JOURNAL OF VIROLOGY, Dec. 1988, p. 4824–4827
0022-538X/88/124824-04$02.00/0

Vol. 62, No. 12

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

MASAHIKO KISHI,[1] HIROSHI HARADA,[1] MITSUYOSHI TAKAHASHI,[1] AKIKO TANAKA,[1] MASANOBU HAYASHI,[1] MEIHAN NONOYAMA,[1] STEVEN F. JOSEPHS,[2] ABY BUCHBINDER,[2] FRANCOIS SCHACHTER,[2] DHARAM V. ABLASHI,[3] FLOSSIE WONG-STAAL,[2]* S. ZAKI SALAHUDDIN,[2] AND ROBERT C. GALLO[2]

Department of Virology, Showa University Research Institute for Biomedicine in Florida, St. Petersburg, Florida 33716,[1] and Laboratory of Tumor Cell Biology[2] and Laboratory of Cellular and Molecular Biology,[3] National Cancer Institute, Bethesda, Maryland 20892

Received 6 April 1988/Accepted 25 August 1988

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 (DR_2), present in the IR_S-IR_L 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-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 (IR_L and TR_L; IR_S and TR_S) which, respectively, flank a long unique sequence (U_L) and a short unique sequence (U_S) (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 *Bam*HI, whereas the same probe failed to hybridize to noninfected-cell DNA. Many fragments of HBLV-infected-cell DNA hybridized to

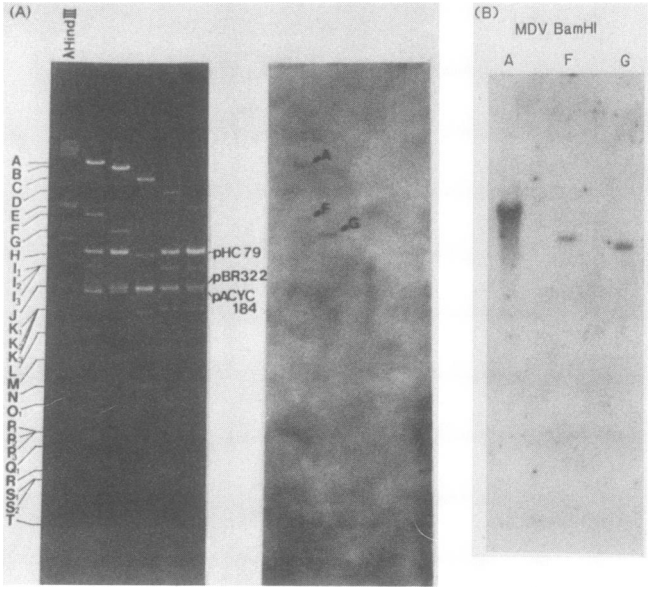

FIG. 1. Hybridization of the HBLV ZVH14 probe to MDV. (A) The left panel shows the ethidium bromide-stained agarose gel which contained the fragments of the MDV *Bam*HI genomic library and which was blotted onto nitrocellulose filters as described previously (10). After hybridization at 65°C in 6X SSC (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 *Bam*HI A, F, and G fragments, as discussed in the text. (B) Hybridization of the individual fragments from a separate gel run.

* Corresponding author.

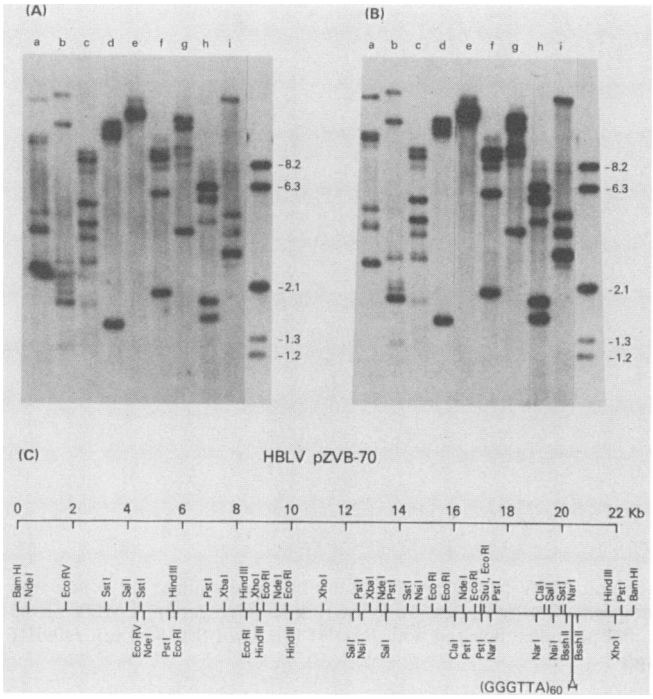

FIG. 2. Hybridization of HBLV DNA with *Bam*HI A and $(GGGTTA)_5$. (A) HBLV DNA was digested with various restriction enzymes and transferred to a nitrocellulose filter after gel electrophoresis as previously described (10). Hybridization was carried out with the MDV *Bam*HI A fragment probe at 37°C 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 *Bam*HI (a), *Xho*I (b), *Sal*I (c), *Hin*dIII (d), *Eco*RI (e), *Pst*I (f), *Xba*I (g), *Sst*II (h), and *Sst*I (i). (B) HBLV DNA was hybridized as described above with the $(GGGTTA)_5$ probe. (C) Restriction map of the 23-kb HBLV *Bam*HI B fragment (ZVB70 [= pZVB-70]) which contained the regions hybridizing to the *Bam*HI 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 *Bss*HII sites, as indicated by the bar.

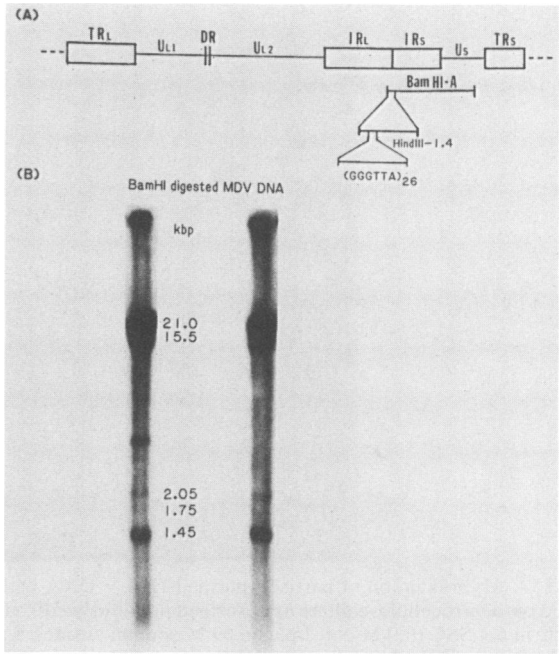

FIG. 3. MDV genomic structure and hybridization of MDV DNA with the $(GGGTTA)_5$ probe. (A) Schematic map of the MDV genome (10). IR_S and IR_L, Short and long inverted repeat regions, respectively; TR_S and TR_L, short and long terminal repeat regions, respectively; U_S and U_L, short and long unique regions; DR, direct repeat. (B) 1.4-kb *Hin*dIII (left lane) and $(GGGTTA)_5$ (right lane) were used as probes for hybridization to *Bam*HI-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.

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 *Bam*HI 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 *Bam*HI A fragment was used as a probe against HBLV-infected-cell DNA under stringent conditions (Fig. 2A). Furthermore, the terminal 1.4-kilobase (kb) *Hin*dIII subfragment of the MDV *Bam*HI A fragment, which contains the IR_L-IR_S junction region (Fig. 3A) (10; M. Hayashi, K. Fukuchi, A. Tanaka, and M. Nonoyama, Microbiol. Immunol., in press), hybridized similarly to HBLV-infected-cell DNA (Fig. 3B). The 1.4-kb *Hin*dIII subfragment of the MDV *Bam*HI A fragment hybridized to several *Bam*HI fragments of purified genomic HBLV DNA (Fig. 3B). Among the hybridizing fragments, the 22-kb HBLV *Bam*HI B fragment was cloned, and the map is shown in Fig. 2C. DNA sequencing of a 6-kb subclone of the HBLV *Bam*HI B fragment showed that the GGGTTA sequence is repeated 60 times in the region indicated by the bar (Fig. 2C) between the two *Bss*HII sites. 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 *Bam*HI 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 *Bam*HI A probe and the 1.4-kb *Hin*dIII 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 *Bam*HI A fragment. Of the several bands detected by the $(GGGTTA)_5$ probe against *Bam*HI-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 *Hin*dIII 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

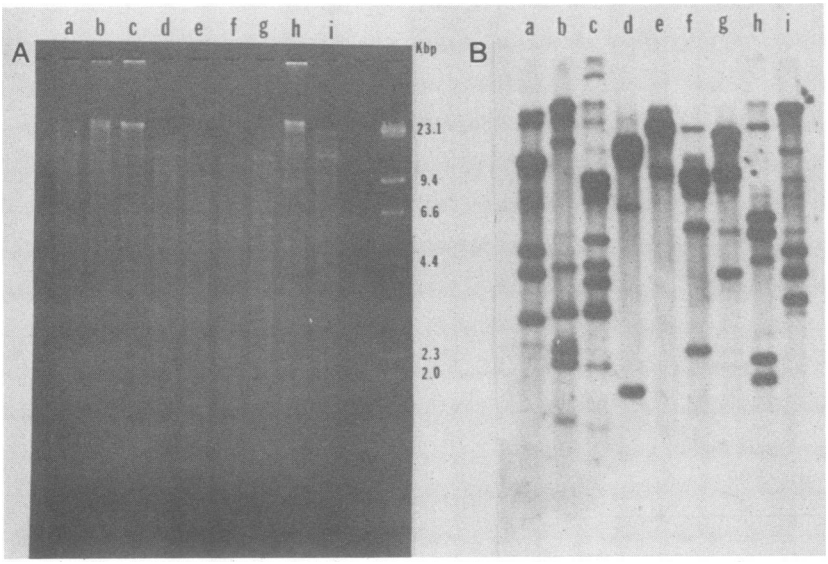

FIG. 4. Hybridization of partially purified HBLV DNA with a total genomic MDV probe. (A) Ethidium bromide-stained gel which was blotted onto nitrocellulose filters as described previously (10). (B) Autoradiogram after hybridization to the radiolabeled genomic MDV probe at 65°C in 6× SSC (0.9 M NaCl plus 0.09 M sodium citrate). Lanes contained samples digested with *Bam*HI (a), *Xho*I (b), *Sal*I (c), *Hin*dIII (d), *Eco*RI (e), *Pst*I (f), *Xba*I (g), *Sst*II (h), and *Sst*I (i). Kbp, Kilobase pairs.

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 $(GGGTTA)_5$ probe was demonstrated by detection of at least two additional *Bam*HI 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 $(GGGTTA)_n$ 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 HVT 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 etiological 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.

We thank Linda Jagodinsky (Biotech Research Laboratories, Rockville, Md.) for technical support.

LITERATURE CITED

1. **Ablashi, D. V., S. Z. Salahuddin, S. F. Josephs, F. Imam, P. Lusso, R. C. Gallo, C. Hung, J. Lemp, and P. D. Markham.** 1987. HBLV (or HHV-6) in human cell lines. Nature (London) **329**:207.
2. **Balachandran, N., D. E. Oba, and L. M. Hutt-Fletcher.** 1987. Antigenic cross-reactions among herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus. J. Virol. **61**: 1125–1135.
3. **Biberfeld, P., B. Kramarsky, S. Z. Salahuddin, and R. C. Gallo.** 1987. Ultrastructural characterization of a new human B lymphotrophic DNA virus (human herpesvirus 6) isolated from patients with lymphoproliferative disease. JNCI **5**:933–941.
4. **Biggs, P. M.** 1973. Marek's disease, p. 557–594. *In* A. Kaplan (ed.), The herpesviruses. Academic Press, Inc., New York.
5. **Calnek, B. W.** 1986. Marek's disease—a model for herpesvirus oncology. Crit. Rev. Microbiol. **12**:293–320.

6. **Downing, R. G., N. Sewankambo, D. Serwadda, R. Honess, D. Crawford, R. Jarrett, and B. E. Griffin.** 1987. Isolation of human lymphotrophic herpesviruses from Uganda. Lancet **ii**:390.
7. **Efstathiou, S., U. A. Gompels, M. A. Craxton, R. W. Honess, and K. Ward.** 1988. DNA homology between a novel human herpesvirus (HHV-6) and human cytomegalovirus. Lancet **i**:63–64.
8. **Evans, D. L., J. W. Barnett, J. M. Bowen, and L. Dmochowski.** 1972. Antigenic relationship between the herpesviruses of infectious bovine rhinotracheitis, Marek's disease, and Burkitt's lymphoma. J. Virol. **10**:277–287.
9. **Fabricant, C. G.** 1985. Atherosclerosis: the consequence of infection with a herpesvirus. Adv. Vet. Sci. Comp. Med. **30**:39–66.
10. **Fukuchi, K., M. Sudo, Y. S. Lee, A. Tanaka, and M. Nonoyama.** 1984. Structure of Marek's disease virus DNA: detailed restriction enzyme map. J. Virol. **51**:102–109.
11. **Fukuchi, K., M. Sudo, A. Tanaka, and M. Nonoyama.** 1985. Map location of homologous regions between Marek's disease virus and herpesvirus of turkey and the absence of detectable homology in the putative tumor-inducing gene. J. Virol. **53**:994–997.
12. **Igarashi, T., M. Takahashi, J. Donovan, J. Jessip, M. Smith, K. Hirai, A. Tanaka, and M. Nonoyama.** 1987. Restriction enzyme map of herpesvirus of turkey DNA and its colinear relationship with Marek's disease virus DNA. Virology **157**:351–358.
13. **Josephs, S. F., A. Buchbinder, H. Z. Streicher, D. V. Ablashi, S. Z. Salahuddin, H.-G. Guo, F. Wong-Staal, J. Cossman, M. Raffeld, J. Sundeen, P. Levine, R. Biggar, G. R. F. Kruegger, R. I. Fox, and R. C. Gallo.** 1988. Detection of human B-lymphotropic virus (human herpesvirus 6) sequences in B cell lymphoma tissues of three patients. Leukemia **2**:132–135.
14. **Josephs, S. F., S. Z. Salahuddin, D. V. Ablashi, F. Schachter, F. Wong-Staal, and R. C. Gallo.** 1986. Genomic analysis of the human B-lymphotrophic virus (HBLV). Science **234**:601–603.
15. **Lee, Y. S., A. Tanaka, S. Silver, M. Smith, and M. Nonoyama.** 1979. Minor DNA homology between herpesvirus of turkey and Marek's disease virus. Virology **93**:277–280.
16. **Leib, D. A., J. M. Bradbury, C. A. Hart, and K. McCarthy.** 1987. Genome isomerism in two alphaherpesviruses: herpesvirus saimiri-1 (herpesvirus tamarinus) and avian infectious laryngotracheitis virus. Arch. Virol. **93**:287–294.
17. **Lusso, P., S. Z. Salahuddin, D. V. Ablashi, R. C. Gallo, F. Di Marzo-Veronese, and P. D. Markham.** 1987. Diverse tropism for HBLV (human herpesvirus 6). Lancet **ii**:743.
18. **Makari, J. G.** 1973. Association between Marek's herpesvirus and human cancer. I. Detection of cross-reacting antigens between chicken tumors and human tumors. Oncology **28**:164–176.
19. **Makari, J. G.** 1973. Association between Marek's herpesvirus and human cancer. II. Detection of structural viral antigen in chicken tumors and human tumors. Oncology **28**:177–183.
20. **Mocarski, E. S., and B. Roizman.** 1981. Site-specific inversion sequence of the herpes simplex virus genome: domain and structural features. Proc. Natl. Acad. Sci. USA **78**:7047–7051.
21. **Mocarski, E. S., L. E. Post, and B. Roizman.** 1980. Molecular engineering of the herpes simplex virus genome: insertion of a second L-S junction into the genome causes additional genome inversions. Cell **22**:243–255.
22. **Naito, M., K. Ono, T. Doi, S. Kato, and S. Tanabe.** 1971. Antibodies in human and monkey sera to herpes-type virus from a chicken with Marek's disease and to EB virus detected by the immunofluorescence test. Biken J. **14**:161–166.
23. **Naito, M., K. Ono, S. Tanabe, T. Doi, and S. Kato.** 1970. Detection in chicken and human sera of antibody against herpes type virus from a chicken with Marek's disease and EB virus demonstrated by the indirect immunofluorescence test. Biken J. **13**:205–212.
24. **Nazerian, K.** 1980. Marek's disease: a herpesvirus-induced malignant lymphoma of the chicken, p. 665–682. *In* G. Klein (ed.), Viral oncology. Raven Press, Publishers, New York.
25. **Okazaki, W., H. G. Purchase, and B. R. Burmester.** 1970. Protection against Marek's disease by vaccination with a herpesvirus of turkey (HVT). Avian Dis. **14**:413–429.
26. **Ono, K., S. Tanabe, M. Naito, T. Doi, and S. Kato.** 1970. Antigen common to a herpes type virus from chickens with Marek's disease and EB virus from Burkitt's lymphoma cells. Biken J. **13**:213–217.
27. **Roizman, B., S. Carmichael, G. B. de The, M. Masic, A. Nahmias, W. Plowright, F. Rapp, P. Sheldrick, M. Takashi, M. Terni, and K. Wolfe.** 1978. Provisional classification of herpesviruses, p. 1079–1082. *In* G. de The, W. Henle, and F. Rapp (ed.) Oncogenesis of herpesviruses III. Part II. International Agency for Research on Cancer, Lyon, France.
28. **Ross, L. J., J. A. Frazier, and P. M. Biggs.** 1972. An antigen common to some avian and mammalian herpesviruses, p. 480–484. In P. M. Biggs, G. de The, and L. N. Payne (ed.) Oncogenesis and herpes virus. International Agency for Research on Cancer, Lyon, France.
29. **Salahuddin, S. Z., D. V. Ablashi, P. D. Markham, S. F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B. Kramarsky, and R. C. Gallo.** 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **234**:596–601.
30. **Sharma, J. M., R. L. Witter, B. R. Burmester, and J. C. Landon.** 1973. Public health implications of Marek's disease virus and herpesvirus of turkeys. Studies on human and subhuman primates. J. Natl. Cancer Inst. **51**:1123–1127.
31. **Sheldrick, P., and N. Berthelot.** 1974. Inverted repetitions in the chromosome of herpes simplex virus. Cold Spring Harbor Symp. Quant. Biol. **39**:667–678.
32. **Tedder, R. S., M. Briggs, C. H. Cameron, R. Honess, D. Robertson, and H. Whittle.** 1987. A novel lymphotropic herpesvirus. Lancet **ii**:390–392.
33. **van Zaane, D., J. M. A. Brinkhof, and A. L. J. Gielkens.** 1982. Molecular-biological characterization of Marek's disease virus. II. Differentiation of various MDV and HVT strains. Virology **121**:133–146.
34. **van Zaane, D., J. M. A. Brinkhof, F. Westenbrink, and A. L. J. Gielkens.** 1982. Molecular-biological characterization of Marek's disease virus. I. Identification of virus specific polypeptides in infected cells. Virology **121**:116–132.
35. **Vlazny, D. A., A. Kwong, and N. Frenkel.** 1982. Site-specific cleavage/packaging of herpes simplex virus DNA and the selective maturation of nucleocapsids containing full-length viral DNA. Proc. Natl. Acad. Sci. USA **79**:1423–1427.
36. **Wadsworth, S. C., R. J. Jacob, and B. Roizman.** 1985. Anatomy of herpes simplex virus DNA. II. Size, composition, and arrangement of inverted terminal repetitions. J. Virol. **15**:1487–1497.
37. **Yamanishi, K., K. Shiraki, T. Kondo, T. Okuno, M. Takahashi, Y. Asano, and T. Kurata.** 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet **i**:1065–1067.