This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2011.005a-cV***	(to be completed by ICTV officers)

Short title: Recognition of *Human herpesvirus 6* variants A and B as distinct herpesvirus species
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☒	**8** ☒	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Dharam Ablashi (dharam_ablashi@hhv-6foundation.org
Dario Diluca (ddl@unife.it)
Philip Pellett (ppellett@med.wayne.edu)

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at http://www.ictvonline.org/subcommittees.asp . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	*Herpesvirales* Study Group

ICTV-EC or Study Group comments and response of the proposer:

Background comments from the Herpesvirales Study Group to the ICTV-EC:

Human herpesvirus 6 includes two closely related viruses, which have been referred to as HHV-6 variants A and B since 1993. The question of whether the HHV-6 variants should be formally recognized as herpesvirus species was considered by the Herpesvirus Study Group several years ago, but was not approved, in part because of the weight given at the time to quantitative measures of similarity of genes conserved across the herpesvirus family. In the interim, additional data accumulated that is consistent with the HHV-6 variants representing distinct replicating lineages.

A non-ICTV Ad Hoc Committee on HHV-6A & HHV-6B Genomic Divergence submitted a proposal that the HHV-6 variants be recognized as distinct herpesvirus species. The proposal from the ad hoc committee (20 authors, two of whom offered dissenting views), plus portions of documents previously published by Dr. Pellett were submitted to the Herpesvirales Study Group for discussion (Appendix 1).

As part of the discussion phase, most Study Group respondents indicated that they were in favor of the species recognition proposal. Two members made the point that this

recognition is not being made simply on the basis of a particular threshold of sequence difference, but encompasses the breadth of what is known of their biology. One member voiced concerns about the possibility that the HHV-6 variants can recombine in the wild. One member disagreed with the proposal, based on the two dissenting views that accompanied the proposal.

Dr. Pellett prepared a document (Appendix 2) summarizing the data related to the possibility of in vivo recombination, which was included with the ballot. All SG members responded. The measure was passed with no dissenting votes (one abstention).

In addition to the question of species recognition, formal recognition of the HHV-6 variants as distinct herpesvirus species would necessitate giving them formal names. What to call them has been a long-standing major sticking point: HHV-6 and HHV-9 (and which variant should retain the "6"), HHV-9 and HHV-10, etc. With the Stability Principle in mind, and given that the HHV-6A and HHV-6B designations are well accepted and are embedded in the literature, the Study Group has elected to retain the "6A" and "6B" designations. Thus, this is a proposal to abolish *Human herpesvirus 6* as a virus species, and to create two new species: *Human herpesvirus 6A* and *Human herpesvirus 6B*.

Date first submitted to ICTV:	January 15, 2010
Date of this revision (if different to above):	June 17, 2011 – reformatted into a single file 4 January 2012 – addition of assigning *Human herpesvirus 6A* as the type species

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	***2011.005aV***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Roseolovirus***
Subfamily:	***Betaherpesvirinae***
Family:	***Herpesviridae***
Order:	***Herpesvirales***

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Human herpesvirus 6A	NC_001664
Human herpesvirus 6B	NC_000898

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Because of its length and complexity, the rationale for recognizing the HHV-6 variants as distinct herpesvirus species is provided as an Appendix.

The proposal from the Ad Hoc Committee was framed in the context of the long-standing definition of herpesvirus species: "Related herpesviruses are classified as distinct species if (a) their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and (b) they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts." Since the submission of the proposal, and during its discussion, the Herpesvirales Study Group concluded a lengthy discussion and revised the species definition to read "A herpesvirus may be classified as a species if it has distinct epidemiological or biological characteristics and a distinct genome that represents an independent replicating lineage." The new definition is included in the Herpesvirales chapter of the Ninth Report of the ICTV. The rationale for recognizing the HHV-6 variants as distinct herpesvirus species is consistent with the old and the new definitions.

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:
- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2011.005bV***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Human herpesvirus 6

The present taxonomic position of these taxon/taxa:

Genus:	***Roseolovirus***
Subfamily:	***Betaherpesvirinae***
Family:	***Herpesviridae***
Order:	***Herpesvirinae***

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	**YES**

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

This Module is part of a proposal to recognize the *Human herpesvirus 6* variants as distinct herpesvirus species.

Human herpesvirus 6 has encompassed two closely related viruses, known since 1993 as HHV-6 variants A and B. It has become clear that the HHV-6 variants represent independently replicating virus lineages that meet the definition of herpesvirus species. The new species will be known as *Human herpesvirus 6A* and *Human herpesvirus 6B*, thus *Human herpesvirus 6* should be abolished.

Part (b) re-assign to a higher taxon

Code		(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	
Subfamily:	
Family:	
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

MODULE 8: **NON-STANDARD**

Code	***2011.005cV***	(assigned by ICTV officers)

To re-designate the following as the type species of the genus

Human herpesvirus 6A	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

Reasons to justify the designation of a new type species:

With the removal of the existing type species, *Human herpesvirus 6*, from the genus *Roseolovirus* (see 2011.005bV, above), it becomes necessary to designate a new type species.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

References are provided in the Appendices

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Appendix 1. Proposal package as submitted to the *Herpesvirales* Study Group

Appendix 2. Summary of data related to the possibility of in vivo recombination between HHV-6A and HHV-6B

Appendix 1 Proposal package as submitted to the *Herpesvirales* Study Group

Cover letter from the Chairs of the ad hoc committee

Proposal from the ad hoc committee

Comments from the committee related to naming the viruses

Figure illustrating the extent of sequence similarity between HHV-6A and HHV-6B from Dominguez et al. (J. Virol. 73:8040-8052,1999)

Relevant text from HHV-6 review by Braun et al. (Clinical Micro. Rev. 10:521-567,1997)

Cover letter for the proposal from the Ad Hoc Committee on HHV-6A and HHV-6B Genomic Divergence

From: Dharam Ablashi [dharam_ablashi@hhv-6foundation.org]
Sent: Friday, January 15, 2010 11:29 AM
To: Pellett, Philip
Cc: Dario Di Luca; Kristin Loomis
Subject: Proposal to ICTV on HHV-6A & HHV-6B

Dear Phil,

As you know, we assembled an Ad Hoc Committee on HHV-6A and HHV-6B Genomic Divergence in 2008 to consider the question of whether HHV-6A and HHV-6B should be recognized as two separate viruses.

Of the 20 members of the Ad Hoc Committee on HHV-6A and HHV-6B Genomic Divergence, 18 are in favor of recognizing the "variants" as two separate viruses. We have summarized the key points in the attached petition to the ICTV. There is a minority opinion for the two who disagree.

While we have fairly good consensus on the question of recognizing HHV-6A & B as two viruses, we do not have a consensus on how the viruses should be renamed. Most of the HHV-6 experts on our Committee did not favor the two options you proposed, which are consistent with ICTV rules. The majority felt that given the long passage of time, and the fact that the medical community has associated HHV-6 with HHV-6B, the two most practical solutions would be either rename HHV-6A as HHV-9 or continue to call them HHV-6A and HHV-6B.

We look forward to hearing your response.

Best,

Dharam Ablashi & Dario Di Luca
Co-Chairs, Ad Hoc Committee for Recognition of HHV-6A and HHV-6B Divergence

Petition to the ICTV:
Recognition of HHV-6A and HHV-6B Divergence

To: Phil Pellett, Chairman, Herpesvirales Study Group

From: Dario Di Luca & Dharam Ablashi, Co-Chairs,
Ad Hoc Committee on HHV-6A & HHV-6B Genomic Divergence

Date: January 11, 2010

Human herpesvirus 6 (HHV-6) was first identified in 1986,[1] and shortly thereafter, several clinical strains were isolated in many laboratories. It became gradually apparent that all HHV-6 isolates can be unambiguously included in one of two groups, differing for molecular, epidemiological and biological properties.[2-4] In the early 1990s, the scientific community debated whether the two groups reflected a normal heterogeneity of the virus population, with the uniting links still to be identified, or HHV-6 strains should be reclassified as different viruses within the nomenclature system of the International Committee for Taxonomy of Viruses (ICTV).[2]

Since their discovery, it was clear that the two groups show different *in vitro* tropism for T cell lines, specific immunological reactions to monoclonal antibodies, distinct patterns of restriction endonuclease sites, and specific and conserved interstrain variations in their DNA sequences. Moreover, there was the unconfirmed suggestion that they might have different epidemiology and disease association.

In 1992, at the 17th International Herpesvirus Workshop held in Edinborough, a satellite symposium on HHV-6 was attended by 65 scientists, and a consensus was reached that considering the two groups different viral species was still premature.[5] This decision was based on two main reasons: i) the interspecific divergence of nucleic acids was remarkably low and ii) differential epidemiology and pathogenic potential were still unknown. It was therefore proposed to designate the groups as "HHV-6 variant A" and HHV-6 variant B", and that these issues would be further discussed.[5]

Since then, new evidence has been provided, but the new evidence has not been critically assessed by the scientific community in the context of HHV-6 classification. However, individual reviews suggested the opportunity to designate the two variants as different species.[6-9]

The initial descriptions from the early 1990s that HHV-6 variants have conserved genomic differences and distinct biological properties have all withstood the test of time, and further evidence has accumulated. So far, all clinical isolates have been unambiguously characterized as A or B variants.

Studies of genomic sequencing have confirmed the indisputable distinction between HHV-6A and HHV-6B. The genomes of HHV-6 variants are co-linear and share an overall identity of 90%, but intervariant divergence of specific sequences (i.e. the IE1 region) is higher than 30% [10, 11] and there are differences in function between IE1A and IE1B.[12] Interestingly, even though the IE1 region differs substantially between HHV-6A and HHV-6B variants, this region is remarkably conserved (>95%) within clinical and laboratory isolates of the same group.[13]

Analysis of different viral strains shows that even highly conserved sequences with homology higher than 95%, such as gH, gB and U94, are characterized by specific amino acid signatures, permitting the unambiguous distinction between variants.[14, 15]

Several reports describe that the splicing pattern and temporal regulation of transcription of selected genes are different.[10, 11, 16-18]

Finally, the lack of a genetic gradient and the absence of evidence of intervariant recombination suggest that the two groups *in vivo* occupy different ecological niches.[6] However, it is important to note that the molecular methods currently employed to detect and characterize HHV-6 variants in *ex vivo* samples do not permit a clear differentiation between intervariant recombination and co-infection, and that intervariant recombinants have never been isolated *in vitro*.

Biological Properties & Disease Associations

HHV-6A and HHV-6B show consistent differences in specific biological properties.

1. HHV-6 variants have a differential distribution in human tissues. HHV-6B is significantly more prevalent in peripheral blood mononuclear cells of healthy adults, and transplant patients than HHV-6A.[7, 25-29] HHV-6A is detected more frequently than HHV-6B in the plasma of bone marrow transplant patients.[30, 31] HHV-6A has been associated with adult infections associated with neurological disease and increased neurotropism, as well as syncytial-giant cell hepatitis in liver transplant patients.[32-40]

2. Although both HHV-6A and HHV-6B have been reported to have a strong CD4+ T-lymphocyte tropism both *in vitro* and *in vivo*,[19, 20] there are some important differences in their ability to infect cytotoxic effector cells. While HHV-6A has been shown to productively infect CD8+ T cells, natural killer cells and gamma/delta T cells, inducing *de novo* expression of CD4 that is otherwise not expressed in these cell subsets,[21-23] HHV-6B can infect these cells very inefficiently, if at all.[24]

3. Although HHV-6A and HHV-6B are supposed to stimulate cross reactive T cell responses because they share more than 88% sequence homology, it has been reported that at least 7% of the T cell clones that are reactive to HHV-6 show specific and distinct pattern of proliferation either to variant A or variant B *in vitro*.[41]

4. In the US and Japan, 97-100% of primary infections are HHV-6B and occur between the ages of 6 and 12 months.[42-45] Although very little is known about HHV-6A, infection is acquired later in life and the primary infection is typically without clinical symptoms.[46] HHV-6A is the predominant variant associated with viremic infection in a pediatric population of Sub-Saharan Africa.[47] Contrary to HHV-6B, HHV-6A is rarely found in the saliva, and it has been found in 54% of the lungs of healthy adults.[48]

5. While the overwhelming percentage of transplant reactivations is HHV-6B, HHV-6A DNA is found more frequently than HHV-6B in patients with neuroinflammatory diseases such as MS[37, 49] and rhomboencephalitis.[50] HHV-6A has been found predominantly in the CNS of a subset of patients with Multiple Sclerosis, despite common latent HHV-6B persistence in peripheral blood.[37, 49, 51-54]

6. HHV-6A and HHV-6B are both neurotropic but there is evidence for increased severity of HHV-6A.[29, 46, 47, 50]

7. HHV-6B, but not HHV-6A, has been associated with mesial temporal lobe epilepsy and status epilepticus.[55]

Cell Tropism & Monoclonal Antibodies

Other significant differences between variants include:

1. HHV-6B, but not HHV-6A, infects and induces CPE in Molt-3 cells, and HHV-6A, but not HHV-6B, infects and induces CPE in HSB-2 cells.[4, 5, 56, 57] HHV-6A, but not HHV-6B, efficiently replicates in CD8+ lymphocytes,[21, 24] natural killer cells and gamma/delta T cells[23] inducing de novo expression of CD4 messenger RNA and protein.[21, 22] HHV-6A, but not HHV-6B, successfully replicates in human progenitor derived astrocytes.[58] HHV-6B infection in the astrocytic cell line U251 leads to abortive infection whereas with HHV-6A, it leads to replication.[58, 59] HHV-6A, but not HHV-6B, supports productive replication in oligodendrocyte progenitor cells.[57, 58, 60]

2. Several monoclonal antibodies are variant-specific. For example, 2E2 (reacting with gp110), 2-D6 (reacting with gp82/105), p6H8 (reacting with IE-2)[61, 62] and gp110 reacting with 2E2[63] are specific for HHV-6A while OHV-3 (reacting with p98)[64] and C3108-103 (reacting with 101K/U11) are specific for HHV-6B.[65]

3. There are functional differences in cells infected with different variants. In fact, HHV-6A, but not HHV-6B, induces syncytia formation with a "fusion from without" mechanism.[66]

Receptor Induction & Glycoproteins

1. Although both HHV-6A and HHV-6B have been shown to utilize CD46 as a cellular receptor,[67] the modality and/or affinity of receptor interaction seem to differ between the two viruses. This finding is suggested by the observation that HHV-6A (U1102 or GS) but not HHV-6B can induce CD46-mediated cell-cell fusion without viral replications[66] through a tripartite complex encompassing glycoproteins gH, gL, and gQ.[68]

2. The HHV-6A and HHV-6B gO gene products have 76.8% amino acid identity, which is much lower than the identity between other glycoproteins. The lower identity suggests that the gH–gL–gO complex, as well as the gH/gL/ gQ1/gQ2 complex, may confer at least some of the different biological properties on the HHV-6A and -6B variants that cause them to target different cells.[69]

3. The glycoprotein-encoding genes that encode gQ (U97, 98, 99 and 100) of HHV-6A and HHV-6B share only 72.1% sequence identity.[10] This glycoprotein may therefore have a role in the differential effects of HHV-6A and HHV-6B infections. gQ1, along with gB and gH, contains epitopes recognized by neutralizing antibodies and represents a target for variant-specific neutralizing antibodies.[16, 17] The gH/gL/gQ complex is an important target for virus-neutralizing antibodies.[69]

Definition of herpesvirus species

Do these differences warrant the designation of HHV-6 variants as different viral species?

To answer this question, it is necessary to consider the definition of herpesvirus species, as defined by the Herpesviridae Study Group of the International Committee on Taxonomy of Viruses (ICTV): "Related herpesviruses are classified as distinct species if (a) their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and (b) they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts." (http://talk.ictvonline.org/files/folders/vert04/entry174.aspx)

We suggest that HHV-6 variants fulfill the requirements of both items. Concerning item (a), the conserved differences between the genomes of variants are easily and reliably assessed by molecular analysis, and there is no genetic gradient between variants, as shown by conservation of differences. The variant-specific differences in cell tropism, tissue distribution, and disease association support distinct epidemiology and pathogenesis.

We suggest that, 17 years after the consensus position,[5] there is enough new evidence to critically reassess the nomenclature for HHV-6. The issues at stake are relevant because a good classification system allows researchers and clinicians to anticipate the properties and pathogenic potential of new isolates.

Distinction of HHV-6A vs. HHV-6B in publications

In spite of suggestions originally advanced by Braun et al.,[8] authors of many HHV-6 related papers have failed to clearly specify which variant was studied. The lack of distinction between variants makes it difficult to properly assess epidemiological differences and pathogenic associations. We call on the ICTV to assist by asking all scientists to differentiate between HHV-6A and HHV-6B in their publications, regardless of the formal names.

All on the committee except two agree that HHV-6A and HHV-6B should be declared separate viruses. For the dissenting opinion, please see the attached comments in Exhibit A. Although there is consensus that HHV-6A and HHV-6B are separate viruses, there is no consensus on a recommendation for how the viruses should be re-named. We have summarized our comments on that subject in a separate document.

Thank you for your consideration.

Signed,

Ad Hoc Committee on HHV-6A & HHV-6B Genomic Divergence

Co-Chairs:
Dharam Ablashi, DVM, HHV-6 Foundation, Santa Barbara, USA
Dario DiLuca, PhD, University of Ferrara, Ferrara, Italy
Henri Agut, MD, PhD, Hospital Pitie-Salpetriere, Paris, France
Yoshizo Asano, MD, PhD, Fujita Health University, Toyoake, Aichi, Japan
Roberto Alvarez-LaFuente, MD, Hospital Clinico San Carlos, Madrid, Spain
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Duncan Clark, PhD, Royal Free & University College Medical School, London, UK
Steve Dewhurst, PhD, University of Rochester, USA
Louis Flamand, PhD, University Laval, Quebec, Canada
Niza Frenkel, PhD, Tel Aviv University, Tel Aviv, Israel
Robert Gallo, MD, Institute of Virology, University of Maryland, USA
Ursula Gompels, PhD, London School of Tropical Medicine, London, UK

Caroline Hall, MD, University of Rochester, Rochester, USA
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Koichi Yamanishi, MD, PhD, National Institute of Biomedical Innovation, Osaka, Japan
Tetsushi Yoshikawa, MD, Nagoya University School of Medicine, Toyoake Aichi, Japan

Exhibit A. Dissenting views: HHV-6A and HHV-6B should not be classified as separate virus species using the current ICTV nomenclature.

1. Henri Agut, MD, PhD, Hospital Pitie-Salpetriere, Paris, France

"On one hand, I completely agree that, regarding HHV-6A and HHV-6B, their nucleotide sequences are unambiguously distinct across the entire genome, they do not recombine with each other (at the current state of our knowledge), they can be readily recognized by means of specific reagents (in particular monoclonal antibodies) or PCR-derived techniques, their cell tropism in experimental cell cultures is different (extended for HHV-A as compared to HHV-B).

On the other hand, I am not sure that they occupy so different ecological niches. Both are present in organs containing mononuclear cells derived from lymphoid tissues and, at least in adult tissues, the frequency of mixed infections (HHV-6A + HHV-6B) in these organs (lungs, lymph nodes) is high. It is clear that, for yet unknown reasons, the detection of HHV-6A in peripheral blood and CSF is less frequent than that of HHV-B. But, to be honest, we must confess that we do not know the epidemiology of HHV-6A infection very well and we lack a specific serological tool for investigating this question. The question of a different pathogenicity is also unclear: a possible role of HHV-6A in some cases of exanthem subitum cannot be ruled out currently; the impact of the two variants on CNS infections is a complex picture (HHV-6B is the most frequent from CSF virological studies but HHV-6A would exhibit a higher severity as concluded from clinical cases)."

2. Ursula Gompels, PhD, London School of Tropical Medicine, London, UK

"I certainly agree with the distinctions between the strain variant groups, HHV-6A and HHV-6B, as our results highlight key biological differences between these groups. We have characterised the only hypervariable gene which is specific to HHV-6, the viral chemokine U83, hence the prime candidate for strain differences. This work shows migration towards the different chemokines from HHV-6A or B (U83A or U83B) of distinct cellular subsets for lytic or latent infection, consistent with differences observed in cellular tropisms of the strain variants.[70] This is also key for HIV interactions as we also show that only HHV-6A U83A can inhibit CCR5 HIV infection.[71]

However, our results also show that in a country, Zambia, with prevalent infant primary infection with HHV-6A, the symptoms, fever, and complications, minor rash, seem consistent with HHV-6B primary childhood infections seen elsewhere (febrile illness or fever with rash 'exanthema subitum').[47] Further, this study shows in sequence analyses, evidence for recombination between HHV-6A and B strains in selected genes.

Therefore, I would support the current nomenclature HHV-6A and HHV-6B for the strain groups, and ask that the ICTV accept or develop nomenclature for strain variant distinctions present in this and other virus species. The designation of new HHV numbers do not seem apt."

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Comments for the ICTV Committee on the issue of renaming HHV-6A and HHV-6B

Choice 1:

HHV-6A would become HHV-6, HHV-6B would become HHV-9
Committee members in favor: Flamand, Gallo, Lusso, Luppi

Choice 2:

HHV-6 would be retired, HHV-6A would become HHV-9 and HHV-6B would become HHV-10
Committee members in favor: None

Choice 3:

HHV-6A would become HHV-9, HHV-6B would become HHV-6 (inconsistent with ICTV rules):
Committee members in favor: Asano, Clark, Frenkel, Jacobson, Kondo, Mori, Yamanishi, Yoshikawa,

Choice 4:

Keep the names the same (inconsistent with ICTV rules):
Committee members in favor: Ablashi, Agut, Alvarez- La Fuente, Carrigan, Di Luca, Hall

INDIVIDUAL COMMENTS:

Dharam Ablashi:
Even though HHV-6A was discovered before HHV-6B, my feeling is that ICTV should leave the names the same and recognize them as two distinct species based on the data gathered and presented in the document. Renaming HHV-6B as HHV-9 would create confusion which could be harmful to clinical and basic researchers. If ICTV will not change its rules and only gives two choices, my vote would be for Choice 1.

Dario Di Luca:
I think that the ICTV rules need to be updated and that they should be adapted to the new situation that has taken shape in the past 20 years. In fact, rules on herpes nomenclature are not necessarily implemented. For example, the official ICTV name is HHV-8. and yet many researchers publish their results as KSHV, and to my knowldege no scientific journal enforces the ICTV rules. Nobody objects to the common use of EBV and CMV, instead HHV-4 and HHV-5, or to VZV, or to HSV. After all, considering all human herpesviruses (and also most veterinary ones) only HHV-6 and HHV-7 follow the official nomenclature. Even herpesviruses discovered more recently than HHV-6 have a name that does not follow the official nomenclature (i.e. PLHV-1 and PHLV-2 porcine lymphotropic HV, should be SuHV suid HV, if I am not wrong).

The question is: what do we want to achieve? My idea is that an official differentiation between A and B would be of great help in future studies. The problem goes back to Edinborough, when it was decided to call them variants, without a definition for "variant" – variants as HIV clades, as HCV genotypes, as HPV types, or what else? Under this respect, in my opinion, a good result would be to recognize that they are separate entities, with different epidemiology, different pathogenic potential. It would be a great result if we get a community consensus on this, and if

ICTV recognizes this reality. However, I feel that a change of name would create a lot of confusion. Therefore, even if it is not in accordance with the
official rules, I favor the idea to keep the current names (HHV-6A and HHV-6B), as long as "officially" they are recognized as distinct species. If ICTV gives only the first two choices, I favor Choice 1.

Carolyn Hall
Clear distinctions exist in the genomic and biologic properties, but the "ecological niche" of the two viruses is less clear, geographically variable, and, importantly, rapidly evolving.

Thus, I suggest that a reasonable consensus is that the two should be clearly differentiated, as stated in the letter, as two viruses, but that they should not be renumbered, but currently should retain their known appellations, HHV-6A and HHV-6B.

Henri Agut:
I must acknowledge my main concern, as a medical virologist, is the impact of the taxonomy changes in the monitoring of HHV-6 infections in clinical settings as well as in the development of applied research on these viruses. Taxonomy, I think everybody will agree with that, is a pure convention the usefulness of which is: (i) to clarify the evolutionary links between biological entities; (ii) improve the accuracy of data exchanges between scientists by means of a specific vocabulary (nomenclature) ; (iii) strengthen the structure and the visibility of a biological discipline ; (iv) promote the interest of both scientists and general population on the classified entities. Personally, I have some difficulties to interest my clinician colleagues and fund providers in HHV-6 infections despite my absolute conviction that this (these) virus(es) are real pathogens for humans. I am really afraid that a dramatic change of nomenclature would have detrimental effects on the future of HHV-6 research. No matter HHV-6A and HHV-6B will be considered either as two separate species or variants of a single species, in my opinion, the essential point is to maintain the current names of HHV-6A and HHV-6B and to go on forward. In that sense, the two choices proposed by Phil (according to current taxonomic rules, I agree, Phil) seem both inadequate to me.

Roberto Alvarez-La Fuente:
My opinion is that a change in the name (overall with choices 1 and 2) is going to generate more confusion in the people in general, and in the clinicians in particular. I agree with Dario Di Luca: the objective should be that the ICTV recognizes that HHV-6A and HHV-6B are separate entities… although their names continue being the same ones.

Don Carrigan
We believe that changing the name of either HHV-6A or HHV-6B to HHV-9 is a very bad idea. Such a change would greatly disrupt the literature and cause significant confusion both in and out of the field. The names should remain the same.

Niza Frenkel:
With regards to the two alternative ways of renaming of the variants I would like to add a third option, which I would like you and others to consider: Designate HHV-6B as HHV-6 and HHV-6A as HHV-9. The reason is the epidemiology. Thus far HHV-6B is the major disease-causing virus and there are many publications concerning diseases associated with HHV-6: Roseola Infantum, convulsions, encephalitis, complications in bone marrow and other transplantations. Many of the publications just state HHV-6 and not the variant name. Furthermore, as far as I know, many clinicians send nowadays samples for diagnosis of HHV-6, without specifying the variant type. It would cause tremendous confusion to designate HHV-6B as HHV-9 or HHV-

10.Of course I can follow the reason for suggesting that HHV-6A will be renamed as HHV-6 and HHV-6B as HHV-9 (or the viruses become HHV-9 and HHV-10, respectively), since the HHV-6A (GS) was discovered before HHV-6B (Z29) was isolated. However, (i) The ICTV guidelines of designating the viruses with Arabic numbers according to the discovery date is justified when the designation is close to the time of virus discovery. For HHV-6A and HHV-6B we are 23-25 years after discovery, with numerous published papers on molecular virology and disease associations of HHV-6, without relating to variant type. The disease-causing virus has been mostly HHV-6B; so let HHV-6B be referred to HHV-6. (ii) As far as I know there are previous cases where the designations of Human Herpesvirus nomenclature were not by the chronology of discovery. Thus, EBV was discovered and first isolated in 1964 by Epstein and Barr and now it is designated as HHV-4, whereas CMV was first isolated by Smith in 1956, Rowe and coworkers in 1956, and Weller et al in 1957 independently. It is now designated as HHV-5. So let us reduce the confusion as much as possible and designate HHV-6B as HHV-6.

Duncan Clark:
I would though like to register my support for Niza's proposal in renaming HHV-6B as HHV-6 and HHV-A as HHV-9. As has been commented, we have considerable knowledge on the epidemiology, biology, and importantly the pathogenic potential of HHV-6B such as exanthem subitum (ES) and disease post-transplantation including bone marrow suppression and encephalitis. To maintain the now established association of HHV-6B with these clinical outcomes (in particular ES), I would support the proposal to rename HHV-6B as HHV-6.

Steve Jacobson:
"I agree with the recommendations of Niza."

Yasuko Mori:
"I agree with the recommendations of Niza."

Koichi Yamanishi:
"I totally agree you [Niza]. The data of HHV-6B have been accumulated and this virus hopefully can be renamed as HHV-6."

Tetsushi Yoshikawa:
"I think that Niza's plan is the best."

Yoshizo Asano:
"I believe HHV-6B should be HHV-6 and HHV-6A would become HHV-9.."

Kazuhiro Kondo:
"I agree with Drs. Frenkel, Hall, Jacobson, Mori, Yamanishi, and Yoshikawa.
HHV-6A would become HHV-9, HHV-6B would become HHV-6."

Louis Flamand
My choice was that HHV-6A be HHV-6 and that HHV-6B be HHV-9. I can however understand and somewhat agree with Niza's comment to the effect that renaming HHV-6B by HHV-9 will cause a lot of confusion in the medical world. I think that the point she is trying to make is more practical than anything else.

Mario Luppi:
In principle, I agree with Dharam that the most relevant issue is to make the two viruses separate. The epidemiologic arguments raised by Niza Frenkel are reasonable, but I think we should give more weight to the fact that HHV-6A was discovered first. Thus, if we want to make the point that the two viruses are classified as distinct, HHV-6B should become HHV-9.

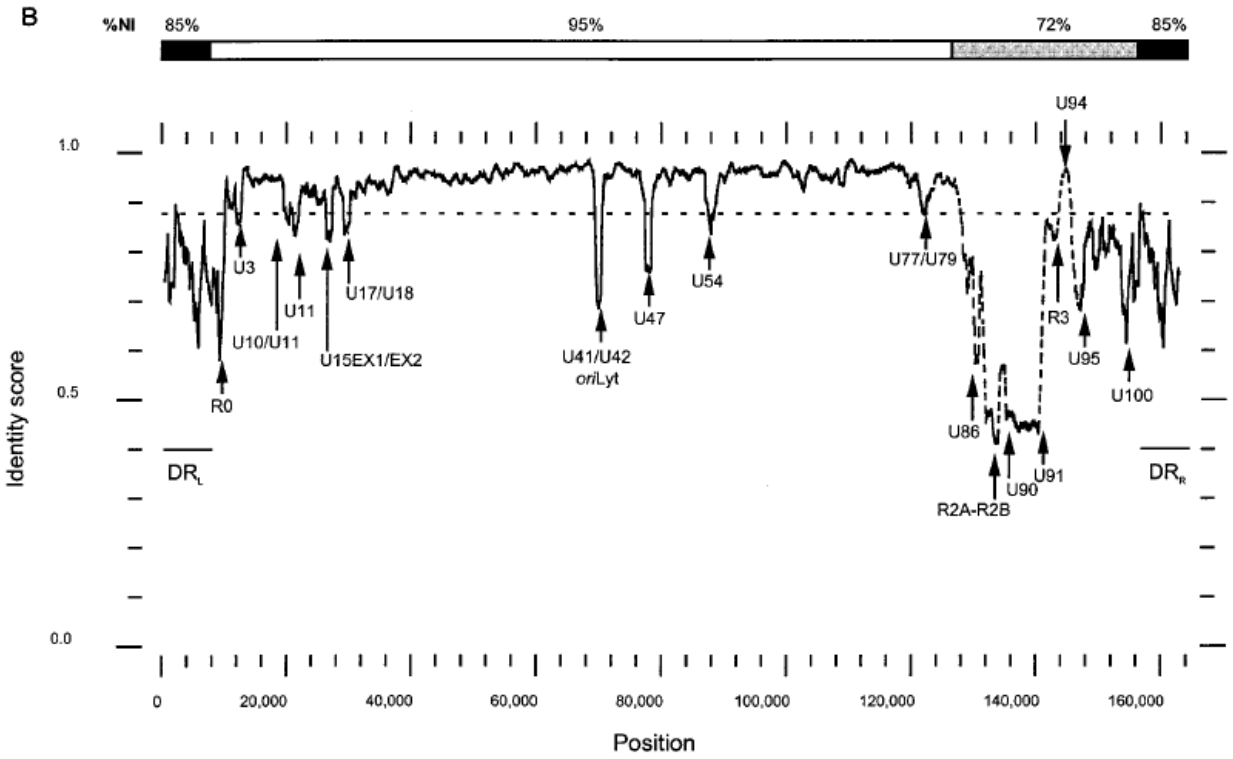
Bob Gallo:
Choice 1. HHV-6B would become HHV-9. "Exactly what I recommended in my Barcelona opening talk three years ago."

Paolo Lusso:
I agree, in principle, that HHV-6A and B should be considered as separate species. My suggestion is to opt for choice #1 (A remains HHV-6 and B becomes HHV-9).

Gave no opinion on name change:

Steven Dewhurst

Figure illustrating the extent of sequence similarity between HHV-6A and HHV-6B from Dominguez et al. (J. Virol. 73:8040-8052,1999)



Nucleotide sequence comparison between HHV-6A and HHV-6B genomes. Genomes were aligned in segments by using GAP with gap and length weights of 50 and 3, respectively, except for the region spanning residues 124000 through 144500 (dashed line), for which weights of 25 and 1, respectively, were used to maximize the alignment. After concatenation of the aligned segments, identities between the aligned sequences were plotted by using PLOTSIMILARITY with a window of 1,000 residues. The horizontal dashed line represents mean identity of 88% across the whole alignment. Several regions with scores less than the mean are labeled; variable intergenic regions are identified by their flanking genes, e.g., U10/U11; the region spanning R2A and R2B is indicated as R2A-R2B. Nucleotide identity (NI) for the indicated regions was determined using GAP for aligned degapped sequences, with gap and length weights of 50 and 3, respectively, except for the segment spanning the right end of U, where weights 250 and 25, respectively, were used in order to omit gaps.

by several laboratories, and isolates were obtained from specimens collected on several continents, indicating that infection with the virus is widespread (11, 49, 144, 324, 504, 556). Results from several of these laboratories indicated that the newly described virus was most likely to be found and to grow in $CD4^+$ lymphocytes (49, 144, 324, 343, 497, 504). The name of the virus was changed to human herpesvirus 6 (HHV-6), a name independent of the cell tropism of the virus and in accordance with guidelines established by the International Committee on Taxonomy of Viruses (6).

As the cellular and molecular biologic properties of independent isolates of HHV-6 were compared, the segregation of isolates into two groups became apparent. The groups differ with respect to epidemiology (3, 34, 35, 61, 128, 131, 139, 178, 254, 405, 455, 551), growth properties (3, 89, 128, 545), reactivity with panels of monoclonal antibodies (MAb) (3, 34, 89, 128, 455, 545), restriction endonuclease profiles (3, 34, 35, 180, 196, 250, 455), and nucleotide sequences (34, 35, 100, 101, 196, 197, 200, 505, 554). Nonetheless, they are very closely related, with some genes being over 95% identical. After considerable debate in the literature and at international scientific meetings, a decision was made to formally recognize the differences as sufficient to define the two groups as HHV-6 variants A and B (2). HHV-6A is exemplified by strains GS and U1102, and HHV-6B is exemplified by strains Z29 and HST. The presence of the two groups raises several questions. What are the differences between the viruses with regard to disease spectrum and epidemiology? Can a variant-specific serologic test be developed? Can intertypic recombinants be formed? Answers to these and many related questions should be forthcoming in the next few years.

The early literature describing HHV-6 serology shows several inconsistencies. In some reports, as few as 3% of people were described as seropositive for the virus, and in others over 90% were seropositive. Age-related increases as well as declines in seropositivity were noted. Correlations were and were not seen between HHV-6 antibody status and the progression of HIV-related disease. Much of this confusion can be attributed to the lack of sensitivity of some of the earliest serologic tests, as well as to the use of very conservative cutoffs to avoid nonspecific results. Current methods indicate that more than 95% people over 2 years of age are seropositive for either HHV-6A or HHV-6B or both.

The search for diseases associated with HHV-6 infections has been complicated by the high viral seroprevalence, but the early age of acquisition of infection noted by some workers suggested that the virus might be associated with a common childhood disease. Yamanishi et al. found that HHV-6B could be cultured during the febrile phase of the common childhood illness ES (roseola infantum) and, further, that the children demonstrated seroconversion to the virus within weeks of the acute illness (556). Primary HHV-6B infection normally produces mild or even subclinical disease, but more severe presentations have been observed. As described in detail below, these include hepatitis, hemophagocytic syndrome, fatal disseminated infection, and neurologic manifestations (e.g., febrile convulsions and encephalitis). Illness associated with primary HHV-6B infection accounts for 10 to 40% of febrile admissions of young children to pediatric emergency departments. No isolates of HHV-6A independent of HHV-6B have been obtained from immunocompetent children with roseola or other febrile illness.

There may be negative consequences of the failure to diagnose routine HHV-6B infections. One scenario (51, 236, 474) involves a febrile child who is being treated with antibiotics and then breaks out in a rash after a day or two. This might be interpreted as an adverse reaction to the antibiotic and duly noted as such, with that antibiotic and its relatives being stricken from the list of antibiotics available for treating that person for the rest of his or her life. The antibiotic reaction may in fact have been a normal presentation of primary HHV-6B infection. Thus, even though primary infection with HHV-6B has few sequelae, definitive diagnosis of the infection is in the patient's best interest.

In addition to the study of primary infection in children, HHV-6A and HHV-6B have been studied as possible cofactors in other clinical situations. Herpesviruses are common opportunistic pathogens in immunocompromised individuals, and HHV-6A and HHV-6B activity has been detected following renal and liver transplants and bone marrow transplants (BMT). HHV-6 antigens and nucleic acids are widely disseminated in autopsy tissues from AIDS patients. The clinical consequences of these findings are under study. Segments of HHV-6 DNA can transform cells to a malignant phenotype in vitro, and it is possible that the virus plays an etiologic role in Hodgkin's disease (HD) and other malignancies.

An underappreciated aspect of HHV-6 biology is its commensal presence in brain tissue. The implications of this observation are not known. Several recent reports indicate that HHV-6 activity and distribution in the central nervous system (CNS) are altered in multiple sclerosis (MS) patients (87, 540).

NOMENCLATURE AND CLASSIFICATION

The brief history of HHV-6 nomenclature provides a clear reminder that science is done by humans. There have been changes in names and suggested classification, and there have been spirited discussions and arguments about what to call the variant groups. Some issues remain to be settled.

HHV-6A and HHV-6B are members of the *Betaherpesvirinae* subfamily, in the *Roseolovirus* genus along with HHV-7 (Fig. 1). This classification is based on the relatively high level of sequence conservation and general genetic colinearity between these viruses and HCMV (a well-characterized betaherpesvirus), in comparison to the generally lower level of genetic similarity to alpha- or gammaherpesviruses. The genetic relationships of HHV-6A and HHV-6B with the other herpesviruses are discussed in more detail in the section on molecular biology, below.

Shortly after the discovery of HHV-6, several laboratories described the isolation of viruses that appeared to be different strains of the same virus, in that their DNA hybridized specifically with a cloned DNA fragment from one of the primary isolates. Early on, it was noted that in hybridizations of this fragment with *Hin*dIII-digested viral DNA, either of two profiles were detected: a single 8.5-kb band or a 5.4-kb band plus a 23-kb band (50, 244, 268), but the significance of these differences was not evident. In a comparison between two isolates, strains Z29 and U1102 reacted differently with some MAb, and while strain U1102 replicated in the T-cell lines J JHAN and HSB-2, strain Z29 did not (545). Given that only two isolates were compared, the only conclusion possible was that the strains differed in these properties.

Three important papers on the subject of HHV-6 strain grouping were published independently in 1991. Each reported comparisons of several properties across collections of HHV-6 strains. All three papers reported that the collections of HHV-6 isolates segregated into two groups that were consistent from marker to marker. Aubin et al. (35) compared restriction endonuclease profiles from large genomic segments and nucleotide sequences from a 163-bp segment and found two groups, typified by strains SIE and HST. Strains included

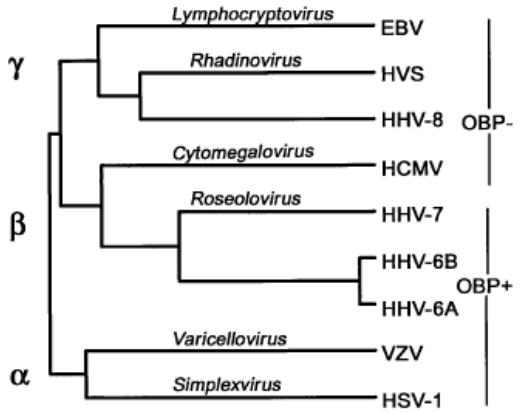


FIG. 1. Genetic relationship of HHV-6A and HHV-6B to other herpesviruses. gH amino acid sequences were aligned by PILEUP (127) with a gap weight of 3 and a length weight of 0.1. The dendrogram represents clustering of the sequences based on their similarity; vertical branch lengths are proportional to the distance between the sequences. α, β, and γ are the herpesvirus subfamilies. Genera within each subfamily are indicated. The close relationship between HHV-6A and HHV-6B is evident, as are the progressively larger distances from these viruses to the other betaherpesviruses, HHV-7, and then HCMV. The viruses indicated as OBP+ encode homologs of the HSV-1 UL9-encoded origin binding protein; their origins of lytic DNA replication are highly conserved. The viruses indicated as OBP− do not encode homologs of this protein, and their origins of lytic DNA replication share little similarity with those of the OBP+ viruses. References for the sequences are as follows: EBV, 39; HVS (herpesvirus saimiri), 16; HHV-8, 368; HCMV, 92; HHV-7, 381; HHV-6B, 317; HHV-6A, 200; VZV, 123; HSV-1, 357.

in group 1 were isolated from immunocompromised adults, and those in group 2 were isolated from children. Ablashi et al. (3) found that isolates that grew in HSB-2 and Sup T1 but not Molt-3 cells reacted with all MAb directed against strain GS and shared identical restriction endonuclease profiles, while the isolates that did not grow in HSB-2 and Sup T1 cells but grew in Molt-3 cells reacted with the same subset of strain GS-derived MAb and shared identical restriction endonuclease profiles that differed from those of the other group. The authors proposed that HHV-6 isolates be divided into group A (GS-like) and group B (Z29-like). Schirmer et al. (455) compared whole-genome restriction endonuclease profiles and reactivity with a panel of MAb among a collection of HHV-6 isolates. The isolates that shared restriction endonuclease profiles with strain Z29 had similar reactivity with the panel of MAb. With the exception of strain Z29, which was isolated from an AIDS patient, the Z29-like viruses were all obtained from ES patients. The authors suggested that reclassification of HHV-6 strains be considered.

There was sufficient overlap in the collections studied in these papers to suggest that the two groups of isolates described in each of the three papers were in fact the same groups. Subsequent studies confirmed this and extended the results to much larger collections (34, 89). As it stands, over 100 HHV-6 isolates have been analyzed by a combination of restriction endonuclease analysis, cell tropism, and reactivity with panels of MAbs (3, 128, 131, 137, 455); except for isolates that appear to be mixtures of the two variants (3, 131), all have been unambiguously assigned to one of the two groups.

While it is clear that HHV-6 isolates segregate into two distinct groups, it is also clear that the two groups are closely related. Members of both groups infect primary CD4-positive T cells. Although a subset of MAb derived against a member of one or the other group do not cross-react between the groups, many MAb do. Much of the human immune response is cross-reactive between the groups, and polyclonal rabbit antibodies to either cells infected with a member of one group or a purified structural protein from a member of one group react strongly with their counterparts from the other group (89, 406). The genomes cross-hybridize efficiently (316, 455). The nucleotide sequence identity ranges from 75 to 97%, depending on which gene is being compared, with identities being in the vicinity of 95% for most genes (34, 35, 101, 197, 200, 315, 317, 323, 406, 554). Notably, both groups encode closely related homologs of the parvovirus *rep* gene at equivalent genomic locations (425, 509); the next most closely related herpesvirus, HHV-7, does not encode such a gene (381).

The clear evidence for two closely related yet distinct groups of HHV-6 isolates has led to a nomenclature conundrum. Do the two groups warrant distinct names? Do the two groups constitute distinct herpesvirus species? The issue was discussed in special sessions at international herpesvirus meetings, and a consensus letter from the community on the subject was published in 1993 (2). The letter described the available evidence and concluded that the groups should be recognized as HHV-6 variants A (strain GS- and U1102-like) and B (strains Z29- and HST-like). It was suggested that it was premature to recognize the variants as distinct species because of their close relationship (closer than any other pair of recognized herpesvirus species) and the gaps in our understanding of the epidemiology of the variants.

In the intervening 4 years, much has been learned, and we would like to describe our reasons for proposing that the issue of recognizing the variant groups as distinct species be reconsidered. We preface the discussion with the definition of herpesvirus species as put forth by the Herpesvirus Study Group of the International Committee on Taxonomy of Viruses (ICTV):

> Related viruses could be classified as distinct species if (a) their genomes differ in a readily assayable and distinctive manner across the entire genome (e.g., restriction endonuclease cleavage site patterns obtained with many enzymes) and not merely at a specific site (e.g., small number of genes or small number of restriction endonuclease sites) and (b) if the virus can be shown to have distinct epidemiologic and biologic characteristics (436).

Has this definition been met? With respect to item "a" from the ICTV definition, which relates to genomic differences, we think the answer is, "Yes." Evidence for this includes the identification of variant-specific nucleotide sequence differences in fragments from diverse segments of the respective genomes and the manifestation of these differences in the markedly distinct restriction endonuclease fragment profiles of each variant. These are perhaps most easily appreciated in the whole-genome restriction endonuclease profiles presented in the studies of Schirmer et al. (455). The sequence variation is logically the source of the variation in reactivity with panels of MAb directed against a variety of viral proteins.

An important question to consider, given the high degree of similarity between the HHV-6 variants, is whether there is any evidence for natural recombination between them, which would suggest overlapping or shared biologic niches and the absence of speciation. Points of comparison would be the EBV variants and interstrain variation among HCMV isolates. In the case of EBV, viruses have been identified that are EBV-1 at some loci and EBV-2 at others (314), suggesting that there is a biological gradient between the variants that precludes their recognition as distinct species. For HCMV, the evidence when considered in its parts (single genes) appears to indicate

the presence of defined strain groups, but when the totality of evidence (multiple genes) is considered, strains that sort into a given group when the sequence of one gene is analyzed, sort into other groups when other genes are analyzed, there being no constancy of the sorting from marker to marker (97, 98, 304). There is no reported evidence of a genetic gradient between HHV-6A and HHV-6B; all isolates that have been characterized for more than one marker have been unambiguously assignable to one or the other variant.

Do the variants have "distinct epidemiologic and biologic characteristics" (item "b")? As mentioned above, A variants replicate preferentially in HSB-2 and J JHAN cells and B variants replicate preferentially in Molt-3 cells. There is a nearly absolute association of B variants with ES (128, 131, 137, 405, 455); the two A variants found were each present in the ill child simultaneously with the B variant (131). Of HHV-6 strains detected in BMT recipients, 70 (87.5%) of 80 were B variants, 6 (7.5%) were A variants, and 4 (5%) were mixtures of HHV-6A and HHV-6B (147, 178, 254, 458, 528, 539). As recently elegantly summarized by Di Luca et al. (135), there are significant variant-specific differences in the frequencies of detecting HHV-6 DNA in (i) peripheral blood mononuclear cells (PBMC) of healthy donors, CFS patients, and HIV-infected patients; (ii) lymph nodes from patients with various conditions; and (iii) various tissues. In most of the examples, the B variant was detected most frequently, with HHV-6A being detected more frequently than HHV-6B in healthy skin, KS lesions, KS-derived cell lines, and primary fibroblast cultures, suggesting a preferential tropism of HHV-6A for skin. The distinctions between the variants were less marked in lung tissue specimens, where 22 (65%) of 34 specimens were PCR positive for both variants, 2 (6%) were positive for HHV-6A, and 10 (29%) were positive for HHV-6B (115). Although there is some overlap in tissue distribution and disease associations, the variant-specific differences in cell tropism, disease associations, and tissue distribution seem sufficient when considered together to constitute "distinct epidemiologic and biologic characteristics."

We are of the opinion that sufficient new information relating to the biology and epidemiology of the HHV-6 variants has accumulated to allow reconsideration of the classification of the HHV-6 variants as distinct species. As we were reminded by Di Luca et al. (135), the herpesvirus classification system is intended to allow researchers and clinicians to anticipate the properties and pathogenic potentials of new isolates (436). Thus, whether or not the species threshold is deemed to have been met, the differences between the variants are real and require recognition for us to fully appreciate the subtleties of the specific niches that the viruses occupy. In this review, we have highlighted areas where variant-specific differences have been noted. In addition, to the extent that we could manage, we have treated the variants as separate entities, even in areas for which variant-specific differences have not been (and might not be) identified. Although this has led to some instances of more difficult phraseology, we do not consider the absence of evidence for differences to mean the absence of differences, and we hope to stimulate continued study of the similarities and differences between the variants. We also hope that a by-product of this and other work describing variant-specific differences will be motivation of authors of HHV-6-related papers in the future to be clear in specifying which variant was studied, isolated, or otherwise detected.

CELLULAR BIOLOGY

Ultrastructure and Morphogenesis

Virions of HHV-6A and HHV-6B, as for all herpesviruses, have four main structural elements: an electron-dense core, a capsid with icosahedral symmetry, a tegument (the less highly structured material occupying the space between the capsid and the envelope), and an outer envelope in which virally encoded glycoproteins and integral membrane proteins are embedded (reviewed in reference 437). The assembly process leading to mature HHV-6 particles begins in the nucleus, where nucleocapsids are assembled. HHV-6 capsids have a diameter of 90 to 110 nm (53, 562). After capsid assembly, the tegument is acquired while still in the nucleus by a series of envelopment and deenvelopment steps which have not been fully characterized (385, 434). The tegument may be acquired in a novel structure, the tegusome, which is a membrane-bounded spherical compartment, apparently of cytoplasmic origin, that is present in the nuclei of HHV-6-infected cells (434). Fully tegumented capsids, with a diameter of approximately 165 nm, appear to be released into the cytoplasm via fusion of the tegusome with the nuclear membrane (434, 562). HHV-6 morphogenesis has the unique feature of cytoplasmic accumulation of fully tegumented capsids with a prominent and well-demarcated tegument; these tegumented cytoplasmic capsids appear to acquire their envelope by budding into cytoplasmic vesicles. Mature virions are approximately 200 nm in diameter (53, 562) and are released by exocytosis (434).

Cell Tropism

In vitro. HHV-6A and HHV-6B replicate most efficiently in vitro in activated primary T cells, and several isolates have been adapted to grow efficiently in continuous T-cell lines. Of the two most widely used strains of HHV-6A, strain GS is most commonly propagated in the T-cell line HSB-2 and strain U1102 is most commonly propagated in J JHAN cells. HHV-6B(Z29) is grown most often in primary lymphocytes and has been adapted for growth in the Molt-3 T-cell line. While T cells are most widely used for propagation of HHV-6A and HHV-6B, cell lines of neural, epithelial, and fibroblastic origin have different levels of permissivity for HHV-6 growth in vitro (6, 86, 94, 213, 325), although none of these cells are in common use for routine propagation of the virus. With few exceptions, virus isolation procedures have relied on cultivation of patient PBMC or cocultivation with primary adult PBMC or umbilical cord blood lymphocytes. HHV-6 can infect primary natural killer (NK) cells harvested from PBMC (341).

In vivo. The in vivo host tissue range of HHV-6 is broader than its in vitro host range might suggest and includes lymph nodes (309), lymphocytes (112, 119, 243), macrophages and monocytes (288), kidney tubule endothelial cells (388), salivary glands (174, 296, 424), and CNS tissues, where viral gene products have been localized to neurons and oligodendrocytes (87, 329, 330).

Takahashi et al. (497) sorted lymphocytes obtained from children with primary infections during episodes of ES and found that virus was recovered most abundantly from $CD4^+$ cell populations. Because these isolates were obtained during ES, they were probably HHV-6B. Four weeks following primary infection, virus could be recovered only from macrophages (288). As discussed below, this finding suggests that macrophages are a potential repository for latent infection.

Appendix 2. Summary of data related to the possibility of in vivo recombination between HHV-6A and HHV-6B

Prepared by P. Pellett for second round of Study Group discussion, in response to a question raised during the initial discussion.

Data related to the possibility of HHV-6 intervariant recombination

P. Pellett

January 24, 2011 – modified June 17, 2011 to remove copyrighted material

Dr. Gompels dissented from the HHV-6 Working Group proposal that the HHV-6 variants should be recognized as distinct herpesvirus species. Dr. Gompels's opposition is based on (i) HHV-6A being detected in some febrile children from Africa, and (ii) the possibility of intravariant recombination. With respect to the first issue, HSV-1 and HSV-2 can cause similar disease, but this does not preclude their recognition as separate species. Thousands of children with illness due to primary HHV-6 infection have been examined by Dr. Hall in Rochester; most of the illness is associated with HHV-6B, with very few being positive for HHV-6A. These viruses have distinct epidemiology, even if overlaps or can be identified.

Evidence is related to the recombination question

Aubin et al. evaluated 10 strains from France, Japan, Ivory Coast, and Uganda (4 HHV-6A and 6 HHV-6B) by PCR-sequencing of portions of the U31 (LTP) and U57 (MCP genes), plus reactivity with six monoclonal antibodies, one of which (variant-specific) is against the product of the U100 gene (2). Thus, the markers examined spanned at least 90 kb of the genome. All viruses were consistently either A or B for the three loci examined.

My lab has published a number of whole genome restriction profiles:

9 from Osaka children vs.HHV-6B(Z29) (5)[a figure from this paper is included below]
15 from Rochester children vs. 1 Japanese isolate and HHV-6B(Z29) (6)
6 from Pittsburgh BMT recipients (4)

I do not see evidence in that data for recombination. Some of the restriction endonucleases and probes used were chosen to highlight the interstrain differences (we wanted to show that the viruses represented independent isolates). Digests were also done with enzymes that do not accentuate the length heterogeneities present at the ends of the HHV-6 DR sequences. Thus, the HindIII and ClaI digests in the Adv. Exp. Med. Biol. paper (5) show a clear absence of divergence among that collection of the sort that would suggest the possibility of intravariant recombination. The BamHI digests shown in Fig. 2B of the NEJM paper accentuate visibility of the terminal fragment length heterogeneity. If the fragments illuminated in Panel B by a terminal fragment probe are ignored, the remainder of the profiles shown in panel 2A are very homogeneous (6). ClaI digests were also done for these isolates and are similar in their homogeneity to the ClaI digests from (5) that are shown below. In Kadakia et al. (4), the most informative profiles are from the PstI digests.

I will not argue that the work described above was exhaustive, but the collection of viruses examined came from diverse sources internationally, the comparisons spanned all or much of the genome, and the sort of recombination events most likely to have been missed would have been relatively short double crossovers.

I have copied two paragraphs verbatim from a paper from Dr. Gompels's laboratory (Bates et al. (3)) that includes information about possible recombination, plus Fig. 3 from the paper and its legend.

From the Results section, pp. 783-784 of Bates et al.:

> **Multiple Infections and Recombination**
>
> Most coding differences were in the U47 gene, which had been previously analyzed in earlier febrile infant cohorts. Thus these differences in the U47 gene from this current study were analyzed now in comparison to previous results in order to tabulate the range of variation and examine evidence for recombination [Kasolo et al., 1997; Gompels and Kasolo, 2006]. Analysis of representative sequences from laboratory reference strains or from European/American clinical isolates at this locus, shows conserved amino acid changes, clearly differentiating variants A and

B (Fig. 3). In two laboratory reference strains however (AJ and GS), whilst the selected amino acids are predominantly those of variant A, they contain **two and one** *[emphasis added]* ''B-like'' residues respectively that belong to the HHV-6B reference strains (Z29, HST, and KF). Analysis of the U47 gene among Zambian samples expands this further, identifying sequences that are either A, B, A/B and now also B/A (primarily B but with some A-like residues). The Zambian sequences also demonstrated some novel amino acid substitutions (at positions 1, 2, 3, 5, 6, 10, 11, 12, 17, and 18), indicating further complexity. This further variation, together with high prevalence of HHV-6A, support further distinctions for primary HHV-6 infections in Southern Africa compared to that seen in North America, Europe, and Japan. Further analyses of the codon substitutions show that although the overall patterns for A and B variants are distinct, no single amino acid substitution is specific for a single variant. Some sequences also show evidence for complex recombination events, primarily from HIV-1 positive febrile infants.

From the Discussion section, p. 786 of Bates et al. (JMV 81:779-789,2009):

Sequence analysis of the hypervariable U47 locus identifies viruses with distinct grouping to either HHV-6A or B reference strains, but also identifies novel sequences that appear to be chimeras of both strain variants. This raises the possibility suggested previously for U47 (gO) [Kasolo et al., 1997; Gompels and Kasolo, 2006], of the existence of recombinants between HHV-6A and B, **which has recently been reported for HHV-6B strains at the U39 (gB) locus [Achour et al., 2008]** *[emphasis added – as quoted below, Achour et al. were more cautionary on this point]*. Such recombinants are detected commonly in HCMV for which more sequence data are available [Haberland et al., 1999], as has been suggested for UL74 (HHV-6 U47/gO homologue), although the time-frame for these events are most likely ancient as shown by molecular modeling [Mattick et al., 2004; Bates et al., 2008]. This supports the view that recombination is an important mechanism of evolution in herpesviruses in general [Gompels et al., 1995; Umene, 1999; McGeoch et al., 2000, 2006; Thiry et al., 2007; Umene et al., 2008].

In summary, evidence in Bates et al. for recombination consists of a handful of residues within the highly variable gO gene. For three of the viruses listed as recombinants in the figure above from Bates et al., plus three others in the figure that seem to meet similar criteria, the putative recombinational event led to change of a single amino acid residue (single residue red boxes). For two viruses (Zam317 and Zam12), two and four residues are affected. For these viruses, there is no information for downstream markers to know whether this might have been the product of a single crossover.

Quote from pp. 1219-1220 of Achour et al., JMV 80:1211-1221,2008 (1). This paper was cited by Bates et al. as having evidence in support of HHV-6 intravariant recombination.

To date, recombination between HHV-6A and HHV-6B has been considered very infrequent but has yet been suggested from the study of U47 gO gene in an epidemiological context in which HHV-6A and HHV-6B were present equally [Kasolo et al., 1997; Gompels and Kasolo, 2006]. The present study does not provide a clear answer to that question. Regarding the crucial variant-specific amino acid changes defined both in gB and gH (26 and 39 changes respectively), it provides no example of an exchange between these markers which might suggest a recombination between HHV-6A and HHV-6B, this conclusion being alleviated by the relatively low number of HHV-6A isolates studied. On the other hand, some gB changes such as A323T, P324S, E326D, K344N, and E348D are ambiguous in the sense they reflect a difference both between HHV-6A and one HHV-6B subgroup, and between the two HHV-6B subgroups, as shown in Figure 3. Hence, it is difficult either to rule out or to prove the hypothesis that, in the case of gB, the region encompassing the positions 323–348 may result from a complex intervariant recombination process. The arguments for intravariant recombination within gB gene are much more convincing.

If these sequences are the product of recombination, as opposed to the possibility that they reflect a small set of allowable changes in this restricted amino acid sequence context, such highly restricted recombinational kisses are very different from the sort of intertypic recombinational mating that could be expected within a species, e.g., the left half of a genome is derived from one lineage and the right half from another. Indeed, Achour et al. provide evidence for such intravariant recombination. The Bates et al. paper cites the Achour et al. paper as being in support of the intervariant recombination idea. However, Achour et al. actually said that their data "provides no example of an exchange between these markers which might suggest a recombination between HHV-6A and HHV-6B." They acknowledge that for the region spanning gB residues 323-348, an available interpretation involves complex intervariant recombination.

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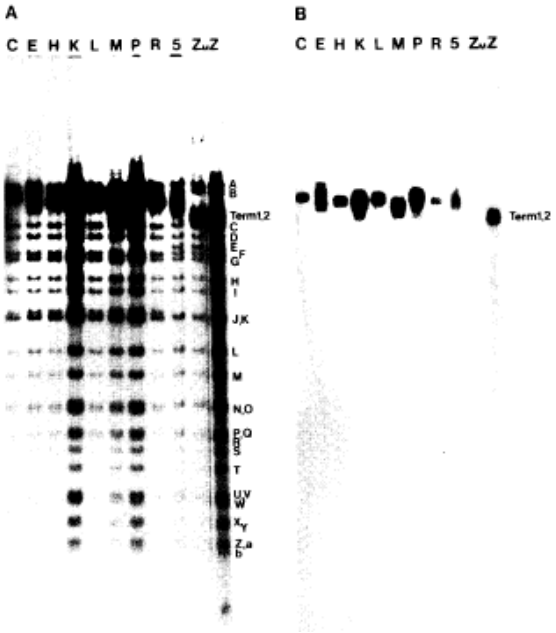

Fig. from (5). ClaI digests of whole cell DNA from cells infected with HHV-6B isolates from Osaka (C, E, H, K, L, M, P, R) and Africa (5, Z29). After digestion, the DNA was separated in a 0.8% agarose gel and then blotted to a membrane. In panel A, the membrane was hybridized with purified HHV-6B nucleocapsid DNA. The hybridization in panel B was with a plasmid containing a fragment from within the terminal direct repeat (DR). Although not visible in this scanned image, the patterns for the smaller fragments (P,Q and below) are identical for all of the viruses. The DR probe illuminates terminal fragments that vary in length between strains and on passage in cell culture.