A HOST GENE REGULATES THE STRUCTURE OF THE TRANSMEMBRANE ENVELOPE PROTEIN OF MURINE LEUKEMIA VIRUSES

BY MICHAEL A. COPPOLA AND CHRISTOPHER Y. THOMAS

From the Departments of Internal Medicine and Microbiology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Retroviruses carry two envelope proteins that are formed by proteolytic cleavage of a common precursor molecule (1, 2). The major surface protein (SU)\(^1\) functions to bind the virion to specific receptors on the cell membrane. The minor transmembrane protein (TM) has several functions, including binding to the SU protein, anchoring both envelope proteins to the virion surface via a membrane-spanning segment near the COOH terminus, and fusing viral and cellular membranes to allow entry of the virion core into the cytoplasm. This latter function is probably mediated by a group of hydrophobic residues near the NH\(_2\) terminus (3). These and other functional domains of the envelope proteins may also influence the pathogenicity of certain retroviruses. For example, the SU and TM proteins of many retroviruses, including HIV, carry epitopes that elicit a strong immune response from the host and are targets for neutralizing antibodies or cytotoxic T cells (4, 5). A small domain within the TM protein of the feline leukemia virus has been reported to have immunosuppressive effects (6). In vivo or in vitro passage of the simian immunodeficiency virus (SIV) results in selection for or against truncated forms of the TM protein (7, 8). The pathogenic effects seen in other lentivirus infections may also depend upon the generation of viruses with altered envelope proteins (9, 10).

The development of spontaneous or retrovirus-induced lymphomas in high leukemia strains of inbred mice is accompanied by the generation of recombinant murine leukemia viruses (MuLVs), which are formed when the endogenous or exogenous ecotropic viruses recombine with envelope gene sequences encoded by a family of defective endogenous polytropic proviruses. The genomes of these recombinant viruses are presumably generated during reverse transcription by a mechanism involving strand invasion or template switching by the viral polymerase to yield hybrid cDNAs, and, ultimately, integrated recombinant proviruses (11, 12). The tumor-associated recombinant viruses recovered from AKR, C58, HRS, and CWD mice invariably contain ecotropic virus-related sequences in the 5' portion of the genome, but the

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\(^1\)Abbreviations used in this paper: AKV, AKR endogenous ecotropic murine leukemia virus; LTR, long terminal repeat; MuLV, murine leukemia virus; SIV, simian immunodeficiency virus; SU, retrovirus surface protein; TM, retrovirus transmembrane protein.
receptor-binding region of the SU protein, gp70^{env}, is encoded by sequences that are derived from the endogenous polytropic viruses (13-16). These polytropic envelope gene sequences allow the viruses to bind a cellular receptor distinct from the ecotropic virus receptor (17) and confer a broader in vitro host range (18).

We have previously reported differences in the envelope genes of recombinant viruses recovered from spontaneous lymphomas arising in CWD and HRS/J mice, although these two strains carry and express the same endogenous ecotropic proviruses, Env-1 and Env-3 (15, 16, 19, 20). Specifically, the sequences that encode the TM protein, p15E^{env}, differ between HRS/J and CWD recombinants. HRS/J recombinants retain ecotropic sequences in the 5' end of the TM coding region and are referred to as class I env recombinants (15). In contrast, the envelope genes of CWD recombinants contain polytropic virus sequences in this region and are designated class II env recombinants (16). Because of this difference in the extent of polytropic sequence substitution within env, the two classes of virus can be readily distinguished by hybridization to a probe from the 5' portion of the ecotropic p15E TM gene (21). This strain-specific difference in the structure of the TM gene is also seen in recombinants present in tumors induced by the highly leukemogenic AKR ecotropic retrovirus SL3-3 (22, 23).

Nucleotide sequence analysis has shown that the HRS/J class I and CWD class II recombinant proviruses acquire envelope gene sequences from the same family of endogenous polytropic proviruses (Coppola, M. A., S. C. Lawrenz-Smith, A. C. Massey, and C. Y. Thomas, manuscript in preparation). Thus, the differences in the origin of the p15E coding sequences of the HRS/J class I and CWD class II recombinants are probably not the result of differences in the structure of the polytropic virus parents, since all inbred strains contain multiple copies of these endogenous polytropic viruses (24, 25). To better understand what host factors might be involved in the selection for the class I or class II TM proteins, we determined the envelope gene structure of recombinant viruses in leukemic (CWD × HRS)F₁, F₂, and (CWD × HRS)F₁ × CWD backcross mice. In these studies the selection for class I recombinants in the HRS/J strain was a dominant trait controlled by a single genetic locus that cosegregated with RFLPs within the MHC on chromosome 17. CBA/J mice, which share the H-2^k haplotype with HRS/J, also carried the dominant allele of this gene.

Materials and Methods

Mice. CWD breeding stock (cw/d/ d, a/a and cw d/cw d, a/a) was obtained from The Jackson Laboratory, Bar Harbor, ME, and maintained at the University of Virginia vivarium by brother-sister matings (cw/cw to cw/+). These mice are now available as an inbred strain under the designation CWD/Lc. The CBA/J and HRS/J strains were also obtained from Jax. HRS/J mice were maintained in the heterozygous state by brother-sister matings of Hr/Hr and hr/+ animals. CWD and HRS mice were mated to generate progeny designated CHF₁. For simplicity's sake, all of the F₁ mice are referred to in this way, regardless of the identity of the male and female parents. We observed nothing indicative of sex-linked effects in any of our experiments. CHF₂ mice were the progeny of brother-sister matings of the CHF₁ animals, and CxBc mice were the progeny of the backcross of CHF₁ to the parental CWD strain. Similarly, CWD × CBA F₁, F₂, and backcross mice are designated CCF₁, CCF₂, and CxCc, respectively. All animals were housed in the University of Virginia Medical School vivarium. Those mice that had been inoculated with the SL3-3 virus were observed daily for signs of disease (lymphadenopathy, hepatosplenomegaly, lethargy or failure to thrive).
Animals found to be ill were killed by carbon dioxide asphyxiation and subjected to necropsy. Animals which died in the cages were refrigerated until autopsied within 24 h of death.

**Virus Stocks.** The leukemogenic ecotropic SL3-3 virus was the generous gift of Jack Lenz (Albert Einstein Medical School, Bronx, NY), and was originally derived by transfection of NIH-3T3 cells with an infectious proviral clone (26). Filtered tissue culture supernatants from NIH-3T3 cells chronically infected with this virus were stored at −70°C until used to inoculate neonatal mice intraperitoneally with 0.05 to 0.1 ml per animal. Mice were injected within 48 h of birth.

**Hybridization Probes.** The subcloned fragment of the 5′(pAKV5) portion of the AKV p15E TM gene was the generous gift of Winship Herr (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) (21). The AKVSX pol probe was the 800-bp Sac I-Xho I fragment from the AKV 623p provirus clone (27), which was a gift from Doug Lowy (NIH, Bethesda, MD), as was the plasmid pBenv, which contains the 334 bp Sma I fragment of the Akv gp70 gene and hybridizes only to ecotropic MuLV. The plasmid pGemI-Ad was a gift from Ronald Germain (NIH, Bethesda, MD) (28). The class I MHC probe was the 1.0-kb Bam HI–Hind III fragment from the plasmid pD5-4, which is a pBR327 subclone of the Eco RI–Pvu II fragment of the H-2Dd genomic clone pD1-1 (29), and was provided by Victor Engelhard (University of Virginia, Charlottesville, VA). The T25PB p15E TM probe was the 162-bp Pst I–Bgl II fragment from the plasmid pT25P, which contains the 656 bp Pst I p15E/LTR fragment of the CWN-T25 class II recombinant provirus subcloned in pUC13 (Coppola, M. A., et al., manuscript in preparation). All probes were excised from the plasmids by digestion with the appropriate restriction enzymes and purified from low mol wt agarose gels after electrophoresis. The fragments were labeled with 32P by the random primer-extension method (30) using a kit from Boehringer Mannheim (Indianapolis, IN). Specific activities greater than 5 × 10^8 dpm/μg were routinely obtained by this method using 30 μCi of either α-[32P]dATP or dCTP (New England Nuclear, Boston, MA).

**Southern Blotting and Hybridization.** High molecular weight DNA was isolated from tumor tissues which had been rapidly frozen at the time of autopsy. The tissues were homogenized in 5 ml of TEN buffer (20 mM Tris-HCl, pH 7.5; 20 mM EDTA; 150 mM NaCl; 1% SDS) in ground glass tissue grinders, digested with proteinase K (80 μg/ml) at 50°C for 4 h and extracted three times each with neutralized phenol saturated with 10 mM Tris-HCl (pH 7.5)/1 mM EDTA (TE), phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1). The volume of the sample was readjusted to 5 ml with TE, and the RNA degraded by treatment with 100 μg/ml RNase A at 37°C for 1 h. After repeating the proteinase K digestion and extractions, the DNA was precipitated by the addition of 2–3 vol of absolute ethanol. The precipitate was collected by centrifugation, dried, and resuspended in TE at a concentration of between 0.2 and 2.0 mg/ml. 5–10 μg of DNA were digested with the appropriate restriction enzymes under the conditions recommended by the suppliers (New England Biolabs, Beverly, MA; Bethesda Research Laboratories, Gaithersburg, MD; International Biotechnologies Incorporated, New Haven, CT; United States Biochemical, Cleveland, OH). The samples were electrophoresed on 0.6% to 1.2% agarose gels in TAE buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.1). The gels were treated with 0.25 M HCl for 10 min before transfer of the DNA to nylon membranes (Zeta-probe; Bio-Rad, Richmond, CA) in 0.4 M NaOH. After prehybridization for 2 h at 65°C in 4× SSCP (1× SSCP is 120 mM NaCl, 15 mM sodium citrate, 15.4 mM dibasic sodium phosphate, 5.3 mM monobasic sodium phosphate, pH 7.0), 1× BFP (0.2 mg/ml BSA, 0.2 mg/ml ficoll type 400, 0.2 mg/ml polyvinylpyrrolidone), 1% SDS, and 125 μg/ml denatured salmon sperm DNA, the membranes were hybridized to 0.5–10 × 10^6 dpm of the appropriate 32P-labeled probe in 4× SSCP, 1× BFP, 1% SDS, and 10% dextran sulfate for 18–36 h at 65°C. The final high stringency wash was in 0.1× SSCP, 0.1% SDS at 65°C for 15–60 min, depending on the particular probe used. Kodak X-omat RP5 film was exposed to the filters at −70°C with the aid of intensifying screens. Satisfactory exposures were generally obtained after 1–10 d. In some experiments, the probe was stripped from the filters after autoradiography by incubation in 0.4 M NaOH at 42°C for 30 min, followed by neutralization in 0.2× SSCP, 0.2 M Tris-HCl (pH 7.5), 0.02 M EDTA, and 0.1% SDS. The filters were stored at 4°C in prehybridization solution until re-hybridized to a new 32P-labeled probe.
Results

To study the host genes that determine the structure of tumor-associated envelope gene recombinant MuLVs, we inoculated neonatal F1 progeny of CWD x HRS crosses (CHF1 mice) with the highly leukemogenic ecotropic virus SL3-3. This virus rapidly induces lymphoma and the formation of class I and class II env recombinants, respectively in the HRS/J and CWD parental strains (22, 23). The structure of the envelope gene of the tumor-associated recombinant retroviruses was determined by Southern blot analysis of the tumor DNAs. As shown in Fig. 1, the endogenous ecotropic and the SL3-3 proviruses lack Eco RI sites, but contain Pst I sites in the U3 portion of the long terminal repeats (LTRs). Recombinant proviruses that have acquired polytropic envelope genes contain a single Eco RI site in the gp70-coding region of env. The 1.4-kb Eco RI-Pst I env/LTR fragment from a recombinant provirus will hybridize to the pAKV5 probe only if ecotropic sequences are present in the 5' portion of the p15E TM gene. Therefore, only recombinants with class I env genes are detected in this assay. The proviruses of the endogenous or acquired SL3-3 ecotropic MuLVs are seen as an 8.2-kb Pst I band. Fig. 2 A shows that DNAs from 22 of 24 CHFI tumors had acquired a 1.4-kb Eco RI-Pst I band that was indicative of a class I envelope gene recombinant provirus. Tumors CHFI-26 (lane n) and CHFI-46 (lane w) lacked this band, but did display amplification of a 0.6-kb band. This smaller species most likely represented class I recombinants that had acquired a Pst I site in the p15E TM coding region, but retained sufficient ecotropic 5' p15E sequence to hybridize to the probe. Southern blots of Eco RI-Xba I, Bam HI-Xba

Figure 1. Restriction maps of ecotropic, endogenous polytropic and recombinant proviruses. The location and origin of hybridization probes used in these studies is shown, as well as the sizes of hybridizing bands diagnostic of the presence of envelope gene recombinant proviruses. Polytropic-related sequences are indicated by the stippled pattern. Note that the precise breakpoints of recombination in individual proviruses can vary. This is represented by the diagonal boundaries of the polytropic substitutions in the recombinant viruses. Many endogenous polytropic proviruses contain an insertion of cellular DNA, shown here in black, in the LTR. These proviruses may also carry deletions in the 5' portion of the genome, as indicated by the breaks in this part of the map. Restriction sites in the proviral DNAs: Bg, Bgl II; Bs, BsaH II; E, Eco RI; P, Pst I; X, Xba I.
FIGURE 2. Presence of class I, but not class II envelope gene recombinant proviruses in DNAs of tumors induced by SL3-3 in (CWD × HRS)F₁ mice. (A) Southern blot of control and tumor DNAs digested with Eco RI and Pst I endonucleases and hybridized to the pAKV5 probe. Lanes: AKR, spontaneous thymic tumor of an AKR/J mouse; HRS, HRS/J placenta; CWD, CWD embryo; (a-o) DNAs from tumors of individual CHF₁ mice injected with SL3-3. The sizes in kilobase pairs of the major bands are shown at left. (B) Southern blot of tumor DNAs digested with Eco RI and BssH II and hybridized to the pAKV5 probe (top panel) or T25PB probe (bottom panel). Lanes: (a) DNA from mink cells chronically infected with the class I HRS/J recombinant MuLV PTV-1; (b) DNA from mink cells chronically infected with the class II CWD recombinant MuLV CWM-P15; (d-o) DNAs from tumors of individual CHF₁ mice injected with SL3-3. The approximate size of the bands in kilobase pairs is shown at left.
I, and Eco RI–BssH II proviral fragments confirmed this interpretation (data not shown).

The class I env proviruses were also detected by the pAKV5 probe as 1.8–2.0-kb bands on Southern blots of Eco RI–BssH II fragments (Fig. 2 B, top panel). The differences in the migration of these bands resulted from variation in the size of the U3 region. To determine if proviruses with class II envelope genes were also present, this blot was hybridized to the T25PB probe. This probe contains p15E gene sequences that anneal to both acquired class II env recombinant and endogenous tropic proviruses but not to class I or ecotropic proviruses (see Fig. 1) (31, 32). As shown in the lower panel of Fig. 2 B, lane b, the 1.7-kb Eco RI–BssH II fragment from the class II env recombinant virus could be distinguished from the larger fragments that contained endogenous tropic virus sequences. The CHF1 tumors that contained class I env recombinants lacked detectable class II env recombinants (a faint 1.7–1.8-kb band is seen in lane f). Taken together, these data indicated that the host gene(s) that promotes the association between class I env recombinants and HRS/J lymphomas was dominant with respect to the gene(s) responsible for the association of class II env recombinants with CWD lymphomas.

To determine the number of loci controlling this phenotype, CHF1 mice were intercrossed or backcrossed to the recessive CWD parent and the progeny of these matings were inoculated as neonates with SL3-3. DNAs from the resultant tumors were again assayed by Southern blotting for the presence of class I and class II proviruses. Fig. 3 A is an autoradiogram of a representative Southern blot of CHF2 and CHxC tumor DNAs digested with Eco RI and Pst I and hybridized to the pAKV5 probe. In this experiment, only some of the tumor DNAs contained 1.4-kb (or 0.6-kb) bands diagnostic of class I envelope gene recombinant proviruses. To confirm that recombinant viruses were present in those tumors that lacked class I viruses, the same samples were digested with Eco RI and Hind III and hybridized to the AKVSX pol probe. This assay will detect a 3.9 kb pol/env fragment of either class I or class II recombinants (see Fig. 1), although the probe hybridizes to multiple provirus sequences. This assay allowed us to infer the presence of class II recombinants in those tumors that lacked class I recombinants. As shown in Fig. 3 B, all but one of the tumor DNAs contained an acquired 3.9-kb Hind III–Eco RI band (lanes d–s). Tumor CHF2-61 DNA (lane j) was negative in this assay, but contained class I proviruses as shown in Fig. 3 A. This recombinant may not have inherited the Hind III site in pol from the SL3-3 ecotropic parent. These Southern blot assays revealed that 53 of 66 CHF2 tumors induced by SL3-3 contained class I envelope gene recombinants. The remaining 13 contained class II recombinants. Of 25 backcross tumors examined, 14 carried class I recombinants and 10 contained class II viruses (Table I). We were unable to detect envelope gene recombinant proviruses in two backcross tumors. The observed numbers of animals with each type of recombinant were very close to the 1:1 and 3:1 ratios expected for a single dominant gene segregating in backcross and intercross animals, respectively. Examination of the phenotypes of individual mice indicated that the dominant HRS/J gene was not linked to the hr mutation on chromosome 14, to the normal gene allelic to the cw mutation on chromosome 9, or to the albino locus on chromosome 7 (33).

We next determined the MHC genotypes of the leukemic CHxC backcross and CHF2 animals by analysis of Pst I RFLPs near the I-Aβ locus (Fig. 3 C) and H-
FIGURE 3. Structure of envelope gene recombinant proviruses in tumors from (CWD × HRS)F2 and (CWD × HRS) × CWD backcross mice correlates with the segregation of RFLPs near the I-Aβ gene of the MHC. (A) Southern blot of control and tumor DNAs digested with Eco RI and Pst I and hybridized to the pAKV5 probe. Sizes in kilobase pairs of major bands are shown at the left. Lanes: (a) CWD embryo; (b) normal CHFI liver; (c) HRS/J placenta; (d–k) DNAs from tumors of CHF2 mice injected with SL3-3; (l–s) DNAs from tumors of (CHFI × CWD) backcross mice injected with SL3-3. (B) Southern blot of the same DNAs digested with Eco RI and Hind III and hybridized to the AKVSX pol probe. Lanes same as in A. (C) Southern blot of the same DNAs digested with Pst I and hybridized to the pGemI-Aβd probe. Fragment sizes in kilobase pairs are indicated at left.

2D-related loci (data not shown). Fig. 3 C shows the hybridization of the pGemI-Aβd probe to Pst I-digested DNAs from the same representative tumors in Fig. 3, A and B. There is an apparent correlation between the inheritance of HRS/J I-Aβ alleles and the presence of class I env recombinants (Fig. 3 A). As summarized in Table I, tumors from mice that had inherited one or two HRS/J MHC alleles contained class I env recombinants, while tumors from nearly all animals that were homozygous for the CWD MHC genes contained recombinants with class II env genes. Tumor DNAs from two CHF2 animals homozygous for CWD MHC alleles contained class I env recombinants, but these tumors also contained somatically acquired recombinant proviruses that hybridized to the T25PB probe (data not shown). In these cases, our assays did not distinguish between the presence of both class I and class II recombinants and the presence of a single recombinant with an envelope gene capable of hybridizing to both the pAKV5 and T25PB probes. The only other exception was a CHF2 mouse that was heterozygous at I-Aβ, but generated a class II env recombinant. However, RFLPs detected with the H-2D probe sug-
Table I
Correlation of MHC Genotype with the Envelope Gene Structure of Tumor-associated Recombinant Retroviruses in Crosses between CWD and HRS Mice

| Cross          | Proviral envelope gene structure | MHC genotype |
|----------------|---------------------------------|--------------|
|                |                                 | CWD | F1 | HRS |
| (CWD x HRS)F1  | 24 class I                       |     | 24 |    |
| (CWD x HRS)F2  | 53 class I (80.3%)               | 2*  | 31 | 20 |
|                | 13 class II (19.7%)              | 12  | 14 | 0  |
| (CWD x HRS)F1 x CWD | 14 class I (58.3%)              | 0   | 14 |    |
|                | 10 class II (41.7%)              | 10  | 0  |    |

The envelope gene structure of recombinant proviruses integrated in tumor DNAs of SL3-3-injected mice was determined by Southern blotting as illustrated in Figs. 1, 2, and 3. The MHC genotypes were determined by analysis of Pst I RFLP adjacent to both the I-A\(\beta\) gene (Fig. 3C) and H-2D-related genes (not shown).

* Hybridization to both pAKV-5 and T25PB probes.

\(\dagger\) The MHC genotype of this mouse was CWD by analysis of Pst I restriction site polymorphisms adjacent to H-2D-related genes.

It suggested a homozygous CWD MHC genotype for this individual, which indicated the possibility of a crossover within the MHC. Thus, the HRS/J gene responsible for the generation or selection of class I env recombinants was linked to the MHC on chromosome 17.

This result was not peculiar to HRS/J mice. CBA/J is a low leukemia strain that shares the H-2\(^k\) haplotype with HRS/J (34). SL3-3 rapidly induces lymphoma and the formation of class I env recombinants in CBA/J mice (22). Table II summarizes data from studies of (CWD x CBA)F1, F2, and (CWD x CBA)F1 x CWD backcross mice inoculated at birth with SL3-3. Again, we found the class I env recombinant-

Table II
Correlation of MHC Genotype with the Envelope Gene Structure of Tumor-associated Recombinant Retroviruses in Crosses between CWD and CBA Mice

| Cross          | Proviral envelope gene structure | MHC genotype |
|----------------|---------------------------------|--------------|
|                |                                 | CWD | F1 | CBA |
| (CWD x CBA)F1  | 12 class I (92.5%)              |     | 12 |    |
|                | 1 class II (7.7%)               |     | 1  |    |
| (CWD x CBA)F2  | 25 class I (71.4%)              | 1*  | 16 | 8  |
|                | 10 class II (28.6%)             | 7   | 3* | 0  |
| (CWD x CBA)F1 x CWD | 9 class I (34.6%)              | 0   | 9  |    |
|                | 17 class II (65.4%)             | 15  | 2* |    |

The envelope gene structure of recombinant proviruses integrated in tumor DNAs of SL3-3-injected mice was determined by Southern blotting. The MHC genotypes were determined by analysis of Pst I RFLP adjacent to both the I-A\(\beta\) gene and H-2D-related genes (data not shown).

* Discordant event.
forming phenotype to be dominant and controlled by a single locus linked to the H-2 complex on chromosome 17, although there were more discordant events. 12 of 13 F1 tumors contained class I proviruses, as did 25 of 36 F2 and 9 of 26 backcross tumors. With only one exception, all animals that generated class I recombinants had inherited CBA/J MHC genes.

Discussion

These studies revealed that HRS/J and CBA/J mice carried a gene or genes on chromosome 17 that mediated the selection of class I recombinant MuLV that contained ecotropic virus sequences in the 5' portion of the p15E (TM) gene. The HRS/J and CBA/J alleles were dominant with respect to the gene(s) of CWD mice that selects for class II recombinants that contain polytropic virus sequences in this region of the TM gene. In crosses between HRS/J or CBA/J and CWD mice, there was a strong, but not absolute, correlation between the presence of class I recombinants in tumor DNAs and the inheritance of at least one copy of HRS/J or CBA/J H-2k alleles. The few discordant events could reflect the limitations of the Southern blot assays used to detect and distinguish between class I and class II recombinants. Alternatively, the gene of interest may be located on chromosome 17 but at some distance from the marker RFLPs, or the gene may have less than total penetrance or variable expressivity.

It is unlikely that the gene we have identified represents a specific endogenous polytropic provirus which donates env sequences to the class I recombinant viruses. First, 9 of the 10 endogenous polytropic proviruses shared by HRS/J and CBA/J mice have been mapped to chromosomes other than 17 (25). (The chromosomal location of the tenth polytropic provirus has not yet been determined). Second, analysis of the env sequences of class I HRS/J and class II CWD recombinants indicates that these viruses were generated by recombination with closely related members of the same family of endogenous polytropic viruses (Coppola, M. A., et al., manuscript in preparation). Finally, we have observed selection against the class II-specific domain of the TM protein of a leukemogenic CWD-derived recombinant in HRS/J mice (Coppola, M. A., et al., manuscript in preparation), suggesting that the gene responsible for the association of class I recombinants with HRS/J and CBA/J tumors exerts its effect at some point after the generation of the recombinants.

Fig. 4 compares the deduced amino acid sequences of class I and class II p15E TM proteins (Coppola, M. A., et al., manuscript in preparation). Of the 12 amino acid differences, 10 are clustered within a 23 amino acid region. The molecular or cellular basis for the selection of this segment of the p15E molecule is not clear. The incorporation of ecotropic virus-derived sequences into the 5' portion of the TM gene of class I recombinants may reflect an in vivo selection that acted directly on these specific nucleic acid sequences, but it seems more likely that there was a selection for class I or against class II TM proteins. This selection probably did not involve the interaction between the viral envelope and the cellular receptor or differences in the ability of viruses to fuse with cellular membranes, since the portions of the envelope proteins responsible for these functions do not differ between class I and class II viruses. Moreover, the polytropic virus receptor is located on chromosome 1 (35), not 17, and the amino acid differences between class I and class II TM
proteins are located to the COOH-terminal side of the putative membrane fusion domain (3).

It is tempting to speculate that the selection for class I or against class II recombinants resulted from a specific interaction between the H-2^k gene products and the NH2-terminal portion of the p15E TM protein. Both HRS/J and CBA/J carry the H-2^k haplotype, as do two other high leukemia mouse strains, AKR and C58, that also produce class I env recombinant MuLVs as well (13, 14, 21, 34). Genes within or linked to the MHC are known to influence the susceptibility of mice to certain murine leukemia viruses (36-43), and the expression of an endogenous xenotropic provirus (44). However, none of these genes have been shown to influence the structure of the viral envelope gene or proteins.

How retroviruses adapt to the environment encountered in a newly infected host has not been well studied. Immune responses to retroviral envelope proteins are regulated by MHC genes (45-47). Passage of the Gross MuLV in BALB/c-H-2^k mice results in the generation of variant viruses that have lost specific gp70^env epitopes (48). Although the structural differences between Gross virus and these variants have not yet been analyzed in detail, some MHC-controlled immunoselective pressure seems to be involved, in that sera from immunized H-2^k mice fail to neutralize the variants, while immune sera from congenic H-2^b mice can neutralize both wild-type and variant viruses. Alterations in the structure of the envelope proteins of the ovine and equine lentiviruses may also result from selection for virus variants that avoid host responses (9, 10), but it has been difficult to determine if these differences are related to an in vivo selection or simply the result of random variation of the virus population. Similarly, the biologic significance of envelope gene heterogeneity in the human and primate immunodeficiency viruses is unclear (49, 50). The hetero-
geneity in the envelope genes of lentiviruses such as HIV most likely results from point mutations or small deletions and duplications rather than recombination with endogenous virus sequences as is seen with the murine or feline leukemia viruses.

Whatever mechanisms may be involved in the generation of envelope gene diversity, the studies reported here demonstrate that a specific host gene can influence the structure of the envelope proteins of pathogenic retroviruses. To our knowledge, the only host gene previously found to influence the structural proteins of a retrovirus after infection is Fo-1, which is located on mouse chromosome 4 (51, 52). In that case, there is a selection for mutated or recombinant MuLVs that substitute one or two amino acids within a specific region of the p30 envelope protein (53). The elucidation of the mechanism of action of the gene defined in the present study will likely provide new insights into the complex interactions between the envelope proteins of pathogenic retroviruses and the host.

Summary

Heterogeneity in the structure of the envelope proteins has been observed in many human and animal retroviruses and may influence pathogenicity. However, the biological significance of this heterogeneity and the mechanisms by which it is generated are poorly understood. We have studied a mouse model in which the envelope gene structure of lymphoma-associated viruses appears to be controlled by a single host gene. The inoculation of HRS and CWD mice with a leukemogenic murine leukemia virus (MuLV) results in recombination between the injected virus and envelope gene sequences of endogenous retroviruses. The genomes of HRS (class I) env recombinants and CWD (class II) env recombinants differ in the sequences encoding the NH2-terminal portion of the transmembrane envelope protein (TM). We have shown that an HRS gene linked to the MHC on chromosome 17 mediates a dominant selection for recombinant retroviruses with the class I envelope gene structure. CBA mice, which share the H-2k haplotype with HRS, also carry the dominant allele at this locus. This system provides a useful model for studies of host factors involved in the selection of specific variants of pathogenic retroviruses.

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