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No association between human herpesvirus 6 reactivation and cryptococcosis in human immunodeficiency virus-infected patients

Human herpesvirus 6 (HHV-6) is a part of the virus subfamily betaherpes. A recent *in vitro* study suggested that HHV-6 may dysregulate monocyte-mediated anti-cryptococcal defences with an overall pro-cryptococcus result (Cermelli *et al.*, 2006). Briefly, co-cultures of THP1/JJHAN cell lines were tested for anti-cryptococcal activity by c.f.u. inhibition assay. THP1/JJHANmock and THP1/JJHAN$_{HHV-6}$ co-cultures were exposed to *Cryptococcus neoformans* for 2, 3, 4 and 6 h. The co-cultures showed negative levels of anti-cryptococcal activity that were more marked for THP1/JJHAN$_{HHV-6}$ at 6 h. The results demonstrated that macrophages exposed to HHV-6 have reduced anti-cryptococcal activity.

Cryptococcosis is a major cause of opportunistic infections in Cambodian human immunodeficiency virus (HIV)-infected patients with severe immunodepression (Chhin *et al.*, 2004; Senya *et al.*, 2003). Indeed, the prevalence of cryptococcal infection among HIV-infected patients with a CD4$^+$ count <200 cells mm^{-3} was 18.0 % (59/327) in a recent Cambodian study (Micol *et al.*, 2007).

The HHV-6 seroprevalence is up to 90 % in children less than 2 years old in industrialized countries (Irving & Cunningham, 1990). No data are available concerning the HHV-6 seroprevalence in the Cambodian population. However, in Thailand the seroprevalence of HHV-6 was 88.1 % (185/210) in children under 12 years old (Bhattarakosol *et al.*, 2001). HHV-6 reactivation has been observed in HIV-infected individuals (Astriti *et al.*, 2006; Qavi *et al.*, 1995). Thus, we compared the prevalence of HHV-6 reactivation among 53 cases of cryptococcal infection and 105 controls without cryptococcal infection to validate or not *in vivo* data obtained *in vitro* by Cermelli *et al.* (2006).

In 2007, stored sera (Institut Pasteur du Cambodge) were used for HHV-6 and cytomegalovirus (CMV) DNA detection by quantitative PCR. DNA was extracted from 200 µl serum with the MagNA Pure Compact (Roche) following the MagNA Pure Compact nucleic acid isolation kit I protocol. We used the HHV-6 quantitative in-house PCR from the Virology Laboratory of the Pitié Salpêtrière Hospital (Paris, France) (Gautheret-Dejean *et al.*, 2002). The CMV real-time PCR was conducted according to the protocol used in the Unité de Virologie of the Hôpital Necker-Enfants Malades since 2001 (Leruez-Ville *et al.*, 2003). HHV-6 replication was defined as the presence of HHV-6 DNA in serum. Cryptococcal infection was defined by a positive cryptococcal polysaccharide agglutination test [in serum or cerebrospinal fluid (CSF)] and/or positive *C. neoformans* culture (blood, CSF or urine), or positive India ink direct examination of CSF. Continuous variables were presented with their median and interquartile range (IQR). Variables were compared between groups using the Mann–Whitney *U* test for continuous variables, and the chi-square or Fischer's exact test for categorical variables.

The median (IQR) age of patients was 34 years (31–37 years). The median CD4$^+$ count (IQR) was 14 cells mm^{-3} (6–32 cells mm^{-3}). The two groups presented no difference in age (median 35 versus 34 years), gender (males/females 34/19 versus 62/43) and CD4$^+$ count [14 cells mm^{-3} (IQR 6–24 cells mm^{-3}) versus 15 cells mm^{-3} (IQR 6–47 cells mm^{-3})]. The prevalence of positive HHV-6 PCR was 3.8 % (2/53) in the cryptococcal infection group and 0 % in controls (Fisher exact test $P=0.1$). The CMV DNAaemia prevalence was 56.6 % (30/53) and 55.2 % (58/105) in cases and controls, respectively ($P=0.9$). The viral loads of the two HHV-6 positive sera were high (5.4 and 6.0 log copies ml^{-1}) suggesting either reactivation with high replication or a possible chromosomal integration (Ward *et al.*, 2006). In addition there was no more CMV replication among patients with cryptococcal infection than among those without infection.

In conclusion, there was no *in vivo* association between HHV-6 replication and cryptococcal infection in Cambodian HIV-infected patients. Severely immunodepressed HIV-infected patients with HHV-6 or CMV DNA in their serum do not present an increased risk of developing cryptococcosis than appropriate controls.

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