

Serological and Molecular Detection of HHV-8 in Brazilian Populations

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Little is known about human herpesvirus 8 (HHV-8) distribution in Brazil. We used indirect immunofluorescence serological assays to determine HHV-8 seroprevalence in two Amerindian tribes from the Amazon region, and blood donors and Kaposi's sarcoma (KS) patients from the Campinas/SP (Southeastern region). Anti-HHV-8 antibodies were detected in 56.8% of the Amerindians (558/982), in all ages (0-81 years old) and both sexes. In these populations, high prevalence in children younger than 2 years old (44.4%) and children from 2 to 9 years old (35.0%) suggests non-sexual routes of HHV-8 transmission, through vertical transmission or contact to contaminated secretions. HHV-8 seroprevalence in blood donors from Campinas/SP was low (2.8%) and all positive cases were male (9/319) in the fourth and fifth decades of life. Curiously, these individuals were negative to routine serological tests applied in blood banks. Every KS patient assessed in our study was male, with average age of 37 years (27 to 79 years) and anti-HHV-8 positive assays in all cases.

In order to determine HHV-8 molecular prevalence, we analyzed DNA from three Amerindian tribes from the Amazon region, KS patients from Campinas/SP and HIV patients from Salvador/BA (Northeastern region). We used Nested-PCR to amplify HHV-8 ORF-26 region by molecular screening. Every ORF-26 amplified sample was also amplified to hypervariable ORF-K1 region for HHV-8 genotyping. We analyzed 384 DNA samples from Amerindian tribes and detected HHV-8 sequences in 3.8% (13/384). KS patients had all DNA samples from skin biopsies and 45.5% from peripheral blood (PBMC) amplified. DNA samples from 148 HIV positive patients were analyzed and HHV-8 sequences were detected in 4% of cases (6/148). Almost all positive DNA samples were amplified to ORF-K1 and determined HHV-8 subtypes.

Molecular techniques for amplification and sequencing of two fragments (VR1 and VR2) from ORF-K1 region made possible to build up phylogenetic trees and determine HHV-8 main viral subtypes (A, B, C, D and E) and its variants. Patients with KS from Campinas had subtypes A, B and C detected, with greater frequency of subtype C. Subtypes A and E were detected in Amazon Amerindians. This study is the first to perform genotyping in samples of HIV positive patients from Salvador, detecting subtype B and an unclassified subtype. Thus, it was possible to determine that HHV-8 subtypes A, B, C and E are present in Brazilian populations. As Brazil is a large country with variable population, culture and different geographical characteristics, more HHV-8 epidemiological studies are necessary to establish possible regional differences.

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Innate Immune Immunity and Viral Therapy of Cancer

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Activation of host innate immune responses following virus infection is largely mediated by viral dsRNA, the mechanisms of which remain to be fully determined. We have recently reported that murine embryonic fibroblasts (MEFs) lacking the death adaptor molecule FADD are defective in double-stranded RNA (dsRNA)-activated antiviral gene expression, including Type I interferon (IFN), and thus predisposed to virus infection. The dsRNA signaling pathway incorporating FADD was found to be largely independent of Toll-like Receptor (TLR)-3, tumour-necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and the dsRNA-dependent protein kinase, PKR, though obligated TBK1 activation of IRF3. The requirement for FADD in innate immune responses is evocative of the *imd* pathway in *Drosophila*, which involves an *imd*/dFADD complex that responds to bacteria

infection by activating the transcription of anti-microbial genes. Accordingly, cells lacking the mammalian *imd* homologue, receptor interacting protein 1 (RIP) were also found to be defective in Type I IFN induction and antiviral activity in response to virus/dsRNA, similar to FADD deficient cells. This data indicate the existence of a key intracellular dsRNA/virus recognition pathway in mammalian cells, which is central for the induction Type I IFN and the activation of other important primary innate immune response genes.

In addition, our laboratory has recently shown that vesicular stomatitis virus, VSV, a relatively non-pathogenic, negative-stranded RNA virus, can selectively induce the cytolysis of malignant cells, through the induction of apoptotic cell death. VSV appears able to selectively replicate in transformed cells since these hosts exhibit the hallmarks of a flawed interferon (IFN) system, which is essential for preventing VSV replication. In part, these cellular flaws may include prevalent defects in the regulation of cellular translation. Wild type VSV causes significant tumor regression when administered at sites distal from the tumor, when delivered intravenously, or against syngeneic tumors in immunocompetent hosts. The simple genetic constitution of VSV, lack of any known transforming properties, well studied immunobiology and the ability to genetically manipulate this virus affords an ideal opportunity to further enhance the oncolytic potential of this generally innocuous organism. Accordingly, we have constructed recombinant VSVs that carried cytokines such as IL-4, IL-12 or the Type I or II interferons. We have determined that such viruses were not only viable but synthesized their heterologous products to extremely high levels. In addition, all engineered viruses exhibited greatly increased attenuation, more potent oncolytic activity against metastatic disease in immunocompetent animals than the wild-type virus and were able to stimulate specific anti-tumor CTL responses. Collectively, our data demonstrates that VSV expressing immunomodulatory genes could provide a promising approach to cancer therapy and be useful tools for examining mechanisms of tumorigenesis.

Autologous Hematopoietic Stem Cell Transplantation for HIV-related non-Hodgkin's Lymphomas (NHL)

Henrique Bittencourt

Autologous hematopoietic stem cell transplantation (AHSCT) consist of a myeloablative chemotherapy followed by a rescue with autologous peripheral stem cell transplantation collected and cryopreserved before. The principle is to deliver a maximum tolerated dose of chemotherapy and rescue the bone marrow function with the infusion of hematopoietic stem cells. Data from the CIBMTR show that multiple myeloma, non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD) are the most frequent indications of AHSCT in North America. Specifically for NHL, AHSCT is indicated for patients in second remission or in chemosensitive relapse. A randomized trial published in 1995 showed that AHSCT provided superior overall and disease-free survival when compared to salvage chemotherapy alone. (Philip T, N Engl J Med 1995)

NHL is the second most frequent cancer in HIV patients. It is usually very aggressive, with a worst prognostic compared to non-HIV-related NHL. Difuse large-cell lymphoma and small non-cleaved cell lymphoma comprised more that 95% of the NHL in HIV-patients. Initial approach to treat HIV-related NHL using the same regimens employed in non-HIV patients. Toxicity was for more superior and results inferior. Next step was to decrease intensity of chemotherapy in order to reduce toxicity, maintaining the initial response rate. More recently, aggressive regimens (EPOCH, CDE) associated with HAART, improved response rate and survival, with acceptable toxicity. Nevertheless, more than half of patients eventually relapse. For this patients, salvage chemotherapy only, as in non-HIV patients, is only palliative and associated with high toxicity. Initial attempts using AHSCT for relapsed HIV-related NHL patients failed due to a high frequency of opportunistic infections and AIDS evolution. Furthermore, collection of peripheral blood stem cell (PBSC) was very difficult, due to the use of AZT. More