combination with an A·U base pair at position 3·70, the resulting tRNA was severely damaged for acceptor identity and efficiency of suppression (Table 1), although the amount of tRNA was not reduced (*10*). Interpretation of this result is complicated because in this variant the G·U wobble pair is located nearer the 3′ end of the tRNA molecule, and also because this G·U is surrounded by a distinctive base pair context relative to the G·U in other active variants. We have shown that adjacent base pairs can degrade the function of the G·U wobble pair in wild-type $tRNA^{Ala}$. When wild-type $tRNA^{Ala}$ was changed at position 2·71 and 4·69 from G·C to C·G, both the acceptor identity and the suppression efficiency were depressed (variant C2·G71, G3·U70, C4·G69, Table 1).

The contribution of the G·U wobble pair to the acceptor identity of $tRNA^{Ala}$ has been known for some time. We have suggested that the G·U wobble pair induces an irregularity in the acceptor helix that allows $tRNA^{Ala}$ to fit with the cognate synthetase. Other tRNAs lack this structure and are excluded. The structure could kink the helix, or swing nucleotides outward from the helix, or dissociate the helix to two single strands. In all cases, the thermodynamic instability observed with G·U wobble pairs in helices (*6*) may help form the irregularity. The structure could be important in the initial interaction between $tRNA^{Ala}$ and its cognate synthetase, or in a subsequent kinetic step of the reaction. There is increasing evidence from a number of systems that sequence-dependent conformations of duplex nucleic acids can influence the functions of these molecules (*12*). Furthermore, a possible relation between an imperfection in the acceptor helix and $tRNA^{Gln}$ acceptor identity has been noted previously (*13*).

We are only beginning to understand the relation between structure and function in $tRNA^{Ala}$. We have emphasized the importance of an irregular structure to the acceptor identity of $tRNA^{Ala}$, but have noted that a functional group on the G·U wobble pair may also contribute. In addition, other regions of the $tRNA^{Ala}$ molecule are known to be important for acceptor identity (*2*).

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Infection of the SCID-hu Mouse by HIV-1

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SCID-hu mice with human fetal thymic or lymph node implants were inoculated with the cloned human immunodeficiency virus–1 isolate, HIV-1_{JR-CSF}. In a time- and dose-dependent fashion, viral replication spread within the human lymphoid organs. Combination immunohistochemistry and in situ hybridization revealed only viral RNA transcripts in most infected cells, but some cells had both detectable viral transcripts and viral protein. Infected cells were always more apparent in the medulla than in the cortex of the thymus. These studies demonstrate that an acute infection of human lymphoid organs with HIV-1 can be followed in the SCID-hu mouse.

MULTIPLE LINES OF EVIDENCE suggest that the etiologic agent of the acquired immunodeficiency syndrome (AIDS) is human immunodeficiency virus (HIV) (*1*). Definitive proof and direct analysis of infection by HIV in vivo has been hampered by the lack of a suitable small animal model. Thus, we know little about the events which occur immediately after infection or about the subsequent progression of disease (*2*). A number of experimental animal models have been advanced. All are approximations with severe limitations: either an animal retrovirus other than HIV is used to infect a host other than man (*3*), or HIV is used in a context beyond that of its natural tropism (for example, in primates or rabbits) (*4*). The ideal system would allow the study of the interactions that occur during the natural course of disease, that is, those of HIV with the human lymphoid system. The SCID-hu mouse (*5*) was constructed precisely for this purpose. We present evidence now that this mouse model supports infection by and replication of HIV-1.

Implantation, growth, and interactions between the human lymphoid organs of the SCID-hu mouse have been described (*5*). In many respects, the environment of this hematochimeric mouse is similar to that encountered by HIV in man. After parenteral inoculation or sexual contact, it seems likely that HIV drains to and is sequestered within lymphoid organs such as lymph nodes, Peyer's patches, or spleen; during perinatal infection, the developing thymus may also be a major target organ for infection (*6*). These lymphoid organs present full complements of interactive cells which are permissive for infection, including $CD4^+$ T cells and myelomonocytic cells. The human fetal thymus and lymph node implants which grow in the SCID-hu mouse have microscopic features and cellular compositions that are essentially indistinguishable from those of their normal human counterparts (*5*). We anticipated that parenteral inoculation of these organs would provide a previously unobtainable view of acute infection with HIV in vivo.

A molecular clone of HIV-1 was used in these studies (*7*). Derived from the cell-free cerebrospinal fluid of a patient with subacute encephalopathy and Kaposi's sarcoma,

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HIV-1_{JR-CSF} was propagated in short-term culture with phytohemagglutinin-stimulated human peripheral blood lymphocytes and then cloned into the bacteriophage vector EMBL4 (*8*). This isolate is infectious for mitogen-stimulated human T cell blasts and for glial cell explants, but not for primary monocyte-macrophage cultures. It has not been passaged and does not grow in continuous human T or monocyte-macrophage cell lines (H9, Jurkat, MOLT4, HUT-78, U937). Such tropism marks a potentially important difference between it and other isolates which have been cloned and adapted to growth in vitro (for example, LAV, HTLV-III_B, HXB2, and ARV) (*9*).

SCID-hu mice were infected within the confines of a specially adapted animal biosafety level (BSL) 3 facility (*10*). A flank incision exposed the growing human thymus or lymph node implant of anesthetized SCID-hu mice. Graded doses of HIV-1_{JR-CSF} were introduced by direct intrathymic or intranodal inoculation. The mice were maintained in micro-isolator cages, inside a sealed glove box, within the BSL3 facility. At various time points, biopsy specimens of the infected lymphoid organs were obtained, fixed in 4% paraformaldehyde, and assayed for signs of acute infection.

Inoculation of SCID-hu mice with HIV-1_{JR-CSF} generated detectable signs of viral replication. The most direct assay was in situ hybridization of infected tissue sections with the RNA probe pG4 (Fig. 1) (*11*). This probe hybridizes to the 3′ end of the genomic HIV-1 transcript (see legend to Fig. 1) accordingly, all viral RNA transcripts (genomic and subgenomic) are detected without discrimination. Under the conditions of the assay, viral DNA is not detected. Infection of the human thymus implants was time-dependent. At 1 week after intrathymic inoculation of 400 to 4000 infectious units (IU), no cells in the section hybridized with the pG4 probe (Fig. 1A). At 2, 4, and 8 weeks (Fig. 1, B, C, and D, respectively), more cells hybridized (see below, Fig. 3). This suggests that ongoing rounds of infection occurred in vivo. The infected cells were scattered throughout the cortex and medulla of the injected human thymus and across the injected human lymph node (Fig. 1F). Discrete foci of infection, involving more than one contiguous cell, were only rarely observed (see below, Fig. 2B).

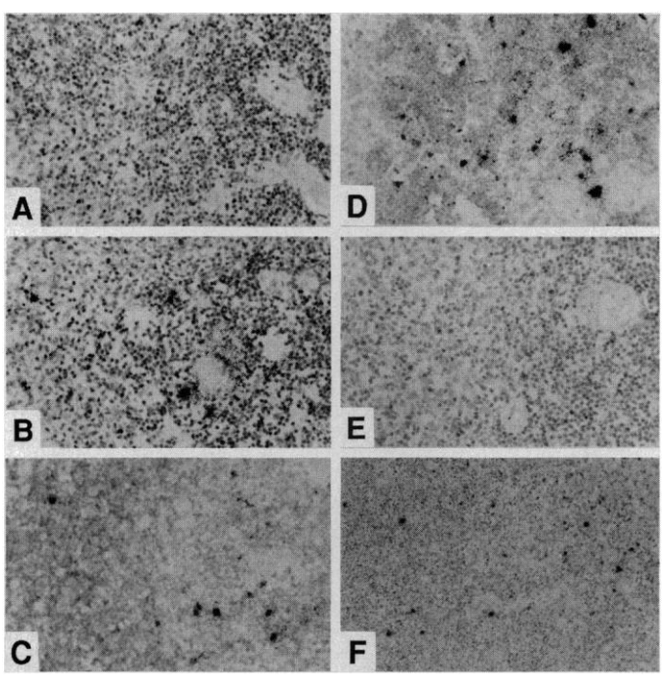

Fig. 1. Infection of the SCID-hu fetal thymus and lymph node implants with HIV-1. Eight weeks after implantation of human fetal thymus (**A** to **E**) or lymph node (**F**) into SCID mice, 400 to 4000 IU of HIV-1_{JR-CSF} (in 20 μl) were introduced by direct intrathymic or intranodal injection. At intervals of 1 week (A), 2 weeks (B, E, and F), 4 weeks (C), or 8 weeks (D) thereafter, the implants were harvested, fixed in 4% paraformaldehyde in phosphate-buffered saline at 4°C for 4 to 8 hours, washed with phosphate-buffered saline for 24 hours, and then frozen in O.C.T. embedding compound (Miles Laboratories, Inc.). Six-micrometer sections were subjected to in situ hybridization (*15*) with a ^{35}S-labeled antisense (A to D and F) or sense (E) RNA probe to the 3′ end of HIV-1, pG4. This probe was prepared by subcloning the 1.1-kb Bam HI–Hind III fragment of HXB2 [nucleotides 8054 to 9199, according to (*16*)] into the multicloning site of pGEM1. Transcription was from the SP6 promoter (anti-sense, A to D and F) or T7 promoter (sense, E) in the presence of ^{35}S-labeled UTP. Under the conditions used, neither probe hybridized to uninfected mouse or human cells, or to HIV-1 DNA; therefore, cells with grains in the autoradiograph are presumed to be infected with transcriptionally active HIV-1. All micrographs of thymus (A to E) are aligned such that the medulla is on the right and the cortex on the left.

The events described in Fig. 1 are specific for HIV-1 infection. In infected tissue no cells hybridized with pG4 RNA probes in the "sense" orientation (Fig. 1E); no cells in uninfected tissues hybridized to pG4 probes in either orientation. Thymic or lymph node implants inoculated with heat-inactivated virus (80°C, 1 hour) did not hybridize after 4 weeks. The infection was also dose-dependent. Thus, a 10^{-1} dilution of HIV-1_{JR-CSF} was infectious, but dilutions of 10^{-3} and 10^{-5} were not. Using estimates of viral titers determined by in vitro assays, the 10^{-1} input dose corresponded to 400 to 4000 IU. (*12*).

We also applied immunohistochemical stains to the tissue sections. The polyclonal antiserum GB, selected for its high-titer reactivity with viral env, gag, and pol determinants, was used. Many cells in the infected thymus were reactive with this antiserum. In all cases examined, the number of reactive cells was fewer than the number reactive by in situ hybridization with the pG4 probe (*11*). To explore this observation further, thymus tissue sections 2 weeks after HIV-1_{JR-CSF} infection were stained first with immunohistochemical stains and then by in situ hybridization (Fig. 2). In this manner, the presence of viral protein could be detected in cells making viral RNA transcripts; in addition, the cell surface phenotype of infected cells could be determined. In both the medulla and the cortex of the thymus, as well as in the lymph node, cells were found to produce either detectable amounts of viral protein and RNA (arrows, Fig. 2A) or only viral RNA transcripts (all other reactive cells, Fig. 2A). The latter population represented the majority of total infected cells in either compartment (Fig. 3). No cells were observed to produce only viral protein in the absence of detectable viral RNA. The basis for this observation is not clear. An artifact related to the procedure itself appears unlikely: cells with each mode of expression (viral RNA and protein, or viral RNA alone) are randomly dispersed and frequently adjacent (see Fig. 2, A and B). More likely, patterns of transcriptional and translational control after infection may differ in distinct cell types, differentiative stages, or both. Complex patterns of this sort have been documented in vivo after infection with other lentiviruses (for example, visna virus and canine arthritis-encephalitis virus) (*13*). Their existence is consistent with recent experiments on HIV-1 in vitro (*14*). The regulatory mechanisms by which they occur may now be explored with HIV-1 in

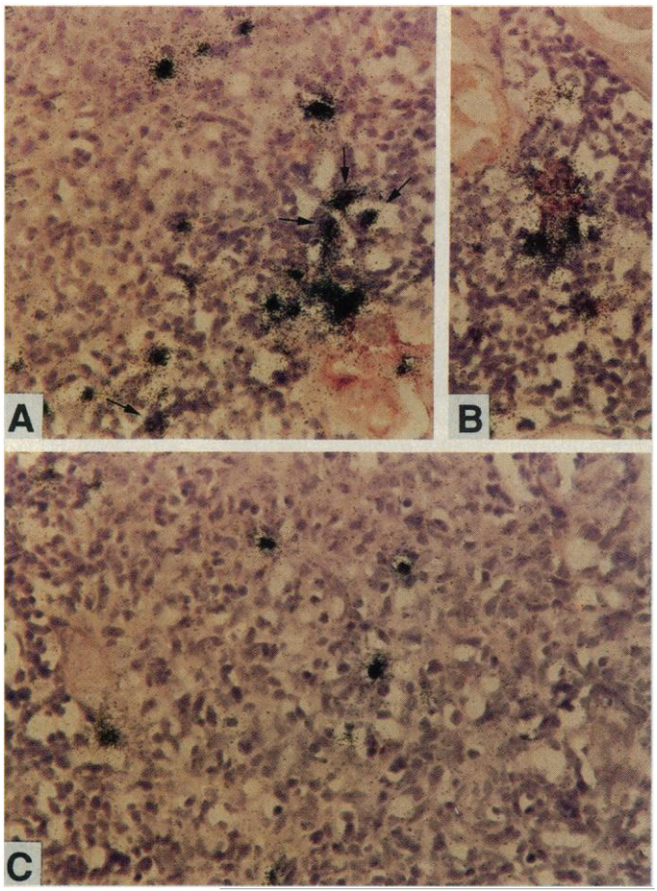

Fig. 2. Simultaneous visualization of HIV-1 viral protein and RNA within infected SCID-hu thymus. A SCID-hu mouse was infected by intrathymic injection of HIV-1_{JR-CSF} (400 to 4000 IU). Individual 6-μm thymic sections were analyzed 2 weeks later by successive applications of immunohistochemical stains and in situ hybridization. They were first immunostained with the human polyclonal antiserum GB (1:300 dilution) and then with peroxidase-labeled goat antibody to human immunoglobulin G (1:100) (CALTAG Laboratories). The peroxidase activity was developed with 3-amino-9-ethylcarbazole. Second-stage antibody alone was unreactive with infected thymus. In uninfected thymus, no reaction products were seen using this combination of antibodies. The immunostained tissue sections were then fixed again with 4% paraformaldehyde (20 min, 20°C), and subjected to in situ hybridization with the antisense pG4 RNA probe (as described in Fig. 1). Representative areas are shown of thymus medulla (**A**) and cortex (**C**). Cells reactive with both antibody and RNA probes are marked by arrows in (A). Medullary cells of the appearance shown in (**B**) were occasionally observed; at all times, they showed evidence of both viral protein and RNA.

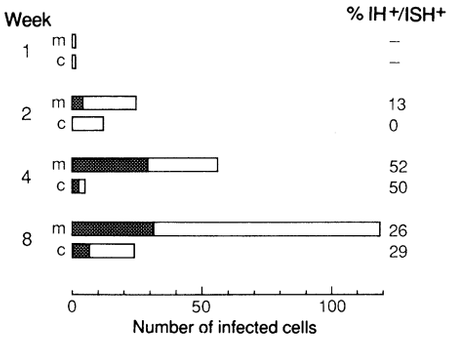

Fig. 3. Quantitation of HIV-1 infection of the SCID-hu thymic cortex and medulla. The SCID-hu thymic sections of Fig. 1 were subjected to immunohistochemistry and in situ hybridization in parallel to detect HIV-1 viral protein and RNA transcripts. At each time point, two observers counted the number of cells in five to ten high-power (400×) fields that stained with the antibody GB or with the RNA probe pG4. The bar graphs represent the mean number of infected cells in either the thymus medulla (m) or cortex (c) that were detected by in situ hybridization. The mean number of cells that stained with antibodies to HIV-1 is shown as a darkened area of each plot. To the right, the number of cells immunohistochemically stained (IH) is expressed as a percentage of the number of cells positive by in situ hybridization (ISH): %IH^+/ISH^+.

vivo, within the SCID-hu mouse.

More cells were found to be infected in the medulla (Fig. 2, A and B) than in the cortex (Fig. 2C), even though there was an abundant representation of $CD4^+$ thymocytes in each compartment (*5*). A similar situation was found at weeks 4 and 8 after infection, throughout multiple planes of observation (Fig. 3). Thus, within any given section at any given time, 70 to 90% of the infected cells were medullary in location. Multinucleated giant cells, a common feature of HIV infection in vitro, were occasionally seen (Fig. 2B).

In summary, human thymus and lymph node transplanted into SCID mice support the in vivo initiation and spread of infection by HIV. The SCID-hu mouse thus appears to provide a fertile environment for further investigations into the course of infection by HIV in vivo. To the extent that one may observe the onset of infection within complex lymphoid organ systems following parenteral inoculation with HIV, it should be possible to dissect subsequent interactions systematically, after infection, on both a cellular and molecular level. Such analyses, initiated in vivo within a small animal model with human lymphoid organs, might more closely approximate the course of infection in man and more readily lead to meaningful and useful paradigms.

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10. The design of the BSL3 facility and procedures used with animals therein were developed according to guidelines set forth by the NIH/CDC and approved by the Stanford Panel on Biosafety and the Stanford Administrative Panel on Laboratory Animal Use. To provide additional containment within the BSL3 facility, all mice were housed inside a sealed glove box equipped with high efficiency particulate-free air filters (Isotec Systems).
11. Similar results to those shown in Figs. 1 and 3 were obtained with another RNA probe, pG3. This probe was constructed in pGEM1 by subcloning the Hind III–Bgl II fragment between nucleotides 5610 and 6622 of HXB2 (*16*); it should predictably hybridize to viral genomic RNA and to envelope mRNA.
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