EXPANSION OF NATURAL KILLER CELLS BUT NOT T CELLS IN HUMAN INTERLEUKIN 2/INTERLEUKIN 2 RECEPTOR (Tac) TRANSGENIC MICE

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IL-2 is a potent growth factor for T lymphocytes in vitro (1). Stimulation of T cells by an antigen induces expression of IL-2 and/or IL-2-R L chain (Tac) and allows proliferation of T cell clones recognizing the particular antigen (2). The controlled expression of IL-2 and IL-2-R L chain is thus speculated to be involved in antigen-specific clonal expression of peripheral T cells and possibly in the development of the T cell repertoire in the thymus (3, 4). On the other hand, the deregulated expression of IL-2-R L chain is suggested to result in some T cell malignancies such as adult T cell leukemia (ATL)[1] (5–7).

We have already reported some properties of transgenic mice bearing cDNA for human IL-2 or IL-2-R L chain (8, 9). The expression of these cDNAs is directed by the constitutive H-2K^d promoter. Unstimulated spleen cells of the IL-2-R transgenic mice had ~120 high affinity binding sites for IL-2 per cell, and proliferated in vitro in the presence of exogenous human rIL-2, indicating the constitutive expression of the H chain of IL-2-R in unstimulated normal spleen cells (9). We have not observed any abnormality in this transgenic mouse, probably because endogenous murine IL-2 has orders of magnitude weaker binding affinity to the human IL-2-R than to the murine counterpart. By contrast, alopecia and pneumonia were observed in the IL-2 transgenic mice whose sera contained 30–500 pM human IL-2 (8). The IL-2 transgenic mice died within 1 yr after birth.

The constitutive expression of both human IL-2 and IL-2-R L chain in a single transgenic animal could induce autocrine stimulation of lymphocytes, giving rise to several abnormalities as follows. First, the antigen-independent activation of the IL-2/IL-2-R pathway might cause polyclonal T cell proliferation, which results in nonspecific immune responses including autoimmunity. Second, the constitutive ex-

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[1] *Abbreviations used in this paper:* ATL, adult T cell leukemia; LGL, large granular lymphocytes; PE, phycoerythrin.

pression of IL-2 and its receptor in the thymus could somehow affect the T cell development. Third, some kind of T cell leukemia/lymphoma-like ATL may arise in the animal. Fourth, unusual expansion of the cells bearing the H chain of IL-2-R may be observed.

To test these possibilities, we produced the hybrid mice that carried both human IL-2 and IL-2-R L chain transgenes by crossing the two parental strains (8, 9). The hybrid mice showed severe growth retardation and died by 4 wk of age. The spleens of the mice contained an unusually large number of Thy-1$^+$/CD3$^-$ large granular lymphocytes (LGL), which bore the elevated NK activity. In addition, we found unexpectedly the selective loss of Purkinje cells in the cerebellum of the mice. However, we have not observed any signs of typical autoimmunity or incidence of leukemia/lymphoma in the hybrid mice.

Materials and Methods

Animals. Construction and properties of the C57BL/6 mice bearing either human IL-2 or IL-2-R L chain cDNA driven by the H-2K^d promoter were described previously (8, 9). The IL-2 and IL-2-R transgenic mice were crossed to produce the mice carrying both of the transgenes. C57BL/6 mice were purchased from Shizuoka Laboratory Animal Co., Shizuoka, Japan.

Flow Cytometry and Cell Sorting. Single cell suspensions were prepared from thymuses and spleens as described (9). For staining, the following reagents were used: FITC-labeled anti-Thy-1.2 mouse mAb (ICN ImmunoBiologicals, Lisle, IL), phycoerythrin (PE)-labeled anti-CD4 mAb (anti-mouse L3T4 mAb; Becton Dickinson & Co., Mountain View, CA), FITC-labeled anti-CD8 mAb (anti-mouse Lyt-2 mAb, Becton Dickinson & Co.), FITC-labeled anti-human IL-2-R L chain mAb (anti-Tac mAb; reference 10), FITC-labeled anti-mouse IL-2-R L chain mAb (clone 7D4; reference 11), FITC-labeled anti-mouse Mac-1 mAb (clone M1/70; reference 12), biotinylated anti-mouse CD3 mAb (clone 145.2C11; reference 13), and PE-conjugated streptavidin (Becton Dickinson & Co.). Washed cells were stained with optimal concentrations of the fluorescein-labeled mAbs or the biotinylated mAb followed by PE-streptavidin. Finally, cells were analyzed for two-color fluorescence by an Epics Profile flow cytometer (Coulter Electronics, Inc., Hialeah, FL) with a single argon laser and logarithmic intensity scales. 3×10^4 viable cells were analyzed in each sample. Dead cells were excluded from analysis using a combination of low-angle and sideways light scatter. For cell sorting, spleen cells of the hybrid mice were stained first with biotinylated anti-CD3 mAb and second with PE-streptavidin and FITC-anti-Thy-1 mAb. They were sorted into the Thy-1$^+$/CD3$^-$ and Thy-1$^+$/CD3$^+$ populations separately by using FACStar cell sorter (Becton Dickinson & Co.). Reanalysis of the sorted cells revealed that the purity of each population was >95%.

Cytotoxicity Assay. 5×10^6 target YAC-1 (H-2^a), P815 (H-2^d), or BW5147 (H-2^k) cells were labeled with 100 μCi ^{51}Cr (New England Nuclear, Boston, MA) for 45 min at 37°C, and extensively washed before use. Graded doses of effector spleen cells were added in duplicate to 10^4 target cells in V-bottomed wells in a final volume of 200 μl. After incubation for 4 h at 37°C, 100 μl of supernatant was collected from each well to count the released radioactivity. We determined spontaneous release of the radioactivity in wells without effector cells, and the maximal release in wells in which cells were lysed by the addition of 0.1% Triton X-100 (100 μl) for 30 min before collecting supernatant. Percent specific lysis was calculated as: 100× (experimental − spontaneous release)/(maximal − spontaneous release).

Cell Cultures. Spleen cells from the normal, IL-2-R L chain transgenic, or hybrid mice were cultured at 10^6 cells/ml in the complete RPMI 1640 medium (9) supplemented with 100 U/ml human rIL-2 (Takeda Chemical Industries, Osaka, Japan). On day 3, the cultures were fed with fresh medium containing IL-2.

Southern Blot Analysis. DNAs from the liver and cultured spleen cells were prepared using a nucleic acid extractor (Applied Biosystems Inc., Foster, CA). 10 μg of each DNA was digested with restriction enzymes, subjected to electrophoresis on a 0.8% agarose gel, transferred to

a nitrocellulose filter, and hybridized with ^{32}P-labeled cDNA probes for the TCR genes. The cDNA probes for Cβ (an Eco RI fragment of clone 86T5) and for Cγ (a Pvu II fragment of clone pHDS203-205) were provided by Dr. M. Davis (Stanford University, Stanford, CA) and Dr. S. Tonegawa (Massachusetts Institute of Technology, Cambridge, MA), respectively.

Histological and Cytological Examinations. Organs were fixed in Bouin's solution. Sections were stained routinely with hematoxylin and eosin. Lungs of the normal, IL-2-R L chain transgenic, or hybrid mice were removed, and cut into fine pieces. They were suspended in PBS containing 0.05% of collagenase (type IV; Sigma Chemical Co., St. Louis, MO) at 37°C and gently stirred by a magnetic stirrer for 5 min to release interstitial cells. The recoveries of single cells were 1.5–8.0 × 10^6 cells per hybrid mouse and <0.2 × 10^6 cells per normal or IL-2-R L chain transgenic mouse. Morphology of hematopoietic cells was assessed after depositing cells on microscope slides with a cytocentrifuge and staining with Wright and Giemsa's stain.

Results

Expansion of Thy-1$^+$/CD3$^-$ LGL in the Spleen of Human IL-2/IL-2-R L Chain Transgenic Mice. The most striking cellular immunological abnormality found in the hybrid transgenic animals was the increase in the proportion and number of Thy-1$^+$/CD3$^-$ cells in the spleen, as shown in Fig. 1. About 18% of the spleen cells of the 2-wk-old hybrid mice were phenotypically Thy-1$^+$/CD3$^-$, whereas the age-matched normal spleen did not contain a significant proportion of cells with this phenotype. The Thy-1$^+$/CD3$^-$ cells were also rare in the spleen of the parental (IL-2 or IL-2-R) transgenic mice. Another staining experiment showed that the spleen of the 2.5-wk-old hybrid mice contained a larger proportion of Thy-1$^+$ cells (~28%) than that of normal mice (Table I). However, the sum (~11%) of CD4$^+$ and CD8$^+$ cells was far less than the total Thy-1$^+$ cells, indicating that there were a large number of Thy-1$^+$/CD4$^-$8$^-$ cells (~17%) in the spleen of the hybrid mice (Table I). As mature CD4$^+$ or CD8$^+$ T cells always express the CD3 complex on their surface (14), the two populations (Thy-1$^+$/CD3$^-$ and Thy-1$^+$/CD4$^-$8$^-$ cells) detected independently in the spleen of the hybrid mice are most likely to be identical. The hybrid mice had almost normal proportions of thymocyte subsets as compared with the age-matched normal C57BL/6 mice, although the number of thymocytes in the hybrid mice was significantly smaller (Table I).

We also performed morphological analysis on the hybrid spleen cells. As shown in Table II, the spleen of the 2.5-wk-old hybrid mice contained a larger proportion of LGL (~28%) as compared with the normal control (~7%). It was then examined whether the expanded Thy-1$^+$/CD3$^-$ cells were morphologically LGL. We collected the Thy-1$^+$/CD3$^-$ cells and Thy-1$^+$/CD3$^+$ cells separately from the spleen of the hybrid mice by using a FACS, and assessed the morphology of each population. More than 95% of the Thy-1$^+$/CD3$^-$ cells were morphologically LGL (Fig. 2 *A*).

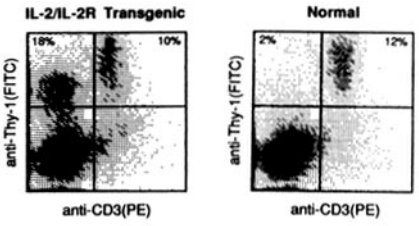

FIGURE 1. Expansion of Thy-1$^+$/CD3$^-$ cells in the spleen of human IL-2/IL-2-R L chain transgenic mice. Whole spleen cells of the mice (2 wk old) were stained with optimal concentrations of FITC-anti-Thy-1.2 mAb and biotinylated anti-mouse CD3 mAb followed by PE-streptavidin, and analyzed as described in Materials and Methods. Fluorescence intensities of cells are indicated as dot matrices with logarithmic scales.

TABLE I

Distribution of Lymphocyte Subsets in Human IL-2/IL-2-R L Chain Transgenic Mice

Organ	T cell	Percent of cells	
		Transgenic	Normal
Thymus	$CD4^-8^-$	4.0 ± 0.1	2.7 ± 0.5
	$CD4^+8^+$	87.1 ± 0.8	85.1 ± 2.2
	$CD4^+8^-$	7.1 ± 0.7	7.7 ± 0.8
	$CD4^-8^+$	1.8 ± 0.3	4.4 ± 1.8
Spleen	Thy-1^+	28.2 ± 3.9	11.5 ± 0.1
	$CD4^+8^-$	4.6 ± 0.4	5.2 ± 0.4
	$CD4^-8^+$	6.3 ± 0.5	3.2 ± 0.2

Mean percentages from three mice (2.5 wk old) are shown ± SDs. Mean numbers of nucleated cells per organ of these mice were 4.5 ± 0.2 × 10^7/transgenic thymus, 11.3 ± 0.9 × 10^7/normal thymus, 3.6 ± 0.4 × 10^7/transgenic spleen, and 4.3 ± 0.9 × 10^7/normal spleen. Washed cells were stained with the fluorescein-labeled antibodies and analyzed by flow cytometry.

By contrast, the Thy-1^+/$CD3^+$ cells in the hybrid spleen were not LGL, but ordinary lymphocytes without granules (Fig. 2 *B*).

Expanded Thy-1^+/$CD3^-$ LGL Have the Strong NK Activity. When analyzed functionally, spleen cells of the hybrid mice showed the markedly elevated NK activity (Fig. 3). Not only a common NK target (YAC-1 cells [Fig. 3 *A*]), but also P815 cells (Fig. 3 *B*) were killed by the hybrid spleen cells. Such NK activity of the hybrid spleen cells is extraordinarily elevated as compared with normal spleen cells. Spleen cells of the normal mice (including nude mice) at the age of 2–3 wk usually have only marginal NK activity, and they can not kill P815 cells at all.

We next examined which of the two Thy-1^+ populations (Thy-1^+/$CD3^-$ or Thy-1^+/$CD3^+$ cells) in the spleen of the hybrid mice bore the strong NK activity. As shown in Table III, the sorted Thy-1^+/$CD3^-$ LGL showed very strong NK activity, whereas the Thy-1^+/$CD3^+$ cells, which represent ordinary T cells, had only negligible NK activity.

TABLE II

Increased Proportion of LGL in the Spleen of Human IL-2/IL-2-R L Chain Transgenic Mice

Mouse	Differential count of leukocytes in the spleen			
	Granulocytes	Monocytes	Lymphocytes	LGL
Transgenic				
1	13	10	50	27
2	2	5	67	26
3	5	1	62	32
4	2	8	63	27
Normal*	7 ± 4	4 ± 2	82 ± 6	7 ± 4

Individual percentages and mean percentages ± SDs are shown for the transgenic and normal mice, respectively. All of four transgenic and six normal mice analyzed were 2.5 wk old.

* n = 6.

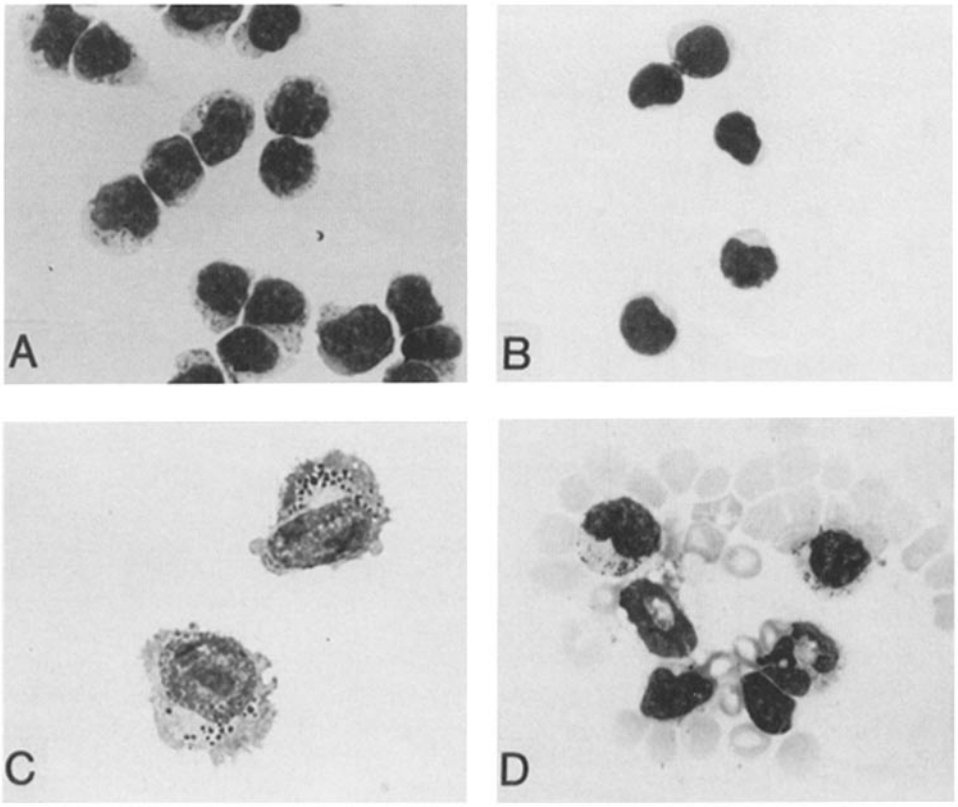

FIGURE 2. Morphology of expanded cells in human IL-2/IL-2-R L chain transgenic mice. Thy-1$^+$/CD3$^-$ cells (*A*) and Thy-1$^+$/CD3$^+$ cells (*B*) collected separately from the spleen of the transgenic mice. (*C*) Spleen cells of the transgenic mice enriched for Thy-1$^+$/CD3$^-$ cells by the in vitro culture with IL-2. (*D*) Cells isolated from the lung of the transgenic mice.

Preferential IL-2-dependent Expansion of Thy-1$^+$/CD3$^-$ LGL In Vitro from the Spleen of Human IL-2/IL-2-R L Chain Transgenic Mice. We next examined whether the spleen cells of the hybrid mice were able to proliferate in vitro without any supplement of growth factors. Though Thy-1$^+$/CD3$^-$ LGL might have expanded in vivo by the autocrine mechanism, none of the hybrid spleen cells could survive for more than several days in the ordinary culture medium (data not shown).

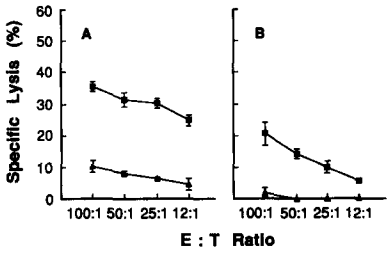

FIGURE 3. Elevated NK activity of spleen cells of human IL-2/IL-2-R L chain transgenic mice. The NK activity of unfractionated spleen cells of the transgenic mice (■) and normal controls (▲) was examined using either NK-sensitive YAC-1 cells (*A*) or relatively NK-resistant P815 cells (*B*) in various E/T ratios. Means of data from three mice (2 wk old) are plotted with SDs (*vertical bars*).

TABLE III

NK Activity of Spleen Cell Subpopulations of a Human IL-2/IL-2-R L Chain Transgenic Mouse

Effector cells	Target cells	
	YAC-1	BW5147
	%	
Transgenic		
Whole spleen cells	27	5
Thy-1$^+$/CD3$^-$ spleen cells	49	6
Thy-1$^+$/CD3$^+$ spleen cells	5	2
Normal		
Whole spleen cells	0	1

Thy-1$^+$/CD3$^-$ and Thy-1$^+$/CD3$^+$ cells were collected separately from the spleen of the transgenic mouse, and analyzed as described in Materials and Methods. Values are specific lysis, and are the average of duplicate determinations. E/T ratio was 12:1. Mice analyzed were 2.5 wk old.

When supplemented with IL-2 (100 U/ml), however, Thy-1$^+$/CD3$^-$4$^-$8$^-$ cells preferentially expanded in vitro among the whole spleen cells of the hybrid mice with 650% viable cell recovery of the input on day 6 (Fig. 4 *C*). The surface phenotype of the in vitro expanded cells was indistinguishable from the in vivo expanded Thy-1$^+$/CD3$^-$ cells sorted from the hybrid spleen. They were also morphologically LGL (Fig. 2 *C*). In contrast, when the spleen cells of the IL-2-R L chain transgenic mice were cultured in vitro with IL-2, Thy-1$^+$/CD3$^+$4$^-$8$^+$ cells preferentially expanded among the whole spleen cells (Fig. 4 *B*). Both of the two expanded popula-

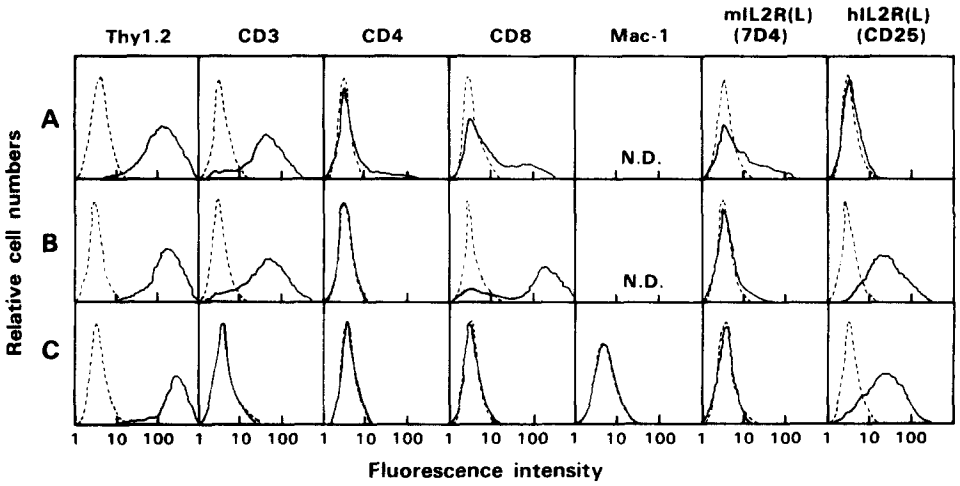

FIGURE 4. Phenotypical analysis of the spleen cells cultured with IL-2. Spleen cells from the normal (*A*), IL-2-R L chain transgenic (*B*), or hybrid (*C*) mice were cultured in the presence of 100 U/ml IL-2 for 6 d. Cultured cells were then analyzed with various mAbs by a flow cytometry as described in Materials and Methods. (*Dashed lines*) Controls that were not stained or stained with only the second reagent; (*m* and *h*) mouse and human, respectively.

tions of the hybrid and IL-2-R transgenic mice expressed human IL-2-R L chain antigen, while endogenous murine IL-2-R L chain was almost undetectable (Fig. 4, *B* and *C*). Normal (nontransgenic) spleen cells hardly grew in vitro in response to IL-2, with <20% cells remaining viable on day 6, and they were mostly Thy-1$^+$/CD3$^+$ cells (Fig. 4 *A*). These results suggest that Thy-1$^+$/CD3$^-$ LGL of the hybrid mice might have intrinsic proliferative dominance over the CD3$^+$ T cells in response to IL-2.

Thy-1$^+$/CD3$^-$ LGL in Human IL-2/IL-2-R L Chain Transgenic Mice Have Unrearranged TCR Genes. The absence of the CD3 complex on the cell surface of the expanded LGL in the hybrid mice suggests that they have neither the $\alpha\beta$ nor $\gamma\delta$ type of the TCR. To confirm this observation, we analyzed the rearrangement of TCR genes in the LGL of the hybrid mice that were enriched in vitro by the culture with IL-2. With the use of the Cβ probe, only discrete germline bands of the expected intensity were detected in the Thy-1$^+$/CD3$^-$ LGL-enriched hybrid spleen cells (Fig. 5, lanes *4*), while germline bands with the decreased intensity were seen in the CD3$^+$ cell-enriched spleen cells of the normal or IL-2-R transgenic mice (Fig. 5, lanes *2* and *3*). In addition, the Cγ probe detected only germline bands in DNAs of liver and the hybrid spleen cells (Fig. 5, lanes *1* and *4*), whereas the rearranged 16-kb Eco RI band (the uppermost one) was detected with the decreased intensity of 10.5- and 10.8-kb Eco RI germline bands in DNAs of CD3$^+$ cell-enriched spleen cells (Fig. 5, lanes *2* and *3*). These results indicate that the expanded LGL in the hybrid mice have not rearranged the TCR genes and, as a consequence, do not express TCR molecules on their surface.

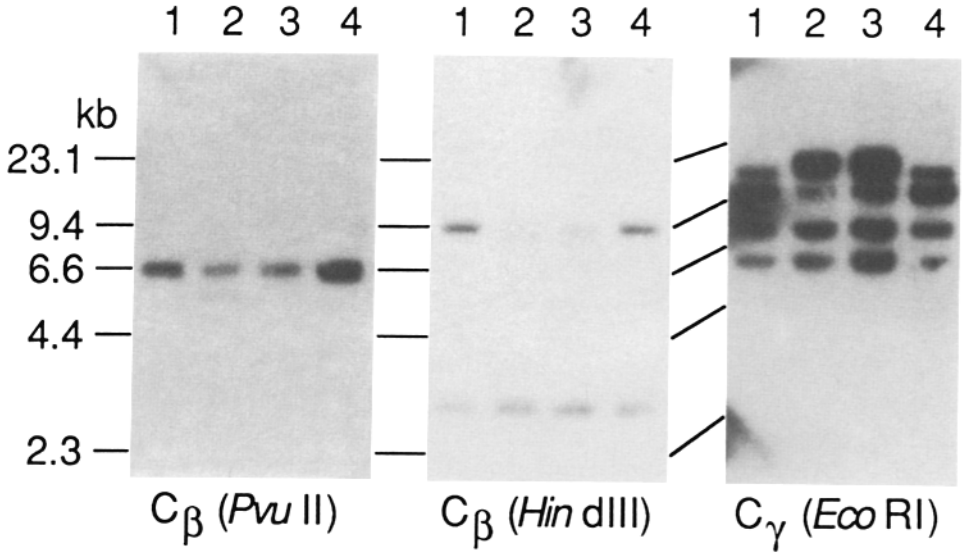

FIGURE 5. Southern blot analysis of TCR genes of the spleen cells cultured with IL-2. DNAs were isolated from the spleen cells cultured with IL-2 for 6 d. Origins of DNA are C57BL/6 liver (lanes *1*), normal spleen cells (lanes *2*), IL-2-R L chain transgenic spleen cells (lanes *3*), and hybrid spleen cells (lanes *4*). DNA was digested with restriction enzymes indicated. Southern blot filters were hybridized with nick-translated, ^{32}P-labeled cDNA probes for Cβ and Cγ. The Cγ probe contains a portion of a Vγ gene.

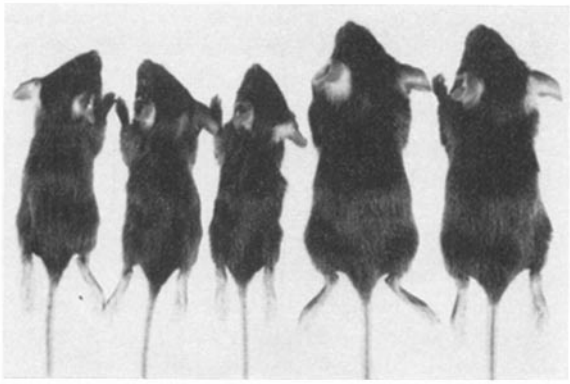

FIGURE 6. Growth retardation of human IL-2/IL-2-R L chain transgenic mice. Left three transgenic and right two normal mice were all 3 wk old.

Other Abnormalities in Human IL-2/IL-2-R L Chain Transgenic Mice. The hybrid mice always showed severe growth retardation, which became apparent ~2 wk after birth (Fig. 6). In addition, their gait was disturbed or ataxic (i.e., widebased and staggering), and they fell down easily while walking. These hybrid mice died by 4 wk of age without exception.

To elucidate the primary cause of the early death of the hybrid mice, we analyzed their tissues histologically. The 3-wk-old hybrid mice showed severe interstitial pneumonia with focal infiltration of lymphocytes and other inflammatory cells (Fig. 7, *A* and *B*). The majority of infiltrating lymphocytes in the lung of the hybrid mice were probably expanded Thy-1$^+$/CD3$^-$ LGL because cells collected from the collagenase-treated lungs of the hybrid mice contained a large number of LGL (Fig. 2 *D*). The thymus and spleen of the 3-wk-old hybrid mice were far smaller than those of the control mice, and the profound lymphocyte depletions were found histologically in the cortex of the thymus (Fig. 7, *C* and *D*) and in the red as well as white pulp of the spleen (Fig. 7, *E* and *F*). To our surprise, the 3-wk-old hybrid mice selectively lost 40–70% of Purkinje cells in the cerebellum without any sign of inflammation (Fig. 7, *G* and *H*). This change might be associated with the ataxic gait of the mice.

Histological studies on 2-wk-old hybrid mice did not show evident sign of damage in the thymus, spleen, and cerebellum although interstitial pneumonia was already seen at this age. The results indicate that the damage in the thymus, spleen, and cerebellum took place mainly after birth.

Discussion

Expansion of NK Cells but not T Cells in Human IL-2/IL-2-R L Chain Transgenic Mice. As LGL that expanded in the spleen of the hybrid mice had the Thy-1$^+$/CD3$^-$4$^-$8$^-$ phenotype, the germline configuration of TCR genes, and the strong NK activity, they can be regarded as classical NK cells of non-T-cell lineage (15, 16). Recent studies (9, 17, 18) showed that both LGL (or NK cells) and ordinary T cells (especially CD8$^+$ T cells) expressed the H chain of IL-2-R constitutively even at the resting state. According to these findings, T cells as well as NK cells of the hybrid or IL-2-R L chain transgenic mice are expected to express high affinity binding sites for IL-2 and to proliferate efficiently in response to IL-2. Indeed, unstimulated spleen cells of the IL-2-R L chain transgenic mice had ~120 high affinity binding sites for IL-2

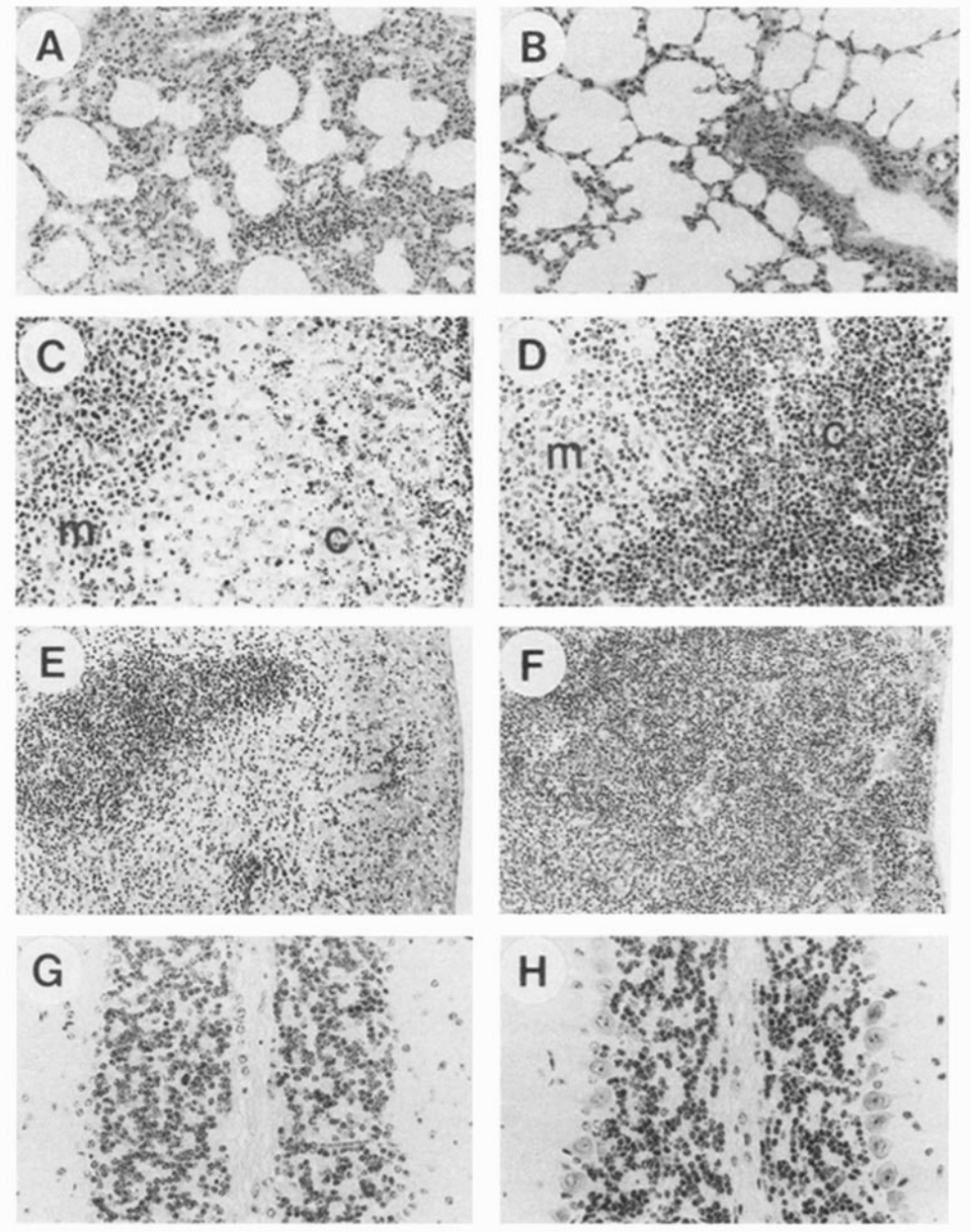

FIGURE 7. Histological abnormalities in human IL-2/IL-2-R L chain transgenic mice. (*A*) Histology of pneumonia in the transgenic mice. (*B*) Normal lung histology. (*C*) Histology of the lymphocyte depletion in the cortex of the transgenic thymus. (*D*) Normal thymus histology. (*E*) Histology of the lymphocyte depletion in the transgenic spleen. (*F*) Normal spleen histology. (*G*) Histology of the selective loss of Purkinje cells in the cerebellum of the transgenic mice. (*H*) Normal cerebellum histology. In *C* and *D*, c and m indicate cortex and medulla, respectively. Mice analyzed were 3 wk old (140×, *A* and *B*; 225×, *C*, *D*, *G*, and *H*; and 110×, *E* and *F*).

per cell, and proliferated in vitro in response to the supplemented IL-2, yielding the enriched population of Thy-1$^+$/CD3$^+$8$^+$ cells in 6 d of the culture (9) (Fig. 4 *B*). Therefore, we first expected the expansion of ordinary Thy-1$^+$/CD3$^+$ T cells in the hybrid mice carrying the IL-2 as well as IL-2-R L chain transgenes. Unexpectedly, however, we could not detect the expansion of the ordinary T cells in the hybrid mice, but instead observed the remarkably increased number of Thy-1$^+$/CD3$^-$ NK cells in the spleen. The absence of nonspecific activation of T cells was supported by the finding that the sera of the hybrid mice did not contain a detectable amount of autoreactive antibodies as assessed by the immunohistochemical technique (data not shown). These results suggest that the NK cells in the hybrid mice have apparent advantages for selective proliferation as compared with ordinary T cells in these mice.

The continuous activation of the IL-2/IL-2-R pathway in vivo in the hybrid mice might have triggered negative feedback reactions in T cells but not in NK cells. It is also possible that T cells but not NK cells might require an additional signal for their long-term proliferation in vivo, which could be transduced by the TCR complex. T cells may require a growth factor other than IL-2 for their proliferation in vivo.

Since we constructed IL-2 or IL-2-R L chain transgenic mice by using the H-2K^d promoter (8, 9), the transgenes should have been expressed early in the fetal stage of the hybrid mice before the normal T cell development took place. This might have caused the earlier extrathymic induction of NK cells in the hybrid mice than in the normal controls, resulting in the dominant expansion of NK cells. We have already reported that the in vitro IL-3/IL-2 culture induced CD3$^-$ LGL lines from fetal liver cells while the same procedure induced CD3$^+$ LGL lines from postnatal spleen cells (19). These results further support the notion that the NK cells in the hybrid mice might have been induced extrathymically in the fetal stage. The results also suggest that functional IL-2-R H chains might be expressed on certain lymphoid cells at an early embryonic stage.

We observed the expansion of NK cells only in the IL-2/IL-2-R L chain hybrid mice, but not in the IL-2 transgenic mice (8). These results clearly indicate that the induction of the IL-2-R L chain is important for in vivo proliferation of NK cells, which were reported to proliferate in vitro in the presence of high concentrations of IL-2 without the IL-2-R L chain expression (20–22).

We have never observed any incidence of leukemia or lymphoma in the hybrid mice. This may be partly due to the shortened life span of the hybrid mice.

Histological Abnormalities and Activation of NK Cells. Every hybrid mouse examined showed typical interstitial pneumonia with similar time course, suggesting that bacterial or viral infections are unlikely as the cause. The majority of the lymphocytes infiltrating the lungs of the hybrid mice were the activated NK cells. Since the lungs of the normal mice, which were administered with a high dose of human rIL-2, were reported to be infiltrated with asialo-GM1$^+$ lymphoid cells (23), it is reasonable to assume that so called IL-2-activated cells, such as the NK cells in the hybrid mice, may have some affinity to the lung tissue. Alternatively, the NK cells might have been activated and expanded in situ in the lung of the hybrid mice, because we could easily detect mRNA of human IL-2 in the lung of IL-2 transgenic mice (8). In any case, interstitial pneumonia in the hybrid mice seems to be the primary cause of their early death.

We found that the spleens of the hybrid mice at the age of 3 wk were smaller than

the normal ones, which was probably due to the lymphocyte depletion from the organ. As NK cells were reported to have a capacity to kill syngeneic lymphocytes (24), ordinary lymphocytes in the spleen of the hybrid mice might have been killed by the expanded Thy-1$^+$/CD3$^-$ LGL with the elevated NK activity. Though the profound lymphocyte depletion was also observed in the cortex of the thymus of the 3-wk-old hybrid mice, we could never detect the expansion of Thy-1$^+$/CD3$^-$ LGL in the thymus of the mice (data not shown). This suggests that the mechanisms of the lymphocyte depletion in the spleen and thymus of the mice might be different. The stressed condition of the hybrid mice at the age of 3 wk, just before their death, might have resulted in the increased level of serum cortisone and the depletion of cortisone-sensitive cells in the hybrid thymus cortex.

The ataxic movement of the hybrid mice is most likely due to the decreased number of Purkinje cells. Though the histology of the cerebellum of the hybrid mice at the age of 2 wk was almsot normal, we found that the hybrid mice at the age of 3 wk selectively lost more than a half of Purkinje cells in their cerebella.

Patients treated with high-dose rIL-2 occasionally showed psychological reactions such as confusion (25), suggesting that IL-2 may have some effects on the central nervous system. In fact, astrocytes or oligodendrocytes in the brain were shown to proliferate and to mature in response to IL-2 (26, 27). Though we could not detect mRNA of human IL-2 or IL-2-R L chain in the brain of the transgenic mice (8, 9), a localized expression of the transgenes could allow the activation of the IL-2-responsive cells in the brain of the hybrid mice, which may somehow result in the loss of Purkinje cells. Another possibility is that Purkinje cells may be extremely susceptible to the NK-specific factors (28) produced by the increased Thy-1$^+$/CD3$^-$ LGL in the hybrid mice. At this stage, however, it is fair to say that we have no reasonable explanation for the specific loss of Purkinje cells in the hybrid mice.

Summary

Transgenic mice expressing both human IL-2 and the L chain of IL-2-R constitutively had an unusual expansion of Thy-1$^+$/CD3$^-$4$^-$8$^-$ large granular lymphocytes, which bore the elevated NK activity. Unexpectedly, the transgenic mice had neither T cell expansion nor autoreactive antibodies. The increase in number and activity of NK cells seems to be responsible for both the severe interstitial pneumonia and lymphocyte depletion in the spleen that we found in these transgenic mice. In addition, we found the selective loss of Purkinje cells in the cerebellum of the mice, which gave rise to their disturbed gait. All the transgenic mice died by 4 wk of age.

We thank Dr. K. Kuribayashi for his help in the NK assay and Dr. Y. Nishizuka for the pathological diagnosis. We also thank Ms. S. Okazaki and J. Kuno for excellent technical assistance, and M. Sugiura for preparing the manuscript.

Received for publication 30 May 1989 and in revised form 20 June 1989.

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