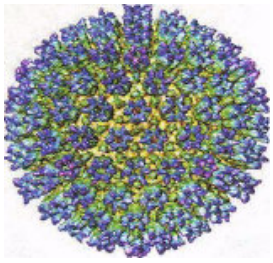

HUMAN HERPESVIRUS-6

A Pictorial Atlas

GERHARD R.F. KRUEGERM.D., Ph.D.

Professor of Pathology & Laboratory Medicine, Department of Pathology & Laboratory Medicine; Adjunct Professor of Internal Medicine, The University of Texas - Houston Medical School, Houston, Texas 77030, USA;

Professor & Dean Emeritus, Cologne University Medical School; Head, Laboratory of Computational Cell Biology, Department of Anatomy II, University of Cologne, 50924 Cologne, Germany

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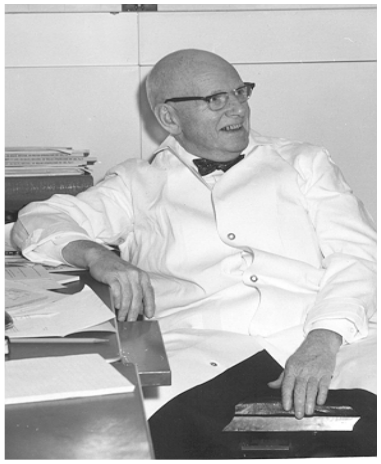
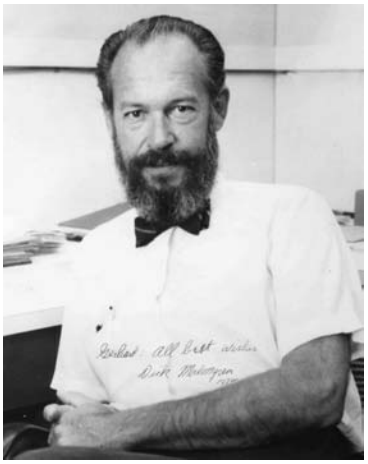
In Memoriam of my teachers

Johann Wilhelm Masshoff



Harold Leroy Stewart

Thelma Brumfield Dunn



Richard Albert Malmgren

Robert Fischer

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FOREWORD

Photos shown in the present atlas are obtained from some 20 years working with human herpesvirus-6 at the Immunopathology Laboratory, Institute of Pathology, The University of Cologne, Germany, and the Department of Pathology & Laboratory Medicine, The University of Texas - Houston Medical School, USA. The author hopes that information he collected on the clinical pathology of the virus may not be lost after his retirement. Much of this work was done in collaboration with my good friends Dharam V. Ablashi, James E. Whitman, Albert M. Ramon, Axel Hoffmann, Gerhard Bertram, Julieta Rojo and Guanyu Wang. The ever present "good spirit" of our laboratory was our chief medical lab technician Brigitte Koch Schneider. Her expertise in tissue culture, serology and molecular techniques, as well as in classical histology and immunocytochemistry deserves special mentioning. Eleven doctoral theses at the University of Cologne resulted from these HHV-6 studies and our enthusiastic medical candidates Ulrike A. Habermann, Michael Schonnebeck, Tessa Koenig, Cecylia Schinke, Jochen Ketterer, Uwe Klueppelberg, Andreas Guenther, Frank A.W. Eichler, Sabine Boehmer, Marie Louise Huetter and Patricia Simoes. Last not least, all work would not have been possible without the gracious support by several diagnostic and pharmaceutical companies including AB1 Advanced Biotechnology Inc., Columbia, Maryland, USA, Organon Teknika BV, Boksel, The Netherlands, Du Pont de Nemours Europe, Homburg / Taunus, Germany, and Ortho Diagnostics Division, Heidelberg, Germany. We gratefully mention also Elsevier Science Publishers, Amsterdam, who readily published our findings and symposia and thus fostered significantly further collaboration.

Disease associations and causality in HHV-6 infection is still a matter of dispute and probably will remain so for quite a while. It is a consequence of the large variety of testing methods in use, their in part limited reproducibility and — unfortunately ~ some limitations in communication and understanding between clinicians, virologists and molecular biologists. This atlas is not thought as an attempt to solve such discrepancies and beliefs. It should rather provide observations under various conditions of infection and disease, and leave the interpretation to the reader. It will be a success, if further studies can be stimulated this way that may finally help our patients.

In all case materials presented here, we followed a stepwise diagnostic approach to prove and to classify HHV-6 infection as shown below:

Stepl

Screening of patients' sera for HHV-6 IgG antibodies by IFA using HSB2 cells infected by

HHV-6A, GS or Co6 isolates.

If titers are 1:20 or higher, **Step 2**

Screening for active infection by

a) sera for IgM antibody b) virus isolation

using HSB2 cells

c) Antigen-capture ELISA for p41 antigen (ABI advanced Biotechnologies Inc., Columbia,

Maryland, USA)

Step 3

Confirmatory procedures:

a) if biopsy available: immunohistochemistry for HHV-6 antigens (p41, p!35, gp 110/64, gpl 16/64/54; ABI Advanced Biotechnologies Inc) and in situ hybridization (ISH; probes pZVH14) for HHV-6 DNA

b) if biopsy available: Western blot for HHV-6 proteins

c) blood for PCR (nested PCR) d) only in exceptional cases quantitation of DNA copies (single round hotstart PCR)

Step 1 was always done and combined in individual cases by at least one procedure of steps 2 and 3. The most frequent combination of techniques was IgG serology, antigen capture ELISA and/or virus isolation, biopsy immunohistochemistry and ISH.

Interpretation: Acute primary infection IgG+, IgM+, p41+ in biopsy, serum (antigen-capture), virus isolation+.

Active (non-primary infection) IgG+, IgM-/+, p41+, virus isolation+ Latent infection: IgG+, non-p41 antigens+, ISH+, PCR (low level)+

Non-primary infection may consist in a) reactivated infection *(endogenous re-infection)* or b) secondary infection *(exogenous super infection).* The latter is proven only by showing differences in HHV-6 strains in primary and non-primary infections.

Selective References for Diagnostic Testing

Krueger GRF, Ablashi DV, Gallo RC (eds). Persistent herpesvirus infections. Current techniques in diagnosis. J Virol Methods 21, 1-326, 1988 (entire volume; several chapters)

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1. GENERAL INTRODUCTION

The human herpesvirus-6 (HHV-6), first described in 1986 by Salahuddin et al, is a member of the *$ -herpesviridae* (like human cytomegalovirus and human herpesvirus-7) which may become a serious pathogen in man. Similar to other herpesviruses (except for HHV-8), HHV-6 infections commonly occur early in life with lifelong persistence. It is thus a prime candidate for *opportunistic infections,* i.e. it may cause serious disease when reactivated in immunodeficient persons. In addition, such opportunistic infections of HHV-6 frequently occur in coincidence with other reactivated viruses such as Epstein-Barr virus (EBV; HHV-4) and human cytomegalovirus (HCMV; HHV-5) with the possibility of mutual activation.

The characteristics of HHV-6 were recently summarized by Caroline B. Hall (2006) as

• ubiquitous and worldwide infection
• infection is acquired in early life • antibody titers generally persist throughout life

The antibody prevalence of HHV-6 averages between 70% and 100% with variable mean titers in different countries **(Tables 1 a,b).**

	D	B	P	ISR	SA	J	AUS	MEX	USA
M/F ratio	1.5	1.2	3.6	NS	4.3	2.4	NS	4.5	1.2
Age range	18-58	19-69	18-47	18-28	16-68	0.1-91	NS	19-54	21-73
mean age	35.5	38.9	24.6	NS	30.5	28.7	NS	29.6	39.4

Table la: Characteristics of blood donor population for determination of HHV-6 antibody prevalence (from: Krueger et al., Vox Sang 75: 193-197, 1998) D: Germany, B: Belgium; P: Poland; ISR: Israel; SA: South Africa; J: Japan; Aus: Australia; MEX: Mexico.
NS: not stated

Country	Region	Samples	Percent Antibody Titers of						Ar. Mean
			40	80	160	320	640	>640	
D	Cologne	200	2	4	15	37	37	5	415.2
B	Brussels	231	1	12	13	32	7	15	274.6
P	Szczecin	119	5	23	20	20	10	1	299.6
ISR	Beer Sheva	100	0	5	16	34	24	19	539.8
J	Hokkaido	171	0	6	12	20	25	29	89.2
AUS	Brisbane	295	1	7	15	30	17	26	576
USA	Minneapolis	200	3	7	13	23	21	25	393.3
MEX	Mexico City	202	0	2	15	32	15	36	445.1
SA	Durban	227	0	3	17	44	20	16	411.3

Table Ib: HHV-6 IgG inverse antibody titers in different areas. All titrations use HHV-6A (Co6 strain) infected HSB2 cells and IFA (from: Krueger et al., Vox Sang 75: 193-197, 1998). Ar.Mean: arithmetic mean titer

1.1 Further Reading

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