A Repeat Sequence, GGGTTA, Is Shared by DNA of Human Herpesvirus 6 and Marek’s Disease Virus

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Some regions of the genomes of human B-lymphotropic virus (HBLV), also designated as human herpesvirus 6, and Marek’s disease virus were found to hybridize to each other under moderate to stringent conditions, scoring from 10 to 30% base-pair mismatch. Nucleotide sequence analysis showed that a 6-base-pair repetitive sequence, GGGTTA (DR2), present in the IRx-IRy junction region of the Marek’s disease virus genome, was also reiterated in the HBLV genome. The function(s) of such a sequence is unknown, but this is the first report of homology between HBLV and a nonhuman herpesvirus.

A new human herpesvirus, human B-lymphotropic virus (HBLV) or human herpesvirus 6, was recently isolated from patients with lymphoproliferative diseases (6, 29, 32) and infants with roseola infantum (exanthem subitum) (37). Some of these patients were also seropositive for human immunodeficiency virus type 1. HBLV has been found to be distinct from other human herpesviruses, such as Epstein-Barr virus, cytomegalovirus, herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus, by immunologic, molecular, and morphological analyses (3, 14, 29), but it bears some sequence homology to cytomegalovirus (7). HBLV has also been found to be distinct from other nonhuman primate B- and T-lymphotropic herpesviruses by immunologic analyses (29). Since cross-reactivities between various herpesviruses of mammalian and avian origins have been detected by immunologic assays or molecular hybridization (2, 8, 16, 23, 26, 28, 30), we extended our studies to determine whether HBLV bears any homology to Marek’s disease virus (MDV).

MDV, an avian herpesvirus, induces T-cell lymphoma and other manifestations in chickens (4, 24). Marek’s disease was once a significant economic problem in the poultry industry; however, the development of vaccines consisting of either attenuated MDV or herpesvirus of turkeys (HVT), a serologically related avian herpesvirus (33, 34), virtually solved this problem (4, 24, 25). Because MDV induces tumors in its natural host, it serves as a model for viral oncogenesis (5).

The structure of the MDV genome has been studied in detail, and restriction enzyme maps of the viral DNA have been constructed (10). The viral genome contains two sets of inverted repeat regions (IRL and IRR; IRx and IRy), which, respectively, flank a long unique sequence (U1) and a short unique sequence (U2) (Fig. 1A). This arrangement is similar to that of the HSV type 1 genome (31).

We report here the hybridization of MDV and HBLV DNAs by Southern blot analyses. We found that HBLV and MDV share sequence similarities in certain regions and a 6-base-pair repeated sequence, GGGTTA, which is reiterated 60 and 26 times within these genomes, respectively.

Initially, Southern blot hybridizations between MDV DNA and HBLV DNA yielded two interesting results. (i) A labeled MDV total genomic DNA probe hybridized to DNA from HBLV-infected cells digested with BamHI, whereas the same probe failed to hybridize to noninfected-cell DNA. Many fragments of HBLV-infected-cell DNA hybridized to

FIG. 1. Hybridization of the HBLV ZVH14 probe to MDV. (A) The left panel shows the ethidium bromide-stained agarose gel which contained the fragments of the MDV BamHI genomic library and which was blotted onto nitrocellulose filters as described previously (10). After hybridization at 65°C in 6X SSC (0.9 M NaCl plus 0.09 M sodium citrate) to the radiolabeled ZVH14 probe and autoradiography, three bands were detected, as indicated by the arrows in the right panel. These were the MDV BamHI A, F, and G fragments, as discussed in the text. (B) Hybridization of the individual fragments from a separate gel run.

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the MDV DNA probe under hybridization conditions which would score for a 30% base-pair mismatch. (ii) One of the cloned HBLV DNA fragments, ZVH14 (14), hybridized to three fragments, A, F, and G, from the BamHI library of MDV DNA (Fig. 1A and B). The same probe also hybridized to certain restriction fragments of HVT DNA (data not shown). This is the first observed hybridization of HBLV DNA probes to DNA of another nonprimate herpesvirus.

Hybridization was also observed when the MDV BamHI A fragment was used as a probe against HBLV-infected-cell DNA under stringent conditions (Fig. 2A). Furthermore, the terminal 1.4-kilobase (kb) HindIII subfragment of the MDV BamHI A fragment, which contains the IRS-IR5 junction region (Fig. 3A) (10; M. Hayashi, K. Fukuchida, A. Tanaka, and M. Nonoyama, Microbiol. Immunol., in press), hybridized similarly to HBLV-infected-cell DNA (Fig. 3B). The 1.4-kb HindIII subfragment of the MDV BamHI A fragment hybridized to several BamHI fragments of purified genomic HBLV DNA (Fig. 3B). Among the hybridizing fragments, the 22-kb HBLV BamHI B fragment was cloned, and the map is shown in Fig. 2C. DNA sequencing of a 6-kb subclone of the HBLV BamHI B fragment showed that the GGGTTA sequence is repeated 60 times in the region indicated by the bar (Fig. 2C) between the two BssHII sites.

This sequence is reiterated 26 times in the junction region between IRS and IR5 in the MDV genome (Hayashi et al., in press). It is also found in the HVT genome (unpublished data). To determine the extent to which this sequence accounted for the hybridization results seen with the MDV BamHI A fragment, we synthesized an oligonucleotide probe with the GGGTTA sequence reiterated five times, (GGGTTA)n. With various restriction enzymes, this probe yielded patterns of hybridization identical to those seen with the MDV BamHI A probe and the 1.4-kb HindIII subfragment in HBLV-infected-cell DNA (Fig. 2A and B and 3B). Thus, the shared GGGTTA sequence alone can account for the hybridization results with the BamHI A fragment. Of the several bands detected by the (GGGTTA)n probe against BamHI-digested MDV DNA, the two largest bands contained TRS and the junction region sequences, while the three smallest bands contained the terminal region of TRS (Fig. 3B) (Hayashi et al., in press). The fragments detected between 2.05 and 15.5 kb by the HindIII 1.4-kb probe have not been characterized.

The tandem GGGTTA repeat sequence in the IRS-IR5 junction region of MDV (Hayashi et al., in press) is analogous to a 12-base-pair tandem repeat (DR3) found in the "a" sequence in the junction region of HSV DNA (20). The HSV "a" sequence contains 19 tandem repeats of DR3 which are bracketed by two 20-base-pair repeats of DR1 (20). Although there is no sequence homology between the "a" sequences of the MDV and HSV junction regions, their structural
similarities suggest functional similarities, such as possible involvement in the mechanism of inversion of viral DNA at the junction site (20, 21) or the cleavage of viral DNA from head-to-tail concatemers and packaging of unit-length genomes (35). Whether the GGGTTA repeat is present in the junction region of HBLV remains to be determined. Recently, the GGGTTA repeat sequence was found to comprise the telomeric sequences of humans and other species (R. K. Moyzis, J. N. Buckingham, L. S. Cram, M. Dani, L. L. Deaven, M. D. Jones, J. Meyne, R. L. Ratliff, and J. R. Wu, Proc. Natl. Acad. Sci. USA, in press). It would be of interest to determine whether there is any shared structure or function of the viral and cellular sequences, including possible protein interactions. Another question is whether the shared sequences arose by recombination events between the viral and host genomic DNAs.

Cross-hybridization of MDV and HBLV DNAs other than that seen with the (GGGTTA) probe was demonstrated by detection of at least two additional BamHI fragments in digested HBLV DNA by the MDV genomic probe (Fig. 4) and by the hybridization of ZVH14 with MDV DNA. In our previous studies of the relationship between HVT and MDV DNAs, the use of total viral DNA as a probe revealed a small number of homologous fragments in Southern blot hybridizations (15), whereas the use of specific cloned fragments as probes revealed extensive homology between the two viral DNAs so we were able to establish that the two genomes had the same genetic organization and were colinear (12). A similar approach is being used to determine the relationship of the HBLV genome to that of MDV.

The 6-base-pair (GGGTTA), repeat sequence identity found in the MDV and HBLV genomes and the cross-hybridization of additional subgenomic fragments suggested a phylogenetic relationship between the two viruses. On the basis of the tropism so far studied (1, 17, 29), HBLV would tentatively be assigned to the Gammaherpesviridae subclass, to which MDV, HTV, and Epstein-Barr virus have been assigned (27). However, in contrast to Epstein-Barr virus, which is transforming, HBLV, MDV, and HTV are highly cytopathic in vitro (17, 29). MDV is associated with T-cell neoplasia, whereas HBLV has not been linked to any cancer. Whether the relationship between MDV and HBLV extends to similarities in aspects of their pathologic spectra remains to be explored. For example, MDV has been linked to neurological disease and atherosclerosis (4, 9, 24), but nothing much is known of the potential role of HBLV in these diseases in humans. The possibility of HBLV involvement in T-cell neoplasia should be considered, since HBLV infects fresh immature and mature T cells and established cell lines in vitro (17). Recently, we found HBLV sequences in some B-cell tumors (13), but an etiologic role for HBLV in these or other hematopoietic neoplasias remains to be determined. It would also be of interest to determine whether the finding of a serologically cross-reactive glycoprotein from leukocytic lesions of chickens infected with MDV and antigens prepared from a variety of human lymphomas, leukemias, and carcinomas (18, 19) can be explained by the presence of an HBLV-encoded protein.

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