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Herpes virus in Alzheimer's disease: relation to progression of the disease

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ABSTRACT

Studies regarding different viruses of the herpes family, such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), or human herpes virus 6 (HHV-6) in Alzheimer's disease (AD) are scarce. DNA from peripheral blood leukocytes (PBL) and brain samples were analyzed for the presence of CMV, EBV, or HHV-6. All samples were negative for CMV. EBV positivity was 6% in AD brains, whereas 45% of PBL samples from AD patients and 31% from controls were positive for EBV ($p = 0.05$). HHV-6 showed a 23% positivity in PBL samples from AD and 4% from controls ($p = 0.002$). 17% of AD brains were HHV-6 positive. Within a group of elderly individuals, followed up for 5 years, EBV-positive or HHV-6–positive PBL increased in those who developed clinical AD. Virus serological positivity was also investigated, and IgG levels for CMV and EBV antigens were also increased in those subjects who developed AD during the follow-up. Our findings suggest that EBV and HHV-6 may be environmental risk factors for cognitive deterioration and progression to AD in elderly persons.

1. Introduction

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder and the most common cause of dementia. According to the World Health Organization, it is estimated that nearly 35.6 million persons worldwide currently experience dementia. This number is expected to double by 2030 and to more than triple by 2050. Dementia affects individuals in all countries; with more than 50% of affected individuals living in low- to middle-income countries; by 2050, this figure is likely to rise to more than 70% (http://www.who.int/mediacentre/news/releases/2012/dementia_20120411/en/). Because of this urgency for effective preventive and therapeutic measures, extensive research has focused more on pathogenetic mechanisms of AD; however, until now, no therapy has been found. In recent publications (Licastro et al., 2011; Porcellini et al., 2010), we discussed genetic data from 4 genome-wide association (GWA) studies on AD (Harold et al., 2009; Hollingworth et al., 2011; Lambert et al., 2009; Naj et al., 2011). From these investigations, a set of single-nucleotide polymorphisms (SNPs) associated with AD emerged, and we suggested that the concomitant presence of these SNPs might result in a genetic signature predisposing to AD, via complex and diverse mechanisms each contributing to an increased individual susceptibility to herpes virus infection (Licastro et al., 2011; Porcellini et al., 2010). AD and control populations included in this study were all genotyped in the above-mentioned GWA studies. However, the limited number (AD patients, n = 100; controls, n = 300) precluded a separated genetic analysis of these 2 cohorts.

A viral etiology, especially involving herpes virus in AD, has already been investigated, and most studies have shown an association of Herpes simplex virus type 1 (HSV-1) with AD (Burgos et al., 2006; Carter, 2008; Itzhaki and Wozniak, 2008; Mori et al., 2004; Wozniak et al., 2009). It is interesting to note that other herpes viruses share the ability to become latent in the infected host and only latently infect neurons. However, investigations focused on different viruses of the herpes family, such as human cytomegalovirus (CMV), Epstein–Barr virus (EBV), or human herpes virus 6 (HHV-6) in AD are scarce.

CMV is ubiquitously distributed in the human population. This virus is also the most frequent cause of brain infection in infants by

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congenital virus transmission or in immune compromised patients (Tsutsui et al., 2008). Postnatal acute CMV infection is usually asymptomatic; but, once established, the virus remains latent in blood monocytes (Pawelec et al., 2009). CMV has also been associated with other chronic aging diseases, including cardiovascular disease, cognitive decline, and cancer. The specific mechanisms responsible for these associations have not been fully understood, although an immune and inflammatory component is likely (Simanek et al., 2011). Sero-conversion to positive CMV may vary over the years, ranging from 0.5 to 1.5% per year. It has been suggested that CMV is responsible for age-associated immune changes in elderly individuals, leading to a reduction in the number of naive T cells (Koch et al., 2006; Rymkiewicz et al., 2012). An increased rate of cognitive decline over a 4-year period in subjects with elevated CMV antibody levels has also been reported (Aiello et al., 2006). Previous studies on brain frontal and temporal cortex samples found that both AD patients and elderly healthy subjects were positive for CMV, without a statistically significant difference (Lin et al., 2002). However, CMV was found in the brain of a greater proportion of patients with vascular dementia than of normal elderly individuals, suggesting a role for this virus in the disease (Itzhaki et al., 2004).

EBV infects more than 95% of human beings within the first years of life. The virus causes acute (infectious) mononucleosis in a minority of immune-competent subjects, whereas the majority develop a lifelong asymptomatic infection, and the virus remains latent in B-lymphocytes. EBV is also involved in the development of several diseases such as Burkitt lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma (Kutok and Wang, 2006). Moreover, EBV seems to be involved in the pathogenesis of various neurological diseases, such as encephalitis, neuritis, myelitis, cerebellits, acute disseminated encephalomyelitis, or central nervous system (CNS) lymphoma in patients with human immunodeficiency virus (HIV) infection (Kleines et al., 2011) or multiple sclerosis (Lassmann et al., 2011). To the best of our knowledge, no data regarding the presence of EBV in AD are on record.

HHV-6 is a neurotropic virus and has been associated with multiple neurological diseases and disease conditions including seizures, encephalitis, mesial temporal lobe epilepsy, and multiple sclerosis (Yao et al., 2010). HHV-6 has been found in a higher proportion in AD brain than in age-matched controls (Lin et al., 2002). However, these findings were not confirmed in another investigation (Hemling et al., 2003), which reported higher HHV-6 levels in control brain.

Moreover, we were also interested in the immune response of the host to infections, since with aging the immune system undergoes significant changes after a process called immunosenescence, leading to an increased susceptibility to develop not only infectious diseases but also AD, osteoporosis, autoimmunity, and cancer (Lang et al., 2012). The seropositivity to CMV, EBV, or HHV-6 in general population is very high. However, no data regarding an association of seropositivity to these viruses in AD have been reported. Overall data regarding a possible association of CMV, EBV, or HHV-6 with AD are scarce and conflicting. In fact, it is difficult to find a linear association of serological positivity with AD, because of the high prevalence of seropositivity generally in the elderly population. Therefore, we decided to investigate the presence of CMV, EBV, and HHV-6 in DNA samples extracted from peripheral blood leukocytes (PBL) from a large cohort of patients clinically diagnosed with AD and age-matched controls, and DNA samples from frontal cortex of patients with definitive neurological AD diagnosis. Samples were analyzed by nested polymerase chain reaction (PCR) using specific primers for each virus and/or quantitative real-time PCR (qPCR).

We also investigated virus DNA presence in PBL samples and serological positivity in the same AD patients and healthy elderly controls. Our results indicate an association between DNA positivity and serological data with cognitive decline and AD conversion.

2. Methods

2.1. PBL samples

Patients (23 male, 81.43 ± 6.5 years of age, and 70 female, 84.70 ± 7.02 years of age) with clinical diagnosis of AD and elderly controls (CTR) (88 male, 77.42 ± 5.82 years of age, and 76 female, 77.47 ± 4.62 years of age) were enrolled from the longitudinal "Conselice study" (Ravaglia et al., 2001), as described elsewhere (Licastro et al., 2010a). Cognitive performance was measured according to the Mini-Mental State Examination (MMSE) at the baseline of the study (1999) and at the end of the 5-year follow-up (2004). Clinical diagnosis of AD followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (now called the Alzheimer's Association) (NINCS-ADRDA), as previously reported (Forti et al., 2001). Genomic DNA from circulating leukocytes was purified according to previously described procedures (Licastro et al., 2010a).

2.2. Serology

Plasma samples were collected from AD and CTR enrolled in the Conselice study. The serological assays included enzyme-linked immunosorbent assays (ELISAs) for CMV IgG, EBV Epstein–Barr nuclear antigen (EBNA) IgG, EBV viral capsid antigen (VCA) IgG, and HHV-6 IgG plasma levels. A total of 80 serum samples were assessed, according to the manufacture's recommendations, using commercially available assays, as follows: CMV IgG, ETI-CYTOK-G PLUS (DiaSorin, Saluggia, Italy); quantitative assay (antigens, inactivated HCMV AD 169); EBV EBNA IgG, ETI-EBNA-G (DiaSorin, Saluggia, Italy) quantitative assay (antigens, EBNA-1 synthetic peptides); EBV VCA IgG, ETI-VCA-G (DiaSorin, Saluggia, Italy) quantitative assay (antigens, mainly p18 synthetic peptide); and HHV-6 IgG, HHV-6 IgG ELISA kit (PANBIO, Waltham, MA) qualitative assay (antigens, inactivated HHV6).

We tested 2 different antigens specific for EBV (EBNA and VCA), to improve EBV detection sensibility.

2.3. Brain samples

Autoptic brain samples were collected from the Brain Bank of the Department of Neurosciences and Pathology, University of California–San Diego. Neuropathological diagnosis of AD was performed as previously described (Hansen et al., 1993), and followed the National Institute on Aging (NIA) and the Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria. AD also met the criteria of the DMS III-R and NINCDS-ADRDA (McKahnn et al., 1984). Autopsy was performed within 8 hours of death, as previously described (Corey-Bloom et al., 2000). The left hemibrain was fixed for 5 to 7 days, and the right hemibrain was frozen at −80 °C. Each brain was staged for degree of neuropathology according to modified Braak and Braak criteria (Braak and Braak, 1997). Tissue samples were taken from the midfrontal area of the neocortex. Genomic DNA was obtained from frozen hemibrain samples and was purified according to phenol-clorophorm standard extraction after overnight incubation with proteinase K.

2.4. Genotype assessment

Apolipoprotein E (APOE) genotyping for the ε alleles from leukocytes or brain DNA samples was assessed as previously described (Licastro et al., 1999; Licastro et al., 2007).