ANTIBRAIN ANTIBODIES IN ALCOHOLIC PATIENTS

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Abstract

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Abstract—Sera of 30 chronic alcoholic patients and 30 age-matched and gender-matched controls were examined for antibodies to brain tissues. We performed an indirect immunofluorescence assay using the patients' and controls' sera as first antibodies, and fluorescein-conjugated anti-human-immunoglobulin Ig-A,Ig-G and Ig-M as second antibodies, on frozen sections of normal human brain. Binding to neuronal cell nuclei of frontal cortex and septal area was found in 40% of patients, but only in 6.7% of controls. The antibodies belonged to the IgM, and additionally sometimes the IgA and IgG subclass. The relevance of these antibodies for the development of brain disease in chronic alcoholic patients is discussed.

INTRODUCTION

Immunologic changes are well known in alcoholic patients. For instance, renal IgA-deposits have been described (Bene *et al.*, 1988; Lomax-Smith *et al.*, 1983; Nikolaev *et al.*, 1986), autoantibodies have been found against hepatocytes (Vierna *et al.*, 1985), muscle cells (Cunningham *et al.*, 1985) or parts of the cytoskeleton (Kurki *et al.*, 1984). Antinuclear factors have been described (Faber and Elling, 1967; Gluud *et al.*, 1984; Macgeorge *et al.*, 1984), as well as increases in B-cell numbers (Valenski *et al.*, 1989). Besides these features, there are hints for the central nervous system being involved in immunologic alterations: the blood–brain barrier is impaired in alcoholic patients (Kornhuber *et al.*, 1987) and an immunologic reaction against S-100 protein has been described (Jankovic, 1985). Maternal autoantibodies have been claimed to be responsible for the development of the fetal alcoholic syndrome (Foster, 1986).

When we performed an immunologic study on schizophrenic patients, we found antibrain antibodies not only in the patients, but also in two controls who were suffering from alcoholism (Henneberg *et al.*, 1991). The antibodies were mainly directed to the septal area and frontal cortex. As we were aware of the resemblance in symptoms between alcohol hallucinosis and paranoid schizophrenia (Soyka, 1990), we decided to perform a separate study to look for brain autoantibodies in the sera of alcoholic patients. Thirty patients with chronic alcohol problems and 30 age-matched and gender-matched controls were included using a blind indirect immunofluorescence assay.

MATERIALS AND METHODS

Thirty patients who were admitted to a psychiatric ward with chronic alcoholism (according to DSM-III criteria) were studied after giving their informed consent. The mean duration of disease was 13.4 years (SD = 7.2), additional data are given in Table 1. Ten millilitres of blood were drawn the day after admission, centrifuged at 400 *g*, and the sera stored at −20°C.

Thirty low back pain patients at admission (drug-free) and normal subjects, who were matched in age and gender, served as controls (Table 2). The controls had no drinking or other psychic problems, and neither in patients nor in controls were any autoimmune diseases identified.

Brain tissue was obtained from a 39-year-old

Table 1. Concomitant diseases in the alcoholic patients

Diseases	%
Liver involvement	87
Neuropathy	47
Pyelonephritis	37
Pancreatitis	23
Drug abuse	13
Epilepsy	13
Hallucinosis	7
Lues cerebrospinalis	3
Gastritis	0

The percentage of patients suffering from other diseases beside alcoholism are shown. Most of them showed liver, some pancreas involvement as proven by increase of serum aspartate amino-transferases and amylase levels. One patient suffered from lues cerebrospinalis proven by a lumbar puncture, the other diagnoses were based on clinical features.

patient who died of acute myocarditis. Frontal cortex and septal area were collected 8 hr postmortem, immediately frozen in liquid nitrogen and stored at −80°C. The tissues did not show any macroscopical or histological abnormalities. Brain tissues of two other patients (72 and 68 years of age) dying from pulmonary embolism and sudden heart failure, respectively, were prepared in the same way. The indirect immunofluorescence assay was performed as follows: 7 μm sections of frontal cortex and septal area were fixed in 99% ethanol (15 min, 4°C) and incubated with patients' or controls' sera. After five washings with phosphate-buffered saline (PBS), they were incubated with a second antibody (fluorescein-isothiocyanate-conjugated anti-human-IgA, IgG, IgM, Dakopatts, DK-Kopenhagen). After another five washings, samples were evaluated with a Zeiss fluorescence microscope. Patient and control sera were tested the same day in a blind setting.

The following controls were included in each experiment: routine stain of the brain with hematoxylin-eosin to check histological intactness of tissues. Binding of a monoclonal mouse-anti-neurofilament antibody to the same brain areas to prove preserved antigenicity. Binding of second antibodies to human IgA, IgG or IgM, coupled to cyanogenbromide-activated Sepharose (Pharmacia, Stockholm). Only experiments with 99–100% binding of the second antibodies were evaluated. To exclude nonspecific binding we incubated brain samples with PBS and second antibodies or PBS alone in every experiment. To recognize tissue-independent general antinuclear factors we tested every serum on parietal cells (Viramed, Martinsried, F.R.G.) in parallel to brain tissue, as autoantibodies to these cells had been described to be the most common in alcoholic patients (Forbes *et al.*, 1987). When we tested all of the positive sera and five of the negative sera against septal area and frontal cortex of two different patients we included controls on commercial HEp 2 cells (Viramed, Martinsried, D) to exclude a general antinuclear factor-binding. The data of patients and controls were compared using the Student's *t*-test.

Table 2. Distribution of age and gender in patients and controls

	Gender	Age (years)
Patients	7 f, 23 m	39.4 (2 21)
Controls	7 f, 23 m	39.8 (2.27)

Patients and controls were age- and gender-matched. Mean ages with SEM in parentheses are shown.

Table 3. Antibrain antibodies in alcoholic patients

Patients	Gender	Age (years)
Positive	3 f, 9 m	38 (3.3)
Negative	4 f, 14 m	40 (3.0)

The distribution of age and gender is shown in antibody-positive and antibody-negative patients. The mean ages with SEM in parentheses are shown. There are no statistically significant differences.

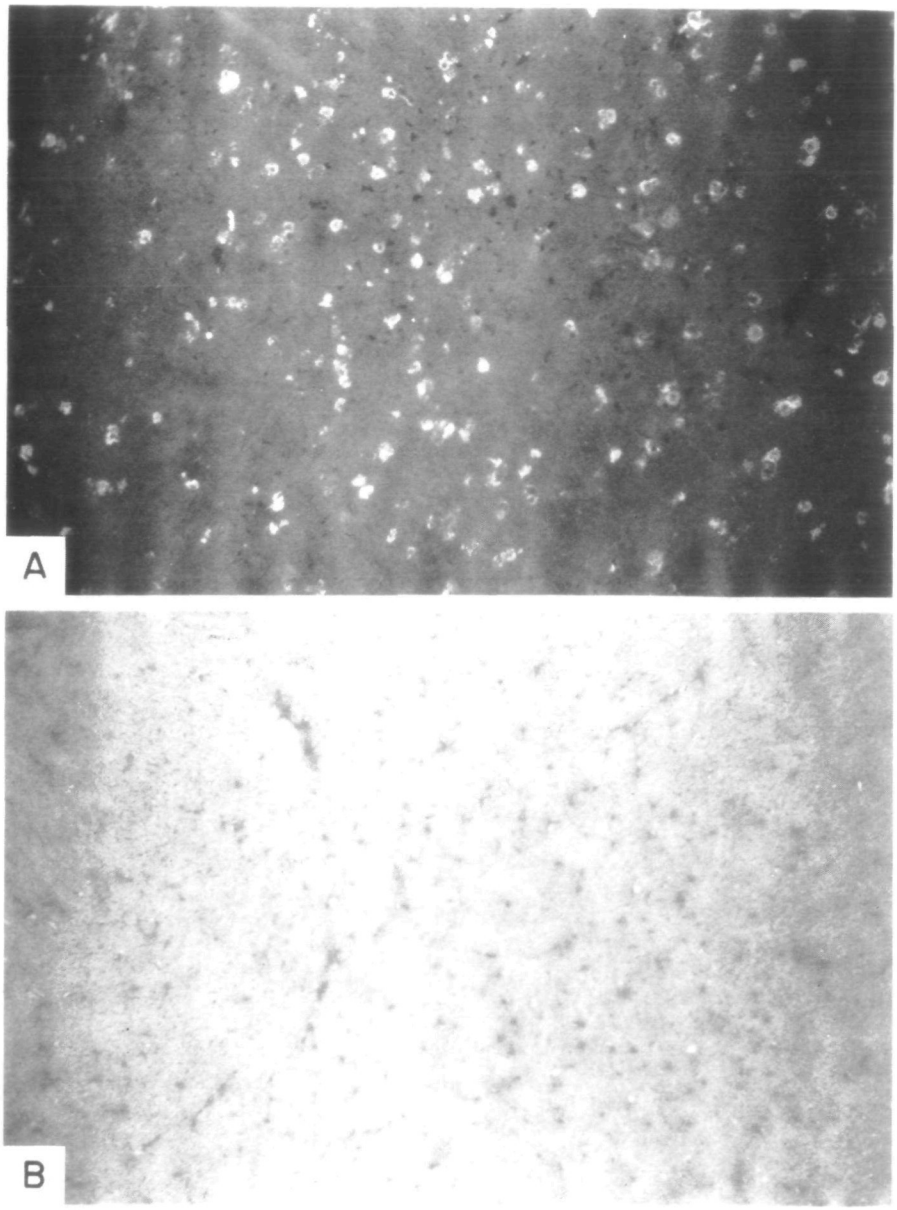

Fig. 2. Frozen sections in the immunofluorescence assay: antibody-binding is shown from a positive patient (a) and a negative control (b) (×160, Ilford 400 negative film, exposure time 2 min).

RESULTS

We found binding to neuronal nuclei in 12 of 30 patients and only 2 of 30 controls ($P < 0.01$; Fig. 1). Binding was directed to neuronal nuclei in frontal cortex (2 patients), septal area (2 patients) or both tissues (8 patients) in the form of sharp-limited spots or rings (Fig. 2). It was mediated by IgM-antibodies in all cases, in four patients additional IgA-binding, in three patients additional IgG-binding was found (Fig. 3). In two positive patients antiparietal cell antibodies also occurred but in one of the two cases they were mediated by IgG-antibodies, while the antibrain antibodies belonged to the IgA/IgM-compartment; in the other case the class of antiparietal cell antibody was not determined. None of the patients showed binding to the HEp 2 cells. When the 12 positive sera and 5 of the negative sera were retested on brain tissues from different patients, they were found to be similarly positive and negative, respectively. Sero-positive and sero-negative patients did not differ significantly in gender or age (Table 3). The two patients with alcohol hallucinosis belonged to the positive group, while the other clinical parameters described in Table 1 could not be correlated with the presence or absence of antibrain antibodies. Interestingly, neither the patients with occasional seizures nor the one with lues cerebrospinalis belonged to the positive group.

DISCUSSION

Immunologic alterations have been described in alcoholic patients (Bene *et al.*, 1988; Cunningham *et al.*, 1985; Faber and Elling, 1967; Gluud *et al.*, 1984; Kurki *et al.*, 1984; Lomax-Smith *et al.*, 1983; Macgeorge *et al.*, 1984; Nikolaev *et al.*, 1986; Valenski *et al.*, 1989; Vierna *et al.*, 1985) and hints for the involvement of brain tissue in autoimmune processes are to be found (Jankovic, 1985; Kornhuber *et al.*, 1987). As we had seen antibrain antibodies in the sera of alcoholic patients who had been used as controls in a previous study (Henneberg *et al.*, 1991), which found antibrain antibodies in patients with paranoid schizophrenia, and as chronic alcoholism sometimes leads to hallucinosis, which in many cases bears a close symptomatic resemblance to paranoid schizophrenia (Soyka, 1990), we decided to examine the sera of 30 alcoholic patients on the same tissues for the occurrence of antibrain antibodies. To exclude bias, 30 age-matched and gender-matched controls were included in a blind indirect immunofluorescence assay.

In 40% of patients and only 6.7% of controls were antibrain autoantibodies found. These were mainly of IgM, but also of IgA and IgG subtypes, and were directed to neuronal nuclei in the septal area and frontal cortex. They could be differentiated from nonspecific binding described by Aarli *et al.* (1975) by shape

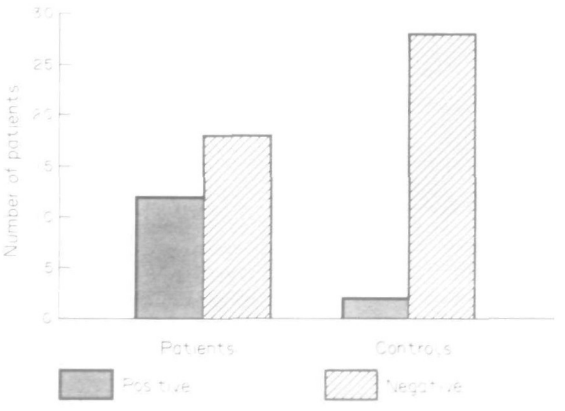

Fig. 1. Antibody-binding in patients and controls

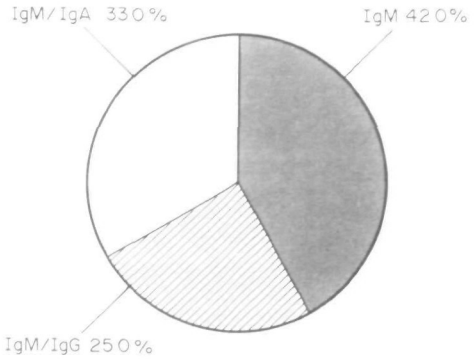

Fig. 3. Distribution of gamma-globulins in positive patients.

and also from the binding we had found in the sera of schizophrenic patients (Henneberg *et al.*, 1991). A nonspecific binding of antinuclear factors can be excluded by the absence of a reaction of positive sera with nuclei of parietal cells in most cases, and negative results on HEp 2 cells in all of the positive patients. Interestingly, both patients with known alcohol hallucinosis belonged to the positive group. Otherwise no correlation to clinical parameters could be drawn.

There are two explanations for the occurrence of antibrain antibodies in alcoholic patients. The first is, that alcohol is toxic to the blood–brain barrier and to neuronal cells and neo-antigenic determinants are recognized by the immune system. This model might explain deficits found in Wernicke's encephalopathy and in Korsakow's syndrome, but it fails to explain the symptoms of alcohol hallucinosis, especially since biochemical disturbances of dopamine receptors have not been found in rats after experimental exposure to alcohol (Hietala *et al.*, 1990). We prefer the other possible explanation: experiments on rats have proven that there are biochemical alterations of hepatocyte surfaces after chronic alcohol intake (Parafita *et al.*, 1989). Another group has described antibodies to various acetaldehyde modified proteins in rats after chronic alcohol administration (Worrall *et al.*, 1989). The presence of gammaglobulins on hepatocytes has been proven in man (Trevisan *et al.*, 1983). Therefore, we conclude that chronic alcohol intake leads to the development of neoantigens in brain areas in man. The emergence of autoantibodies against those structures, which show cross-reactivity with the natural antigens, gradually impairs and later destroys neurones.

This hypothesis must be proven by longitudinal studies using different brain areas, and the number of patients suffering from alcohol hallucinosis must be extended, but it might open an interesting field of therapeutical possibilities in chronic alcoholic patients.

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