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-lymphotrophic 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 IRv-IRl 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-lymphotrophic 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-lymphotrophic 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 (IRi and IRr); IRi and TRR which, respectively, flank a long unique sequence (Ul) and a short unique sequence (Us) (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. 2. Hybridization of HBLV DNA with BamHI A and (GGGTTA)$_5$. (A) HBLV DNA was digested with various restriction enzymes and transferred to a nitrocellulose filter after gel electrophoresis as previously described (10). Hybridization was carried out with the MDV BamHI A fragment probe at 37°C in 50% formamide-3X SSC (0.45 M NaCl plus 0.045 M sodium citrate). The filters were washed at 50°C in 1X SSC-0.1% sodium dodecyl sulfate. Samples were digested with BamHI (a), XhoI (b), SstII (c), HindIII (d), EcoRI (e), PstI (f), XhoI (g), SsrI (h), and SsrI (i). (B) HBLV DNA was hybridized as described above with the (GGGTTA)$_5$ probe. (C) Restriction map of the 23-kb HBLV BamHI B fragment (ZVB70 = pZVB-70) which contained the regions hybridizing to the BamHI A fragment of MDV (the largest band in panel A, lane a). The sequence of GGGTTA is repeated 60 times in the region between the BssHII sites, as indicated by the bar.

FIG. 3. MDV genomic structure and hybridization of MDV DNA with the (GGGTTA)$_5$ probe. (A) Schematic map of the MDV genome (10). IR$_S$ and IR$_L$. Short and long inverted repeat regions, respectively; TR$_S$ and TR$_L$. short and long terminal repeat regions, respectively; U$_S$ and U$_L$. short and long unique regions; DR, direct repeat. (B) 1.4-kb HindIII (left lane) and (GGGTTA)$_5$ (right lane) were used as probes for hybridization to BamHI-digested MDV DNA on the filter at 50°C in 6× SSC (0.9 M NaCl plus 0.09 M sodium citrate). The two hybridization bands at the higher molecular weights represent hybridization of TR$_S$ and the junction region (10), and the three bands at the lower molecular weights represent the terminal end of TR$_L$ (Hayashi et al., in press). kbp. Kilobase pairs.

This sequence is reiterated 26 times in the junction region between IR$_S$ and IR$_L$ 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)$_5$. 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. 2 A 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)$_5$ probe against BamHI-digested MDV DNA, the two largest bands contained TR$_S$ and the junction region sequences, while the three smallest bands contained the terminal region of TR$_L$ (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 IR$_S$-IR$_L$ junction region of MDV (Hayashi et al., in press) is analogous to a 12-base-pair tandem repeat (DR$_2$) found in the "a" sequence in the junction region of HSV DNA (20). The HSV "a" sequence contains 19 tandem repeats of DR$_2$ which are bracketed by two 20-base-pair repeats of DR$_1$ (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 concatamers 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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