD8^+$ double-positive; mature $CD4^+$ or $CD8^+$ single-positive cell populations were also reduced (Fig. 2) (9). The expression of $V_\gamma 2$ on thymocytes of $tg^{-/-}p56^{lck-/-}$ mice was similar to that in normal $tg^{-/-}p56^{lck+/+}$ mice (Fig. 2). In contrast, $tg^{+/-}p56^{lck-/-}$ mice had a larger $CD4^-CD8^-$ double-negative population (70%), almost all of which expressed the tg $V_\gamma 2$ TCR (Fig. 2). The total number of thymocytes in $tg^{+/+}p56^{lck+/+}$, $tg^{+/-}p56^{lck+/-}$, $tg^{+/-}p56^{lck-/-}$, and $tg^{-/-}p56^{lck-/-}$ mice was essentially the same (3.5×10^6 to 10×10^6 versus 1×10^8 in normal $tg^{-/-}p56^{lck+/+}$ mice), which indicates that the lack of $V_\gamma 2^+$ cells in the periphery of $tg^{+/-}p56^{lck-/-}$ mice was not related to a reduction in thymic size or reduced numbers of developing $V_\gamma 2^+$ thymocytes.

During development, 50% of early $CD4^-CD8^-$ precursors transiently express the interleukin-2 receptor (IL-2R) α chain, whereas thymocytes at later maturational stages are negative for IL-2Rα expression (*13*). However, the IL-2Rα chain was expressed on ~30% of thymocytes in $tg^{-/-}p56^{lck-/-}$ mice (Fig. 3). A large percentage (60%) of $CD4^-CD8^-$ thymocytes in $tg^{+/-}p56^{lck-/-}$ mice also displayed surface expression of the IL-2Rα chain (Fig. 3), which suggests that tg $V_\gamma 2^+$ cells are blocked at an early stage of development. In addition, virtually all tg $V_\gamma 2^+$ thymocytes in $p56^{lck-/-}$ mice had intermediate to high heat-stable antigen (HSA) surface expression, another marker of immature thymocytes (*14*), whereas approximately 10% of tg $V_\gamma 2^+$ thymocytes in $p56^{lck+/+}$ mice were phenotypically mature, as defined by expression of small amounts of HSA.

To demonstrate whether the immature

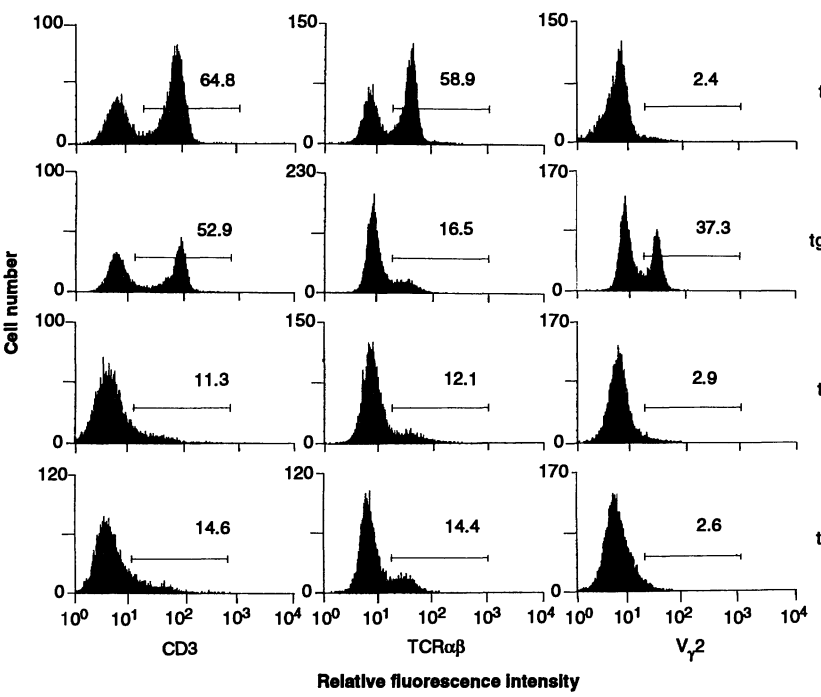

Fig. 1. Flow cytometric analysis of CD3, TCRαβ, and tg $V_\gamma 2$ expression in spleens of $tg^{-/-}p56^{lck+/+}$; $tg^{+/+}p56^{lck+/+}$; $tg^{-/-}p56^{lck-/-}$; and $tg^{+/-}p56^{lck-/-}$ mice. Numbers indicate percentages of CD3-, TCRαβ-, or TCRγδ-positive cells among total splenic lymphocytes. Total numbers of spleen cells were 3.4×10^7 for $tg^{-/-}p56^{lck+/+}$; 3×10^7 for $tg^{+/+}p56^{lck+/+}$; 4.5×10^7 for $tg^{-/-}p56^{lck-/-}$; and 4×10^7 for $tg^{+/-}p56^{lck-/-}$ mice. Immunofluorescence analysis of CD3, TCRαβ, and $V_\gamma 2$ expression in lymph node cells and peripheral blood lymphocytes yielded similar results. For flow cytometry, single-cell suspensions were prepared from red blood cell–depleted spleens, and 1×10^6 cells were stained with directly conjugated mAb to TCRαβ and TCR$V_\gamma 2$ (both fluorescein-labeled; PharMingen) or biotinylated mAb to CD3 (PharMingen) for 30 min at 4°C in immunofluorescence buffer (phosphate-buffered saline, 1% bovine serum albumin, and 0.01% NaN_3). Biotinylated mAbs were visualized with streptavidin-RED613 (Gibco). Samples were analyzed with a Lysis-II program on a FACScan (Becton Dickinson). All animals were screened by Southern (DNA) blotting for the $p56^{lck}$ mutation and integration of the γδ transgene as described (*9–12*).

Table 1. Response of $V_\gamma 2^+$ thymocytes to allogeneic stimulator cells. Thymocyte responders (1×10^5) were incubated with 5×10^5 T cell–depleted (mAb to Thy-1.2 and rabbit complement) and irradiated (2000 rads) spleen stimulator cells from MHC syngeneic (BALB/c, $H\text{-}2^{d/d}$) or MHC allogeneic (C57Bl6, $H\text{-}2^{b/b}$) mice in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum (*12*). After 48 hours, the plates were pulsed with [^{3}H]thymidine (1 μCi per well) for 12 hours. Mean values of triplicate cultures ± SD of the mean are shown. Responder cells were single-cell suspensions of total thymocytes. In tg $p56^{lck+/+}$ and tg $p56^{lck-/-}$ mice, 70% of total thymocytes were $V_\gamma 2^+$, whereas fewer than 1% of nontransgenic control mice were $V_\gamma 2^+$.

Responder cells ($H\text{-}2^{d/d}$)	Stimulator cells	
	$H\text{-}2^{d/d}$	$H\text{-}2^{b/b}$
$tg^{-/-}p56^{lck+/+}$	419 ± 189	567 ± 177
$tg^{+/+}p56^{lck+/+}$	223 ± 10	15,279 ± 3517
$tg^{-/-}p56^{lck-/-}$	153 ± 31	184 ± 25
$tg^{+/+}p56^{lck-/-}$	181 ± 28	231 ± 81

Table 2. Subsets of IELs in $p56^{lck+/+}$ and $p56^{lck-/-}$ mice. IELs were isolated as described (*15*) and triple-stained with mAbs to TCRαβ or TCRγδ (both FITC-labeled; PharMingen), CD8α (Lyt-2; PE-conjugated; PharMingen), and CD8β (Lyt-3; biotinylated; PharMingen); mAbs to TCRαβ or TCRγδ (FITC-labeled), CD8α (Lyt-2; PE-conjugated), and CD4 (biotinylated; PharMingen); or mAbs to TCRαβ or TCRγδ (FITC-labeled), CD4 (PE-conjugated; PharMingen), and CD8β (biotinylated). Biotinylated mAbs were visualized with streptavidin-RED. Expression of TCRαβ and TCRγδ on IELs was mutually exclusive. The presence of $CD4^+CD8^+$ double-positive IELs has been described (*19*). These cells are probably local precursors. Total numbers of IELs were 7.2×10^6 ($\pm 2.1 \times 10^6$) for $p56^{lck+/+}$ and 4.8×10^6 ($\pm 1.4 \times 10^6$) for $p56^{lck-/-}$ mice. Numbers indicate percentages ± SD of T cell subsets among total IELs. At least four mice were included in each group.

IEL subsets	Positive cells (%)	
	$p56^{lck+/+}$	$p56^{lck-/-}$
$TCR\alpha\beta^+CD4^-CD8\alpha\beta^+$	33.2 ± 4.1	20.2 ± 4.9
$TCR\alpha\beta^+CD4^-CD8\alpha\alpha^+$	25.2 ± 4.1	16.3 ± 4.1
$TCR\alpha\beta^+CD4^+CD8\alpha\alpha^+$	5.6 ± 3.4	2.9 ± 0.7
$TCR\alpha\beta^+CD4^+CD8^-$	7.2 ± 2.8	6.7 ± 2.4
$TCR\gamma\delta^+CD4^-CD8\alpha\alpha^+$	23.4 ± 5.9	48.2 ± 8.5
$TCR\gamma\delta^+CD4^-CD8^-$	5.3 ± 0.6	5.7 ± 2.1

phenotype of $V_\gamma2^+$ cells in $p56^{lck-/-}$ mice was also accompanied by functional incompetence, we stimulated $V_\gamma2^+$ thymocytes from *H-2*^{d} mice with allogeneic *H-2*^{b} or syngeneic *H-2*^{d} stimulator cells (*11, 12*). The tg $V_\alpha11V_\gamma2$ TCR is alloreactive to MHC class I Tla^b molecules (*10*), and $V_\gamma2^+$ thymocytes derived from $p56^{lck+/+}$ mice readily responded to *H-2*^{b} stimulators (Table 1). In contrast, $V_\gamma2^+$ thymocytes from $p56^{lck-/-}$ mice did not respond to allogeneic stimulator cells (Table 1).

To test whether extrathymic development of γδ T cells could occur in $p56^{lck-/-}$ mice, we isolated intraepithelial lymphocytes (IEL) from the intestines of $p56^{lck-/-}$ mice (*15*). The intestinal epithelium is a thymus-independent site of lymphopoiesis for both $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells (*15*). Virtually all IELs that develop locally in the intestinal environment express CD8αα (Lyt-2/Lyt-2) homodimers, whereas most thymus-derived IELs express CD8αβ (Lyt-2/Lyt-3) heterodimers (*15*). In $p56^{lck+/+}$ mice, the IEL compartment contained the following T cell subsets: $CD8\alpha\beta^+TCR\alpha\beta^+$ (33%), $CD8\alpha\alpha^+TCR\alpha\beta^+$ (25%), and $CD8\alpha\alpha^+TCR\gamma\delta^+$ (23%) (Table 2). As compared to IELs from $p56^{lck+/+}$ mice, the total number of $CD8\alpha\beta^+TCR\alpha\beta^+$ and $CD8\alpha\alpha^+TCR\alpha\beta^+$ IELs was reduced by more than 50% in $p56^{lck-/-}$ mice (5.1×10^6 in $p56^{lck+/+}$ mice versus 2.2×10^6 in $p56^{lck-/-}$ mice) (Table 2). In contrast to $TCR\alpha\beta^+$ IELs, the total number of $CD8\alpha\alpha^+TCR\gamma\delta^+$ IELs was similar to that in normal mice (2.1×10^6 in $p56^{lck+/+}$ mice versus 2.6×10^6 in $p56^{lck-/-}$ mice) (Table 2). Thus, $p56^{lck}$ is probably not essential for extrathymic maturation of $CD8\alpha\alpha^+\gamma\delta^+$ IELs.

Our data show that $p56^{lck}$ is essential for the thymic development of $\gamma\delta^+$ T cells. Because only a small number (<10%) of tg $TCR\gamma\delta^+$ thymocytes co-express CD4 or CD8 molecules (*11, 12*), it is tempting to speculate that the block in thymic development of TCRγδ and TCRαβ cells in $p56^{lck}$-deficient mice is not CD4- or CD8-dependent but rather involves other signal-transducing molecules that potentially interact with $p56^{lck}$ and are vital for thymic development—for example, the IL-2R (*13, 16*) or phosphatidylinositol glycan–linked molecules such as Thy-1 (*17*). Using a mutant T cell line, others have suggested that $p56^{lck}$ may function independently of CD4 or CD8 or any other receptor molecule (*6*). Mice deficient for both CD4 and CD8 molecules have normal numbers of thymocytes, and thymic differentiation is blocked at a much later stage of development—that is, at the transition from the immature $TCR\alpha\beta^{low}$ stage to the mature $TCR\alpha\beta^{high}$ stage (*18*), which further indicates that the developmental block in $p56^{lck-/-}$ thymocytes is independent of CD4 or CD8 signaling.

Although the protein tyrosine kinase $p56^{lck}$ was crucial for thymic development and function of tg $TCR\gamma\delta^+$ lymphocytes, extrathymic development of $TCR\gamma\delta^+$ T cells probably still occurs within the intestinal epithelium of $p56^{lck-/-}$ mice. Thus, during the development of cells from the same lineage signal transduction by $p56^{lck}$ kinase may be required differentially, depending on the site of maturation.

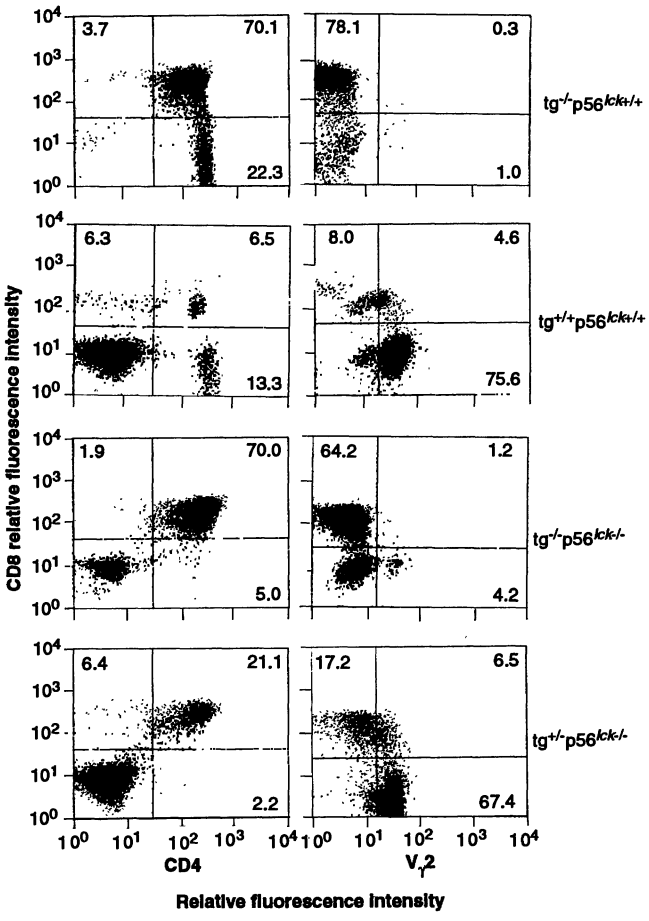

Fig. 2. Immunofluorescence analysis of thymocytes from $tg^{-/-}p56^{lck+/+}$; $tg^{+/+}p56^{lck+/+}$; $tg^{-/-}p56^{lck-/-}$; and $tg^{+/-}p56^{lck-/-}$ mice. Numbers indicate percentages of positive cells within a region. Analysis of $tg^{+/-}p56^{lck+/-}$ heterozygous mice showed essentially the same results. It should be noted that TCRαβ and $V_\gamma2$ expression on thymocytes of all mice were mutually exclusive (*11*). Total numbers of thymocytes in this experiment were 9×10^7 for $tg^{-/-}p56^{lck+/+}$; 5.4×10^6 for $tg^{+/+}p56^{lck+/+}$; 5.1×10^6 for $tg^{-/-}p56^{lck-/-}$; and 3.5×10^6 for $tg^{+/-}p56^{lck-/-}$ mice. Thymic cell suspensions (1×10^6) were double-stained with mAbs to $V_\gamma2$ [fluorescein isothiocyanate (FITC)–conjugated] and CD8 (biotinylated) or mAbs to CD8 (biotinylated) and CD4 [phycoerythrin (PE)-labeled] as indicated. Biotinylated mAbs were visualized with streptavidin-RED613. Staining procedures and analyses were as in Fig. 1.

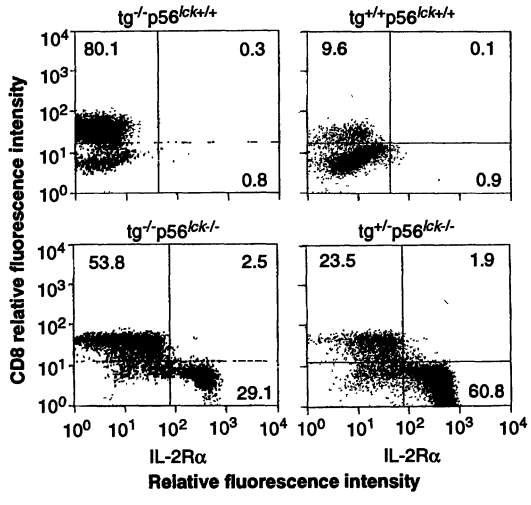

Fig. 3. Expression of the IL-2Rα chain on thymocytes from $tg^{-/-}p56^{lck+/+}$; $tg^{+/+}p56^{lck+/+}$; $tg^{-/-}p56^{lck-/-}$; and $tg^{+/-}p56^{lck-/-}$ mice. Shown are dot blot samples double-stained with mAbs to IL-2Rα and CD8. Double-staining thymocytes with mAbs to IL-2Rα and CD4 also revealed that virtually all IL-2Rα$^+$ thymocytes in $p56^{lck}$-deficient mice did not co-express CD4 or CD8 molecules. Numbers indicate percentages of positive cells within a quadrant. Staining techniques and analysis were as described in Figs. 1 and 2. The mAb to CD8 (PharMingen) was directly FITC-conjugated and the biotinylated mAb to IL-2Rα was visualized with streptavidin-RED613.

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Tumor Cell Growth Arrest Caused by Subchromosomal Transferable DNA Fragments from Chromosome 11

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A fundamental problem in the identification and isolation of tumor suppressor and other growth-inhibiting genes is the loss of power of genetic complementation at the subchromosomal level. A direct genetic strategy was developed to isolate subchromosomal transferable fragments (STFs) from any chromosome, each containing a selectable marker within the human DNA, that could be transferred to any mammalian cell. As a test of the method, several overlapping STFs from 11p15 were shown to cause in vitro growth arrest of rhabdomyosarcoma cells. This activity mapped between the β-globin and insulin genes.

The existence of tumor suppressor genes was first established by genetic complementation, which demonstrated that tumor cells fused to normal cells lose tumorigenicity (*1*). Studies have shown that suppression can also be detected by transfer of monochromosomes into tumor cells (*2*, *3*). However, direct expression cloning of suppressor genes in manageable vectors usually is not possible because growth suppression is normally selected against. Furthermore, although yeast artificial chromosomes (YACs) have been transferred to mammalian cells (*4*), success has been limited to small genes and specific cell types, and assaying for tumor suppression with the thousands of YACs needed for a whole chromosome is impractical. We therefore sought to develop a strategy for transferring subchromosomal fragments intermediate in size between YACs and chromosomes.

Our strategy involves three steps, outlined in Fig. 1: (i) transfection of a mammalian selectable marker gene into mouse cells containing a single independently selectable human chromosome; (ii) transfer of the chromosome by microcell fusion, followed by double selection for both the human chromosome and the marker gene; and (iii) isolation of individual marker-containing chromosomal subfragments by transfer of irradiated microcells from the pooled hybrid panel. Unlike conventional radiation hybrids (*5*), each resulting fragment can then be transferred independently to mammalian cells, owing to the presence of the selectable marker gene.

We isolated 150 separate hybrids of monochromosome 11 that were resistant to selection in hypoxanthine, aminopterin, and thymidine (HAT^R) plus G418 ($G418^R$). We transferred irradiated microcells from 90 of the hybrids, isolating 85 *neo*-containing subfragments of chromosome 11. Of these, 14 were positive by Southern (DNA) blotting for 11p15 sequences, representing 12 independent *neo*-integration sites (a result expected from random *neo* integration in the original transfection). Nine 11p15 subfragments were transferred from A9 cells to Chinese hamster ovary (CHO) cells by microcell fusion, and the pulsed-field gel electrophoresis (PFGE) pattern of donor cell DNA, digested with rare-cutting restriction enzymes, was compared to that of the recipient cells. Hybridization with a human repetitive sequence allowed visualization of the individual human PFGE fragments. The amount of human sequence (sum of the PFGE fragments) could be estimated in seven of nine cases with <10 megabase pairs (Mbp) and ranged from 3.5 to 9.5 Mbp (average, 6.8 Mbp). Eight of nine hybrids showed identical PFGE patterns in donor A9 and recipient CHO cells (Fig. 2), and one showed a different pattern of a single band, possibly resulting from methylation differences or rearrangement. Thus, 61 of 62 PFGE fragments within the chromosomal subfragments remained unchanged after transfer. In addition, pSV2neo always mapped to Alu-positive human PFGE fragments (Fig. 2). Thus, these chromosomal subfragments were intermediate in size between YACs and chromosomal bands, contained a selectable marker within the human DNA, and were stably transferable to mammalian cells. We therefore termed these chromosomal fragments "subchromosomal transferable fragments," or STFs, to distinguish them from conventional nontransferable radiation hybrid fragments.

We used rhabdomyosarcoma and 11p15 for experiments on tumor suppression for the following reasons: (i) 11p15 shows loss of allelic heterozygosity (LOH, implying the presence of a tumor suppressor gene) in many types of tumor, including rhabdomyosarcoma, Wilms tumor, and other embryonal tumors (6–9), as well as tumors of the bladder, lung, ovary, liver, and breast (*10*–

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