JOURNAL OF VIROLOGY, Sept. 1996, p. 6405–6409 Vol. 70, No. 9
0022-538X/96/$04.00+0

Possible Origin of Murine AIDS (MAIDS) Virus: Conversion of an Endogenous Retroviral p12^{gag} Sequence to a MAIDS-Inducing Sequence by Frameshift Mutations

YOSHINAO KUBO,[1] KAZUHIRO KAKIMI,[2] KYOKO HIGO,[1] HIROHIKO KOBAYASHI,[1] TAKESHI ONO,[1] YUKO IWAMA,[1] KAGEMASA KURIBAYASHI,[2] HIROSHI HIAI,[3] AKIO ADACHI,[1] AND AKINORI ISHIMOTO[1]*

Laboratory of Gene Analysis, Department of Viral Oncology, Institute for Virus Research,[1] and First Department of Pathology, Faculty of Medicine,[3] Kyoto University, Kyoto, and Department of Bioregulation, Mie University School of Medicine, Mie,[3] Japan

Received 2 February 1996/Accepted 20 May 1996

The murine AIDS (MAIDS) virus has a unique sequence in its p12^{gag} region, which is responsible for MAIDS development. A transcript hybridizing with this sequence is expressed in normal C57BL/6 mice. The transcript, designated *Edv*, has been previously cloned and sequenced (Y. Kubo, Y. Nakagawa, K. Kakimi, H. Matsui, K. Higo, L. Wang, H. Kobayashi, T. Hirama, and A. Ishimoto, J. Gen. Virol. 75:881–888, 1994). Compared with the nucleotide sequence of the helper LP-BM5 ecotropic virus, the pathogenic replication-defective MAIDS virus has a 16-bp deletion and a 1-bp insertion in the 5′ and 3′ regions of the p12^{gag} sequence, respectively, and the *Edv* transcript contains only a 3-bp deletion. Therefore, the amino acid sequence of the defective MAIDS virus p12^{gag} region is not homologous to that of the helper virus and the *Edv* transcript because of the frameshift. To determine whether the amino acid sequence resulting from the frameshift is critical for MAIDS development, we constructed chimeric viruses that contained the p12^{gag} regions of the helper virus and the *Edv* transcript, respectively, with and without the same frame as the defective MAIDS virus by the artificial frameshift mutations. The mutant viruses with the frameshift mutations induced MAIDS in inoculated mice, but the viruses without the mutations did not. These results suggested that the MAIDS virus was generated by frameshift mutations in the p12^{gag} region of *Edv* or a related sequence.

Murine AIDS (MAIDS) is induced by infection of C57BL/6 mice with the Duplan strain of murine leukemia virus (MuLV) (2, 11, 21). The disease has many similarities to human AIDS and is used as a mouse model of human AIDS (12, 20, 22, 23). The MAIDS virus complex contains a pathogenic replication-defective virus and nonpathogenic helper MuLVs (4). The *pol* and *env* genes are deleted, but the *gag* gene is almost conserved in the pathogenic MAIDS virus. However, the MAIDS virus has unique sequences in the p15^{gag} and p12^{gag} regions (1, 5). Thus, it has been suggested that the unique sequences are responsible for MAIDS induction. Pozsgay et al. reported that the p15^{gag} and p12^{gag} regions are sufficient for MAIDS development (24), and we have shown that these unique sequences are both required for MAIDS induction (14).

Normal C57BL/6 mice express a transcript which hybridizes with the MAIDS virus p12^{gag} sequence (5, 6), and previously we cloned and sequenced this transcript (*Edv*) (15). There are some other lines of evidence indicating that normal mice contain the MAIDS virus-related sequence (3, 7, 10). Since the MAIDS virus was originally isolated from radiation-induced leukemic C57BL/6 mice (18), the *Edv* transcript expressed in C57BL/6 mice might represent the origin of the MAIDS virus. In this study, we examined the pathogenicity of p12^{gag} regions of the *Edv* transcript and the helper virus by constructing the chimeric viruses including these regions. The nucleotide sequence of the p12^{gag} region of the endogenous *Edv* transcript is homologous to that of the MAIDS virus, but the amino acid sequence is not due to frameshift mutations (Fig. 1). Therefore, we constructed mutant viruses containing the p12^{gag} region of the endogenous *Edv*, sequence or the helper virus with or without frameshift mutations resulting in amino acid homology to the pathogenic MAIDS virus to know whether the amino acid sequence of the MAIDS virus resulting from the frameshift mutations is responsible for MAIDS induction.

Clones of the helper LP-BM5 ecotropic MuLV (BM5eco) (5), pathogenic MAIDS virus (G1B) (14), and MAIDS virus-related endogenous sequence (pEDV-2) (15) were used as parental clones for construction of recombinant viruses. The p15^{gag} region of the G1B DNA clone was amplified by PCR with the H-1 and H-2 primers (Fig. 2). The p12^{gag} region of the pEDV-2 or BM5eco DNA clone was amplified by the H-3 and H-4 or H-5 primers (Fig. 2). The G1B DNA clone, but not the pEDV-2 or BM5eco clone, contains a *Sma*I site in its p12^{gag} region. To construct chimeric DNA clones between the G1B clone and pEDV-2 or BM5eco clones, the *Sma*I site was introduced to the pEDV-2 and BM5eco clones by PCR amplification with the H-2 and H-3 primers, which contain *Sma*I sites. The p15^{gag} and p12^{gag} PCR products were ligated at the *Sma*I site, resulting in a *Bst*PI-*Nco*I fragment containing the p15^{gag} and p12^{gag} regions. The *Xba*I-*Sal*I fragment of the BM5eco DNA clone, which contains the 3′ region of the *env* gene, 5′ long terminal repeat (LTR), whole *gag* gene, and 5′ region of the *pol* gene, was subcloned (5). The *Bst*PI-*Nco*I fragment in this plasmid DNA was replaced by the corresponding fragment from the PCR products described above. The *Bst*PI and *Nco*I sites are unique in the *Xba*I-*Sal*I fragment. The *Sal*I-*Spe*I fragment of the BM5eco clone, which contains the 3′ region of the *pol* gene, whole *env* gene, 3′ LTR, and 5′ region of the *gag* gene, was subcloned, and the *Spe*I site was changed to a *Hin*dIII site. The *Sal*I-*Hin*dIII fragment was then inserted into the *Sal*I-*Hin*dIII site of the plasmid containing the *Xba*I-

* Corresponding author.

```
                          10                  20                  30
BM5eco    C C C/G C T/C T T/A C C/C C C/T C T/A T A/A A A/C C C/A G A/
pEDV-2    - - -/- - C/- - -/- - -/- - -/- - -/- - -/- - G/- - -/- A -/
G1B       A - T/- - C/- - A/T A -/- - -/- - -/- - T/- - G/A - -/- A -/

                          40                  50                  60
BM5eco    C C T/T C T/A A A/C C T/C A G/G T T/C T C/T C C/G A C/A A C/
pEDV-2    - - -/C - -/- - G/- - C/- - -/- - -/- - -/C - T/- - T/- G -/
G1B       - - -/C - -/- - G/T - C/- G -/- - -/- - -/C - T/A C T/- - -/
                                 SmaI
                          70                  80                  90
BM5eco    G G C/G G A/C C T/C T C/A T T/G A C/C T T/C T C/A C A/G A A/
pEDV-2    - - -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- - G/
G1B       - - -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- • -

                         100                 110                 120
BM5eco    G A C/C C T/C C G/C C G/T A C/G G A/G A A/C A G/G G A/C C G/
pEDV-2    - - -/- - C/- - -/- - -/- - -/- - -/- C -/- - • • • -/- - T/
G1B       C/- • • • A -/- C -/- C -/- • • • • • • • • • • • • -/- T

                         130                 140                 150
BM5eco    T C C/T C C/C C T/G A C/G G G/G A T/G G C/G A C/A G A/G A A/
pEDV-2    - - -/- - -/T - -/- C -/A - A/- - G/- A -/- - T/G A -/A - -/
G1B       -/- - -/- - T/- - -/T - A/A A -/G G A/C - A/G T T/A - -/- -
                                                  D-2 primer
                         160                 170                 180
BM5eco    G A G/G C C/A C C/T A C/A C T/T C T/G A G/A T C/C C T/G C C/
pEDV-2    - - -/- - G/G - -/G C -/- - C/- - C/- - -/G - T/- - C/C - -/
G1B       -/- - -/- G G/- - G/C - -/- C -/- C -/- - G/- A T/T C C/- -

                         190                 200                 210
BM5eco    C C C/T • C T/C C C/A T G/G T G/T C T/C G C/C T G/C G G/G G
pEDV-2    - - T/- • - -/- - -/- - -/- - -/- - -/- - A/- - -/- - -/- -
G1B       -/- - -/G G -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- -
                                 NcoI
                         220                 230                 240
BM5eco    C/A A A/A G A/G A C/C C C/C C C/G C G/G C A/G A T/T C C/A C
pEDV-2    A/- G G/- - G/- - -/- - T/- - -/- - A/- - G/- - C/- - -/- -
G1B       G/- - C/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- - -/- -

                         250
BM5eco    C/A C C/T C T/C G G/G C T/T T C/
pEDV-2    -/T - -/- - C/- A -/- - A/- - -/
G1B       -/- - -/- - -/- - -/- - -/- - -/
```

FIG. 1. Nucleotide sequence comparison of the p12^{gag} regions between the BM5eco, pEDV-2, and G1B DNA clones. Dashes indicate identity within the pEDV-2 and G1B sequences for the residue in BM5eco. Dots indicate the absence of the corresponding nucleotide. Three nucleotides between slashes correspond to one amino acid codon. The *Sma*I and *Nco*I sites and the location of the D-2 primer are underlined.

*Sal*I fragment. Finally, the fragment containing the 5′ LTR-*gag-pol-env*-3′ LTR was constructed (5, 14).

As shown in Fig. 1, the p12^{gag} region of the defective pathogenic MAIDS virus (G1B DNA clone) (14) has a 16-bp deletion and a 1-bp insertion, and the MAIDS virus-related endogenous sequence, *Edv* (pEDV-2 DNA clone) (15), isolated from normal C57BL/6 mice has only a 3-bp deletion, compared with that of the helper ecotropic LP-BM5 virus (BM5eco DNA clone) (5). Therefore, the amino acid sequence of the MAIDS virus p12^{gag} region (positions 89 to 184 in Fig. 1) is less homologous to either the BM5eco virus or *Edv* sequence because of the frameshift in the region. To determine whether the frameshift in the MAIDS virus is essential for the development of MAIDS, chimeric viruses containing the p12^{gag} region of *Edv* or the BM5eco virus with or without a 1-bp deletion (position 89 in Fig. 1) and a 1-bp insertion (position 184 in Fig. 1) were constructed (Fig. 2B). The insertion mutation was prepared by PCR-mediated mutagenesis with the H-3 and H-4 primers for the helper BM5eco virus and the E-1 and E-2 primers for the *Edv* sequence (Fig. 2B and C). The H-4 and E-2 primers contained the 1-base insertion (Fig. 2C). The deletion mutation was prepared by site-directed mutagenesis with the H-M and E-M primers (Fig. 2B and C) for the helper BM5eco virus and *Edv* sequences, respectively. Site-directed mutagenesis was performed with a Transformer Site-Directed Mutagenesis Kit (Clontech Co., Ltd.) according to the method of Kunkel (9, 17). The nucleotide sequence of a selection primer, which exchanges the *Xho*I site of the pBluescript vector for a *Pst*I site, was 5′-GATACCGTCGACCTGCAGGGG GGGCCCGGTACC-3′. The mutations were confirmed by sequencing of the resulting DNA clones. The frameshift mutations resulted in no termination codon.

A chimeric virus, HGB-2, was constructed by replacing the p15^{gag} and p12^{gag} regions (*Bst*PI-*Nco*I fragment) of the helper virus with the corresponding region of the pathogenic G1B DNA (Fig. 2A) (14). The HGB-2 chimeric virus is defective and requires a helper virus to produce virions. Chimeric viruses constructed in this study contained structures with replacement of the *Sma*I-*Nco*I region of the HGB-2 virus by the corresponding region of BM5eco or *Edv* with or without the frameshift mutations. HGB-4 and HED-1 chimeric viruses contained the p12^{gag} regions of the helper BM5eco and *Edv* sequences without the mutations, respectively. HGB-4M and HED-1M carried the modified p12^{gag} regions from BM5eco and *Edv*, respectively. The resulting amino acid sequences of the p12^{gag} region of the mutant viruses are shown in Fig. 3. Chimeric viruses were constructed so as to contain the p15^{gag} region of G1B and the p12^{gag} region with and without the frameshift mutation (Fig. 2B), since the p15^{gag} and p12^{gag} regions of the MAIDS virus are both required for MAIDS development (14).

We reported previously that the chimeric viruses containing either the p15^{gag} or p12^{gag} region of the pathogenic MAIDS virus are replication defective (14). The amphotropic packaging cells GP+envAm12 (19) were cotransfected with the chimeric viral DNA and pSV$_2$neo, and neomycin-resistant colonies were selected in the presence of G418, since the chimeric viruses constructed in this study containing the p15^{gag} region of the defective MAIDS virus were also expected to be replication defective. The best producer was selected by its ability to express the highest level of viral RNA determined by Northern (RNA) hybridization of virion RNA with the representative probe, which also provided us with data about the rough structure of the generated virus. The producers for the HGB-4, HGB-4M, HED-1, HED-1M, and G1B viruses expressed almost the same levels of viral RNA. One-month-old C57BL/6 mice were inoculated with the transfected cells (10^6 cells per mouse), since the level of pathogenicity of cell supernatant harvested from the packaging cells transfected with the pathogenic G1B DNA was very low (data not shown). However, only 3 of 11 C57BL/6 mice inoculated with the helper-free G1B-transfected GP+envAm12 packaging cells developed MAIDS. Therefore, C57BL/6 mice were inoculated with the mixture of G1B virus-expressing packaging cells (10^6 cells per mouse) and BM5eco-infected SC-1 cells (10^6 cells per mouse) to help the further replication of the defective G1B virus. All inoculated mice (five of five) developed MAIDS this way (Table 1). In the following experiments, C57BL/6 mice were inoculated with a mixture of the chimeric virus-expressing packaging cells and the BM5eco-infected SC-1 cells.

Sixteen of 21 mice inoculated with cells producing the HED-1M virus and 5 of 21 mice inoculated with cells producing the HGB-4M virus developed splenomegaly and lymphadenopathy within the 10-month observation period (Table 1). A functional assay for the T-cell response to concanavalin A showed that in the spleen cells of diseased mice inoculated with the mutant viruses, the level of [^{3}H]thymidine incorporation was lower than that in the spleen cells of HED-1- and HGB-4-infected mice and control mice, which were inoculated with the mixture of nontransfected GP+envAm12 cells and BM5eco-infected SC-1 cells (Table 1). Histological examination of the enlarged spleen revealed that the pathogenicity of the mutant viruses was biologically indistinguishable from that of the wild-type G1B virus (data not shown). We concluded that the mice inoculated with the mutant viruses developed MAIDS. The spleen weight in HGB-4M-infected mice was lower than that in HED-1M-infected mice, and HGB-4M-inoculated mice developed the disease at a lower rate (5 of 21) than the HED-1M-inoculated mice (16 of 21) (Table 1). His-

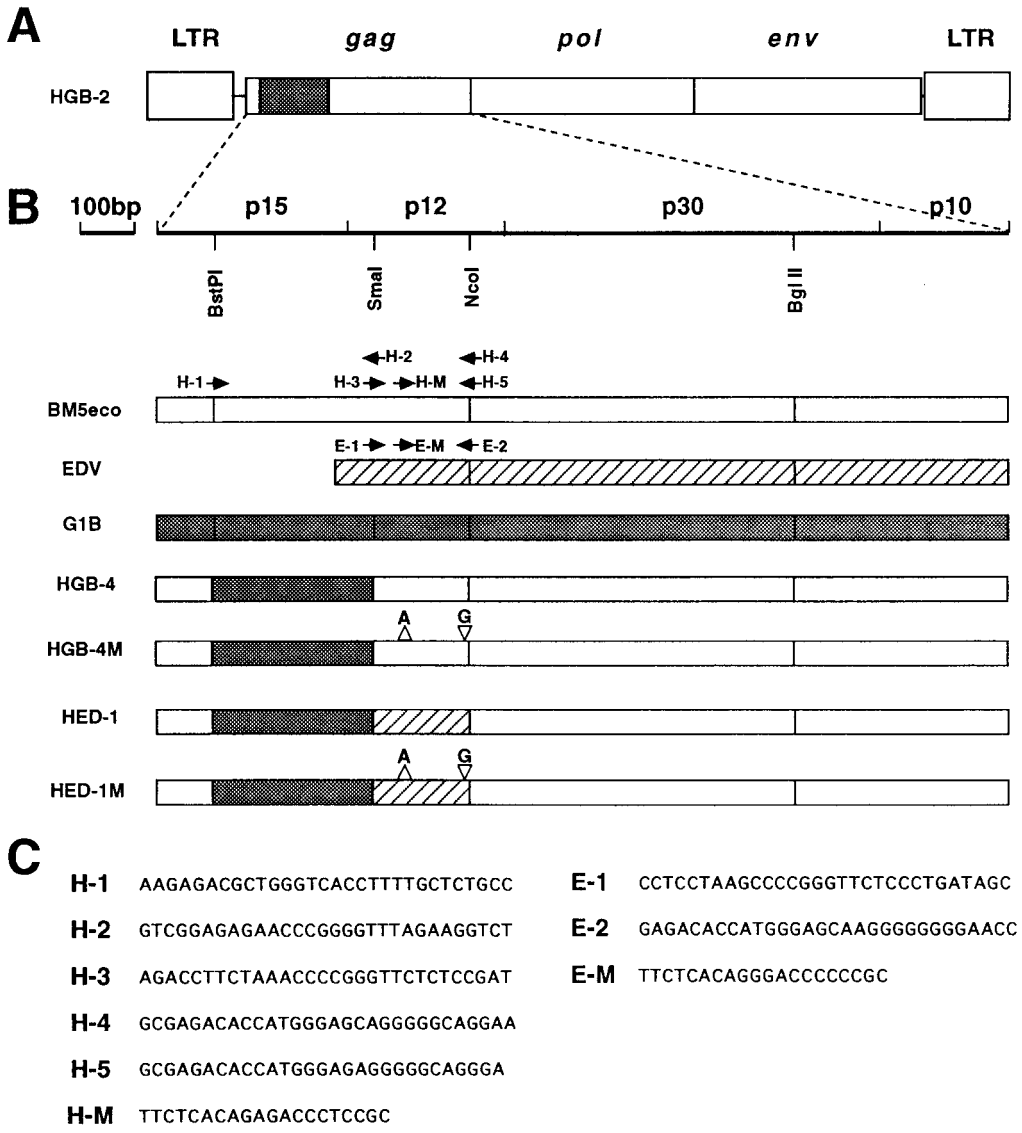

FIG. 2. Structure of the HGB-2 chimeric virus (24) (A), the *gag* structure of the mutant virus constructed in this study and locations of the primers used for mutagenesis (B), and nucleotide sequences of the primers (C). The fragments derived from the BM5eco, EDV, and G1B clones are shown as open, hatched, and shaded boxes, respectively.

tological examination indicated that the diseased mice infected with the HGB-4M virus were in a less-advanced stage of MAIDS than those inoculated with the G1B or HED-1M virus according to the stage classification by Hartley et al. (11) (data not shown). These results suggested that the HGB-4M mutant virus was less pathogenic than the HED-1M mutant and G1B viruses.

To determine whether the inoculated mutant viruses actually induced MAIDS, we examined the proliferation of mutant virus in the inoculated mice by PCR. To avoid PCR amplification of endogenous MuLV-related sequences present in C57BL/6 mice, SC-1 cells in which the endogenous sequences were hardly detected were cocultured with spleen cells isolated from the diseased mice inoculated with mutant viruses. Genomic DNA was then prepared from the infected SC-1 cells after five passages, among which cells from C57BL/6 mice were rare. We then performed PCR of the genomic DNAs with D-1, D-2, and H-6 primers (Fig. 4A); the H-6 primer can hybridize with both MAIDS and helper viruses. Because the D-1 and D-2 primers correspond to parts of the p15^{gag} and p12^{gag} unique sequences of the MAIDS virus, respectively, the primers cannot hybridize with helper sequences. Furthermore, the D-2 primer cannot hybridize with *Edv*, because only 12 among 20 bp of the D-2 primer are conserved (60%) (Fig. 1).

When PCR was performed with the D-1 and D-2 primers,

```
                10        20        30        40        50
HGB-4M  RVLSDNGGPL IDLLTETLRR TENRDRPPLT GMATEKRPPT LLRSLPPAPM
        ||| ||||| |||||::   :    |||:  ::  || |  ::|: ||:||
G1B     RVLPTNGGPL IDLLTDN•PP N••••LPPLS KGPVKKRRPP PPRYSPPGPM
        |||| :|||| ||||| :   :    ||||    :||||||| |||  |::||
HED-1M  RVLPDSGGPL IDLLTGTPRR TEHN•LPPLP ERTMKKRRPP PPRFPPLAPM
```

FIG. 3. Amino acid sequences of the p12^{gag} *Sma*I-*Nco*I regions of the G1B, HGB-4M, and HED-1M mutant viruses. Bars indicate identical amino acid residues. Colons indicate one of the following equivalents: basic amino acids (K, R, and H), acidic amino acids (D and E), uncharged polar amino acids (N, Q, S, T, and Y), or nonpolar amino acids (G, A, V, L, I, P, F, M, W, and C).

TABLE 1. Development of MAIDS by infection of C57BL/6 mice with mutant viruses

Virus	No. of diseased mice/no. of inoculated mice	Avg latency (days)[a]	Avg spleen wt (mg)[a]	[^{3}H]thymidine uptake (cpm [10^4])[b]	
				Non[c]	Concanavalin A
Control	0/5			2.0	10.8
G1B	5/5	104–142 (118)	270–700 (526)	3.4	2.6
HGB-4	0/16			2.6	13.7
HGB-4M	5/21	142–212 (161)	290–570 (424)	3.9	2.8
HED-1	0/17			2.5	15.9
HED-1M	16/21	129–222 (167)	480–2,510 (1,039)	3.5	2.8

[a] Values in parentheses represent the means.
[b] Average value of three mice.
[c] Non, without concanavalin A.

the fragment hybridizing with the MAIDS virus-specific probe (DSP-2) was detected only in the diseased mice inoculated with the G1B virus (Fig. 4B-1). The sizes of the PCR products were confirmed by the λ *Sty*I size marker (data not shown). The DSP-2 probe was derived from the 130-bp *Sma*I-*Nco*I fragment of the p12^{gag} region of the defective virus genome (16). This result excluded the possibility of contamination with the wild-type MAIDS virus in the diseased mice inoculated with the mutant viruses. When PCR was performed with the D-1 and H-6 primers, fragments which hybridized with the DSP-2 probe were detected in the diseased mice inoculated with the HED-1M virus, as in the G1B-infected mice. Furthermore, in one of two animals infected with the HGB-4M mutant virus, the hybridizing fragment was detected (Fig. 4B-2, left lane, HGB-4M) when hybridization was performed at 42°C.

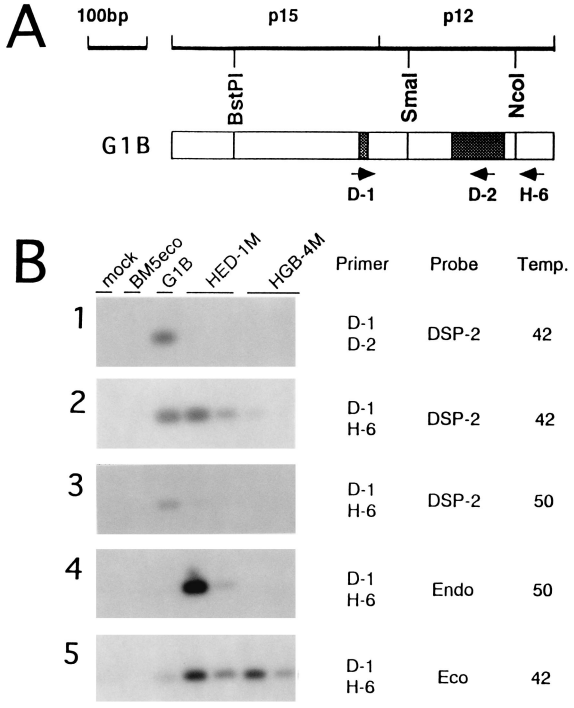

FIG. 4. Southern hybridization of PCR products. Locations of the primers used in this PCR experiment are indicated (A). PCR products with D-1 and D-2 primers were hybridized with the MAIDS virus-specific probe (DSP-2) at 42°C (B-1). Those with D-1 and H-6 primers were hybridized with the DSP-2 probe at 42°C (B-2) or 50°C (B-3), with the endo probe at 50°C (B-4), or with the eco probe at 42°C (B-5). The nucleotide sequences of the primers were as follows: D-1, CCTTTTCCTTTATCGACACT; D-2, CTTCTTAACTGGTCCCTTTG; and H-6, TTTGCCCCGCAGGCGAGACACCATGG.

However, when hybridization was performed at 50°C for high specificity, fragments which hybridized with the DSP-2 probe were detected only in the G1B virus. No hybridizing fragments were detected in the HGB-4M- and HED-1M-infected mice (Fig. 4B-3). Because the p12^{gag} region of the BM5eco virus has about 74% homology to that of the MAIDS virus (15), that of the HGB-4M mutant virus hybridized with the DSP-2 probe at low stringency (42°C).

When the PCR products were hybridized with the *Sma*I-*Nco*I segment of the pEDV-2 DNA clone (endo probe) (15) at 50°C, the fragments were detected in both of the HED-1M-infected mice (Fig. 4B-4). On rehybridization with the *Sma*I-*Nco*I segment of the BM5eco virus (eco probe) (15), the hybridizing fragments were detected not only in the diseased mice inoculated with the HGB-4M virus but also in those inoculated with the G1B or HED-1M virus (Fig. 4B-5). This result suggested the emergence of recombinant viruses containing the p15^{gag} unique sequence of the MAIDS virus and the p12^{gag} sequence of the BM5eco virus in mice inoculated with G1B or HED-1M. Finally, these results showed that the inoculated mutant virus proliferated in the diseased mice. These results indicated that the amino acid sequence resulting from the frameshift mutations is essential for MAIDS development.

It was shown here that virus containing the MAIDS virus p15^{gag} and the BM5eco p12^{gag} sequences appeared in G1B- and in HED-1M-infected mice (Fig. 4B-5). This suggested that recombination occurred between the G1B or HED-1M and helper BM5eco viruses. Such recombination has been described previously (8). Although we tried to determine the nucleotide sequences of several PCR products recovered from the infected mice, no such recombinant virus was detected (data not shown). Therefore, the recombinant virus may be less abundant than the parental viruses.

Recombination between the defective MAIDS virus and an endogenous sequence has been reported, suggesting that the creation of virus variants by the recombination event may play an important role in the pathogenesis and escape from host immune attack (10, 13). However, we observed no such event, although the various changes of nucleotide sequences in p12^{gag} regions from the inoculated mutant viruses were determined by PCR amplification (data not shown).

Recently, Cho et al. (7) reported the presence of a provirus structurally related to MAIDS virus in BXH-2 mice. Although the sequence of the p12^{gag} of the provirus is different from that of MAIDS virus, it may be one of the candidates for the origin of MAIDS virus (7). Some of the mice inoculated with the HGB-4M mutant virus, which contained the BM5eco p12^{gag} region with the frameshift mutations, developed MAIDS. Thus, it was also possible that the MAIDS virus was generated

by frameshift mutations in the p12^{gag} region of the BM5eco viral genome. However, it was shown here that the HGB-4M mutant virus was less pathogenic than the HED-1M virus. Furthermore, the nucleotide sequence of the p12^{gag} region of the MAIDS virus is more homologous to that of *Edv* than that of BM5eco. Therefore, it was likely that the MAIDS virus was generated by frameshift mutations in the p12 sequence of *Edv* or related sequences.

We thank K. Tokunaga and M. Kawamura for valuable technical suggestions and N. Aoyama and T. Ishikawa for technical assistance.

This work was supported by a grant-in-aid to A. Ishimoto for AIDS research from the Ministry of Health and Welfare of Japan and by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

REFERENCES

1. **Aziz, D. C., Z. Hanna, and P. Jolicoeur.** 1989. Severe immunodeficiency disease induced by a defective murine leukaemia virus. Nature (London) **338:**505–508.
2. **Buller, R. M. L., R. A. Yetter, T. N. Fredrickson, and H. C. Morse III.** 1987. Abrogation of resistance to severe mousepox in C57BL/6 mice infected with LP-BM5 murine leukemia virus. J. Virol. **61:**383–387.
3. **Casabianca, A., and M. Magnani.** 1994. A p12 *gag* gene homologue is present in the mouse genome. Biochem. Mol. Biol. Int. **32:**691–696.
4. **Chattopadhyay, S. K., H. C. Morse III, M. Makino, S. K. Ruscetti, and J. W. Hartley.** 1989. Defective virus is associated with induction of murine retrovirus-induced immunodeficiency syndrome. Proc. Natl. Acad. Sci. USA **86:** 3862–3866.
5. **Chattopadhyay, S. K., D. N. Sengupta, T. N. Fredrickson, H. C. Morse III, and J. W. Hartley.** 1991. Characteristics and contributions of defective, ecotropic, and mink cell focus-inducing viruses involved in a retrovirus-induced immunodeficiency syndrome of mice. J. Virol. **65:**4232–4241.
6. **Cheung, S. C., S. K. Chattopadhyay, H. C. Morse III, and P. M. Pitha.** 1991. Expression of defective virus and cytokine genes in murine AIDS. J. Virol. **65:**823–828.
7. **Cho, B. C., J. D. Shaughnessy, Jr., D. A. Largaespada, H. G. Bedigian, A. M. Buchberg, N. A. Jenkins, and N. G. Copeland.** 1995. Frequent disruption of the *Nf1* gene by a novel murine AIDS virus-related provirus in BXH-2 murine myeloid lymphomas. J. Virol. **69:**7138–7146.
8. **Coffin, J. M.** 1992. Genetic diversity and evolution of retroviruses. Curr. Top. Microbiol. Immunol. **176:**143–164.
9. **Deng, W. P., and J. A. Nickoloff.** 1992. Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. Anal. Biochem. **200:**81–86.
10. **Gayama, S., B. A. Vaupel, and O. Kanagawa.** 1995. Sequence heterogeneity of murine acquired immunodeficiency syndrome: the role of endogenous virus. Int. Immunol. **7:**861–868.
11. **Hartley, J. W., T. N. Fredrickson, R. A. Yetter, M. Makino, and H. C. Morse III.** 1989. Retrovirus-induced murine acquired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. J. Virol. **63:**1223–1231.
12. **Jolicoeur, P.** 1991. Murine acquired immunodeficiency syndrome (MAIDS): an animal model to study the AIDS pathogenesis. FASEB J. **5:**2398–2405.
13. **Kanagawa, O., B. A. Vaupel, S. J. Korsmeyer, and J. H. Russell.** 1995. Apoptotic death of lymphocytes in murine acquired immunodeficiency syndrome: involvement of Fas-Fas ligand interaction. Eur. J. Immunol. **25:**2421–2427.
14. **Kubo, Y., K. Kakimi, K. Higo, L. Wang, H. Kobayashi, K. Kuribayashi, T. Masuda, T. Hirama, and A. Ishimoto.** 1994. The p15^{gag} and p12^{gag} regions are both necessary for the pathogenicity of the murine AIDS virus. J. Virol. **68:**5532–5537.
15. **Kubo, Y., Y. Nakagawa, K. Kakimi, H. Matsui, K. Higo, L. Wang, H. Kobayashi, T. Hirama, and A. Ishimoto.** 1994. Molecular cloning and characterization of a murine AIDS virus-related endogenous transcript expressed in C57BL/6 mice. J. Gen. Virol. **75:**881–888.
16. **Kubo, Y., Y. Nakagawa, K. Kakimi, H. Matsui, M. Iwashiro, K. Kuribayashi, T. Masuda, H. Hiai, T. Hirama, S.-I. Yanagawa, and A. Ishimoto.** 1992. Presence of transplantable T-lymphoid cells in C57BL/6 mice infected with murine AIDS virus. J. Virol. **66:**5691–5695.
17. **Kunkel, T. A.** 1985. The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA. Proc. Natl. Acad. Sci. USA **82:**488–492.
18. **Latarjet, R., and J.-F. Duplan.** 1962. Experiment and discussion on leukemogenesis by cell-free extracts of radiation-induced leukemia in mice. Int. J. Radiat. Biol. **5:**339–344.
19. **Markowitz, D., S. Goff, and A. Bank.** 1988. Construction and use of a safe and efficient amphotropic packaging cell line. Virology **167:**400–406.
20. **Morse, H. C., III, S. K. Chattopadhyay, M. Makino, T. N. Fredrickson, A. W. Hugin, and J. W. Hartley.** 1992. Retrovirus-induced immunodeficiency in the mouse: MAIDS as a model for AIDS. AIDS **6:**607–621.
21. **Mosier, D. E., R. A. Yetter, and H. C. Morse III.** 1985. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice. J. Exp. Med. **161:**766–784.
22. **Nakagawa, Y., K. Kakimi, W. Ling, Y. Kubo, K. Higo, T. Masuda, K. Kuribayashi, M. Iwashiro, Y. Komatz, T. Hirama, A. Adachi, and A. Ishimoto.** 1994. Inhibition of murine AIDS (MAIDS) development by the transplantation of bone marrow cells carrying the *Fv-4* resistance gene to MAIDS virus-infected mice. J. Virol. **68:**1438–1441.
23. **Portnoi, D., A. M. Stall, D. Schwartz, T. C. Merigan, L. A. Herzenberg, and T. Basham.** 1990. Zidovudine inhibits characteristic early alterations of lymphoid cell populations in retrovirus-induced murine AIDS. J. Immunol. **144:** 1705–1710.
24. **Pozsgay, J. M., M. W. Beilharz, B. D. Wines, A. D. Hess, and P. M. Pitha.** 1993. The MA (p15) and p12 regions of the *gag* gene are sufficient for the pathogenicity of the murine AIDS virus. J. Virol. **67:**5989–5999.