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A molecular hybridization study for the presence of Herpes simplex, cytomegalovirus and Epstein-Barr virus in brain and blood of Alzheimer's disease patients

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Summary

Among several hypothesis for the development of Alzheimer's disease is a viral hypothesis. The present study was designed to detect nucleic acid sequences for conventional viruses in peripheral blood cells and brain of Alzheimer's disease patients. DNA was isolated from peripheral blood cells and brain tissue from control individuals and Alzheimer's disease patients. Southern blot analysis was performed using radiolabeled probes for various conventional viruses. The results fail to detect the presence of Herpes simplex I (HSV-I), Herpes simplex II (HSV-II), Epstein-Barr virus (EBV) and cytomegalo virus (CMV) at a sensitivity level of detecting 1 genome/100 cells. We exclude conventional viruses as a cause of Alzheimer's disease at this level of detection.

Alzheimer's disease; Viruses; DNA; Dementia

Introduction

Non-reversible dementias, of which approximately half are represented by Alzheimer's disease (AD), are presently estimated to afflict over 4 million elderly individuals in the United States. This number will increase over the next several decades as the proportion of elderly persons in the population increases (Price, 1986). While recognized as a clinical entity for many years, the cause of AD remains unidentified. The possibility of a viral etiology for AD was initially suggested because some of the micropathological alterations in AD resemble those found in

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two chronic slow virus diseases, kuru and Creutzfeldt-Jacob disease (CJD). Despite displaying some clinical similarities, biochemical studies of the amyloid plaques found in the brains of AD patients fail to demonstrate the presence of prion protein that characterizes the plaques of several transmissible dementias (Prusiner, 1987). Non-specific biological indicators of viral infection include the demonstration of pathologic adhesiveness and amyloid phenomenon (Mozar, 1987). Viruses are known to alter cell surface protein and produce cell fusion. Similarly, Down's syndrome fetal tissue fibroblasts show cellular fusion in vitro culture system, as well as Alzheimer's brain extract causes increased cellular fusion (Moreau-Dubois, 1981). The evidence for amyloidotic phenomenon being related to infection comes from the known fact that amyloidosis is associated with chronic infection (Glenner, 1980). More supporting evidence comes from the observation that amyloidosis is seen in transmissible encephalopathies. However, some observations go against the theory of viral infection. There is no suggestion of viral infection in cerebral amyloidosis and also the amino acid composition of Alzheimer's amyloid and scrapie amyloid are quite different. Serological surveys of AD patients and their relatives have failed to demonstrate the presence of higher titer antibodies to common viruses (Renvoize, 1987). However, antibody surveys may not be useful since an infection that initiated damage could have occurred many years prior to the appearance of clinical dementia. While numerous attempts have been made to demonstrate the transmissibility of AD directly, all studies have eventually proven unsuccessful. Manuelidis et al., using a hamster model, reported that certain neuropathologic changes resembling those of CJD could be serially transmitted via the intracerebral injection of buffy coat cells from first degree relatives of AD patients (Manuelidis et al., 1988). In a related area, Pizzo et al. recently reported that the progressive dementias commonly seen in children with human immunodeficiency virus (HIV) infection can be dramatically reversed by treatment with the anti-viral drug 3'-azido-2'-dideoxythymidine (AZT, zidovudine) (Pizzo et al., 1988). Brunetti et al., also studying HIV-induced dementia, reported that AZT improves cortical glucose metabolism and neurologic symptoms (Brunetti et al., 1989). While AD dementia clearly differs from that associated with HIV infection, these latter two encouraging studies have led to the suggestion that AD patients who might have a virus-induced dementia, may benefit from antiviral drug treatment if the viral agent is found.

The findings that AD occurs in one of the five individuals over the age of 80 years suggests the possibility that a common viral pathogen might play a role in its development. For example, chronic central nervous system (CNS) sequelae secondary to rubella or measles is recognized to occur in a small number of genetically susceptible individuals. Herpes viruses can penetrate normal barriers and spread hematogenously or through peripheral nerves to cause acute encephalitis. Latent infections with these viruses have also been described in sensory ganglia. Besides having the ability to interfere directly with host cell functions, neurotropic viruses such as rabies, picornviruses and lymphocytic choriomeningitis virus induce immune and inflammatory responses leading to host cell death.

To explore the possibility that AD might represent an unusual or atypical response to a common virus, we examined peripheral blood and brain tissue from AD patients for the presence of integrated viral nucleic acid. The presence of

Epstein-Barr virus (EBV), cytomegalovirus (CMV) and Herpes viruses types 1 and 2 (HSV) was sought using specific cDNA probes and Southern blot analysis.

Material and Methods

Following informed consent by patients and responsible family members, blood was obtained from 5 patients with dementia of the Alzheimer's type attending the dementia clinic at Francis Scott Key Hospital of Johns Hopkins Institute. All AD patients included in this study met consensus clinical diagnostic criteria (McKhann et al., 1984). Blood from age-matched control individuals without dementia or neurologic symptoms was obtained from participants in the Baltimore Longitudinal Study of Aging at the National Institute on Aging. In addition to blood samples, 5 temporal cortex brain specimens were obtained at autopsy from patients with AD and 5 normal control individuals who had died from non-neurologic causes. The period between death and obtaining the brain tissue ranged from 1 to 24 h. All brain specimens were frozen at -70°C prior to use in this study. DNA was isolated from peripheral blood buffy coat cells and from brain homogenates using a modification of a previous procedure (Kunkel et al., 1977). Ten micrograms of DNA was digested with *Bam*HI (BRL, Gaithersburg, MD) according to the manufacturer's instructions. The digestion products were separated by electrophoresis on 0.8% agarose gels and transferred by capillary flow to a nylon membrane (Zeta-Probe, Bio-Rad, Richmond, CA). The blots were prehybridized in a solution containing $5 \times \text{SSC}$, 50 mM sodium phosphate (pH 6.5), $10 \times \text{Denhardt's solution}$ (0.2% Ficoll, 0.2% polyvinylpyrrolidone and 0.2% bovine serum albumin), 1 mg/ml denatured sheared salmon sperm DNA and 50% formamide at 42°C for 12 h and then hybridized in a solution containing $5 \times \text{SSC}$, 50% formamide, $2 \times \text{Denhardt's solution}$, 100 mg/ml denatured sheared salmon sperm DNA and the ^{32}P random primer translated plasmid containing specific probes for 16–24 h at 50°C . The following probes were used: the pGR30 plasmid containing a 3.5-kilobase (kb) insert including the thymidine kinase gene and corresponding to the *Bam*HI-O fragment from Herpes simplex type I (MP) (HSV-1), the pGR18 plasmid containing a 4.8-kb insert corresponding to the *Sal*II/*Hind*III G fragment of Herpes simplex type 11 (333) (HSV-2) (Reyes et al., 1982), the pSL76 plasmid containing 2 tandem copies of a 3.2-kb insert corresponding to the *Bam*HI-W fragment from Epstein-Barr virus strain B95-8 (Ambinder et al. 1985) and the pRL3 plasmid containing a 16.2-kb insert corresponding to the *Bam*HI-C fragment from CMV (Towne strain) (Lafemina et al., 1980). Neurofilament cDNA (ATCC, Gaithersburg, MD) was used as a control probe. To avoid generating false positive results, the viral probes were specifically selected for lack of homology with normal human genomic DNA (Peden et al., 1982). The sensitivity of the hybridization assay was examined by adding from 1 pg to 100 ng of viral insert containing plasmid (pGR30, pGR18, pSL76, pRL3) to aliquots of genomic DNA prior to *Bam*HI digestion. Viral DNA dilutions served as positive controls. After annealing, the membranes were washed once in $2 \times \text{SSC}$, 0.1% SDS at room temperature for 30 min, twice in $0.1 \times \text{SSC}$, 0.1% SDS at 55°C for 30 min and finally in $0.1 \times \text{SSC}$, 0.1% SDS for 30 min at 65°C . Autoradiography was carried out for 1–14 days at -70°C with enhancing filters.

Results

Southern blot analysis of DNA extracted from blood and brain tissue from AD and control subjects failed to produce a hybridization signal with viral DNA probes specific for HSV-1, HSV-2, EBV and CMV. Because of the uniformly negative hybridization results, the sensitivity of our hybridization technique was examined using a series of controls. Varying concentrations of plasmid containing the viral probes were added to the genomic DNA prior to restriction enzyme digestion. A strong hybridization signal was seen with as little as 1 pg of viral insert containing pBR322 (Fig. 1). The same filter reprobed with control neurofilament probe showed a presence of a band in all lanes. Figure 1 displays the results of a typical experiment with the pGR30 (HSV-1) probe. Similar results were obtained with HSV-2, EBV and CMV probes. In addition, ethidium bromide staining of the agarose gel containing DNA samples showed good quality of DNA.

Discussion

There have been many previous studies attempting to identify virus in brain or blood of AD patients or their relatives. While there continues to be important research examining the transmissibility of neuropathologic brain lesions in AD and other dementias, most recent studies directly searching for viral agents use virus specific cDNA probes, which in addition to being highly sensitive, can detect incomplete or non-infectious viral nucleic acid sequences in latent as well as actively infected tissue. In the present study, Southern blot analysis of *Bam*HI digested DNA from the brain and peripheral blood of AD patients and normal controls failed to

Fig. 1. Representative autoradiograph of a Southern blot of brain DNA from normal and Alzheimer's disease patients. DNA specimens were digested with *Bam*HI and probed with pGR18 plasmid that contains a 4.8-kb insert corresponding to the *Sal*I-*G*/*Hind*III fragment of herpes simplex, type II (333) virus. Lanes A-G demonstrate positive hybridization with dilutions of the probe. Lane A, 100 ng; Lane C, 10 ng; Lane D, 1 ng; Lane E, 10 μ g; Lane F, 10 pg; Lane G, 1 pg of pBR322 containing the pGR18 plasmid. Lanes H-J contain brain DNA from Alzheimer's disease patients and Lanes K-M contain brain DNA from normal control brains. No hybridization was detected with any of the brain specimens.

detect presence of HSV-I, HSV-II, EBV or CMV virus. This contrasts with the abstract of Rogers et al., which reported the presence of herpes virus in 10/10 AD brains but does agree with another recent hybridization study that did not find viral DNA sequences in 18 AD brain specimens (Rogers et al., 1987). Jamieson et al. reported the presence of Herpes simplex virus type 1 (HSV1) DNA in AD brains (Jamieson et al., 1991). Other molecular probe studies including that of Taylor and Crow have failed to detect viral sequences by DNA spot hybridization (Taylor and Crow, 1984; Taylor, 1986). One possible explanation to account for the positive results may be the use of viral DNA probes which have unrecognized homologies to normal human genomic sequences. This problem, if unrecognized, can create the potential for false positive interpretation of hybridization results. To avoid this problem in the present study, we carefully selected viral DNA fragments as probes that do not have homology with normal human genomic sequences.

Despite its high frequency and the large number of studies examining possible etiologies, the cause(s) of AD remains unknown. For many reasons, a viral pathogenesis still appears both attractive and reasonable. Ball et al. suggest 'repeated reactivation of latent or dormant herpes virus from trigeminal ganglia spreading first to mesial temporal lobe, only secondarily to other limbic structures and then to neocortical regions (Ball, 1982, 1986). This may represent an etiological infrastructure for clinical presentation of AD'. Neuropathological changes have been documented in hamsters injected with buffy coat from patients early in the course of AD or from their relatives (Manuelidis et al., 1988). Certain common viruses, particularly herpes viruses that are neurotrophic, produce latent infection and are widely distributed in the population have been repeatedly discussed and experimentally sought as possible etiologic agents in AD. Herpes simplex has a predilection for the mesial temporal lobe, the area also most commonly involved in AD. Also, patients with bilateral temporal and basal forebrain damage, develop multimodal amnesia (Damasio et al., 1985). The histological changes like neurofibrillary tangles, possibly a reflection of previous virus infection, also occur in the hippocampus first. Whereas the studies opposing this theory, summarized by G.W. Roberts refer to inability to find immunologic or molecular evidence for the presence of the virus (Middleton et al., 1980; Esiri, 1982; Mann et al., 1983; Taylor et al., 1984; Roberts et al., 1986; Pogo et al., 1987). Recent studies by Friedland et al. failed to find presence of antibodies against other viruses such as lentiviruses, human immuno deficiency virus, caprine arthritis encephalitis and equine infectious anemia virus in serum and cerebrospinal fluid of AD patients (Friedland et al., 1990).

Although viral etiology is still a possibility for development of AD, we conclude that the conventional viruses such as HSV-I, HSV-II, EBV and CMV are not detectable in AD blood and brain at the sensitivity of detecting virus at less than 1 genome/100 cells. The similarity of neuropathological changes in a variety of transmissible dementias suggest an association that warrants further investigations.

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