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Tissue Selectivity of Murine Leukemia Virus Infection Is Determined by Long Terminal Repeat Sequences

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Here we show that the tissue specificity of murine retrovirus infections is determined by the long terminal repeat (LTR) of an otherwise isogenic set of viruses. The isogenic viruses used for this study contain the coding *gag*, *pol*, and *env* genes of the avirulent Akv virus. Recombinant viruses that contain the LTR of a virus that induces T-cell leukemia lymphoma preferentially infect T lymphocytes. Viruses that carry the LTR of a virus that induces erythroleukemia preferentially infect non-T lymphoblastoid cell lines in the marrow and spleen. The Akv virus itself displays no tissue preference for hematopoietic cells. These experiments suggest that retroviruses that carry appropriate enhancer-promoters can be used to infect selectively specific target cells in animals.

The murine leukemia viruses (MuLVs) compose a large family of retroviruses (15, 18, 19, 21). Individual viral isolates often induce a characteristic disease state when inoculated into susceptible strains of mice. The retroviral genome contains the structural sequences encoding the *gag*, *pol*, and *env* protein products. In addition, the integrated viral genome is flanked at both ends by noncoding long terminal repeat (LTR) sequences. Within the LTR are regulatory signals required for viral transcription, replication, and integration into the host cellular DNA (43). Recent findings show that the ability of these viruses to induce T-cell leukemia and erythroleukemia is determined in large measure by the viral LTR (4, 5, 9–13, 27, 28). Although it has been suggested that the LTR sequences of these viruses confer tissue specificity and, therefore, disease specificity, this hypothesis has not been tested by direct measurement of the extent of infection of hematopoietic tissues. To determine whether the LTR sequences do indeed confer the extent of infection of specific cell types, we constructed a series of isogenic recombinant viruses that differ only in the LTR regions and measured the fraction of thymus, marrow, and spleen cells infected 4 to 8 weeks postinfection of newborn mice.

The structures of the genomes of the recombinant proviruses constructed for this purpose are shown in Fig. 1. The parental viruses used include the avirulent Akv virus (20, 31, 40), the SL3-3 (34, 39) virus that induces a thymic disease, T-cell leukemia lymphoma, and the Friend helper virus (38) that induces primarily splenic erythroleukemia (34, 37, 39, 42). Recombinant viruses that are produced by transfection of NIH 3T3 cells from proviruses that contain the *gag*, *pol*, and *env* genes of the Akv virus and the LTR of either the SL3-3 virus or the Friend helper virus were also used for these experiments. Our previous studies showed that the recombinant SL3-3-LTR-Akv *gag*, *pol*, *env* virus (RSA-1) induces T-cell leukemia lymphomas in AKR, CBA, C3H, SJL, and NFS strains of mice (28).

To determine the organ preferences for infection of each of these viruses, we injected newborn NFS mice with 10^4 to 10^5 infectious units as determined by the XC plaque assay (41) or serological focus assay (7). The ability of virus to replicate in thymus, spleen, and marrow cells was determined by flushing the cells from the organ and measuring the number of infected cells as infectious centers on SC-1 cells. Because SL3-3 itself gave poor XC results on SC-1 cells, the titer of this virus and organ infectious centers were determined by using a serological focus assay.

The number of infectious centers was generally low in the thymus, spleen, and marrow of animals infected with the molecularly cloned Akv (Fig. 2). High viral titers were occasionally observed in some mice. Five of 10 mice tested had no virus in the thymus, spleen, or bone marrow. Three showed low levels (60 to 150 XC infectious centers per 10^7 cells) in the bone marrow only and none in the thymus or spleen. High numbers of infected cells were found in all three organs in two of the animals. Evidently, infection of NFS mice with Akv is variable. Many mice did not maintain infection, and no preferred organ site was observed for animals in which infection was established.

In contrast, with the injection of SL3-3 virus, the fraction of infected cells eluted from the hematopoietic tissue was higher than that in Akv-inoculated mice tested 4 to 8 weeks postinfection. Moreover, the number of virus-positive cells in the thymus was consistently higher than those in the spleen and bone marrow. The increased infectivity of the virus for all hematopoietic organs and the preference for thymocytes is also a property of the recombinant RSA-1, which contains the LTR sequences of SL3-3 and the *gag*, *pol*, and *env* coding genes of Akv. Early after infection, the RSA-1 virus was found in thymus and bone marrow cells but not spleen cells. In older mice there was a considerable preference for infection of thymocytes as compared with either marrow or spleen cells. We conclude that preferential infection of thymocytes is determined by the SL3-3 LTR sequences of the RSA-1 recombinant virus.

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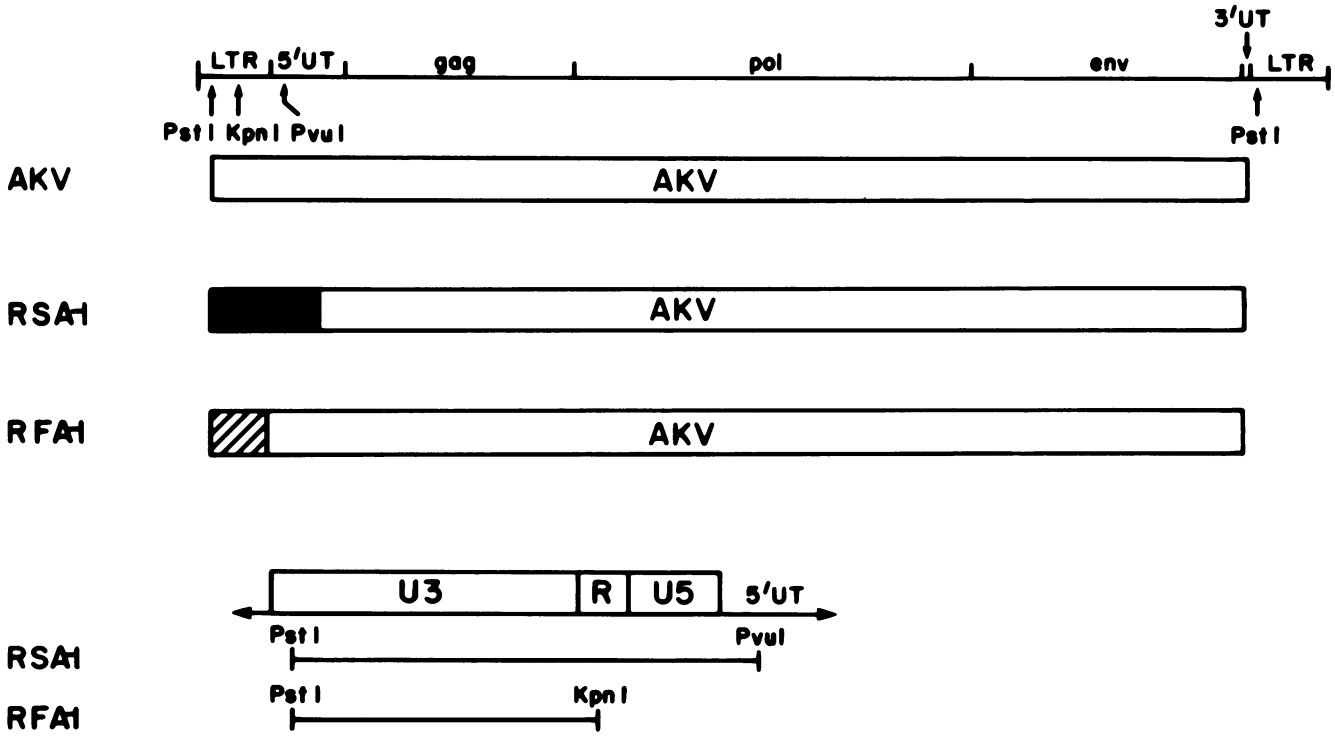

FIG. 1. Diagram identifying the origin of genomic sequences present in the recombinant viruses. The line drawing at the top details the genomic organization of a typical MuLV. The open bar represents the genome of Akv virus. Regions derived from the SL3-3 and Friend genomes are indicated by solid and crosshatched bars, respectively. A more detailed diagram showing the actual amount of SL3-3 and Friend LTR sequences present in the recombinants is shown at the bottom. Construction of plasmid RSA-1 (previously RECAS 115) has been described previously (26). To construct plasmid RFA-1, a molecular clone of Fr-MuLV 57 was cleaved with *Pst*I-*Kpn*I, and the 450-base-pair fragment encompassing the indicated portion of the Friend LTR was used to replace the corresponding segment present within the Akv LTR. In each plasmid only one LTR was present. Construction of the final plasmids was always confirmed by extensive restriction enzyme mapping. All recombinant DNA techniques were done by standard established procedures (32). Enzyme digestions were done according to the specifications of the manufacturers.

The organ tropism of a recombinant that contains the LTR sequences of Friend (Fr)-MuLV 57 and the coding genes of Akv differed dramatically from that of the Akv or SL3-3 LTR recombinants (Fig. 2). The fraction of infected cells eluted from the spleen and marrow of animals infected with the RFA-1 virus was consistently higher than the fraction of infected cells eluted from the thymus. The fraction of infected cells was one to two orders of magnitude greater in the spleen and bone marrow and about two orders of magnitude smaller in the thymus in mice infected with the RFA-1 recombinant than it was for mice infected with the RSA-1 recombinant.

The organ tropism of the RFA-1 virus was similar to that of Fr-MuLV 57, the strain from which the LTR sequences were derived. However, the extent of infection of the thymus, marrow, and spleen of Fr-MuLV 57-infected animals was considerably higher than that observed with infection with the Friend LTR recombinant RFA-1. Evidently, regions of the genome other than the LTR contributed to the overall level of virus infection.

Our results show that the specificity of Akv-like viruses to infect preferentially the thymus, spleen, or bone marrow cells of NFS mice is largely a property of the LTR and is not principally determined by the *gag*, *pol*, and *env* genes of the virus. We postulate that the ability to infect a significant number of cells in a given organ is a prerequisite for disease induction by the nonacute retroviruses. This hypothesis is in agreement with previous studies implicating the LTR regions of the MuLVs as a major determinant of disease phenotype (1, 4, 5, 9–14, 26–28). For example, recombinant viruses that contain the LTR sequences of the thymic leukemia viruses SL3-3 and Moloney leukemia virus and the coding sequences of Akv or Friend virus induce a T-cell leukemia (4, 26). The reciprocal recombinant that contains the LTR of Friend and the coding genes of Moloney induces a disease typical of the Friend virus (5). The high level of infection of thymocytes by Fr-MuLV 57 observed here (Fig. 2) was in accord with the observation that although most of the mice inoculated with this virus contracted erythroid tumors, a small number of T-cell tumors were also found. We note that a small number (less than 10%) of NFS mice injected with RFA-1, the Friend-Akv recombinant virus, developed an erythroproliferative disease (data not shown). Thus, although viral replication in the proper tissue is probably necessary for induction of hematopoietic neoplasms, other factors that determine the overall level of infection of a tissue are likely to influence the leukemogenic outcome. It is also of interest that the incidence of specific types of disease correlates very well with the numbers of infected cells in an organ. For SL3-3 and the RSA-1 recombinant, the incidence of T-cell leukemia (the only disease noted in these animals) was comparable, corresponding to comparable titers of infected thymocytes (26). The incidence of erythroleukemia in mice injected with the recombinant that contained Friend virus LTR and Akv coding genes was about one-tenth that observed for the Friend virus itself, in accord with the lower

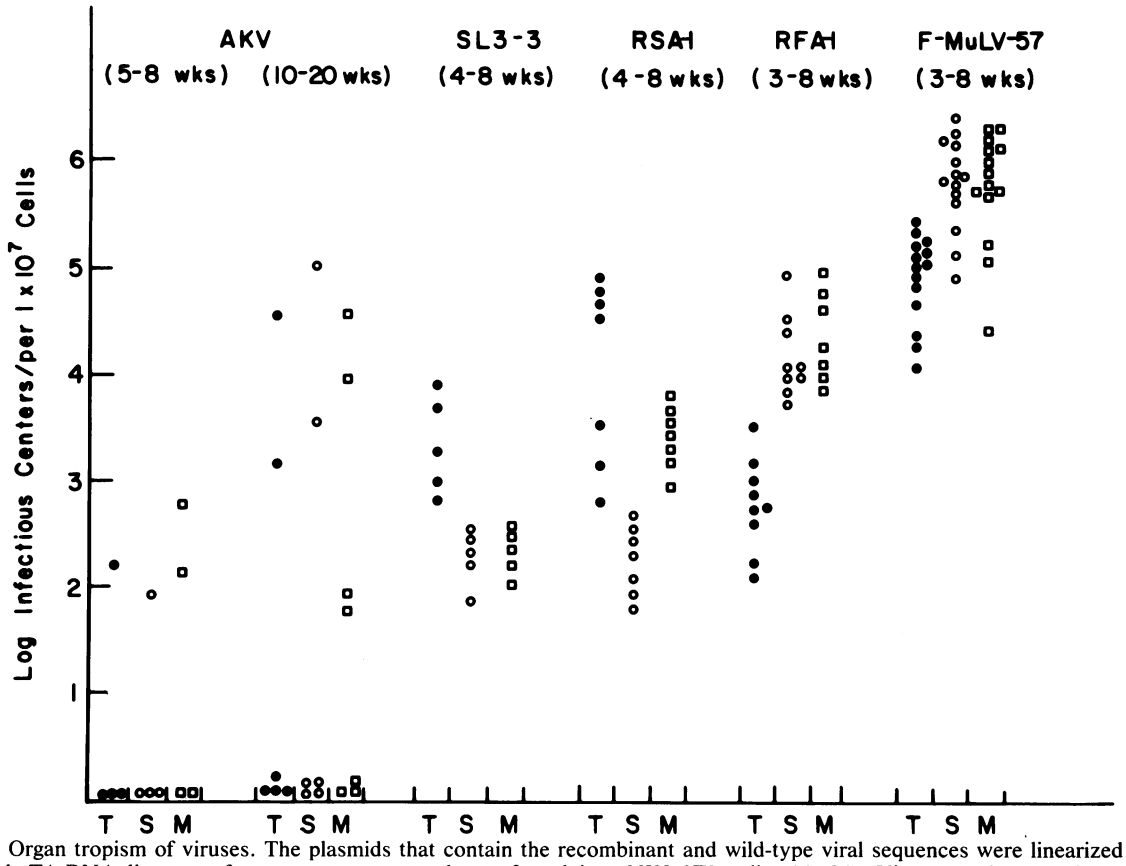

FIG. 2. Organ tropism of viruses. The plasmids that contain the recombinant and wild-type viral sequences were linearized with *Pst*I, ligated with T4 DNA ligase to form concatemers, and transfected into NIH 3T3 cells (16, 26). Virus was harvested 3 to 4 weeks posttransfection, and newborn (less than 2 days old) NFS mice were inoculated with 10^4 to 10^5 PFU. At the indicated times, mice were sacrificed, and 10^7 cells were flushed from the isolated organs and plated onto a lawn of SC-1 cells. An XC plaque assay was performed (41). Viral titers shown here are expressed as log infectious centers per 10^7 cells. Each symbol represents the number of infectious centers obtained from 10^7 thymus (●), spleen (○), or bone marrow (□) cells of an individual mouse. Viral titers of mice inoculated with SL3-3 were determined by a serological focus assay (5).

titer of infected bone marrow and spleen cells observed for the recombinant when compared with the parental virus (data not shown). The data suggest that together the type and incidence of disease induced by these viruses represent a continuum dependent on the efficiency of infection of the target tissues.

The recent studies of Oliff et al. with recombinants of Fr-MuLV also indicate that genomic sequences other than the LTR influence the frequency of virally induced leukemias (35–37). The extent of infection notwithstanding, it is evident that the Friend LTR confers a tissue tropism on the recombinant different from that of either the SL3-3 or the Akv LTR.

Recent evidence suggests that a necessary step for disease induction by some nonacute transforming viruses is transcriptional activation of a cellular gene via nearby integration of a provirus (7, 8, 25, 30, 44). Integration within the host genome is thought to be a random process; thus, the probability for activation of a cellular gene will be greatly increased if virus replicates efficiently in particular tissues.

What property of the LTR might determine the tissue specificity of infection? A sequence comparison of the Akv, SL3-3, and Fr-MuLV LTR U3 tandem repeat regions is shown in Fig. 3. As noted previously, with the exception of a single point mutation, the only difference in the LTR sequence between Akv and SL3-3 is in the tandem repeat region (27, 28). The sequence of the Fr-MuLV U3 region also differs from those of Akv and SL3-3 in a similarly placed tandem repeat region. The sequence of the LTR of Fr-MuLV also differs from those of the Akv and SL3-3 viruses in the region 3′ to the tandem repeat elements. However, in this region the sequence of the Friend LTR is similar to that of the thymotropic Moloney MuLV (22). Thus, it is likely that the arrangement of sequences within the tandem repeat determines the tissue tropism of the virus. The tandem repeat elements of Moloney MuLV, Akv, and SL3-3, together with the tandem repeats of several papovaviruses, function as transcriptional enhancer elements (3, 23, 24, 29, 33). In addition to directing high levels of transcription, enhancer elements often show a specific tissue preference (2, 16, 45). In particular, recent studies show that the transcriptional elements of the SL3-3 LTR exhibit a marked preference for in vitro activity in T cells when compared with the corresponding region of the Akv virus (3).

In conclusion, we suggest that differences in the ability of the enhancer elements to promote transcription and thereby promote viral replication in specific, differentiated tissues are major determinants of both tissue tropism and pathogenesis of the MuLVs. In this respect, the enhancer element must be considered as one of several determinants that may affect the replication capacity of a virus in a specific tissue and therefore affect the tissue tropism of disease induction.

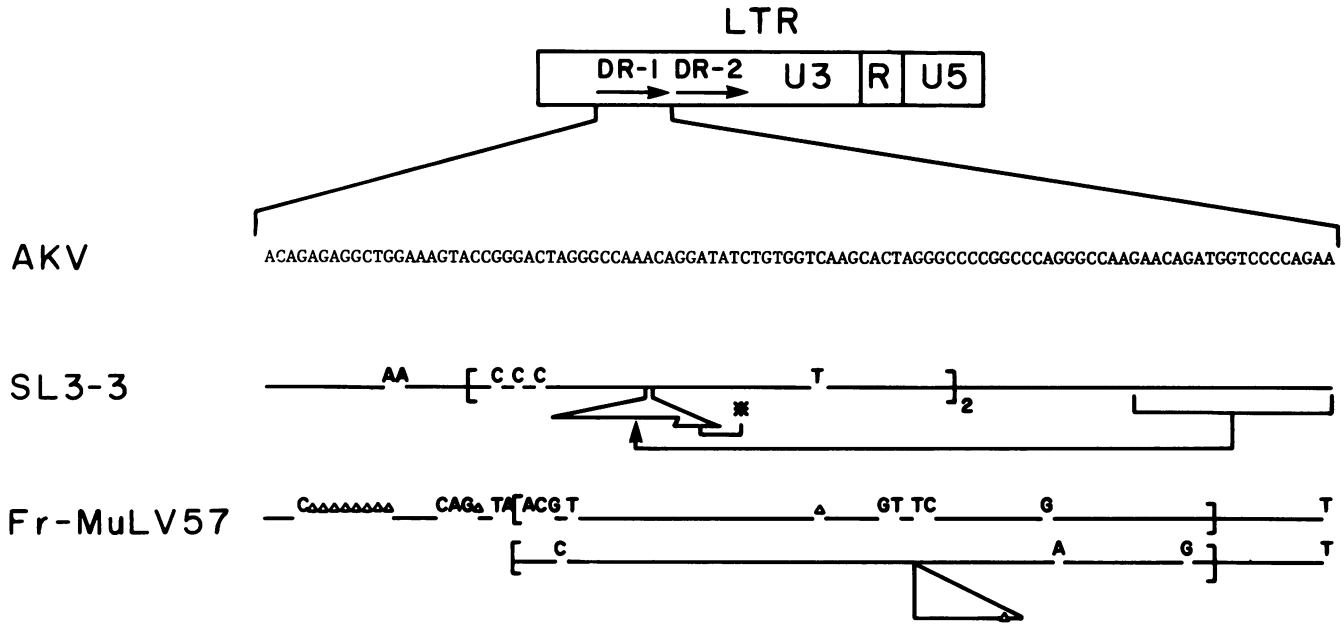

FIG. 3. Comparison of the sequence organization within the tandem repeat regions of the Akv (31), SL3-3 (39), and Fr-MuLV 57 (22) LTRs. The sequence of a single 99-base-pair repeat element present within the Akv LTR is shown at the top. The solid lines shown below represent regions of homology between the Akv repeat sequence and the 72- and 66-base-pair repeats of SL3-3 and Fr-MuLV 57, respectively. The number after the bracketed region is the number of copies of this sequence present within the LTR. Deletions (△) and substitutions relative to the Akv sequence are as shown. The sequence CCGCTAACG indicated by the asterisk is not present in the Akv sequence shown.

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