HIGH SERUM ANTIBODY TITERS TO HUMAN HERPESVIRUS-6 IN MELANESIAN POPULATION

J. RAJČÁNI[1], R. YANAGIHARA[2]

[1]Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovak Republic;
[2]Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA

Received November 2, 1993

Summary. – IgG antibodies to human herpesvirus-6 were found by indirect immunofluorescence in the sera of 29 out of 45 tested healthy adults from Bratislava, and in 33 out of 49 healthy Melanesians from Solomon Islands and Papua New Guinea. Although antibody seropositivity was similar in both population groups (64.5 % and 67.4 %, respectively), the geometric mean titer was significantly higher in the sera of Melanesians ($p < 0.0001$).

Key words: human herpesvirus-6; serum antibody titer; seroepidemiology

Human herpesvirus-6 (HHV-6) is distributed worldwide (reviewed by Lopez and Honess, 1990; Pellet *et al.*,1992; Oren and Sobel, 1992; Yamanishi, 1992). Significant differences in prevalence of antibodies to HHV-6 were not found in USA (60 – 70 %) and highly developed European countries, e.g. United Kingdom (60 – 85 %). A somewhat higher positive rate (95 %) was reported for the population in Japan (Yanagi *et al.*, 1990). In contrast, a lower seropositivity reported for Malaysia (Yadav, 1990), was confirmed later for Malaysian Chinese (42 %) as well as for Malaysian Indians (35 %) (Levine *et al.*, 1992). In Africa, the prevalence of HHV-6 infection as tested by immunofluorescence in pregnant women was least common (20 %) and with low geometric mean titer (GMT) in Morocco, while in subsaharan countries (Niger, Mali, Ivory Coast, Togo, Congo) the seroprevalence was as usual (60 – 90 %) and with higher GMT (34 – 229) (Ranger *et al.*, 1991). Antibodies against HHV-6 were readily detected in children aged 3 – 5 years (61 – 95 %) with and age-dependent decrease in seropositivity and GMT (Brown *et al.*, 1988; Leach *et al.*, 1992; Yanagi *et al.*, 1990). Virus-specific antibody titers as determined for different geographic areas depend also on the methods used. By indirect IF, the antibody titers to HHV-6 usually range from 10 to 160 (Okuno *et al.*, 1989) regardless whether the strain GS (variant A) or strain Z29 (variant B) is used as antigen. By anticomplement IF or ELISA the antibody titers are higher, but the preparation of an ELISA antigen of high quality may require more sophisticated techniques such as affinity chromatography columns with bound monoclonal antibodies to the major proteins of HHV-6 (Iyengar *et al.*, 1991). In a survey of 500 randomly selected sera from healthy blood donors in USA, Saxinger *et al.* (1988) reported 81 – 97 % seropositivity by ELISA with titers ranging from 100 to 10,000. The majority of seroepidemiologic studies still relies on indirect IF using infected cell smears because of the ease of preparation; the smears may be stained also by immunoperoxidase technique (Shavanas *et al.*, 1992).

In this study we compared the antibody titers to HHV-6 by indirect IF in sera of 49 Melanesians from Papua New Guinea and Solomon Islands, and of 45 subjects from Bratislava, Slovakia. Serum samples were collected from healthy adults of both sexes ranging in age from 20 – 60 years; the sera were shipped on dry ice and stored at –20 °C before testing. For indirect IF test HSB-2 cells and HHV-6 strain GS-infected HSB-2 cells (Ablashi *et al.*, 1991) were grown in RPMI-1640 medium supplemented with 10 % foetal calf serum, 2 mmol/l L-glutamine and gentamicin (50 μg/ml). The cells were counted twice weekly by trypan blue dye-exclusion method and the cultures were replenished with uninfected HSB-2 cells to keep their concentration at 10^6/ml. For preparation of smears, cells were pelleted, resuspended to a concentration of 10^7/ml in PBS containing 1 % BSA and spotted onto 10-well

microscopic slide (Cell-line Associates, Newfield, NJ, USA). The slides were air dried, fixed in cold acetone for 10 mins and stored at –20 °C. After blocking with normal goat serum (diluted 1:20) for 20 mins the smears were incubated for 45 mins at 37 °C with test sera diluted from 1:20 to 1:640 washed tree times with PBS for 15 mins each, and finally incubated with FITC-labelled goat anti-human IgG (Capel) diluted 1:50 and containing Evan's blue at concentration of 1:10,000. After three washes in PBS the slides were mounted in Aqua-mount (Lerner Labs, Pittsburgh, PA, USA) and viewed in Leitz epifluorescence microscope.

To ensure correct and reproducible end-point readings a commercially available (Universal Biotechnologies, Rockwille, MD, USA) standard anti-HHV-6 serum with a titer of 1:160 was included in each test at dilutions of 1:40 (scored + +), 1:160 (scored +) and 1:640 (scored negative). Smears incubated with PBS and/or conjugate only served as negative controls. Each staining was performed with both HHV-6-infected and uninfected HSB-2 cells, and, each serum was repeatedly used at highest and lowest dilutions for staining of commercially available HHV-6-infected slides (Universal Biotechnologies). Infected control cells were regularly checked for presence and absence of HHV-6 DNA by polymerase chain reaction (Rajčáni *et al.*, 1994).

HHV-6 antigen scored + + showed a briliant positive staining in nuclei and cytoplasm of approximately 10 – 20 % of infected cells. Cells showing typical CPE were enlarged with swollen nuclei and cytoplasm. The intensity of fluorescence scored + corresponding to the standard serum dilution 1:160 was clearly distinct from the negative background of smears stained with PBS and conjugate only. The sera exhibiting nonspecific fluorescence were adsorbed to noninfected cells and retested.

Of the 45 healthy adults from Bratislava 29 were positive with a titer of 20 – 80 (positive rate 64.5 %), while of the 49 Melanesians 33 showed positive staining at dilutions ranging from 20 to 640 (positive rate 67.5 %). The seroprevalence of HHV-6 infection among the Solomon Islanders was slightly higher (69.5 %) than that among a smaller group of individuals from Papua New Guinea (61.5 %). The antibody GMT of the sera from Bratislava was 36.6 while that of the Melanesian sera was 257 (Table 1), a highly significant difference ($P < 0.0001$, unpaired t-test).

Levine *et al.* (1992) found high antibody GMT to HHV-6 in the sera of individuals from Ghana. Though variations in results of the indirect IF test may occur due to interlaboratory methodologic differences, we favor the interpretation that in some remote populations with lower hygienic standard and with higher parasitic burden, the serum IgG antibody levels against ubiquitous agents such as HHV-6 may be higher due to intensive oral contact between adults and infants and higher prevalence of virus shedders. A very high rate of early acquisition of antibodies to cytomegalovirus and Epstein-Barr virus was found among inhabitants of the Solomon Islands and the Eastern Highlands of Papua New Guinea (Lang *et al.*, 1977). Nevertheless, the explanation of this observation remains speculative. Our preliminary studies with detection of HHV-6 DNA in extracts of peripheral blood mononuclear cells by PCR revealed a higher incidence of detectable latent HHV-6 DNA in Melanesians (latency rate 24.2 %) than in individuals from Bratislava (latency rate 3.5 %) (Rajčáni *et al.*, 1994).

Table 1. Anti-HHV-6 serum IgG antibody titers in Slovakia and Melanesia

Geographic origin	Sera with anti-HHV-6 antibody titer							Positive/ total sera
	>640	320	160	80	40	20	<20	
	Number of sera							%
Solomon Islands	6*	7	5	3	2	2	11	25/36
Papua New Guinea	0	3	0	2	3	0	5	8/13
Melanesia (total)	6	10	5	5	5	2	16	33/49 (67.4%)
Slovakia	0	0	0	6	6	17	16	29/45 (64.5%)

*Antibody titer determined by the indirect IF test and expressed as reciprocal of highest serum dilution resulting in specific fluorescence. Sera with titers <20 considered as negative.

References

Ablashi, D.V., Balachandran, N., Josephs, S.F., Hung, C.L., Kruger, R.F., Kramarsky, B., Salahuddin, S.Y., and Gallo, J. (1991): Genomic polymorphism, growth properties, and immunologic variations in human herpesvirus-6 isolates. *Virology* **184**, 545–552.

Brown, N.A., Sumaya, C.V., Liu, C.R., Ench, Y., Goroncsi, M., and Kaplan, M. (1988): Fall in human herpesvirus seropositivity with age. *Lancet* **ii**, 396.

Iyengar, S., Levine, P.H., Ablashi, Dh., Neequaye, J., and Pearson, G.R. (1991): Sero-epidemiological investigations on human herpesvirus 6 (HHV-6) infections using a newly developed early antigen assay. *Int. J. Cancer* **49**, 551–557.

Lang, D.J., Garruto, R.M., and Gajdusek, D.C. (1977): Early acquisition of cytomegalovirus and Epstein-Barr virus antibody in several isolated Melanesian populations. *Am. J. Epidem.* **105**, 480–487.

Leach, Ch.T., Sumaya, C.V., and Brown, N.A. (1992): Human herpesvirus-6. In B. N. Fields and D.M. Knipe (Eds): *Fields Virology*. Raven Press, New York, 2nd edition, pp. 2055–2062.

Levine, P.H., Neequaye, J., Yadav, M., and Connelly, R. (1992): Geographic/ethnic differences in human herpesvirus-6 antibody patterns. *Microbiol. Immunol.* **36**, 169–172.

Okuno, T., Takahashi, K., Balachandra, K., Shiraki, K., Yamanishi, K., Takahashi, M., and Baba, K. (1989): Seroepidemiology of human herpesvirus-6 infection in normal children and adults. *J. clin. Microbiol.* **27**, 651–653.

Oren, I., and Sobel. (1992): Human herpesvirus-6: Review. *Clin. inf. Dis.* **14**, 741–746.

Pellet, P.A., Black, J.B., Yamamoto, M. (1992): Human herpesvirus-6: the virus and search for its role as a human pathogen. *Adv. Virus. Res.* **41**, 1–52.

Rajčáni, J., Yanagihara, R., Godec, M., Nagle, J.W., Kúdelová,M., and Asher, D.M. (1994): Low incidence of latent infection with variant B or roseola type herpesvirus-6 in leukocytes of healthy adults. *Arch. Virol.* **134**, 357–368.

Ranger, S., Patillaud, S., Denis, F., Himmich, A., Sangare, A., M' Boup, S., Itoua-N' Gaporo, A., Prince-David, M., Chout, R., and Agut, H. (1991): Seroepidemiology of human herpesvirus-6 in pregnant women from different parts of the world. *J. med. Virol.* **34**, 194–198.

Saxinger, C., Polesky, H., Eby, N., Grufferman, S., Murphy, R., Tegtmeyr, G., Parekh, V., Memon, S., and Hung, C. (1988): Antibody reactivity with HBLV (HHV-6) in U.S. populations. *J. virol. Methods.* **21**, 129–208.

Shanavas, K.R., Kala, V., Vasudevan, D.M., Vijayakumar, T., and Yadav, M. (1992): Anti-HHV-6 antibodies in normal population and in cancer patients in India. *J. exp. Pathol.* **6**, 95–105.

Yadav, M., Ponniah, K., and Umamaheswari, S. (1990): IgG antibodies to human herpesvirus-6 in Malaysians. *J. Biosci.* **1**, 59–63.

Yanagi, K., Harada, S., Ban, F., Oya, A., Okabe, N., and Tobina, K. (1990): High prevalence of antibody to human herpesvirus-6 and decrease in titer with increase in age in Japan. *J. infect. Dis.* **161**, 153–154.

Yanagihara, R., Jenkins, C.L., Alexander, S.S., Mora, C.A., and Garruto, R.M. (1990): Human T lymphotropic virus type I infection in Papua New Guinea: high prevalence among the Hagahai confirmed by Western analysis. *J. infect. Dis.* **162**, 649–654.

Yanagihara, R., Ajdukiewicz, A.B., Garruto, R.M., Sharlow, E.R., Wu, X.Y., Alamaena, O., Sale, H., Alexander, S.S., and Gajdusek, D.C. (1991): Human T lymphotropic virus type I infection in the Solomon Islands. *Am. J. Trop. Med. Hyg.* **44**, 122–130.

Yamanishi, K. (1992): Human herpesvirus-6. Minireview. *Microbiol. Immunol.* **36**, 551–561.