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Immunology

Murine leukemia virus sequences are encoded in the murine major histocompatibility complex

(viral integration/class I gene/TL region/cosmid library/gene expression)

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ABSTRACT **The studies reported here localize murine leukemia viral sequences to the *TL* region of the major histocompatibility complex, *H-2*. We examined a battery of 38 cosmids, isolated from two large genomic libraries constructed from C57BL/10 spleen DNA, that define 25 class I gene sequences. The viral probes used hybridized with only four cosmids, containing overlapping mouse sequences, that define four class I gene-related sequences in a region of 90 kilobases of DNA. The data show that two distinct viral envelope sequences are contained in the cluster. One of these sequences is situated with its 3′ end next to the 3′ end of a class I sequence. The other sequence, which does not contain the entire viral envelope, is proximal to the 3′ end of a different class I sequence. Hybridization of the viral probes with the *H-2* cosmid clones does not appear to be due to homology between viral and *H-2* sequences. Rather, the viral sequences detected appear to be linked to or inserted amid class I genes. These findings may be significant in understanding molecular mechanisms involved in the generation of *H-2* class I gene diversity.**

The murine major histocompatibility complex (MHC), *H-2*, encodes the most polymorphic group of genes known in eukaryotes (10–50 alleles for each of its members) (1). The complex is comprised of the classic transplantation (class I) genes, immune response (class II) loci, and complement-related (class III) genes (2–4). Several features of the complex have hitherto drawn attention: extensive polymorphism (1), gene duplication (5, 6), gene dispersion (7), gene conversion (8), extraordinary mutation rates (9), and the association of class I and class II genes with susceptibility or resistance to a myriad of diseases (10, 11). A recurrent theme of many hypotheses to explain these characteristics has been the potential role of viruses (7, 12, 13). The studies reported here lend credence to this concept by localizing murine leukemia virus sequences within the *H-2* complex.

The role of viruses in the above-mentioned properties of MHC genes became interesting to us when it was shown that transformation of thymus cells by an RNA type C leukemia virus, radiation-induced leukemia virus (RadLV), led to shutdown of synthesis and expression of antigens encoded by class I genes (14). Also, it was later shown that this effect of RadLV on MHC antigen expression probably plays a critical role in the escape of RadLV-transformed cells from the surveillance mechanisms of the immune system (15).

After several hypotheses had been tested (16, 17), it was concluded that viral integration may play a role in the observed alterations of antigen expression and that one of the critical integration sites of the virus may be adjacent to a MHC gene or a gene that regulates the expression of MHC antigens. The present studies locate viral sequences adjacent to class I genes in normal mice but do not address the potential location(s) of RadLV sequences within the MHC. However, they add further weight to the hypothesis that viruses such as RadLV may integrate within the MHC cluster of genes. They also set the stage for understanding the distinction between potential RadLV integration sites (if found) and endogenous viral sequences previously integrated next to class I genes in normal cells.

MATERIALS AND METHODS

Cloned Viral Probes. A p15E-specific probe was subcloned from a cloned 8.8-kilobase AKR-ecotropic (pAKR-Eco) murine leukemia virus DNA sequence (18), and pAKR-Eco was kindly provided by Malcolm Martin (National Institutes of Health).

DNA Procedures. DNA was digested with the appropriate restriction endonuclease (New England BioLabs or Bethesda Research Laboratories) as recommended by the supplier. Gel electrophoresis and hybridization were done by the method of Southern (19). Only stringent conditions were used. After transfer of DNA to the filters was complete, they were washed with 6× standard saline citrate (NaCl/Cit; 1× NaCl/Cit is 0.15 M NaCl/0.015 M Na citrate, pH 7), air dried, baked for 5 hr at 80°C under vacuum, and then each was submerged in 100 ml of prehybridization fluid (6× NaCl/Cit/0.02% bovine serum albumin/0.02% Ficoll and 0.02% polyvinylpyrrolidone, prewarmed to 65°C). After incubation for 5 hr in a water bath at 65°C with shaking, the filters were removed from the blocking solutions, air dried, and placed in heat-sealable plastic bags. Then, the hybridization solution [2× NaCl/Cit/0.01 M EDTA, ^{32}P-labeled denatured probe DNA (labeled to at least 2×10^8 cpm/μg), and denatured calf thymus DNA at 5 μg/ml] was added and hybridization was continued overnight at 65°C. The filters were then washed three times for 20 min each in a tray containing 2× NaCl/Cit/0.05% $NaDodSO_4$/20 mM NaH_2PO_4/0.06% $Na_2H_2P_2O_7$ at 65°C, once with 1× NaCl/Cit, once with 0.4× NaCl/Cit, and finally numerous times with 0.1× NaCl/Cit until the Cerenkov count was <100 cpm/5 ml).

RESULTS

Detection of Viral Sequence in *H-2* Cosmids. Because *H-2*-linked resistance to RadLV-induced thymomas involves viral interaction with *H-2* at the DNA level (14–17) we tested the possibility that viral DNA sequences may be located within the MHC. Preliminary experiments suggested that viral sequences are integrated in the region of chromosome 17 containing the *H-2* genes in RadLV-induced tumors as well as in tissues from normal mice. Digestion of DNA from thymocytes of several inbred mouse strains with *Bam*HI, *Xba* I,

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Abbreviations: MHC, major histocompatibility complex; RadLV, radiation-induced leukemia virus; NaCl/Cit, standard saline citrate; kb, kilobase(s).

or *Kpn* I produced fragments that appeared to hybridize with *H-2* and viral probes. However, because many genomic fragments hybridize with sequences that are detected by the broadly crossreactive *H-2* probes we used (20), the significance of hybridization of one of these fragments with a viral probe was unclear and these results might be merely coincidental. Greater certainty would result if the fragment containing the *H-2* sequences and cross-hybridizing with the viral probe could be isolated and cloned. For this reason, we examined 38 cosmids isolated from two large genomic libraries constructed from C57BL/10 ($H\text{-}2^b$ haplotype) spleen DNA (unpublished data; L. Golden, K. Fahrner, A. L. Mellor, J. J. Devlin, H. Bullman, H. Tiddens, and H. Bud, personal communication) for viral sequences. (Taken together, these cosmids defined all class I genes in the C57BL/10 *H-2* complex.) In the experiments reported below, all inserts used as probes were removed from their vectors to eliminate hybridization to nongenomic sequences in the cosmids.

The cosmids were digested with *Bam*HI, which does not cut within the cosmid vectors used [pOPF1 and pTM (21, 22)], and were hybridized under stringent conditions with an ecotropic *env* viral probe, encompassing sequences encoding gp70. The virus probe only hybridized with four cosmids, H6, B1.19, H43, and B1.15 (Fig. 1).

Localization of the Viral Sequences Within the MHC Complex and With Respect to the Specific Class I Genes. The four cosmids studied contain overlapping mouse sequences and belong to a group of 10 overlapping cosmid clones that define five class I gene-related sequences in a region of 90 kilobases (kb) of DNA (Fig. 2). This cluster has been mapped to the *TL* region of the MHC by restriction enzyme polymorphism (unpublished data; K. Fahrner, A. L. Mellor, J. J. Devlin, H. Bullman, H. Tiddens, and H. Bud, personal communication).

Since hybridization with cosmids H43 and H6 shows two bands compared with only a single band in B1.19 (Figs. 1 and 3), one of the two regions hybridizing with our viral probe must be in the left segment of cosmids H6 and H43, which is missing in cosmid B1.19 (Fig. 2). By the same reasoning, the second viral hybridizing fragment, which is the only one present in cosmid B1.19, must be located in the region of DNA common to H6, H43, and B1.19, and been positioned at the rightmost side of H6 and H43.

The leftmost viral hybridizing fragment shown in Fig. 2 was positioned on the basis of two observations. First, H6 and H43 show two bands on hybridization with the *env* probe, while B1.19 yields only one band (Figs. 1 and 3). Therefore, the hybridizing region must be to the left of B1.19. Second, one of the two *Bam*HI fragments in cosmids H43 and H6 hybridizing with the viral p*AKR-Eco* probe does not hybridize with *H-2* probes, p*H-2II* or p*H-2III* (20) (Fig. 3). This fragment cannot be common to B1.19 because the viral hybridizing fragment of the latter cosmid hybridizes with the *H-2* probes (Fig. 3). The H43 and H6 fragments in question must therefore be outside the *H-2* domains and, accordingly (see Fig. 2), can only correspond to the leftmost *Bam*HI fragment. The location of the rightmost viral fragment is derived from the following observations. The fragment is not present in cosmid B1.15. In B1.19 only one *pAKR-Eco*-hybridizing fragment is detected and this fragment appears identical in molecular weight to one of the *Bam*HI fragments of cosmid H6 (Figs. 1 and 3). In addition, the common viral fragment in cosmids B1.19 and H6 hybridizes with both p*H-2II* and p*H-2III*. The equivalent fragment in cosmid H43 is smaller by about 1.5 kb because this is the right terminal *Bam*HI fragment of H43, which is truncated. This fragment of H43 hybridizes with p*H-2II* but not with p*H-2III*, which is consistent with the cosmid map because *H43* does not contain the 5′ region of the third rightmost *H-2* gene. The location of the p*15E*-hybridizing fragment was determined as follows, using a p*15E* probe derived by digestion with *Pst* I and *Xba* I from λAKR (23). This viral fragment is not present in B1.19 and must therefore be in the leftmost portion of cosmids H6 and H43. The p*15E* fragment does not hybridize with either *H-2* probe (p*H-2II* or p*H-2III*; Fig. 3). The 4.2-kb *Bam*HI p*15E*-hybridizing fragments in both cosmids are of identical molecular weight (Fig. 3) and too small to contain cosmid vector DNA (because *Bam*HI does not cut the 8-kb vector) and as expected does not hybridize with pBR322 (Fig. 3). Thus p*15E* sequences must lie between those hybridizing with p*AKR-Eco* and the boxes containing *H-2*-hybridizing sequences. B1.15 is also expected to contain p*15E* sequences, but it was not hybridized with the probe and therefore a p*15E* location is not shown in Fig. 2. The different nomenclature used for the various cosmid clones (H vs. B) reflects the fact they were derived from independent cosmid libraries.

Cosmids H6, H43, and B1.19 were also hybridized under stringent conditions (Fig. 1) with a probe derived from the

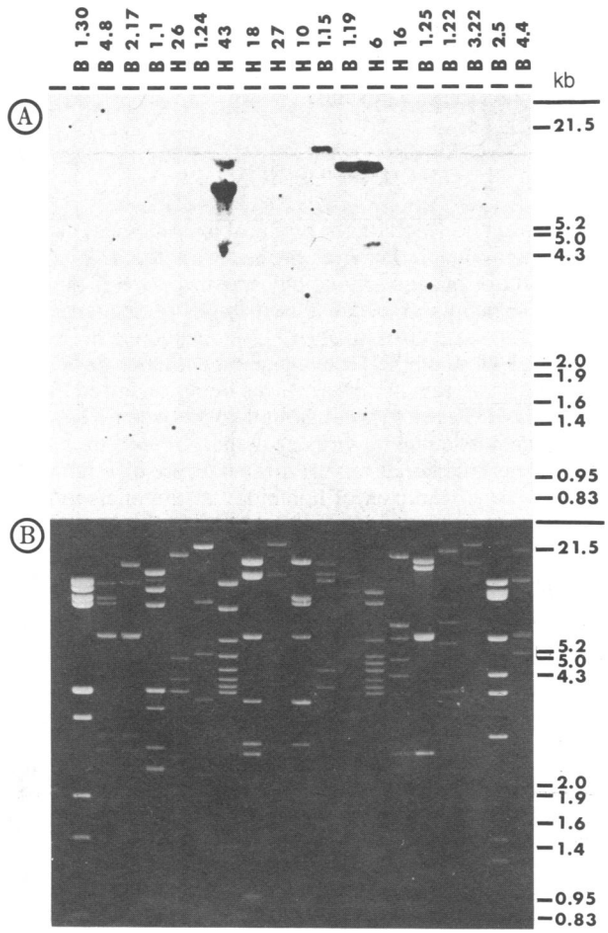

FIG. 1. Viral envelope sequences are found within *H-2* cosmid clones. Southern blots (19) of *Bam*HI-digested DNA of 19 *H-2* cosmid clones are shown. (In addition to the cosmids shown, two other filters containing an additional 19 *H-2* cosmid clones were hybridized but no other viral hybridizing sequences were found.) Each cosmid DNA (1 μg) was digested overnight with a 10-fold excess of *Bam*HI and electrophoresed on 0.8% agarose gels (Bethesda Research Laboratories) containing ethidium bromide. After electrophoresis, the gels were visualized under UV light and photographed (*B*). The DNA was then denatured and transferred to nitrocellulose as described (19). The probe used was a pAKR-Eco 2.7-kb *Sal* I/*Bam*HI fragment within the envelope (*env*) gene (4.3–7.0 kb) of complete infectious AKR provirus cloned probe, λAKR 623 (23, 24). Nick-translation was done using New England Nuclear kit NEK 004B. (*A*) Results after autoradiography. Molecular weight markers run on the same gel were *Hin*dIII/*Eco*RI-digested phage λ DNA.

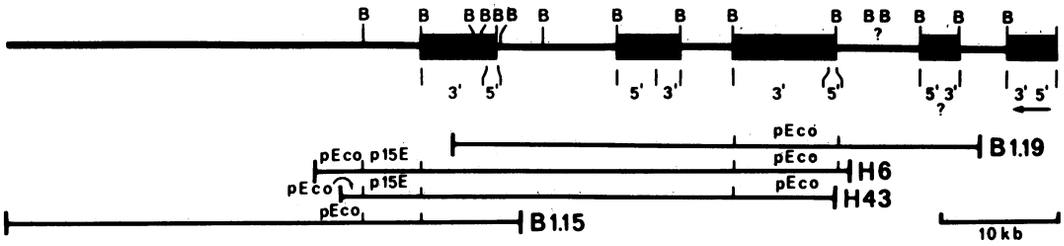

FIG. 2. Cosmid map of the *H-2* cluster encompassed by cosmid clones H6, H43, B1.15, and B1.19, which contain overlapping sequences. Regions hybridizing to the 5′ or 3′ end of *H-2* genes, detected with probes pH-2III and pH-2II, respectively, are indicated by the dark boxes. cDNA clone pH-2II codes for amino acids 167–352 and 600 nucleotides of the 3′ untranslated region of *H-2* (20), which have been removed from our probe. Clone pH-2III codes for the 5′ region of *H-2* encoding amino acids 63–160 (20). The extent of the hybridizing gene sequences is defined only by the restriction sites shown [for endonuclease *Bam*HI (B)]. The orientation was determined from additional restriction analysis with *Sac* II, *Kpn* I, *Hpa* I, and *Cla* I (data not shown). The positions of viral sequences most consistent with data in Figs. 1 and 3 are shown.

p15E region of λAKR (23, 24), which is encoded on the 3′ side of the viral envelope region coding for gp70. The *p15E* probe hybridizes with sequences in cosmids H6 and H43 but not B1.19 (Fig. 3). The absence of *p15E* hybridizing sequences in B1.19 suggests that a defective genome is encoded in this class I cosmid.

DISCUSSION

An important question raised by the above findings is whether hybridization of the viral probes with the *H-2* cosmid clones occurs because homology exists between the viral and *H-2* segments or because both types of sequences are linked in the same cosmid DNA. This question is even more relevant in view of the recent paper by Clarke *et al.* (25), published while this manuscript was being reviewed. Those authors have suggested weak homology between HLA class I and human T-leukemia virus sequences, based on hybridization experiments. However, these data are difficult to explain in view of the lack of homology at the nucleotide sequence level between human T-leukemia virus (26) and HLA.B7 (ref. 27; S. Weissman, personal communication) as determined by computer analysis. Our data do not support a simple homology interpretation for several reasons. First, as pointed out above, for at least one of the viral sequences, some cosmid *Bam*HI restriction fragments hybridize to the *H-2* probe but not to the viral probe (Fig. 3) and vice versa. Similar results are observed after digestion by *Eco*RI, *Hin*dIII, and *Pst* I (data not shown). Further analysis of the second viral sequence by restriction analysis also shows clear separation between the viral and *H-2* sequences (data not shown). Second, the probes themselves do not cross-hybridize; i.e., none of the viral probes hybridize to the *H-2* probes or vice versa under the conditions used. Furthermore, the data in Fig. 1 indicate that the majority of *H-2* genes of the *b* haplotype do not hybridize to the viral probes. Finally, a computer comparison of *H-2* nucleotide sequences for K^b, D^b, and L^d genes (28–31) with the ecotropic envelope sequence (32) indicates no obvious homology and certainly insufficient homology to obtain the degree of hybridization observed in our experiments. For these reasons, we believe that the sequences detected are linked to or inserted amidst class I genes.

A potential caveat to the findings reported here is that the particular mouse used to derive the cosmid libraries may coincidentally have had an inserted viral genome at *H-2*. However, as stated above, the cosmid clones used were derived from independently created cosmid libraries and results with overlapping cosmid clones from the two libraries are internally consistent.

The findings reported herein may have significant implications for the generation of *H-2* class I gene diversity. For example, it has been suggested that the high mutation rate associated with class I genes may result from the incorporation of viral genomes into germinal cells (7, 12) since the spontaneous mutation rate is significantly influenced by environmental conditions during breeding (7, 9). Similarly, the mechanism by which *H-2* mutants arise appears to result from multiple and simultaneous nucleotide substitutions and has been likened to gene conversion (8, 33) as it occurs in yeast in which segments of one gene are replaced by segments of another gene. Such a mechanism appears to be likely since potential donors of the mutant sequence have been detected [some at least a centimorgan away from the acceptor sequences (unpublished results). Several mechanisms might be responsible for this type of gene conversion. One would involve transposon-like structures such as *Alu*-like repeat elements or murine leukemia viruses. These sequences could play a role in insertion or deletion of nonhomologous sequences. A similar mechanism may account for the extensive gene duplication associated with *H-2* genes.

The potential role for viruses in these phenomena is more credible in view of the findings reported herein. Further support for such hypotheses comes from our recent studies on the effects of viruses on *H-2* gene expression and organization. These studies demonstrate associations between RadLV-induced transformation, changes in H-2 antigen expression, and hypermethylation and rearrangements of *H-2* DNA (unpublished results). In this connection, murine leukemia viruses have several properties that could allow them to function as insertional mutagens. Because they can enter many sites in host genomes, they have the capacity to inactivate genes by physical disruption. They also possess regulatory signals capable of altering transcriptional control of flanking host sequences. Likewise, the hypothesis that viruses may play a role in dispersion of histocompatibility loci receives support from recent mendelian genetic studies showing that several polymorphic DNA restriction fragments hybridizing with xenotropic and ecotropic viral probes map adjacent to minor histocompatibility loci (34). For example, viral restriction fragments are associated with *H-30*, *H-3*, and *H-13* on chromosome 2, *H-28* on chromosome 3, *H-16* on chromosome 4, and *H-38* (chromosome location as yet undetermined) (34). In each case, no recombinant has been found between the *H* locus in question and the viral-related restriction fragment, suggesting that linkage is very tight (34). Recent findings in other laboratories further strengthen these hypotheses. For example, it has been reported that transformation of human cells by adenovirus serotype 12 leads to shutdown of the expression of human MHC antigens (35). Presumably this leads to escape of transformed cells from immune surveillance mechanisms (36). In addition, other investigators have also reported that the primary location

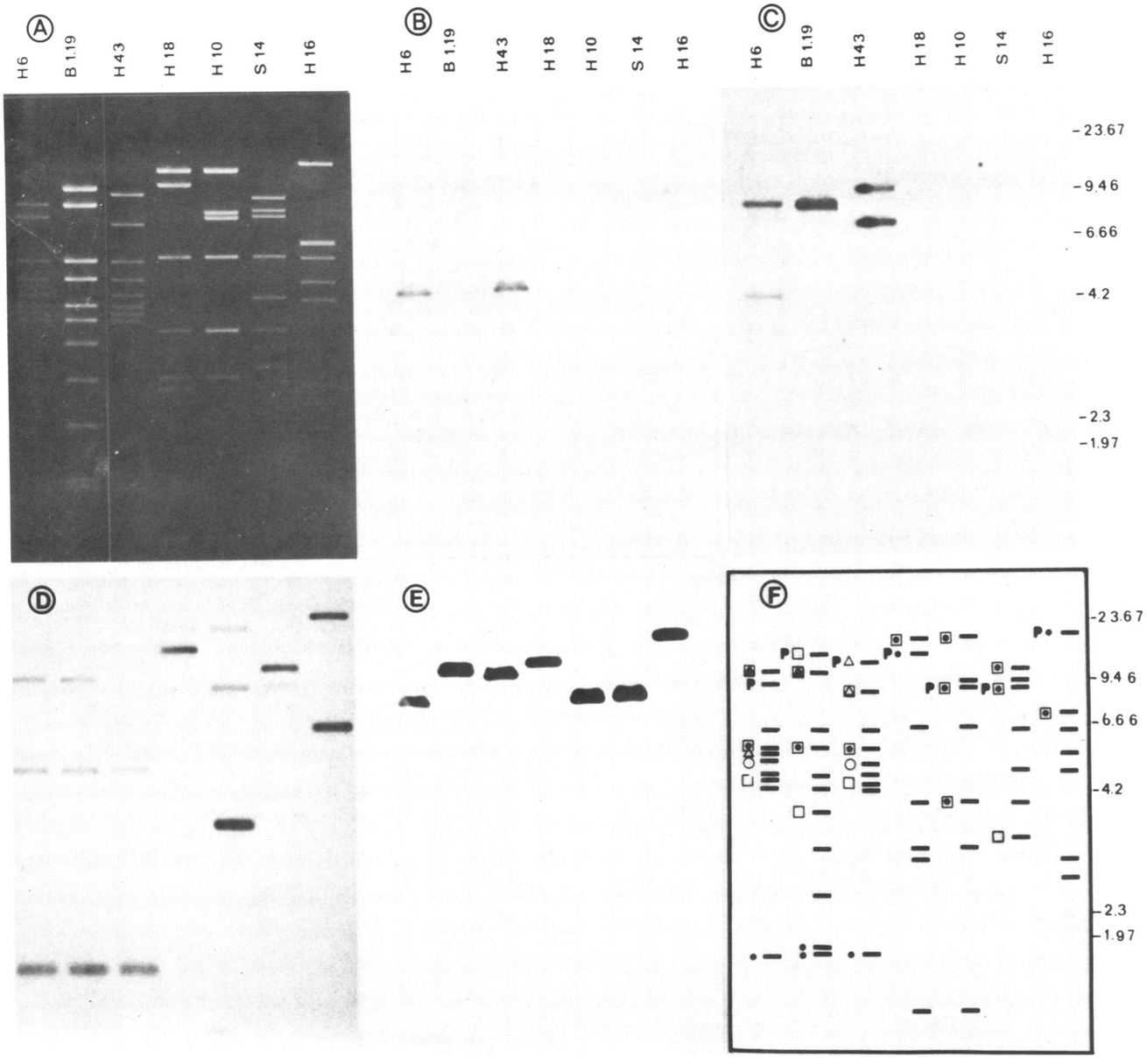

FIG. 3. Hybridization of selected *H-2* cosmid clones with ^{32}P-nick-translated p*15E* (*B*), p*AKR-Eco*, *Sal* I/*Bam*HI fragment (*C*), p*H-2III* (removed from its vector by digestion with *Pst* I) (*D*), and pBR322 (*E*) probes. The ethidium bromide pattern of one such gel under UV light is shown in *A*. Because each gel was run separately, the position of a labeled band with respect to the ethidium bromide pattern can only be evaluated by comparison with its specific ethidium bromide picture. Although all autoradiographs are reduced to approximately the same dimensions, some variability remains. The data from the five separate ethidium bromide gels are summarized in *F*, which also shows the results of hybridizing the seven cosmids with p*H-2II*. Symbols indicating that the fragment hybridizes are as follows: with pBR322, p; with p*AKR-Eco Sal* I/*Bam*HI fragment, △; with p*H-2III*, ●; with p*15E*, ○; with p*H-2II*, □. Digestion, blotting, and hybridization were as described in Fig. 1. Cosmids H6, B1.19, and H43 are from the same cluster region. Cosmids H18, H10, S14, and H16 contain class I gene sequences from other regions of DNA and were not expected to hybridize with the probes used (based on results shown in Fig. 1). They served as controls. Cosmid B1.19 gave additional faint bands of hybridization with pBR322 that did not correspond to any of the bands in *A–F* and did not photograph well. They are assumed to result from contamination or degradation and represent a minor subpopulation of the cosmid DNA. *Hin*dIII fragments of phage λ DNA were used as molecular weight markers (shown × 10^{-3}).

of endogenous viral sequences is in the vicinity of lymphocyte differentiation and histocompatibility loci (37).

The mapping of viral sequences to the *TL* region of the MHC complex is significant in view of the postulate that the *TL* region may encode an integrated viral genome (38). Although this postulate was later withdrawn (39), the data reported here support the original postulate. It remains to be seen whether such viral sequences play a role in the conversion of TL^- strains to TL^+ during leukemogenesis, as originally proposed (38).

Although viral sequences have been found so far in only one *H-2* region, represented by cosmid clones H6, H43, B1.19, and B1.15, detailed analysis of other *H-2* cosmid clusters with additional viral probes should indicate whether viral sequences are associated with more than one group of *H-2* genes. Our findings open the way for a molecular approach to studying the potential role of viruses in the generation of histocompatibility gene diversity.

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