3. Studies of Cancer in Animal Models

No animal model for KSHV/HHV8 has so far been reported; however, several closely related rhadinoviruses, e.g. HVS, *Herpesvirus ateles* (HVA) and murid herpesvirus 4 (MHV68), cause lymphoid malignancies or polyclonal proliferation. In terms of its tropism for B-cells, MHV68 is the most closely similar to KSHV/HHV8; however, angiogenic proliferation is not seen with these animal viruses. Whether any of them will provide in-vivo models for KSHV/HHV8 is unknown. Bovine herpesvirus 4 (BHV-4), also a gamma-2 herpesvirus, has not been associated with specific lymphoid disease but is included in this monograph.

Small DNA fragments of two new primate rhadinoviruses, retroperitoneal fibromatosis herpesvirus of *Macaca nemestrina* (RFHVMn) and retroperitoneal fibromatosis herpesvirus of *Macaca mulatta* (RFHVMm), have recently been obtained from two macaque species in one colony (Rose *et al.*, 1997) and these may be closely related to KSHV/HHV8. The animals are also infected with D-type simian retrovirus type 2 and develop retroperitoneal and subcutaneous fibrosis with progressive fibrovascular proliferation, reminiscent of Kaposi's sarcoma lesions (Stromberg *et al.*, 1984; Bryant *et al.*, 1986).

3.1 *Herpesvirus saimiri* (saimiriine herpesvirus 2)

3.1.1 *Description*

HVS is a non-human primate rhadinovirus; it was first isolated in 1968 from the kidneys of squirrel monkeys (*Saimiri sciureus*), New World primates common to South America (Meléndez *et al.*, 1968). The virus is indigenous to squirrel monkeys and is transmitted horizontally from mother to infant in saliva. There is no evidence that HVS can induce tumours in squirrel monkeys (Jung & Desrosiers, 1994); however, when newborn squirrel monkeys were removed from their mothers immediately after birth and bred in captivity, some also being immunosuppressed with ciclosporin, later inoculation of HVS provoked viraemia and the development of antibody but no illness (Fleckenstein

& Desrosiers, 1982). There are no published reports of the development of tumours in squirrel monkeys in the wild after exposure to HVS.

Squirrel monkeys originating from discrete geographical zones were all found to be infected with HVS. The virus can be recovered routinely from PBMC and other tissues and organs of squirrel monkeys either naturally or experimentally infected with HVS. There is no evidence of seasonal variation in HVS transmission; however, under crowded conditions more virus is shed from the throat (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994). Horizontal transmission of HVS by oropharyngeal secretions from a squirrel monkey to an owl monkey housed in the same isolation unit occurred experimentally (Barahona *et al.*, 1975).

HVS, like other gammaherpesviruses, remains latent in lymphocytes and can cause lymphomas, leukaemias and lymphoproliferative disorders in other species of New World primates — tamarins and marmosets (*Saguinus* and *Callithrix* species), owl monkeys (*Aotus trivirgatus*) and spider monkeys (*Ateles* species) — and in rabbits (*Oryctolagus cuniculus*) (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994).

3.1.2 *Host range, virus isolation and virus multiplication*

HVS was first isolated by chance when it was observed that cultured kidney cells derived from a healthy squirrel monkey degenerated spontaneously, with cytopathic effects reminiscent of herpesvirus-infected cultures (Meléndez *et al.*, 1968). Specifically, hexagonal enveloped virions were found of approximately 140 nm, with capsids of 101–108 nm and cores of 50–60 nm (Deinhardt *et al.*, 1974). HVS can replicate in various monkey and human cell cultures. Higher infectivity titres were obtained in kidney cells from owl monkeys ($\geq 10^{6.2}$/mL) than in those from African green or rhesus monkeys. This experiment also showed that the infection with HVS is highly productive, contrary to most other gammaherpesviral infections *in vitro* (Ablashi *et al.*, 1972).

The first evidence that HVS could infect human fibroblast cells was provided by Ablashi *et al.* (1971a), who showed that infected cells produce infectious virus; however, the infectivity titres obtained were lower than those in owl monkey kidney cells. Simmer *et al.* (1991) showed that HVS persists in T-lymphoblastoid cells of various primate species, including human T cells. The human B-cell line (Raji) containing episomal EBV DNA could be persistently infected with HVS. A human pancreatic carcinoma cell line of epithelial origin could also be infected with HVS, and the cells contained both episomal and linear HVS DNA and produced HVS. Oie *et al.* (1973) reported that the MEST cell line, which is a spontaneously transformed cell line of rhesus monkey embryo origin, showed cytopathic effects after infection with HVS and, after two subpassages, provided a continuous source of HVS. HVS has tropism for T lymphocytes and can immortalize $CD8^+$ and $CD4^+$ cells (Biesinger *et al.*, 1992).

Dahlberg *et al.* (1988) showed that replication of HVS in human cells is semipermissive because of a block in the synthesis of certain late proteins. The studies were conducted with human fibroblasts (HEp-2), other epithelial cells (KHOS) and human T cells and the use of polyclonal and monoclonal antibodies to HVS (Dahlberg *et al.*, 1985).

3.1.3 *Host response: antibody detection*

Klein *et al.* (1973) identified the early and late antigens in HVS-infected Vero cells (an African green monkey continuous cell line) using sera obtained from squirrel monkeys (*Saimiri sciureus*) and HVS-infected white-lipped tamarins (*Saguinus fuscicollis*) and owl monkeys (*Aotus trivirgatus*). Two strikingly different patterns of staining of early antigen were observed, both limited to the nucleus (one trabecular and the other punctate), very similar to EBV EA(R) and EA(D). HVS-infected owl monkeys and white-lipped tamarins behaved quite differently from squirrel monkeys in their early antigen responses, and took two to three times longer to develop antibodies. The early antigen titres lagged behind those of late antigen. Antibodies appeared later in owl monkeys than in white-lipped tamarins; in squirrel monkeys, the virus could be isolated at the same time or somewhat later than the appearance of antibody. In tamarins, HVS was isolated before or at the time of appearance of antibody, whereas in owl monkeys, the virus was usually isolated one to two weeks before antibody was detected.

3.1.4 *Human exposure*

Of 150 animal caretakers who were bitten several times while handling squirrel monkeys or HVS-infected owl monkeys or marmosets, 11 (7.3%) were seropositive (Ablashi *et al.*, 1988). Four of 100 control adult sera (4%) also contained antibody to HVS as tested by immunofluorescence assay. The antibody titre to late antigen in these sera was between 1:10 and 1:80, and intense nuclear fluorescence reactivity was observed in cells in most of the sera. When these sera were analysed by radioimmunoprecipitation, two from the exposed persons were exceptionally reactive and the others weakly reactive. The sera precipitated mainly HVS major capsid protein (160 kDa) but also recognized several other proteins. Unexpectedly, follow-up sera from the caretakers several months later showed no antibodies. Whether these results were due to antibody cross-reactivity with KSHV/HHV8 or another primate rhadinovirus to which these individuals might have been exposed is unclear. Several attempts to isolate HVS from PBMC of the caretakers were unsuccessful.

3.1.5 *Molecular aspects*

HVS has a linear double-stranded DNA genome of about 155 kb with a central unique light region of 112 kb (36% G + C) flanked by variable numbers of 1.4-kb tandem repeats of heavy DNA (72% G + C) (Bankier *et al.*, 1985; Cameron *et al.*, 1989; Albrecht *et al.*, 1992; Jung & Desrosiers, 1994). Earlier studies showed that DNA sequences at the left terminus of light DNA are required for in-vitro immortalization and for the oncogenic phenotypes (Desrosiers *et al.*, 1986). Most HVS genes have ORF sequences that are similar to those of the KSHV/HHV8 and EBV genomes, and most of these genes are arranged in collinear order. Nevertheless, HVS and EBV differ with respect to transformation and latent stage replication (Kung & Medveczky, 1996).

HVS strains have been classified into subgroups A, B and C on the basis of molecular analysis, biological properties and oncogenic and transforming potential. Mutational analysis demonstrates that most of the left ORF of strain 11 (subgroup A) is required to

immortalize common marmoset T lymphocytes, but not for replication of the virus. An ORF designated *STP-C488* (subgroup C) was more potent in transforming Rat-1 cells (Jung *et al.*, 1991). DNA sequencing of the left end of the light strand revealed that HVS contains a gene coding for dihydrofolate reductase (DHFR) with high sequence homology to human *DHFR*. The HVS *DHFR* gene differs from mammalian and avian genes in that it lacks introns, suggesting that it may have been acquired through a process involving reverse transcriptase. The *DHFR* gene is also not required for viral multiplication or for in-vitro transformation (Fleckenstein & Desrosiers, 1982).

Murthy *et al.* (1986) studied RNA derived from 0.0–6.7 map units (7.4 kb) of HVS light DNA by northern blot hybridization and nuclease protection analysis. Although several poly(A)-containing RNAs were found in this region in permissively infected monolayer cells *in vitro*, these RNAs could not be detected in lymphoblastoid tumour cell lines. Instead, transformed T cells expressed four small RNAs of approximately 73, 105, 110 and 135 nucleotides derived from this region; these RNAs were not detected during the course of lytic infection of monolayer cells. HVS and EBV also differ with regard to other genes relevant to transformation and latency. In HVS, there are no *EBNA-1* or *oriP*-like sequences in the region of the genome that corresponds to these sites in EBV (see monograph on Epstein-Barr virus; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994; Kung & Medveczky, 1996). Instead, HVS and other rhadinoviruses appear to have acquired from the host cells a block of genes at the left side of their genomes that is not found in lymphocryptoviruses. Many other viral genes of HVS have been identified which code for cytokines (Yao *et al.*, 1995), as has also been observed in KSHV/HHV8.

3.1.6 *Oncogenicity in non-human primates, rabbits and transgenic mice*

As indicated previously, HVS strains can be divided into A, B and C subgroups. HVS of subgroups A and C are highly oncogenic for a wide spectrum of New World primates, while HVS strains of subgroup B have limited tumour-inducing capacity. In rabbits, only subgroup C has oncogenic potential (Medveczky *et al.*, 1989).

HVS induces malignant lymphoma of the reticulum-cell type in cotton-topped tamarins (*Saguinus oedipus*) and white-lipped tamarins (*Saguinus fuscicollis*) (Meléndez *et al.*, 1969; Deinhardt *et al.*, 1974). Lymphocytic leukaemia was induced in owl monkeys (*Aotus trivirgatus*) inoculated with prototype strain of HVS (295C; Meléndez *et al.*, 1971). Lymphoproliferative disease was induced in cinnamon ringtail monkeys (*Cebus albifrons*) and African green monkeys (*Cercopithecus aethiops*; Meléndez *et al.*, 1972a).

Fleckenstein *et al.* (1978a) induced malignant lymphomas in 3/14 cotton-topped tamarins inoculated with isolated HVS DNA.

Five owl monkeys experimentally inoculated with HVS developed moderately well-differentiated malignant lymphomas with lymphogenous leukaemia within 57–178 days. HVS could be isolated from cells of the spleen, kidney, lung, tumour mass (lymph node), thymus and liver cultured from these organs. HVS could also be isolated from PBMC. In general, the leukocyte counts rose significantly above the baseline levels. Bone-marrow

samples showed eosinophilic hyperplasia and progressive infiltration by lymphocytes. Terminally, the bone marrow was intensely hypercellular, with 70% cells of the lymphogenous series (Ablashi *et al.*, 1971b). The tumour-bearing owl monkeys developed antibodies to HVS early and late antigens (Klein *et al.*, 1973) and membrane antigens (Prevost *et al.*, 1976).

Heat-inactivated HVS strain 295C induced malignant lymphoma in two owl monkeys, suggesting that HVS DNA, even though it may have been fragmented, can induce tumours (Ablashi *et al.*, 1973).

The common marmoset (*Callithrix jacchus*) did not develop tumours after inoculation with HVS, even though the virus was consistently present in the PBMC.

Daniel *et al.* (1974) induced lymphoid tumours in New Zealand white rabbits (*Oryctolagus cuniculus*) by intravenous inoculation of HVS, although not all of the inoculated animals developed tumours. Similar results were reported in an inbred strain III/J (Ablashi *et al.*, 1980). The clinical and pathological findings in these animals were nasal discharge, respiratory disorders leading to death, lymphocyte infiltrates in the nares, enlarged lymph nodes and generalized peripheral lymphoadenopathy. The animals developed antibody to HVS, and the virus could be isolated from lymph nodes, spleen and PBMC. All infected animals showed depressed cell-mediated immune response.

Kretschmer *et al.* (1996) showed that a previously identified ORF (*StpA*) which is necessary for oncogenicity in monkeys induces pleomorphic T-cell lymphomas in transgenic mice expressing StpA in a variety of organs.

3.1.7 *Transformation of mammalian cells* in vitro

Subgroup A and C strains of HVS efficiently transform marmoset PBMC to permanent autonomous growth *in vitro*, whereas cells transformed by subgroup B viruses are IL-2-dependent (Desrosiers *et al.*, 1986; Szomolanyi *et al.*, 1987).

Ablashi *et al.* (1985) established a T-cell line from the spleen of an HVS-infected New Zealand male rabbit which developed a well-differentiated lymphoma. This cell line was IL-2-dependent and was highly oncogenic in rabbits, since it produced well-differentiated lymphomas, and the animals died of lung complications shortly afterwards. The cells (7710 cell line) contained multiple copies of non-integrated circular HVS genome. As in other HVS-transformed non-producer cell lines, a large segment of light DNA was missing from the persistent circular viral DNA present in the 7710 cells.

Medveczky *et al.* (1993) showed that a collagen-like ORF-1 protein is expressed in permanent tumour-derived rabbit T cells and in transformed primate T cells infected *in vitro* with HVS group C strain 484-77. Antibody to ORF-1 protein was also found in rabbits bearing tumours, suggesting that this protein is expressed *in vivo*.

Pacheco-Castro *et al.* (1996) showed that HVS immortalized $\alpha\beta$ and $\gamma\delta$ human T-lineage cells derived from $CD34^+$ intrathymic precursors *in vitro*, and the $\gamma\delta$ lineage was IL-2-dependent.

3.2 *Herpesvirus ateles* (ateline herpesvirus 2)

3.2.1 *Description*

HVA is also a non-human primate rhadinovirus. Strain 810 was first isolated from a degenerating kidney-cell culture from a spider monkey (*Ateles geoffroyii*; Meléndez *et al.*, 1972b). Later, strain 73 of HVA was isolated from circulating lymphocytes of a spider monkey (*A. paniscus*) by co-cultivation with owl monkey kidney cells (Falk *et al.*, 1974). Immunological, biological and molecular analyses indicate that HVA is closely related to HVS but with homology to EBV. HVA is common in species of spider monkeys native to South America and does not cause disease in the host. HVA does not cross-react with other primate herpesviruses. The virus is latent in spider monkeys and can be isolated early from tissue and PBMC (Deinhardt *et al.*, 1974; Fleckenstein & Desrosiers, 1982; Jung & Desrosiers, 1994).

3.2.2 *Host range, cytopathogenicity and viral multiplication*

Although HVA is a T-lymphotropic herpesvirus, it replicates efficiently in monolayer cell cultures of fibroblasts and epithelial cells of animal origin (Deinhardt *et al.*, 1974; Jung & Desrosiers, 1994). HVA in monolayer cultures induces cytopathic effects, with discrete, enlarged, round multinucleated giant cells. Owl monkey kidney cells are an excellent source of high-titre infectious virus. The host range of cells that can be infected with HVA is more distinct than that of HVS: HVA infects primary cultures of African green monkey, owl monkey, marmoset and squirrel monkey kidney cells. The cytopathic effects are slower (more than five days), and HVA titres are 2–3 logs lower than those of HVS. Rabbit kidney cells could not be infected by strain 73 but were infected by strain 810, which also infected cells of hamster heart origin. Lymphocytes from owl monkeys, marmosets and humans could be infected and transformed (Deinhardt *et al.*, 1974; Falk *et al.*, 1974; Ablashi *et al.*, 1976).

Luetzeler *et al.* (1979), using HVA-73, studied the ultrastructural morphogenesis of HVA in owl monkey kidney cells. The replicative cycle paralleled, in general, that of HVS, two morphologically distinct inclusion bodies appearing in the nuclei early in infection.

3.2.3 *Molecular analysis*

Little is known about the molecular biology of HVA. The structural organization of its genome is similar to that of HVS. There has been no sub-classification of its strains. Interestingly, 35% of the light strands of the DNA of HVA and HVS anneal with each other, but with considerable mismatching of base sequences. There is 10% homology between the heavy regions of HVS and HVA, with at least 13% divergence of their base pairs (Fleckenstein *et al.*, 1978b).

3.2.4 *Oncogenicity in non-human primates*

Three cotton-topped tamarins and two owl monkeys were inoculated intramuscularly with HVA strain 810. Two of the tamarins died 28 days after inoculation, and one was

killed when moribund at 40 days; all animals had malignant lymphomas, and generalized enlargement of the lymph nodes and splenomegaly were seen grossly. The two owl monkeys were killed 42 days after inoculation in moribund condition. No significant lesions were seen in one, while in the other focal interstitial collections of lymphoblasts, reticulum cells and eosinophils were present in the kidneys and lung and there was lymphocytic hyperplasia in most lymph nodes (Meléndez *et al.*, 1972b).

Hunt *et al.* (1972) inoculated HVA strain 810 into 12 cotton-topped tamarins. All animals died within about one month with malignant lymphomas of the lymphoblastic type. Leukaemia developed in 11 of the animals. One of four control tamarins in contact with the infected animals also developed lymphoma and leukaemia, indicating horizontal transmission.

Laufs and Meléndez (1973) inoculated partially purified HVA DNA or lymphoid cells derived from tumorous lymph nodes taken from experimentally infected cotton-topped tamarins immediately after death into owl monkeys (*Aotus trivirgatus*) and marmosets (*Callithrix jacchus*), two other New World monkey species, and African green monkeys (*Cercopithecus aethiops*). The two owl monkeys died after 20 and 28 days, respectively, of unknown causes. The two common marmosets died 36 and 104 days after inoculation with generalized malignant lymphoma, which resembled the disease observed in cotton-topped tamarins. The two adult African green monkeys inoculated with tumour cells or with partially purified HVA DNA survived, however, but the virus could not be isolated from their PBMC, indicating that this species of Old World primate is not susceptible to HVA infection.

Three viral isolates of HVA induced fatal malignant lymphomas in six cotton-topped tamarins and two white-lipped tamarins, while one isolate tested in squirrel monkeys did not cause overt disease (Falk *et al.*, 1974).

Two common marmosets infected with HVA strain 73 developed lymphomas of a variety of cell types within 27 days after inoculation. Some of the lymphoma cells were giant cells resembling the Sternberg-Reed cells observed in human Hodgkin's disease (Ablashi *et al.*, 1978).

3.3 Bovine herpesvirus 4 (Movar herpesvirus)

BHV-4 is one of four known bovine herpesviruses. It was first isolated by Bartha *et al.* (1966) under the name 'Movar' strain and has a worldwide distribution. It is the only known bovine gammaherpesvirus and is most probably a rhadinovirus, since it is similar to HVS (Bublot *et al.*, 1992). It has been isolated in a variety of clinical conditions as well as from healthy cattle (reviewed by Thiry *et al.*, 1989, 1990, 1992a,b). The group BHV-4 includes a large number of antigenically related isolates that are distinct from other bovine herpesviruses (Potgieter & Maré, 1974; Staczek, 1990). The role of BHV-4 as the etiological agent of a distinct disease entity is still questionable, although its role in the etiology of some diseases of the eye and respiratory and genital tracts has been suggested (Thiry *et al.*, 1989, 1990).

3.3.1 *Classification*

BHV-4 was initially known as bovine cytomegalovirus and was classified as a beta-herpesvirus because only its biological characteristics were taken into account (Storz *et al.*, 1984). Bublot *et al.* (1992) used molecular data to show that BHV-4 belongs to the Gammaherpesvirinae. Careful examination of the BHV-4 genome showed its close relationship to other gammaherpesviruses such as HVS, which allowed classification of BHV-4 into the rhadinovirus group (Lomonte *et al.*, 1996).

3.3.2 *Description*

The morphology of BHV-4 is typical of that of a herpesvirus. The nucleocapsid is icosahedral, with a dense core within the capsid which is made up of a regular arrangement of short tubular capsomeres (Todd & Storz, 1983). The diameter of the naked nucleocapsid is about 90–100 nm, while enveloped virions have a diameter ranging from 115 to 150 nm (Smith *et al.*, 1972; Munz *et al.*, 1974).

The BHV-4 virion contains double-stranded DNA of approximately 145 kb (Todd & Storz, 1983; Ehlers *et al.*, 1985). The genomic structure of BHV-4, typical of group B herpesviruses (Roizman, 1982), is similar to that of HVS, i.e. it has a unique coding sequence (light DNA) of approximately 110 kb flanked by a (G + C)-rich tandem repeat region of 2.65 kb (in BHV-4 VT strain) called polyrepetitive DNA (Ehlers *et al.*, 1985; Bublot *et al.*, 1990). RNA derived from the heavy strand of DNA has not been detected in infected cells (Chang & van Santen, 1992).

The overall genomic organization of BHV-4 VT strain was determined by sequencing 33 segments of the coding region (light strand) of its genome (Bublot *et al.*, 1992). Twenty-seven sequences showed homology to proteins present in either the three herpesvirus subfamilies or in only the two gammaherpesviruses, EBV and HVS. In the former case, the homology scores were always higher in comparison with EBV and HVS proteins than with the same proteins in alpha- or betaherpesviruses. Twenty-three of the sequenced regions of BHV-4 had homologous counterparts in both HVS and EBV genes. Nineteen of these regions belong to the five blocks of genes that are conserved among gamma- and alphaherpesviruses and/or betaherpesviruses. Five BHV-4 sequences were homologous to genes present only in the gammaherpesvirus genomes. The BHV-4 amino-acid sequences were more closely related to those of gammaherpesviruses than to those of alpha- or betaherpesviruses. Furthermore, the homology of most of these sequences was closer to the homologous products of HVS than to the equivalent proteins of EBV. Therefore, on the basis of the overall conservation of the sequences, BHV-4 is more closely related to HVS than to EBV. Six of the 33 BHV-4 sequences were homologous to neither HVS nor EBV genes nor to any other herpesvirus genes. All of these six sequences were located outside the conserved gene blocks. Genomic regions that were found to vary in size between BHV-4 isolates or strains (Bublot *et al.*, 1990, 1991a; Thiry *et al.*, 1992b) were also located outside these blocks (Bublot *et al.*, 1992).

The presence of five blocks of conserved genes was demonstrated in the BHV-4 genome, as in EBV and HVS. The length of the BHV-4 conserved gene blocks was estimated and found to be more closely related to that of HVS than that of EBV. Only

HVS block 3 was longer than those of BHV-4 and EBV (Lomonte *et al.*, 1996). This difference in size was due to the presence of a repeated region in the HVS gene 48 which causes an expansion of the acidic C-terminal domain of the protein (Albrecht *et al.*, 1992). The space between the BHV-4 gene blocks was also more like that of HVS than EBV, particularly between blocks 3 and 4 and blocks 4 and 5. Finally, as in the HVS genome, no large internal repeats were found between blocks 4 and 5 as is the case in the EBV genome.

No genes were found in region C, located between the second and the third conserved blocks. This region was less than 100 nucleotides long in the HVS and BHV-4 genomes (Lomonte *et al.*, 1996), whereas in the EBV genome this region contains the *BKRF1* gene coding for the EBNA-1 protein (Baer *et al.*, 1984) which is expressed in EBV-immortalized B lymphocytes (Middleton *et al.*, 1991).

A gene coding for viral fas-associated death domain protein interleukin-1β converting enzyme (FLICE)-inhibiting proteins, which prevent apoptosis, has been identified in BHV-4 DNA (Thome *et al.*, 1997).

Glycoprotein gB is a heterodimer and is a major component of the BHV-4 virion, unlike gBs of EBV (gp110) and murine gammaherpesvirus 68 (Lomonte *et al.*, 1997). This glycoprotein corresponds to the gp10/17 described by Dubuisson *et al.* (1989a). BHV-4 gB arises from a 142-kDa precursor which undergoes cleavage to give rise to two covalently linked glycopolypeptides of 128 and 56 kDa, corresponding to the previously identified gp10 and gp17, respectively. The resulting heterodimer has an apparent molecular mass of 210 kDa (Lomonte *et al.*, 1997).

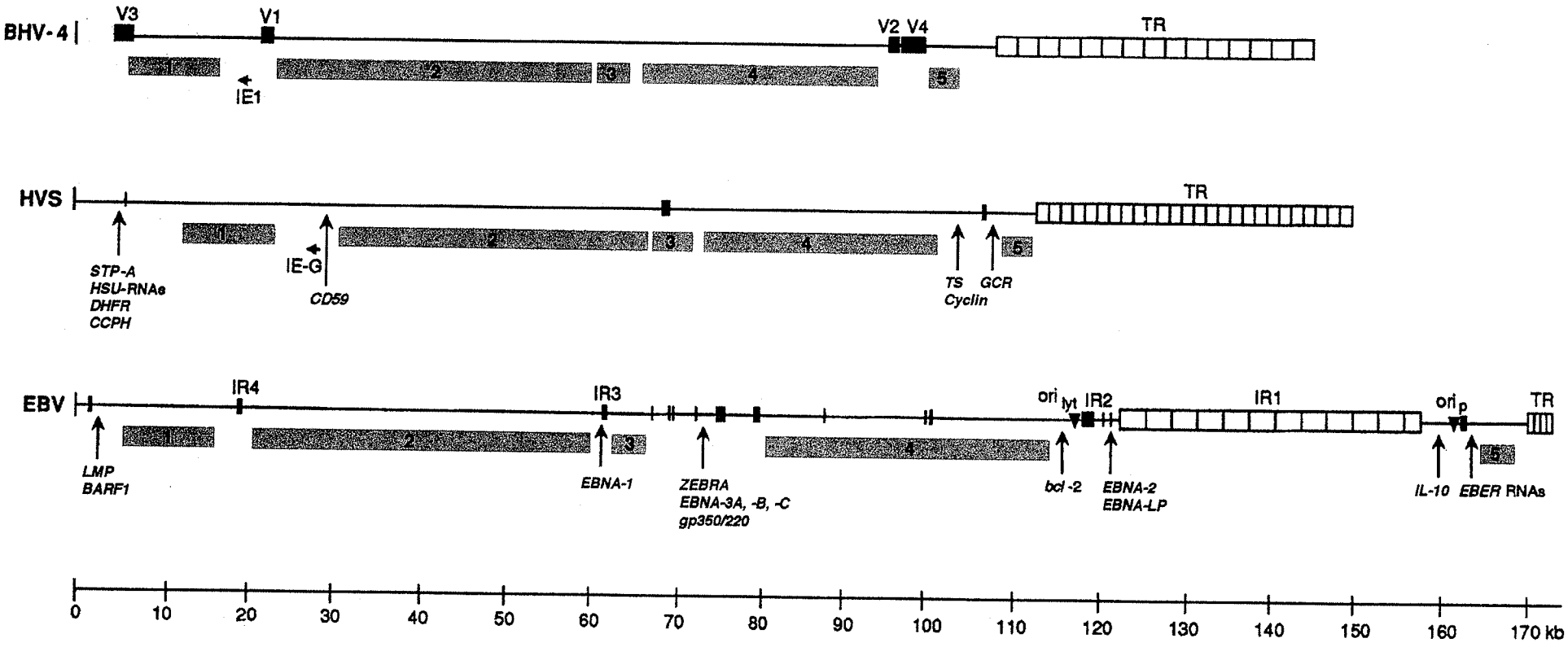
In summary, both genomic and protein analysis show the close homology of BHV-4 to HVS rather than to EBV (see Figure 7); therefore a possible relationship to KSHV/-HHV8 exists.

3.3.3 *Host range*

BHV-4 replicates in a variety of primary and established bovine cell cultures: primary kidney, testicle, lung, skin, spleen and thyroid, Madin Darby bovine kidney, Georgia bovine kidney, embryonic bovine kidney, embryonic bovine trachea, bovine bone marrow (BBM and FB4BM) and calf thymic lymphosarcoma cells (Bartha *et al.*, 1966; Luther *et al.*, 1971; Smith *et al.*, 1972; Parks & Kendrick, 1973; Rweyemamu & Loretu, 1973; Sass *et al.*, 1974; Theodoridis, 1978; Thiry *et al.*, 1981; Storz *et al.*, 1984; Theodoridis, 1985). BHV-4 has also been shown to replicate in cells of other animal species, including sheep, goats, pigs, dogs, rabbits, mink, horses, turkeys, geese, ferrets and potoroo (Luther *et al.*, 1971; Rweyemamu & Loretu, 1973; Kit *et al.*, 1986; Peterson & Goyal, 1988). Although the host range of BHV-4 *in vitro* is very broad, the virus does not replicate in cells of human (HeLa, Hep-2) or mouse (A31B77) origin or in chicken embryo fibroblasts (Luther *et al.*, 1971; Rweyemamu & Loretu, 1973). As in many other herpesviruses, the first receptor of BHV-4 is a heparin-like moiety on the cell surface, and its replication is cell cycle-dependent (Vanderplasschen *et al.*, 1993, 1995).

Of the ruminants, American bison *(Bison bison)* (Todd & Storz, 1983), African buffalo *(Syncerus caffer)* (Rossiter *et al.*, 1989), sheep (Van Opdenbosch *et al.*, 1986)

Figure 7. Order and spacing of gene blocks on the mature, linear genome of BHV-4 (V test strain), *Herpesvirus saimiri* (HVS strain; Albrecht *et al.*, 1992) and Epstein-Barr virus (EBV, B95-8 strain; Baer *et al.*, 1984)



Adapted from Bublot *et al.* (1992)

The five gene blocks that are conserved in gammaherpesviruses are indicated by the large shaded rectangles. Genes conserved in beta- and alphaherpesviruses are located in blocks 1, 2 and 4; blocks 3 and 5 contain only gammaherpesvirus-specific genes. The horizontal arrows indicate the positions of the major immediate-early gene of BHV-4 (*IE1*; van Santen, 1991) and HVS (*IE-G*). The vertical arrows indicate important genes located outside of the gene blocks and which are not conserved between EBV and HVS; the most commonly investigated genes are named: *STP-A*, saimiri transformation-associated protein; *HSU*-RNAs, *Herpesvirus saimiri* U-RNAs; *DHFR*, dihydrofolate reductase; *CCPH*, complement control protein homologue; *CD59*, cluster designation 59 homologue; *TS*, thymidylate synthase; *Cyclin*, cyclin family member homologue; *GCR*, G-coupled receptor homologue; *LMP*, latent membrane protein; *EBNA-2*, *-3A*, *-B*, *-C*, *-LP*, EBV nuclear antigen 2, 3A, 3B, 3C, leader protein; *bcl-2*, *bcl-2* proto-oncogene homologue; *IL-10*, interleukin 10 homologue; EBER, EBV-encoded RNA. EBV origins of replication are indicated by triangles and ori_{lyt} (origin of replication for the lytic cycle) and ori_p (plasmid origin of replication); terminal repeats (TR) and large internal repeats (IR) are represented by open rectangles; small internal repeated sequences are indicated by vertical lines or black rectangles. V1, V2, V3 and V4 are genomic regions which vary in size between BHV-4 isolates (Thiry *et al.*, 1992b). The orientation of the EBV genome is inverted relative to the conventional orientation (Baer *et al.*, 1984).

and goats (Moreno-Lopez *et al.*, 1989) undergo natural or experimental infection with BHV-4. European wild ruminants do not have antibodies to BHV-4 (Thiry *et al.*, 1988). The ability of BHV-4 to infect non-bovine ruminant species is understandable, considering the close phylogenetic relationships between these species.

BHV-4 can also infect species phylogenetically distant from ruminants: it has been isolated from a cat suffering from urolithiasis (Fabricant *et al.*, 1971). The latter isolate, designated 'feline herpesvirus type 2' or 'feline cell-associated herpesvirus', was characterized by restriction analysis (Kit *et al.*, 1986). Even if its role in feline disease is uncertain, the virus was undoubtedly isolated from cats and it has been shown to infect cats (Kruger *et al.*, 1990). The virus was a typical BHV-4 strain. Attempts to infect cats with the Movar 33/63 strain by conventional intranasal and intravenous routes were unsuccessful (Thiry *et al.*, 1991), perhaps due to a lack of adaptation of the virus to the cat rather than to strain specificity. The finding that this virus is a BHV-4 strain led to the disappearance of 'feline herpesvirus type 2' from the International Nomenclature.

The discovery that *Herpesvirus aotus* type 2, isolated from owl monkeys (*Aotus trivirgatus*), is also a strain of BHV-4 extends the range of species that are susceptible to BHV-4 (Bublot *et al.*, 1991b). This virus has been fully characterized; however, the genomic and protein differences from the prototype BHV-4 strains are compatible with the variations detected among BHV-4 strains (Bublot *et al.*, 1991b; Dubuisson *et al.*, 1991a). The antigenic relationship between *Herpesvirus aotus* type 2 and BHV-4 was proven by indirect fluorescence antibody testing with a broad panel of monoclonal antibodies raised against three BHV-4 glycoproteins and three other proteins and rabbit polyclonal sera specific for the two viruses (Bublot *et al.*, 1991b). The protein profile shows some variations between the two: glycoprotein gp6/gp10/gp17 of *Herpesvirus aotus* type 2 has a molecular mass of 160/131/52 kDa, with an extra band of 60 kDa which is probably a duplicate of the 52-kDa component; gp11 has a molecular mass of 109 kDa, while that of gp8 does not vary from those of BHV-4 isolates (Dubuisson *et al.*, 1991a). As a consequence, *Herpesvirus aotus* type 2 disappeared from the International Nomenclature of Herpesviruses (see Introduction, Table 2).

3.3.4 *Natural transmission*

BHV-4 is isolated in bovine species from both the anterior respiratory and genital tracts. Only respiratory infection has been studied experimentally, while other routes of inoculation, i.e. intravenous, intradermal and intratesticular, have been tested in cattle (Osorio & Reed, 1983; Dubuisson *et al.*, 1987, 1989b). Direct and indirect transmission of BHV-4 by means of infected materials is suggested.

3.3.5 *Evidence that bovine herpesvirus 4 causes disease*

After primary infection of cattle, BHV-4 replicates in mucosal cells and infects mononuclear cells, provoking generalized infection (Osorio & Reed, 1983). Viraemia is not always detected, but the virus can be re-isolated from many organs, including brain and spinal cord (Castrucci *et al.*, 1987; Dubuisson *et al.*, 1989b). BHV-4 may also infect the fetus (Kendrick *et al.*, 1976). Thereafter, BHV-4 establishes a latent infection. The spleen

is the main site of latency in rabbits (Osorio *et al.*, 1982, 1985); in cattle, the spleen is also the site where viral DNA is most frequently detected (Lopez *et al.*, 1996; Egyed *et al.*, 1996). In rabbits and cattle, non-B non-T cells and, presumably, monocytes and splenic macrophages are the main cells in which latent infection with BHV-4 is found (Osorio *et al.*, 1985; Lopez *et al.*, 1996); however, this issue is not definitely resolved, because BHV-4 can also replicate in lymphocytes (Egyed *et al.*, 1996). Latent virus can be reactivated by dexamethasone treatment, and re-excretion is shown to occur by the isolation of BHV-4 from peripheral blood leukocytes and nasal swabs (Krogman & McAdaragh, 1982; Dubuisson *et al.*, 1989b).

After viral entry, BHV-4 proteins are expressed in a cascade fashion, as for any herpesvirus. Trancription of two immediate-early genes (*IE1* and *IE2*) is initiated in infected cells (van Santen, 1991; Chang & van Santen, 1992; van Santen, 1993). IE1 and IE2 proteins share homology with IE110 of HSV and EBV R *trans*-activator, respectively (van Santen, 1991, 1993). IE2 was shown to *trans*-activate early (*E*) gene promoters, e.g. thymidine kinase gene (Zhang & van Santen, 1995) and late (*L*) gene (Bermudez-Cruz *et al.*, 1997). Expression of the *L* gene leads to abundant synthesis of a 1.7-kb RNA unique to BHV-4 (Bermudez-Cruz *et al.*, 1997). The precursor of the gB complex is expressed during the early phase and does not require DNA replication. Glycoproteins gp1, gp8 and gp11 are late proteins (Dubuisson *et al.*, 1991b,c, 1992a,b). BHV-4 DNA replication, and consequently the expression of late proteins, are dependent on the S phase of the cell cycle (Vanderplasschen *et al.*, 1995).

The contribution of BHV-4 to disease is speculative. BHV-4 has been designated as a passenger virus when isolated from ethmoidal tumours in Indian cattle (Moreno-Lopez *et al.*, 1989). The virus infects mononuclear blood cells, and this peculiarity allows it to be distributed to the whole body. Therefore, in primary infection and during reactivation of the latent state, BHV-4 can be reisolated from virtually all bovine tissues and organs. It is hard to discriminate between isolation by chance and a direct role of the virus in the observed lesions. BHV-4 has never been identified as a potential cause of tumours either *in vitro* or in cattle or rabbits *in vivo*.

3.3.6 *Isolates*

Two reference strains were isolated from clinical cases of conjunctivitis and respiratory disease: the Movar 33/63 strain in Europe (Bartha *et al.*, 1966) and the DN 599 strain in the United States (Mohanty *et al.*, 1971). Other strains were isolated from cases of respiratory disease (Smith *et al.*, 1972; Evermann *et al.*, 1984). In Africa, several herpesviral strains were isolated from people with a syndrome characterized by epididymitis and vaginitis (Maré & van Resenburg, 1961; Theodoridis, 1978, 1985), which are biologically similar to BHV-4 but have not yet been typed as BHV-4. The two Belgian reference strains, namely V. Test and LVR140, came from cases of orchitis and post-partum metritis, respectively (Thiry *et al.*, 1981; Wellemans *et al.*, 1984). Other BHV-4 strains were isolated from cases of genital disease in Italy (Castrucci *et al.*, 1986) and the United States (Parks & Kendrick, 1973; Evermann *et al.*, 1984). Reed *et al.* (1979) and Wellemans and Van Opdenbosch (1989) isolated BHV-4 from aborted

fetuses, and Reed *et al.* (1977) isolated BHV-4 from cases of mammary pustular dermatitis and ulcers of the udder. A few herpesviral strains biologically similar to BHV-4 were isolated from ruminal tumours (Kaminjolo *et al.*, 1972), tongue lesions (Rweyemamu & Loretu, 1973; Luini *et al.*, 1985) and diarrhoeal faeces (Eugster, 1978/-1979).

BHV-4 was also isolated or serologically suspected in cases of malignant catarrhal fever (Storz, 1968; Todd & Storz, 1983), lumpy skin disease (Alexander *et al.*, 1957; Rweyemamu & Loretu, 1973; House *et al.*, 1990), vesicular stomatitis (Evermann & Henry, 1989) and a variety of neoplasms such as lymphosarcoma (Potgieter & Maré, 1974), ocular squamous-cell carcinoma (Anson *et al.*, 1982) and T-cell lymphoma (Toho *et al.*, 1985).

BHV-4 is regularly isolated from healthy cattle. It has been recognized in renal-cell cultures (Luther *et al.*, 1971; Belák & Pálfi, 1974), in trigeminal ganglia (Homan & Easterday, 1981) and in triturated liver, lung and spleen (Krogman & McAdaragh, 1982).

3.4 Murid herpesvirus 4

Murine gammaherpesvirus 68 (MHV-68) is a naturally occurring rodent pathogen. Intranasal or intravenous administration of MHV-68 results in acute productive infection in B lymphocytes (Sarawar *et al.*, 1997) and lymphoproliferation (Sunil-Chandra *et al.*, 1992a,b, 1994; Sarawar *et al.*, 1997). The virus remains latent in B cells after primary infection (Sunil-Chandra *et* al., 1992b). Sequence analysis of its genome shows that it is most probably a rhadinovirus, more closely related to HVS and KSHV/HHV8 than to EBV (Mackett *et al.*, 1997).

3.5 Retroperitoneal fibromatosis herpesviruses

PCR with consensus gammaherpesvirus primers was used to detect small DNA fragments of two new, closely related rhadinoviruses in captive *Macaca nemestrina* and *M. mulatta* in one colony (Rose *et al.*, 1997). These animals were also infected with D-type simian retrovirus type 2 and suffered from retroperitoneal and subcutaneous fibrosis with progressive fibrovascular proliferation. This condition has some similarities to Kaposi's sarcoma (Tsai *et al.*, 1990; Rose *et al.*, 1997).

Earlier experiments had shown that cell cultures could be established from these lesions, which induced self-limited, transient spindle-cell proliferation, accompanied by pronounced vascularization when inoculated into nude mice (Tsai *et al.*, 1990, 1995).

The short sequences of these retroperitoneal fibromatosis herpesviruses presently available are derived from the polymerase gene and are about 70% identical at the nucleotide level (83–84% at the protein level) to the corresponding KSHV/HHV8 region (Rose *et al.*, 1997). If confirmed by a more extensive sequence analysis, these viruses could represent the closest relatives of KSHV/HHV so far. It may therefore prove to be a useful model for studying some aspects of Kaposi's sarcoma; however, nothing is yet known about the relative contributions of these new rhadinoviruses and simian retrovirus type 2 to the pathogenesis of retroperitoneal fibromatosis.