Antibodies to choroid plexus in senile dementia of Alzheimer's type

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Abstract

Aims: To investigate whether autoantibodies to choroid plexus are present in human senile dementia.

Methods: Serum samples from 40 elderly people presenting with characteristic, diagnostic criteria of senile dementia of Alzheimer's type and 20 age matched healthy controls were tested by indirect immunofluorescence for the presence of autoantibodies to choroid plexuses, using frozen sections of rat or human fetal brain tissue.

Results: Significant labelling of choroid plexus basement membrane was observed in 17 of the 40 samples from patients with senile dementia; in the control series one sample of rat but not human plexus labelled positively ($p < 0.01$).

Conclusions: The antibodies identified in this series of patients with Alzheimer's disease suggest that autoimmune mechanisms might be responsible for some of the changes in cerebrospinal fluid production described in this disorder.

Choroid plexuses are highly vascularised structures that contain numerous capillaries surrounded by a basement membrane which separates them from overlying epithelial cells. They are active in filtration and secretion (vitamins B12, B6, C and E, thyroxine, folates, prealbumin) a process important in the production and delivery of cerebrospinal fluid into the brain. Several changes in the choroid plexus have been described in relation to age.^{1,2} They include a proliferation of the underlying connective tissue, flattened epithelial cells, focal stratification, and the appearance of intraepithelial lipid vacuoles, psammoma bodies, or cysts. These alterations are considered responsible for the decrease in cerebrospinal fluid production from 0.41 ml/mn at age 28 to 0.19 ml/mn at age 77.³ In senile dementia syndromes,^{4,5} mainly of Alzheimer's type, scintiscintigraphic studies have shown a change in cerebrospinal fluid hydraulics, suggesting that the choroid plexus could be altered in these diseases. This notion is also sustained by the lowered secretion of prealbumin reported by Riisøen.⁶ Because autoantibodies increase with age,⁷ and neuron binding antibodies had been described in Alzheimer's disease,⁸ we hypothesised that plexus related antibodies could be involved in

the changes in cerebrospinal fluid production which occur in this disorder.

Methods

Two groups were tested. Group I included 40 elderly people (mean age 84.8, range 74 to 94 years) from a nursing home, presenting with characteristic features of senile dementia of Alzheimer's type (SDAT). There were eight men and 32 women and they fulfilled the diagnostic criteria of the NINCDS-ADRDA work group.⁹

Group II was a control series of 20 age matched (mean age 81.5, range 75 to 90 years) psychologically, and neurologically normal individuals with various non-neurological disorders. All had a Folstein mini-mental status higher than 24.

Serum samples from each individual were forwarded to the immunology laboratory where aliquots were kept frozen at -30°C until tested.

TISSUE SAMPLES

Choroid plexus samples were obtained from two sources. One series was obtained by dissecting the brain of male Wistar rats. The animals had been anaesthetised with barbiturates, killed by cervical disruption, and dissected within a few minutes. Choroid plexuses were readily identified in the lateral ventricles, and were excised and immediately snap frozen in liquid nitrogen. They were then kept at -80°C until used.

Another series of tests was performed using human fetal choroid plexus. This tissue was obtained from a medical abortion product after consent of the local ethics committee had been obtained. The choroid plexus was excised and similarly snap frozen and transferred to the immunology laboratory, where it was then kept at -80°C .

IMMUNOFLUORESCENCE STUDIES

A classic indirect immunofluorescence technique was used. It was applied to all serum samples, using freshly prepared, frozen cut $4\text{ }\mu\text{m}$ thick sections of choroid plexus. These sections were obtained at -30°C , collected on clean glass slides, air dried and rapidly fixed for 45 seconds in a microwave oven. The first section of each sample was checked histologically after rapid staining with toluidine blue. The epithelial and vascular structures of choroid plexus were clearly visible in all cases.

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Serum samples were first diluted 1 in 16 in phosphate buffered saline (PBS), then incubated on choroid plexus sections for 30 minutes at room temperature in moist chambers. This incubation was followed by three washes in PBS. The slides were then covered for 30 minutes with fluorescein isothiocyanate (FITC) conjugated rabbit serum to human IgG, IgA, and IgM (Biosys, Compiègne, France). This second incubation was followed by another series of three washes in PBS. The slides were then mounted on PBS/glycerol (7/3) and observed under ultraviolet light microscopy within a few hours. Examination was performed blind by two different observers using microscopes equipped with Ploem systems of epi-illumination (Olympus BH2, Tokyo, Japan, and Leitz Orthoplan, Weitzlar, Germany). Results were expressed qualitatively.

CONTROLS

The serum samples were further routinely tested for the presence of other autoantibodies, using freshly prepared frozen cut sections of mouse or human tissues. This control series included mouse kidney sections for the identification of anti-nuclear, anti-mitochondrial, and anti-smooth muscle antibodies, as well as human skin, lung, kidney and normal brain.

An enzyme linked immunosorbent assay (ELISA) kit for the detection of antigens to collagen antibodies present in Goodpasture's syndrome was used to test the patients' samples (Biocarb, Lund, Sweden). In this test, ELISA microwells are coated with collagen IV fractions, and the presence of specific IgG is identified by an immunoenzymatic reaction.

The distribution of specific antibodies was compared between patients and controls using the Pearson/Yates correction of the χ^2 test.

Results

The figure shows the characteristic linear fluorescence pattern observed along the base-

Indirect immunofluorescence staining of rat choroid plexus by serum from a patient with Alzheimer's type senile dementia.

ment of choroid plexus in 17 of the patients and one of the controls ($p < 0.01$). These data were observed on sections of rat plexus. The labelling was fainter in a few instances, and more fragmented in five cases. It was, however, not granular, and appeared very similar to the linear fluorescence patterns that are sometimes seen on the glomerular basement membrane in Goodpasture's syndrome or on the skin basement membrane in bullous pemphigoid.

Thirteen of these 17 serum samples yielded the same type of fluorescence pattern on fetal choroid plexus tissue. Four remained negative, as did the control sample which was positive on rat choroid plexus.

Five patients and one control also had antinuclear antibodies. None of the sera tested was positive on skin, lung, or kidney sections. The Goodpasture ELISA test was negative in all cases. Finally, no appreciable labelling was observed on normal brain sections.

Discussion

Immunological disorders are common in elderly people,⁷ and often develop as autoimmune alterations. This was confirmed in our series of patients with SDAT: 12.5% of them had antinuclear antibodies, an incidence similar to that of previous studies. The appearance of anti-basement membrane antibodies is, however, not typically associated with aging. It is probably rare in mentally healthy individuals, as such antibodies were detected in only one of our 20 controls (5%). This suggests that the high rate observed in the group of SDAT patients (42.5%) could be indicative of immunologically mediated alterations of the choroid plexus. The relevance of this observation is strengthened by the high specificity we observed for these antibodies. Basement membranes are complex structures, rich in such macromolecules as type IV collagen, laminin, heparan sulphate, proteoglycans. Their composition varies according to the anatomic structure in which they are found. For instance, the "bullous pemphigoid antigen" is selectively observed in skin samples. In hamster brain, the monoclonal antibody directed against the sulphated glycosaminoglycan (*gag*) chains of heparan sulphate proteoglycan recognises the basement membrane of choroid plexus, but not that of brain capillaries.¹⁰ In Goodpasture's syndrome, three different monomers (respective molecular weights of 24, 26, and 28 kilodaltons) and two dimers (of 44 and 50 kilodaltons) are recognised by the patients' sera in renal glomerules and tubules, as well as in the lung, placenta, and aorta, while only one monomer (28 kilodaltons) and one dimer (50 kilodaltons) are reactive in choroid plexus sections.¹¹ This tissue selectivity was confirmed in our series, where only choroid plexus membranes reacted with the patients' sera. No capillary labelling was seen in brain sections. This suggests that the antibodies identified in this series are different from those reported by Fillit, which reacted with the basement membrane of brain capillaries.¹²

The choroid plexus structure identified in our series also seems to be well conserved, as similar results were obtained using rat or human plexus, and it appears to be an antigen that appears early in development because the fetal brain tissue used was from a 23 week old conceptus.

Choroid plexuses are seldom involved in immunopathological disorders, but the deposition of autoantibodies or immune complexes has been reported before. An experimental model developed in the rabbit was reported by Koss *et al*¹³: injection of bovine serum albumin and Freund's complete adjuvant resulted in the development of acute serum sickness, and linear and/or granular deposits of IgG could be identified along the choroid plexus basement membrane. A lymphoplasmocytic infiltrate was also present in this area. Several disorders in man have been reported to involve the choroid plexus. Sisson *et al* described a specific labeling of the lamina rara interna in Goodpasture syndrome.¹⁴ In 75% of a series of patients with hepatosplenic schistosomiasis Pitella *et al* observed linear or nodular deposits of IgA, IgG, and C3, along the thickened basement membrane of choroid plexus.¹⁵ The same authors reported similar deposits in 10 out of 12 patients with liver cirrhosis,^{16 17} and in a post mortem study of the brain of 25 patients with hypertension, showed segmental granular deposits of immune complexes in five cases. Histological observation of these same samples also showed a periodic acid Schiff (PAS) positive homogeneous substance, located near the thickened choroid plexus basement membrane in 12 cases. These authors concluded that hypertension had increased vascular permeability, permitting the filtration of immune complexes later trapped by immunoglobulin and complement receptors. Deposited immune complexes were also described along the basement membrane between endothelial and epithelial cells in a series of patients with systemic lupus erythematosus (SLE).¹⁸ Transmission electron microscopy showed interstitial dense deposits between the two cell layers, while no lesions were seen in brain tissue. Peress and Levine hypothesised that in SLE the deposition of immunoglobulins resulted in an increased permeability of the blood-brain barrier, allowing the filtration of such macromolecules as brain specific autoantibodies which led to the frequent neuropsychiatric disorders reported in this disease.^{19 20} It is striking, however, that such mental anomalies do not develop in schistosomiasis or Goodpasture's syndrome.

In SDAT several authors failed to demonstrate alterations of the blood-brain barrier.^{21 22} It could be that the autoantibodies we observed had not developed in such patients,

or were not prominent enough to result in measurable alterations. Our hypothesis of an autoimmune component in the development of Alzheimer's disease is supported by several reports, and, indirectly, by a recent report from McGeer *et al*.²³ These authors described the striking absence of SDAT in patients with rheumatoid arthritis, suggesting that patients with SDAT might benefit from the immunosuppressive regimens administered in rheumatoid arthritis or Goodpasture's syndrome.

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