Techniques Used to Demonstrate HHV-6 Antigens in PBMC and Liver tissue samples

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Detection of HHV-6-specific antigens in PBMCs:

PBMCs were isolated from EDTA blood samples by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient with a centrifugation of 400×g for 40 min at room temperature. The isolated cells from the resulting interphase were washed three times in phosphate-buffered saline. After washing, the cells were cytocentrifuged onto microscope slides. The density of the mononuclear cells was estimated to be approximately 10,000 cells per slide. After overnight drying at room temperature, the cellular specimens were fixed in acetone for 5 min at -20°C. The presence of viral antigens was demonstrated by an indirect three-layer immunoperoxidase staining and a monoclonal antibody against an early HH-6-specific antigen detecting both variant A and variant B (MAB8533; Chemicon International Inc., Temecula, CA) and another monoclonal antibody against the HHV-6 variant B virion protein of 101 kDa (MAB8535; Chemicon). The specificity of the antibodies has been described by Chemicon. Normal mouse-IgG was used as a control. A peroxidase-conjugated rabbit anti-mouse (Dako, Copenhagen, Denmark) and a peroxidase-conjugated goat anti-rabbit antibody (Tago Inc., Burlingame, CA) were used as second and third antibodies. The reaction was revealed by 3-amino-9-ethyl carbazole solution containing hydrogen peroxide, and Mayer's hemalum was used for counterstaining.

Detection of HHV-6 antigens in the liver biopsy specimens:

Liver biopsies were performed in the case of graft dysfunction to diagnose allograft rejection. For HHV-6 antigen detection, the liver transplant core needle biopsy material was snap-frozen, and sections (3–4 μm) were cut, acetone fixed, and stored at -20°C until used. Before staining, the sections were treated with chloroform to eliminate nonspecific reactions due to endogenous peroxidase. The presence of viral antigens was demonstrated by an indirect three-layer immunoperoxidase staining and a monoclonal antibody against an early HH-6-specific antigen detecting both variant A and variant B (MAB8533; Chemicon International Inc., Temecula, CA) and another monoclonal antibody against the HHV-6 variant B virion protein of 101 kDa (MAB8535; Chemicon). The specificity of the antibodies has been described by Chemicon. Normal mouse-IgG was used as a control. A peroxidase-conjugated rabbit anti-mouse (Dako, Copenhagen, Denmark) and a peroxidase-conjugated goat anti-rabbit antibody (Tago Inc., Burlingame, CA) were used as second and third antibodies. The reaction was revealed by 3-amino-9-ethyl carbazole solution containing hydrogen peroxide, and Mayer's hemalum was used for counterstaining.

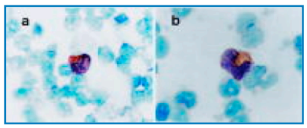

Figure 1. (a) An HHV-6 early antigen-positive lymphocyte in the cytocentrifuge preparation of PBMC demonstrated by indirect immunoperoxidase staining. (b) A monocyte demonstrating positive staining for HHV-6 B antigens. (Original magnification ×1000).