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Antibodies to Brain Tissue in Sera of Schizophrenic Patients – Preliminary Findings

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Summary. The sera of 30 patients suffering from schizophrenia (DSM III) and 30 neurological controls were tested for antibrain antibodies in a blind indirect immunofluorescence assay. We found IgG- and IgM-binding in the sera of 22 patients, but only 4 out of the 30 age- and sex-matched controls. The binding was mainly directed to neurons from the frontal cortex and septal area, areas, which are regarded as important in the development of schizophrenic illness. These preliminary data are presented, to encourage other immunological studies in schizophrenia research.

Key words: Schizophrenia – Pathogenesis – Autoimmune disease

Introduction

In some respects, schizophrenic disorders have the appearance of an autoimmune disease: the relapsing-remitting course, genetic studies, that have shown an autosomal multigenic transmission [9], disturbances of the blood-brain-barrier [3, 27]. Some authors have described alterations in gammaglobulin fractions in sera and cerebrospinal fluid of patients [2, 6, 12, 29, 36, 40, 41], while others did not find them [31, 37]. We like others examined the T-lymphocyte subpopulations in the peripheral blood of schizophrenic patients and found an increase in T-helper cells [18, 34]. Since 1937, when Lehmann-Facius described an antibody to brain tissue in the sera of schizophrenic patients [30], many authors have shown similar phenomena [4, 5, 11, 16, 22, 35]. Their results, however, were often inconsistent, the diagnosis of schizophrenia was not always clear, controls were often not age- and sex-matched, and many studies were not carried out with coded samples. Moreover, other authors did not find binding [7, 13, 32, 38, 47]. These differences have persisted until today [17, 23].

Therefore we decided to look for brain antibodies in the sera of schizophrenic patients with age- and sex-matched controls. Following the recommendations of the WHO for screening tests for autoantibodies, we used an indirect immunofluorescence assay [43], which was performed with coded samples of patients' and matched controls' sera on the same day. Following former immunological, neuropathological and neuroimaging studies [8, 14, 16, 19, 21], we used frontal cortex, hippocampus, putamen, entorhinal area and the septal region as tissue targets; ncl. olivaris and thyroid gland were chosen as tissue controls.

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Patients and Methods

Thirty patients suffering from an acute episode of schizophrenia (diagnosed according to DSM III criteria) were included. The mean duration of the illness was 4.3 years, the mean number of episodes was 2.5. All of the patients but one were on neuroleptic treatment, mostly haloperidol.

Thirty age- and sex-matched neurological or surgical patients without inflammatory disease or known mental disorder served as controls (Table 1). Neither patients nor controls had ever had any autoimmune disease.

Blood was drawn by venipuncture between 8.00 and 10.00 a.m., immediately centrifuged and the sera were stored at −20°C. The brain tissue samples used were obtained from a 39-year-old patient, who died from myocarditis within 24 h. He had never been severely ill before, and there was no history of psychic or autoimmune disease. Most of the sera were tested again on brain tissues of three other patients without neurological disease. The brain areas tested did not show any macroscopical or histological changes. We prepared frontal cortex, putamen, hippocampus, area entorhinalis, area septalis and – as a control for unspecific antinuclear factors – ncl. olivaris and thyroid gland within 8 h post-mortem. Tissues were immediately frozen in liquid nitrogen, and stored at −80°C.

The technique of indirect immunofluorescence assay is well known. In brief, frozen slices 7 µm thick were fixed in ethanol (4°C, for 15 min). The sera of patients and their matched controls

Table 1. Age and sex distribution in patients and controls. Mean age is given in years; standard deviations in brackets

	Female	Mean age	Male	Mean age
Patients	10	35 (3.51)	20	37 (2.37)
Controls	10	35 (3.48)	20	37 (2.35)

were coded by a person who was not involved in the study, to guarantee a blind evaluation of the tests. Afterwards the frozen slices were incubated with the coded sera (4°C, 30 min). After several washings with phosphate-buffered saline (PBS), the second antibody (Rabbit-anti-human-IgG or -IgM; Dako Diagnostics, Hamburg, FRG) was added (4°C, 20 min). Samples were washed again with PBS and immediately evaluated using a Zeiss fluorescence microscope. Patients and their matched controls were tested in blind trials at the same day.

Intactness of tissue samples was proven by routine haematoxylin-eosin staining, antigen binding capacity by using a monoclonal mouse anti-neurofilament antibody and fluorescein-conjugated goat-anti-mouse-IgG as second antibody (Dako Diagnostics, Hamburg, FRG). Binding of rabbit-anti-mouse-IgG and -IgM was verified by using IgG- and IgM-coated sepharose beads (cyanobromide-activated sepharose, Pharmacia, Stockholm) as a target. Only experiments with 100% binding, strong anti-neurofilament binding, and intact brain tissue in the routine stain were included. Unspecific binding of the second antibodies was detected by incubating the brain tissues with PBS and second antibodies, background fluorescence was noted by using only PBS for the two incubations.

The samples were recorded as being "positive" when we found specific structures that represented anatomical correlates in the histological stain.

All of the samples were evaluated separately by two of us (A.H. and S.R.) who were in full agreement on the labelling of the sera as positive or negative.

All of the positive and most of the negative sera were tested again on brain tissues of three different normal persons.

Results

We found binding to brain tissue in the sera of 22 patients, while only 4 of 30 controls appeared to be positive (Fig. 1). The specific binding appeared in cell layers, that according to the haematoxylin-eosin staining represented neuronal cells. The structures which showed fluorescence were of circular shape and sometimes showed a little spot in their middle. The fluorescence thus seemed to represent perinuclear and nucleolar structures of neuronal cells. The binding was detected in the IgG- as well as the IgM-compartment (Fig. 2) and was mainly directed to nuclei of neuronal cells in the frontal cortex and area septalis. Some sera were also positive on hippocampus, putamen and area entorhinalis, while we never found binding to ncl. olivaris or thyroid gland in the patients' specimens (Fig. 3).

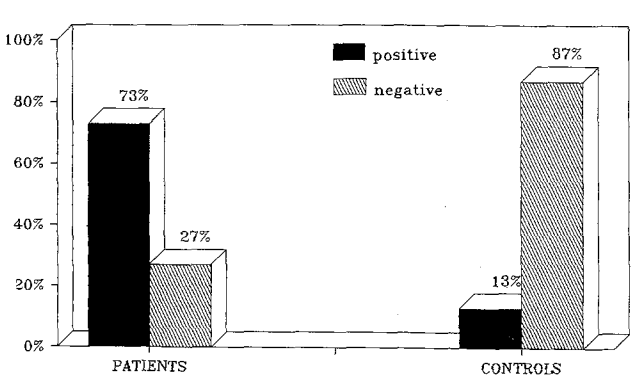

Fig. 1. Antibody-binding in patients and controls

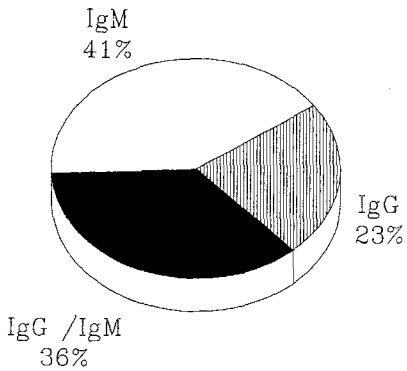

Fig. 2. Distribution of gamma-globulins in positive patients

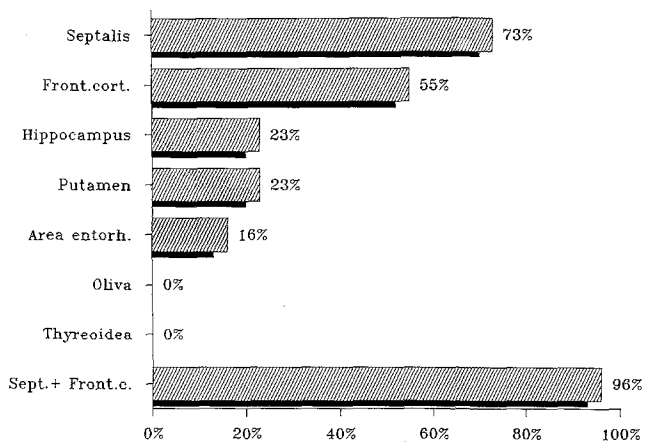

Fig. 3. Areal distribution of antibody binding in sera of positive patients

The one patient who had never received neuroleptic medication belonged to the positive group.

Positive sera that were retested on brain tissues of different patients remained positive, negative sera remained negative. Patients with and without binding did not differ in sex, mean age, duration of disease or number of episodes.

Discussion

Schizophrenia has been hypothesized as being an autoimmune disease by several authors [10, 15, 24, 26]. Some authors had described binding of serum globulins to brain tissue [4, 11, 16, 17, 30, 35], but there were differences in the location of the binding and the number of positive cases, while other authors did not find binding at all [7, 13, 23, 32, 38, 47]. Since most of the experiments did not include age- and sex-matched controls or were not conducted with coded samples, we decided to look again for brain antibodies in the sera of schizophrenic patients. We used a well-controlled indirect immunofluorescence assay, which was carried out with coded samples of patients' and matched controls' sera, which were tested at the same day.

We originally chose this technique, which meets the recommendations of the WHO for searching autoanti-

bodies as cited by Thompson [43], because we had found contradictory statements on the characteristics of the expected binding in the literature. We selected brain areas that had been reported to show neuropathological, neuroradiological or immunological changes even though most of these findings had not been reproduced by other groups so far [8, 14, 16, 19, 21].

We found IgG- and IgM-binding to the nuclei of neuronal cells in 73 per cent of patients' sera and only in 13% of the controls' sera. The binding was mainly directed to the frontal cortex and area septalis. This is in good agreement with the results of Heath and Krupp [16].

There are several reasons why we think that these preliminary findings are reliable: Firstly, the patients were classified according to DSM III, being in an acute state of the disease. Secondly, the experiments were carried out in a well-controlled way in that we were blind for the diagnosis and the treatment of patients tested when performing the experiments, and we tested patients and their matched controls in coded samples on one occasion. Thirdly, the patients' sera did not show affinity to ncl. olivaris or thyroid gland, which excludes a general antinuclear factor, described for example after psychopharmacological treatment [33, 39]. Another study did not find antinuclear factors at all in schizophrenic patients [45], whereas other psychiatric patients showed them in 25% of cases. A general antinuclear factor caused by psychopharmacological treatment is also countered by the positive binding in one patient, who was not medicated at all, and who did not differ from other positive patients. Fourthly, an unspecific binding, as described by Aarli et al. [1], is ruled out by the shape of the binding.

Four control sera showed binding to brain tissue, a fact that we cannot yet explain. We think that these positive controls must be followed up clinically. On the other hand, in myasthenia gravis, an accepted autoimmune disorder, a twin study showed autoantibodies to acetylcholine receptors also in the non-affected twin [28]. Thus autoantibodies without clinical manifestation may not exclude the presence of an autoimmune disorder.

We cannot decide yet whether we have found real autoantibodies to brain tissue or not, although some authors have described autoantibodies to T-lymphocytes in the sera of schizophrenic patients [20, 25, 44, 46], and it is well known that T-lymphocytes and neurons share antigenic components [42].

Obviously, these data are preliminary and further studies have to be conducted in larger numbers of patients. In addition the number of unmedicated patients has to be increased to exclude with certainty an epiphenomenon caused by psychopharmacological treatment. Therefore, further studies are in progress, also to see whether the binding is specific for schizophrenia by testing other psychiatric patients. Also, we have started a longterm study to establish the possible correlation of the appearance of brain antibodies and of psychopathological symptoms.

However, by presenting our preliminary data, which we assume to be valid methodologically, we would like to encourage other groups to consider immunologic aspects of schizophrenia.

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