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Co-purification of soluble membrane cofactor protein (CD46) and human herpesvirus 6 variant A genome in serum from multiple sclerosis patients

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Abstract

The association of human herpesvirus 6 (HHV-6) and multiple sclerosis (MS) has been supported by several immunological and molecular studies. Recently, membrane cofactor protein (CD46) has been identified as the cellular receptor for the A and B variants of HHV-6. Elevated levels of soluble CD46 (sCD46) have been reported in the serum and CSF of MS patients. The aim of this study was to investigate a possible correlation between elevated levels of soluble CD46 and the presence of serum HHV-6 DNA in MS patients. An immunoaffinity column comprised of immobilized monoclonal antibodies to CD46 was developed to isolate sCD46 from cell free body fluids of MS patients and controls. After immunoaffinity purification, DNA was extracted from anti-CD46 column eluates and subjected to PCR amplification. Of the 42 MS samples tested, 4 serum samples were HHV-6 positive, 3 of which were typed as HHV-6A. The co-purification of sCD46 and HHV-6 DNA from MS sera indicates that HHV-6 is tightly connected to its receptor, CD46, in the serum of MS patients.
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Keywords: Human herpesvirus 6; CD46; Membrane cofactor protein; MCP; Multiple sclerosis

1. Introduction

The Human herpesvirus type 6 (HHV-6) is a β-herpesvirus for which seroprevalence rates vary from 72% to 100% in healthy adults worldwide (Clark, 2000). Two variants of HHV-6 (A and B) have been identified (Clark, 2000). These variants have an overall nucleotide sequence identity of 90%. The HHV-6B variant is the etiological agent for *exanthem subitum* (ES) or roseola, while the HHV-6A variant has not been associated with a specific pathology.

Multiple sclerosis (MS) is a chronic neurological disease of unknown etiology. Increased levels of anti-HHV-6 IgM antibodies has been found in MS patients compared to controls (Ablashi et al., 2000; Ablashi et al., 1998; Friedman et al., 1999; Soldan et al., 1997; Tejada-Simon et al., 2002). Additionally, several studies now support a role for the HHV-6A variant in MS. MS patients show presence of HHV-6A DNA in the serum and urine (Akhyani et al., 2000), HHV-6A specific proliferative responses (Soldan et al., 2000), reduced HHV-6A viral load after treatment with beta-interferon (Alvarez-Lafuente et al., 2004), presence of HHV-6A in CSF (Rotola et al., 2004) and in peripheral blood mononuclear cells (Kim et al., 2000). Collectively, these results suggest that active infection with the HHV-6A variant occurs in a subset of MS patients and may contribute to the pathogenesis of this disorder.

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One cellular receptor for HHV-6 has been identified as membrane cofactor protein (CD46), a regulator of the complement system that protects the cell from complement mediated lysis (Santoro et al., 1999). CD46 also serves as the cellular receptor for measles virus (Dorig et al., 1993; Naniche et al., 1993). CD46 belongs to a family of regulators of complement activation (RCA) that also includes decay accelerating factor (DAF; CD55) and protectin (CD59). CD46 acts as a co-factor for factor I cleavage of the activated complement components C3b/C4b. Two groups of the soluble form of CD46 (sCD46) with different molecular weights have been detected in body fluids (Hara et al., 1992; McLaughlin et al., 1996; Seya et al., 1995). The 30 kDa subunit is probably released from the cell membrane by clipping of membrane CD46 while the 45–70 kDa and can be produced by intron retention (Pollard et al., 1998; Seya et al., 1995). The source of serum sCD46 remains unclear, but sCD46 confers protection against complement-mediated lysis, although less efficiently than the membrane bound form (Kawano et al., 1999; Seya et al., 1995). Soluble CD46 (sCD46) is elevated in serum from MS patients (Soldan et al., 2001), patients with other autoimmune diseases (Kawano et al., 1999), and in malignancies (Seya et al., 1995) compared to healthy controls and to patients with other neurological diseases. MS patients with increased levels of sCD46 were statistically more likely to have tested positive for serum HHV-6 DNA (Soldan et al., 2001).

The aim of this study was to investigate the association between the elevated levels of sCD46 and the increased presence of HHV-6 DNA in serum of MS patients, by isolating and detecting HHV-6 DNA from the serum eluates of an anti-CD46 immunoaffinity column. We hypothesize that the concurrence of elevated levels of sCD46 and the presence of HHV-6 DNA in MS patients' sera may represent either HHV-6 bound to its receptor (sCD46) or the incorporation of CD46 into the HHV-6 virion during viral egress.

2. Materials and methods

2.1. Patient serum samples

Serum was obtained from a cohort of 20 healthy individuals and 20 relapsing remitting multiple sclerosis (RRMS) patients from the National Institutes of Health, Bethesda, MD. Of the 20 MS patients, 12 were women and 8 were men, all of Caucasian ethnicity. They had a mean age of 41 years, duration of disease ranging from 1 to 17 years (8 years in average) and a mean EDSS score of 2.4 (range 0–6). All 20 had definitive relapsing remitting MS according to the Poser criteria (Poser and Brinar, 2001).

As additional controls, 41 serum and cerebrospinal fluid (CSF) samples from patients with other neurological disease (OND) from NIH were used. These included 1 spongiform encephalopathy, 1 carential (Vitamin B12) peripheral polyneuropaphty, 2 vascular or degenerative encephalopathies, 1 normotensive hydrocephalus, 2 non inflammatory poliradiculopathies, 2 retrobulbar optic neuritis, 4 Parkinson's disease, 3 epilepsy, 4 amyotropic lateral sclerosis (ALS), 11 HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP), 10 patients with singular motor or sensory symptoms where MS has been ruled out. Serum was also collected from 30 patients with other inflammatory disease (including 1 adult stills, 3 familial mediterranean fever, 2 polymyositis, 3 psoriatic arthritis, 12 rheumatoid arthritis, 8 systemic lupus erythematosus (SLE), 1 TNF receptor associated periodic syndrome).

In addition, 22 serum and CSF was obtained from Italian MS patients (7 paired samples, 5 serum only, and 3 CSF only) and 32 samples from Italian patients with other neurological diseases (7 paired samples, 6 serum only, and 12 CSF only), including 1 normotensive hydrocephalus, 4 inflammatory polyradiculopathy, 1 peripheral polynerurophathy, 2 retrobulbar optic neuritis, 1 autoimmune encephalitis, 1 acute infectious encephalitis, 1 vascular encephalopathy, 1 infectious encephalitis, 2 vascular or degenerative encephalopathy, 1 peripheral and nerve paralysis, and 10 unspecified other neurological disorders. In total 42 MS samples and 123 control samples were tested.

The MS patients in both the NIH and in the Italian cohort were not on corticosteroid treatment or other immunoregulatory therapy at the time of serum sampling. Informed consent was obtained from all individuals under an IRB approved protocol. Ten of the MS patients in the NIH cohort selected for this study were previously found positive for HHV-6 DNA in their serum, while the remaining 10 had been tested negative for serum HHV-6 DNA (Berti et al., 2000; Soldan et al., 1997). The MS samples from the Italian cohort were selected at random and the presence or absence of HHV-6 DNA from these samples is unknown.

2.2. Determination of soluble CD46 in serum

The serum was run on an immunoaffinity column as described previously (Soldan et al., 2001). The pH of the 0.1 M citric acid eluted material captured by the a UV-light treated fraction collector was elevated from 2.5 to 7.5, snap frozen, and stored at –80 °C until DNA extraction was performed.

2.3. Extraction of DNA from the eluates of the CD46 column

The DNA was extracted by the QIAamp Viral RNA Mini Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions, using 560 μl of the serum eluate from the anti-CD46 column. A positive control comprised of HHV-6 DNA, diluted to levels at the detection limits of the primary PCR, but visible with the nested PCR, was run in parallel during the DNA extraction. As negative controls water samples were run in between every two or three samples during the DNA extraction.

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