Development of Disease and Virus Recovery in Transgenic Mice Containing HIV Proviral DNA

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Transgenic mice containing intact copies of the human immunodeficiency virus (HIV) proviral DNA were constructed. Founder animals were not viremic for HIV and remained healthy during a 9-month observation period. After being mated with nontransgenic animals, one founder mouse (No. 13) gave rise to F_1 progeny that developed a disease syndrome characterized by marked epidermal hyperplasia, lymphadenopathy, splenomegaly, pulmonary lymphoid infiltrates, growth retardation, and death by day 25 of life. Infectious HIV, indistinguishable from parental virus by immunoblot analysis, was recovered from the spleen, lymph nodes, and skin of five of five affected animals.

VIRTUALLY ALL OF THE KNOWLEDGE OF THE BIOLOGY AND molecular structure of the human immunodeficiency virus (HIV), the etiological agent of AIDS and AIDS-related disorders, has accrued from studies of tissue culture infections of human peripheral blood lymphocytes (PBL's) (*1*), continuous T cell lines (*2–4*), and, more recently, cultured monocyte or macrophage systems (*5*) and has resulted in the identification and mapping of HIV genes (*6*). Although much effort has been devoted to the analysis of the viral replicative cycle in these tissue culture systems, very little is known about the biology of HIV in vivo.

The pathogenesis of immunosuppression in AIDS may indeed be related to the direct infection of CD4-bearing lymphocytes and monocytes or macrophages (*1–5, 7–9*), but the mechanisms underlying the central nervous system, pulmonary, lymphoproliferative, and cutaneous diseases remain unelucidated. An animal model simulating all phases of HIV infection in man would provide answers to many of these questions. However, only two animal species are susceptible to HIV infection [man and chimpanzee (*10*)] and, to date, only man develops disease.

Since the principal determinant of susceptibility to infection by HIV is the expression, at the cell surface, of the CD4 protein (*2, 11, 12*), it would be logical to construct transgenic animals carrying the human CD4 gene. Previous work had demonstrated that no intrinsic barrier existed in mouse cells for the synthesis of viral proteins and their assembly into infectious virions after transfection of an infectious molecular clone of the HIV provirus (*13*). Since several mouse cell lines synthesizing human CD4 were subsequently shown to be refractory to HIV infection (*12*), the construction of transgenic mice expressing human CD4 did not seem promising as a model for HIV-induced disease. Instead, the construction of transgenic animals containing HIV proviral DNA could model the phase of viral infection subsequent to the integration of HIV proviral DNA and provide important information concerning the in vivo expression of viral proteins. We now show that some transgenic mice, carrying intact copies of the HIV provirus, develop a spontaneous and fatal disease that mimics, in several aspects, features described in human AIDS.

One-cell embryos, obtained from 8- to 12-week-old FVB/N mice, were microinjected with 75 to 150 copies of HIV proviral DNA derived from the infectious molecular plasmids pNL4-3 (*13*) or pNL432 (*14*). Plasmid pNL4-3 was digested with both Sma I and Nru I, which releases a 12.4-kb segment containing 5′ cellular

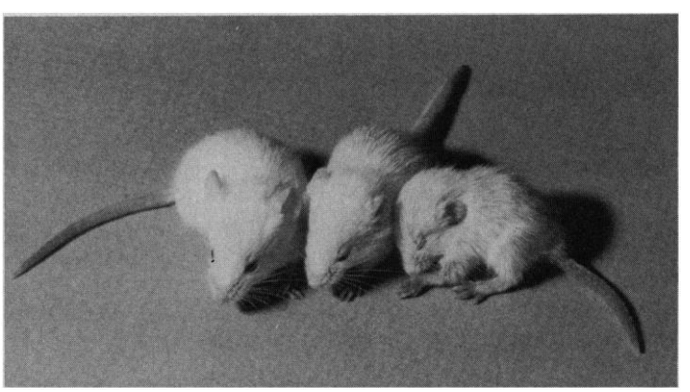

Fig. 1. HIV-affected, transgenic mouse, 20 days old (right), and nontransgenic littermate (left).

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Table 1. F_1 HIV transgenic mice.

Census items	Transgenic founders			
	No. 13	No. 16	No. 62	Nos. 38, 39, 42, 64
Litters (No.)	4	3	3	14
Animals (No.)	41	35	32	96
Affected* (No.)	8 (8)	3 (3)	2 (1)	0
Deaths (No.)†	10	4	2	1
DNA isolated‡	4 (4)	1 (1)	0	0

*Includes growth retardation, skin abnormalities, wasting, moribund. †F_1 animals dying between day 4 and day 28 of life. ‡In several instances, DNA could not be prepared because of tissue autolysis. Numbers in parentheses indicate mice carrying HIV proviral DNA.

flanking (1.2 kb), HIV proviral (9.7 kb), and 3′ cellular flanking sequences (1.5 kb); pNL432 DNA was linearized with Aat II, which cleaves once within pUC 18, generating a 15.1-kb fragment consisting of 5′ cellular flanking (1.2 kb), HIV proviral (9.7 kb), 3′ cellular flanking (1.5 kb), and plasmid (2.7 kb) sequences. After the microinjection procedure, the embryos were inserted into the oviducts of surrogate FVB/N female mice, which were then transferred to a stainless steel glovebox system within a BL4 facility. All transgenic animals were maintained in the glovebox for the duration of the experiment. Animal food, bedding, and waste were introduced or removed from this closed system through a double-door autoclave. Mouse tissues, collected when an animal died, were placed in sealed plastic containers, removed from the glovebox system through the Clorox decontamination tank, and processed in a laminar flow hood in a BL2 facility. The containment and practices followed in the BL4 facility were reviewed and approved by the NIH Biosafety Committee.

Sixty-four of the microinjected ova were successfully carried to term. Of these, 13 were shown by dot blot hybridization of tail DNA preparations to contain from 0.4 to more than 64 copies of HIV proviral DNA per haploid genome. Southern blot analyses indicated that 12 of the 13 founder animals carried full-length (9.7 kb) HIV proviruses in their chromosomal DNA arranged, in all instances, as head-to-tail oligomers. Seven of the 12 founder (F_0) animals, containing potentially intact copies of HIV proviral DNA, transmitted the transgene to their progeny. During a 9-month observation period, all of the F_0 mice were healthy by the criteria of normal fertility, growth, development, and body weight. No HIV was ever recovered from the blood of any F_0 animal after cocultivation with phytohemagglutinin (PHA)-stimulated human PBL's during a 17-day isolation period.

One of the seven founder mice (No. 13), which transmitted the HIV proviral DNA to its offspring, produced serum antibodies to HIV as monitored by enzyme-linked immunosorbent assay; immunoblotting demonstrated reactivity with the gp120 envelope and p64 reverse transcriptase proteins of HIV. Southern blot analyses revealed that female mouse No. 13 contained two copies of proviral DNA at one integration site. Successive matings of mouse No. 13 with nontransgenic FVB/N males resulted in litters that developed a characteristic and fatal clinical syndrome in 45 percent of the pups. At approximately 10 to 14 days of age, affected F_1 progeny were readily distinguishable from their unaffected littermates by their small size (approximately 50 to 80 percent of body weight of the nontransgenic littermates) (Fig. 1) and the presence of a skin disease characterized by dry, thickened, scaling lesions affecting the tail, paws, and ears. Over the ensuing 1 to 2 weeks of life, all of the affected animals nursed normally and displayed no evidence of diarrhea, respiratory distress, gross neurological dysfunction, or other readily observable clinical abnormalities. Only shortly before death did affected animals become sluggish or develop ruffled fur. All affected animals either died or were killed by 25 days of age

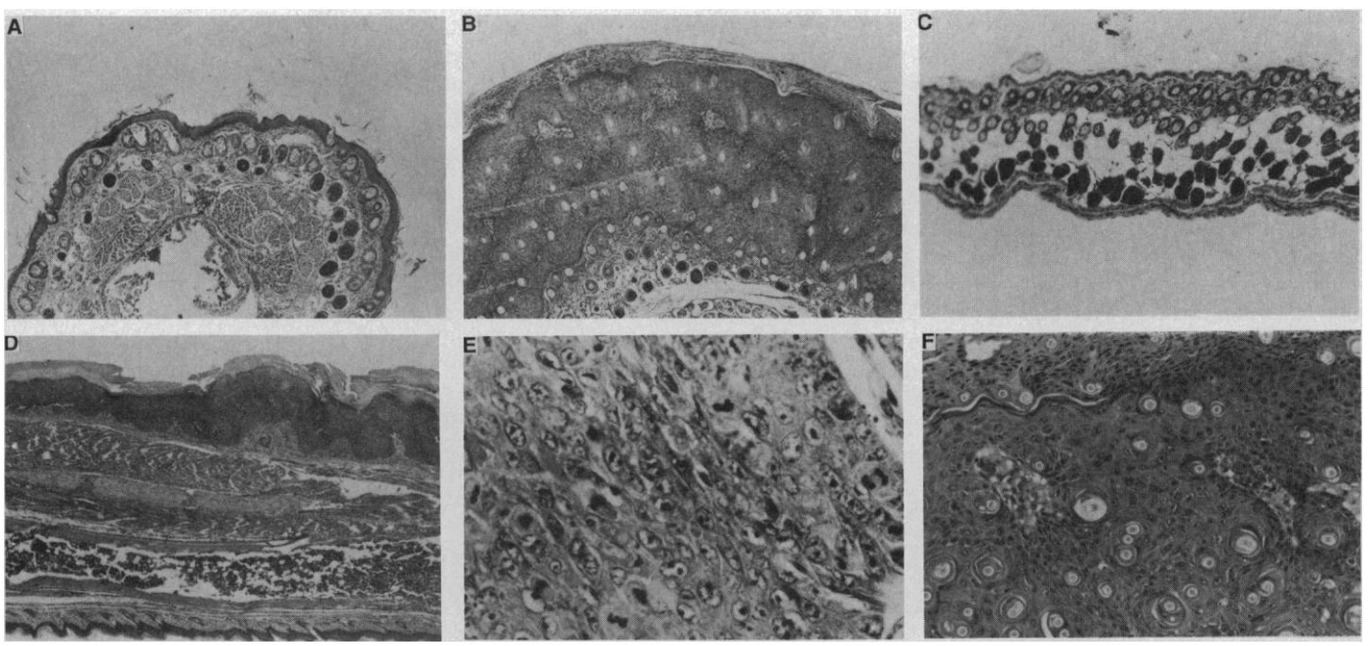

Fig. 2. Histopathology of skin from transgenic mice. (**A**) Tail skin from control mouse with a normal epidermis. (**B**) Tail skin from transgenic mouse is thickened compared to control mouse (A). In addition to marked epidermal hyperplasia, hyperkeratosis and parakeratosis are present. (**C**) Flank skin from transgenic mouse shows lack of pathology seen in tail and ear skin. (**D**) Skin from paw of a transgenic mouse shows epidermal hyperplasia affecting the plantar (top) surface with marked hyperkeratosis and focal parakeratosis. (**E**) Higher power of skin from plantar aspect of paw demonstrates frequent mitoses and moderate keratinocyte atypia. (**F**) A nodular lesion removed from the submandibular region consists of proliferating keratinocytes and hairshafts with associated marked hyperkeratosis and parakeratosis. Original magnification: (A and B) ×16, (C) ×250, (D) ×50, (E) ×750, (F) ×20. All sections were stained with hematoxylin and eosin.

because they were moribund. Attempts to breed strain No. 13 F_1 animals have not been possible because all died before attaining sexual maturity.

Of the 41 strain No. 13 F_1 mice examined by dot blot hybridization, 23 were shown *not* to carry the HIV transgene; all 23 of these animals appeared healthy and displayed none of the abnormalities observed in their transgenic littermates. Of the remaining 18 F_1 animals, 12 suffered growth retardation, developed skin changes, or died spontaneously. Cellular DNA from these 12 animals contained the HIV provirus (Table 1). DNA was not available from the six other strain No. 13 F_1 mice because they had died unexpectedly and tissue autolysis precluded any analysis. Five of these latter deaths (one on day 8 and four on day 14) occurred in the first F_1 litter produced by founder mouse No. 13, alerting us to the existence of a potentially interesting phenotype. The sixth death occurred in a runted animal from the third F_1 litter with the characteristic dermatologic abnormalities. If we assume that all six of these mice, which died or exhibited symptoms during the interval when other strain No. 13 F_1 mice became affected (and from which DNA was unavailable), carried the HIV provirus, then 18 of 41 were transgenic.

Pathologic changes. Necropsies were performed on eight affected strain No. 13 F_1 mice and six nontransgenic, aged-matched littermates that served as controls. In every instance, the runted transgenic animals had severely roughened, scaling, and thickened tails, particularly on the ventral surface. In addition, the four paws, inner surfaces of the ears, and the tips of the nose were all similarly affected. Compared to control animals, the diameter of the tail was twice the size in affected mice, particularly in the proximal, ventral portion (Fig. 2, A and B). Similarly, the paws and ears were enlarged to about double their normal width and thickness, respectively. The haired skin appeared normal except at the base of the tail. At autopsy, enlarged (1 by 1 mm to 3 by 4 mm) axillary, cervical, inguinal, mesenteric, and thoracic lymph nodes were present. No lymphadenopathy was identified in any of six unaffected littermates. The spleens of the affected animals were two to three times larger than normal, as determined by cell count. Thymuses were smaller in severely affected mice compared to those in the nontransgenic littermates. Cutaneous masses present in the submandibular area were noted in three of the affected animals.

Histologic examination of the skin of strain No. 13 F_1 mice revealed grossly apparent lesions of the tail, paws, and ears that were characterized by epidermal hyperplasia (Fig. 2). Compared to the epidermis of nontransgenic animals, normally two to three cells in thickness, the epidermal layer of the affected mice was several times thicker. Dyskaryotic cells and mitotic figures were numerous throughout the epidermis, particularly in the paw (Fig. 2E), and were associated with acanthosis and parakeratosis (Fig. 2, B and D). The granular layer of the epidermis was absent over the severely affected ventral surface of the tail skin. No histopathological changes were observed in the fur-bearing skin (Fig. 2C). Histologic examination of the masses recovered from the submandibular area in three of the mice demonstrated nodular proliferation of mature keratinocytes interspersed with hair shafts with associated hyperkeratosis and parakeratosis (Fig. 2F).

The most characteristic and consistent pulmonary lesions in the strain No. 13 F_1 mice were perivascular cuffs around small arterioles throughout the lung parenchyma which were not associated with other changes (Fig. 3, A to C). The cuffs were composed primarily of tightly packed lymphoid cells with a variable component of polymorphonuclear cells including some eosinophils. The lymphoid cells were mainly small lymphocytes with scattered lymphoblasts; mitotic figures were rare (Fig. 3C). Animals killed at a more advanced age (up to 24 days) displayed a progression of the pulmonary abnormalities with accumulations of lymphocytes, polymorphonuclear leukocytes, and macrophages within the perivascular

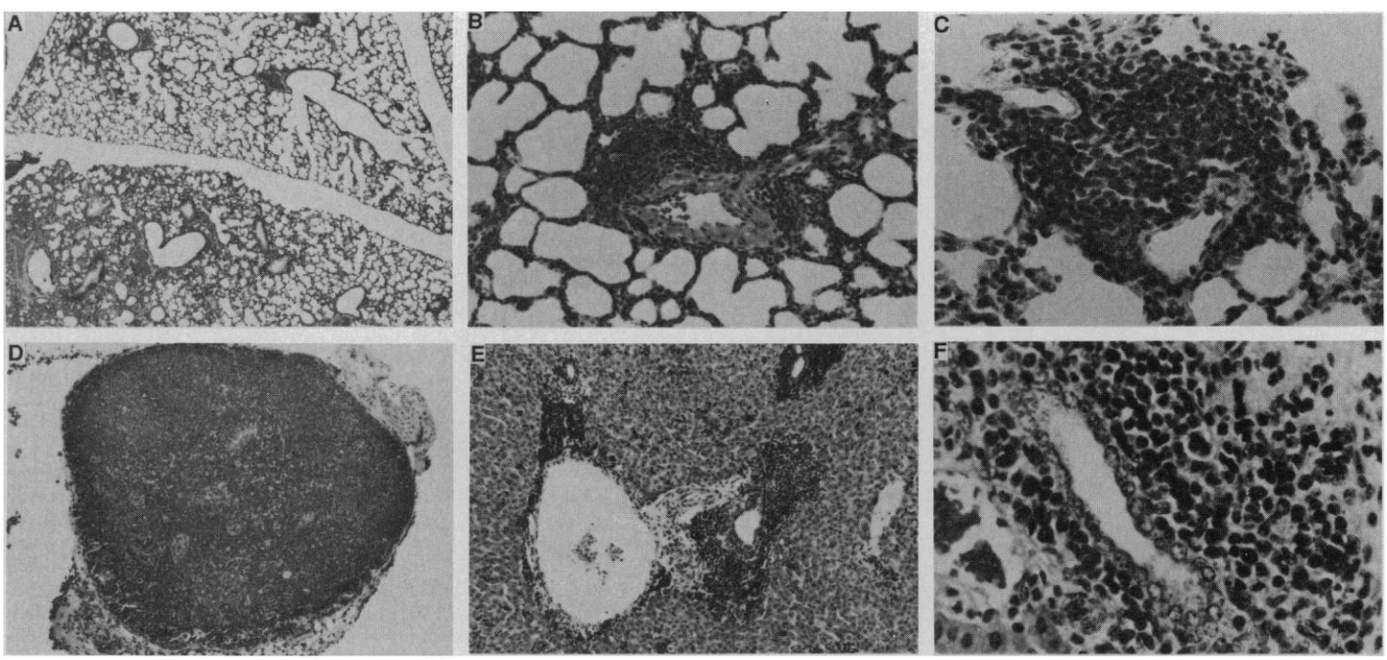

Fig. 3. Histopathology in lung, lymph node, and liver. (**A**) Lung from transgenic mouse shows scattered small lymphoid aggregates. (**B**) Higher magnification of a lung from an affected animal reveals that most of the lymphoid aggregates are situated within the perivascular interstitium. (**C**) The vast majority of the cells in the lymphoid aggregates are lymphocytes with very few plasma cells or immunoblastic cells. (**D**) An enlarged lymph node recovered from a transgenic animal without germinal centers. (**E**) Moderate inflammatory cell infiltrates are present in the portal triads and to a lesser degree in the sinusoids of the lobules of the liver from this transgenic mouse. (**F**) Higher power of (E) shows that the hepatic cellular infiltrates consist of neutrophils and a few lymphoid cells. Original magnification: (A) ×15, (B) ×20, (C) ×160, (D) ×40, (E) ×30, (F) ×500. Sections were strained with hematoxylin and eosin.

interstitium. There was no evidence of a bronchopneumonia that might suggest a bacterial or viral pathogen, and special stains for fungi, acid fast bacilli, and pneumocystis were negative.

Although the lymph nodes were grossly enlarged, histologic examination revealed only reactive changes; no features were clearly different from those in lymph nodes of control animals (Fig. 3D). In the spleen, the borders of the periarteriolar lymphoid sheaths were obscured by the large numbers of lymphocytes filling the red pulp. Infiltrates, composed of both neutrophils and lymphocytes, were found in the liver primarily in portal triads and occasionally in the sinusoids (Fig. 3, E and F). Kupffer cell hyperplasia was also present in the livers of all of the affected mice. Severe cortical atrophy was observed in the thymuses of the most severely affected animals. No pathologic abnormalities were seen in the brain, small intestine, eye, kidney, or bone marrow.

Several tissues (lung, skin from ear and tail, spleen, liver, and gut) from affected strain No. 13 F_1 mice were collected under sterile conditions and cultured for bacterial pathogens. Other than the gastrointestinal tract, which contained normal flora, and the skin, which yielded *Staphylococcus aureus*, all of the other tissues were free of bacteria. At the time of death of the affected animals, no antibodies to Sendai virus, mouse hepatitis virus, LCM virus, pneumonia virus of mice, minute virus of mice, ectromelia, encephalomyelitis virus, *Mycoplasma pulmonis*, or cilia-associated respiratory bacillus were detected in blood. Immunohistochemical analyses of lung specimens from affected animals were negative for Sendai virus, pneumonia virus of mice, and mouse hepatitis virus. None of the nontransgenic strain No. 13 F_1 mice, which were housed in the same cages as their affected littermates, ever developed detectable abnormalities or disease.

HIV expression. In situ hybridization with sense and antisense HIV probes (7) was carried out on several different tissue specimens from affected mice. Sections from affected tail and ear skin and a few scattered cells in liver and the gastrointestinal tract reacted specifically with antisense HIV probes and failed to hybridize with sense probes. No hybridization was detected in sections obtained from brain, lung, spleen, kidney, or lymph nodes from transgenic animals or from the skin of nontransgenic littermates. Reactivity was localized to the outer two-thirds of the epidermis and was associated with both hair follicles and with individual cells interspersed throughout the stratum spinosum (Fig. 4).

To ascertain whether infectious HIV particles were produced in the tissues of affected mice, samples (2 to 5 mm^3) of brain, lung, heart, kidney, skin, liver, spleen, and lymph node were aseptically removed from affected or healthy strain No. 13 F_1 mice at the time of death; these tissues were co-cultivated with PBLs or A3.01 cells (*3*), a $CD4^+$ continuous human T lymphocyte line that is permissive for HIV infection. Progeny virus, which was recovered from several of the mouse tissues, was detected by reverse transcriptase (RT) assays 14 to 21 days later (Fig. 5A). Virus was recovered from skin, lymph nodes, or spleen specimens from five of five affected transgenic mice but not from tissues of nontransgenic littermates. Lysates were subsequently prepared from A3.01 cells infected with the spleen, lymph node, and skin isolates, and analyzed by immunoblotting with pooled serum from HIV-infected individuals. The reactive bands, generated by the mouse-derived viral isolates, comigrated with authentic HIV proteins (Fig. 5B). Attempts to infect two mouse fibroblast cell lines [SC-1 (*15*) and *Mus dunni* (*16*)] with the virus recovered from the transgenic mice were unsuccessful.

Immunologic changes. The development of a fatal disease affecting multiple organs in strain No. 13 F_1 animals immediately raised questions about the status of their immune systems. The most consistent immunologic finding was the splenomegaly (two to three times larger compared to unaffected littermates) and lymphadenopathy observed in all affected animals. Fluorescence-activated cell sorter analysis of lymph node cells from diseased animals indicated an increase in both the number and proportion of Lyt-2^+ cells as well as a reduction in the percentage but not the number of the L3T4 subset (Fig. 6, A and B). The relative proportions of B and T cells in affected spleens was comparable to those of normal mice; however, because of the splenomegaly present in affected mice, the total number of B and T cells was two to three times greater than

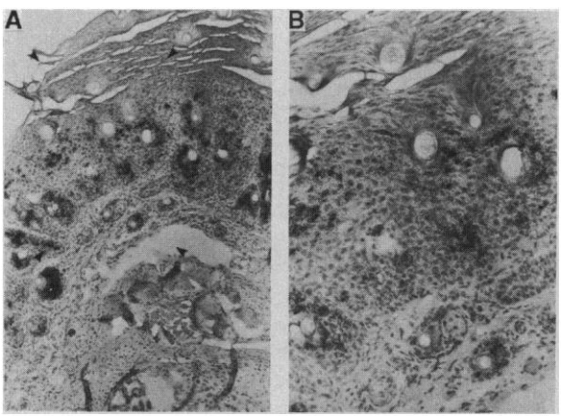

Fig. 4. (**A**) In situ hybridization of transgenic mouse tail with HIV-specific probes. Frozen sections of skin were mounted and treated with proteinase K (1 μg/ml) for 15 minutes and then acetylated; the specimens were then hybridized with ^{35}S-labeled antisense HIV-specific probes (7) for 12 to 24 hours (37°C) as described (*29*). The sections were then washed sequentially in 2× SSC (standard saline citrate) and 0.2× SSC (50 minutes at 48°C), digested with ribonuclease (RNase) (30 μg/ml for 15 minutes at 37°C), and washed in 2× SSC and phosphate-buffered saline (PBS). After they were dehydrated, the specimens were coated with NTB-3 emulsion (Kodak), exposed for 24 to 72 hours, developed, fixed, and then stained with hematoxylin and eosin. Arrowheads indicate the area enlarged (B). Final magnification, ×90. (**B**) High-power view of stratum granulosum and stratum spinosum. Final magnification, ×240.

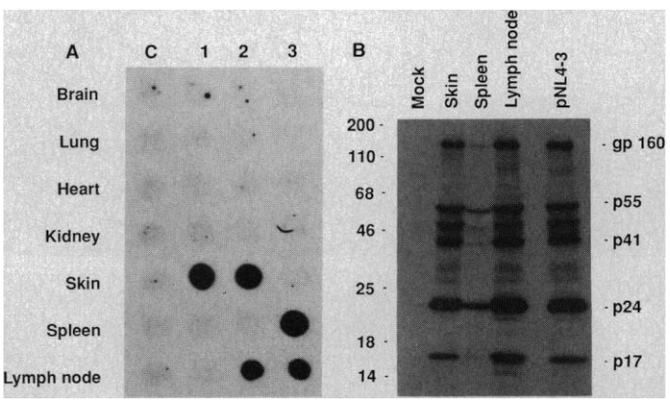

Fig. 5. Recovery of HIV from transgenic mice. (**A**) Tissues from transgenic mice (lanes 1, 2, and 3) and from a nontransgenic littermate (lane C) were collected under sterile conditions at the time of necropsy and cultured with PHA-stimulated human PBL's (2×10^6 cells) maintained in RPMI medium plus 10 percent FCS. Supernatants were collected and assayed for RT activity (*30*) at periodic intervals. The RT activity in 10-μl samples of the culture medium at 3 weeks is shown in the autoradiogram. (**B**) Immunoblot analysis of virus recovered from transgenic mice. Cells (A3.01) were infected with equivalent amounts of virus (as determined by RT activity) from the tail skin, spleen, and lymph nodes of affected transgenic mice as well as with parental virus (pNL-4-3) or RPMI medium (mock). Lysates were prepared on day 15 after infection, proteins were separated by SDS-PAGE (polyacrylamide gel electrophoresis), immobilized on a nitrocellulose membrane, and then treated with pooled AIDS serum as described (*30*). After incubation with ^{125}I-labeled protein A, the membranes were washed and exposed to film.

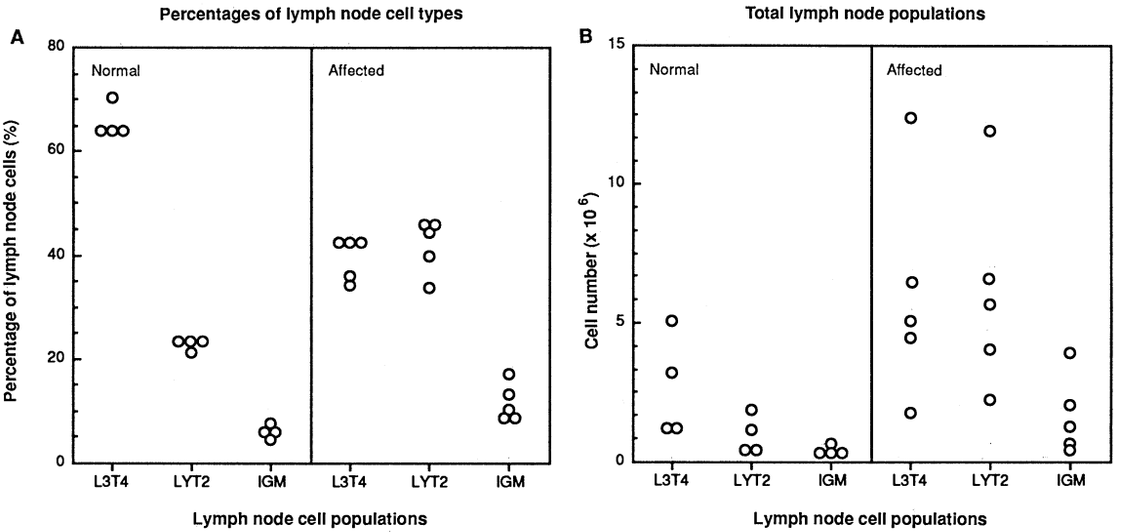

Fig. 6. Characterization of lymphocytes prepared from lymph nodes of HIV transgenic mice. Inguinal, axillary, and cervical lymph nodes of individual transgenic and control mice were pooled and analyzed for lymphocyte populations by means of using FACS analysis (*31*). Data are expressed as (**A**) relative proportion of L3T4, Lyt-2, and immunoglobulin M (IgM) bearing cells and as (**B**) total numbers of these populations in the combined lymph node population. Each data point is the result from an individual mouse. Fluorescein-conjugated antibodies to Lyt-2 were purchased (*32*). Fluorescein-conjugated goat antibody to mouse IgM (μ chain) and biotynilated antibody-L3T4 (clone H129.19) were used (*33*).

that in nontransgenic littermates. In contrast, the thymuses of diseased mice were consistently smaller. In most affected animals, these organs were approximately 40 percent the size of the thymuses in healthy littermates, but contained relatively normal proportions of $L3T4^+$-$Lyt\text{-}2^-$, $L3T4^-$-$Lyt\text{-}2^+$, and $L3T4^+$-$Lyt\text{-}2^+$ thymocytes. In severely affected 20-day-old mice, however, the thymuses were completely involuted with evidence of marked cortical atrophy, and contained only 5×10^6 to 7×10^6 cells (compared to 200×10^6 cells in control animals).

Splenic T cell function was assessed in a few affected animals. The proliferative response to concanavalin A (con A) was moderately but consistently reduced in affected animals; the greatest disparity between transgenic and control animals was in severely affected, older mice (more than 20 days of age). Only modest reduction in lymphocyte proliferation, after stimulation with antibody to CD3, was observed in lymphocytes from affected animals. Likewise, small but consistent decreases in interleukin-2 production, after treatment with con A or after exposure to either autologous or allogeneic cells, were observed in splenic lymphocytes of transgenic mice.

Only one of the seven founder animals (No. 13) gave rise to the phenotype described. The stringent P4 biosafety conditions, the small size of the glovebox facility in which the animals were housed, and restrictions on the total number of transgenic animals maintained at any one time precluded an analysis of a larger number of transgenic strains so that it was not possible to identify other mice displaying a phenotype similar to that observed in strain No. 13; however, two of the six other strains transmitting full-length copies of HIV proviral DNA to their progeny had very high mortality rates. Only 1 death in 96 F_1 offspring of 4 other founder animals was observed (Table 1). In contrast, 7 of 35 F_1 mice from founder No. 16, and 4 of 32 F_1 mice from founder No. 62, either died unexpectedly or became moribund and were therefore killed. Chi-square analyses showed that the latter mortality rates are statistically significant (with Bonferroni correction) at $P \leq 0.0004$ and 0.008, respectively, compared to that of the 96 progeny of founders 38, 39, 42, and 64 (Table 1). DNA prepared from four of the seven strain No. 16 F_1 animals contained HIV proviral DNA; because of autolysis attending some of the spontaneous deaths, no DNA was obtained from the three remaining mice.

Pathogenic mechanisms. At present, we have no explanation for the failure of founder No. 13 to develop disease while all of her transgenic offspring were affected. One possibility is that founder No. 13 is a mosaic animal with germ cells containing the transgene (*17*). However, the expression of an intact HIV provirus in the transgenic F_1 progeny of founder No. 13 results in a consistent and characteristic set of histopathologies. Hyperproliferative skin, perivascular pulmonary lymphoid infiltrates, splenomegaly, lymphadenopathy, growth retardation, and premature death, all occurring in the absence of Leu3 or T4 depletion, comprise the phenotype consistently observed. In the affected lineage, the major histologic abnormalities were found in the skin and lung. While each of the skin lesions is characterized by epidermal hyperplasia, it is difficult to directly equate them with analogous lesions found in man. Various non-neoplastic skin lesions has been reported in AIDS including seborrheic-like dermatitis, psoriasis, and ichthyosis (*18*); none of the skin lesions in these mice completely fits the histologic criteria for any of these diagnoses. The propensity to form nodules in skin is also of interest, since the submandibular lesions most closely resemble a trichofolliculoma, a neoplasm of hair follicles. Therefore, these animals have a distinctive cutaneous condition marked by epidermal hyperplasia. Epidermal hyperplasia and the appearance of dermal sarcomas have been seen in transgenic mice bearing the HIV long terminal repeat (LTR) directing the synthesis of the *tat* gene product (*19*). These tumors were found after observation periods of 1 year or longer and only in male animals. We have seen cutaneous abnormalities in animals of either gender; however, no clinically detectable tumors other than trichofolliculomas have occurred in a shorter observation period (9 months in founder animals and up to 25 days in strain No. 13 F_1 animals).

The pulmonary interstitial lymphoid infiltrates observed in the mice are similar to those present in adult AIDS patients with nonspecific interstitial pneumonitis (*20*). In both the transgenic mice and AIDS patients, the infiltrates occur in the absence of identifiable pulmonary pathogens and may, therefore, represent a nonspecific response to HIV infection. The reduced size of the thymus in all affected mice and the complete thymic involution in two severely affected animals are reminiscent of the precocious thymic involution observed in AIDS patients (*21*); however, thymic involution is also seen as a nonspecific response to stress in mice.

The introduction of the HIV provirus into the germline of mice bypasses the early events in the virus life cycle including the binding of particles to their receptor, the human CD4 molecule; a spreading infection in the newborn mice, unlikely in the absence of human CD4, cannot account for the abnormalities observed. Instead, the syndrome described probably results from the intracellular production of HIV proteins or particles, either of which could elicit the

disease complex observed. The pathophysiological consequences of carrying an integrated HIV provirus resemble some of those in naturally occurring lentivirus infections. Lymphoid pulmonary lesions in visna- or maedi-infected animals (*22*) and dermatologic, pulmonary, and immunologic abnormalities in HIV-infected individuals (*9, 18, 20*) have been reported in the absence of opportunistic infections.

A characteristic of many lentivirus infections is the disparity between the profound clinicopathological findings and the low level of virus expression in affected tissues. HIV replication, for example, can only be demonstrated in circulating mononuclear cells at a frequency of less than 1 in 10,000 (*23*). Although the pathogenic mechanism (or mechanisms) responsible for the syndrome observed in strain No. 13 transgenic mice is not clear, this syndrome is also associated with low HIV gene activity. The absence of detectable HIV RNA in spleen and lymph node samples by in situ hybridization despite the recovery of infectious virus after 2 to 3 weeks of cocultivation, reiterates the pattern of low level lentivirus expression in vivo.

One explanation for the dissociation of HIV expression and severe clinical disease in both man and transgenic mice is that synthesis of very small amounts of viral proteins or progeny particles may be sufficient to elicit the production of cellular proteins which cause deleterious effects. Thus, low level HIV expression in macrophages might disregulate the synthesis or secretion of factors mediating chemotaxis, inflammation, or cell growth and result in the fatal disease observed. The epidermal proliferation observed in the affected transgenic mice, could represent the indirect effects of HIV expression in cells of monocyte or macrophage lineage (*24*). Furthermore, if the transgenic mice are not tolerant to HIV proteins, the observed splenomegaly and lymphadenopathy could be the result of a chronic immune response to the persistent production of viral proteins as observed with lymphocytic choriomeningitis virus (*25*).

Another mechanism potentially responsible for the disease phenotype of the F_1 transgenic animals is the interruption of an essential mouse gene as a result of proviral DNA integration. Insertional mutations in transgenic animals, however, tend to be recessive traits that are expressed in offspring homozygous for the defective gene (*26*). The uniformly heterozygous state of affected mice, the recovery of virus only from diseased tissues of affected transgenic animals, and the parallels between the syndrome described here and those reported in naturally occurring lentiviral diseases make insertional mutagenesis an unlikely mechanism. Furthermore, two other strains of transgenic mice that do not display the histological changes described for strain No. 13 die at rates significantly greater than control animals (Table 1). Perhaps the early deaths or morbidity observed in strains No. 16 and No. 62 animals reflects incomplete penetrance of the "full-blown" syndrome present in strain No. 13 mice. Differential expression of endogenous retroviral sequences has been observed in vivo (*27*), and transgenic mouse strains carrying Moloney leukemia virus in their germlines have been shown to express virus in utero, perinatally, late in life, or not at all (*28*).

Transgenic mice such as these prove useful for studying the pathogenesis of HIV-related diseases in man. These animals could also be used to identify compounds that abrogate disease onset or progression if they are administered before onset.

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