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AN EXPERIMENTAL STUDY OF GLIAL ANTIGENS: SOME PATHOPHYSIOLOGICAL DISORDERS AND CHANGES IN CSF PROTEINS INDUCED BY THEM IN MONKEYS

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The role of glial antigens and pathophysiological disorders induced by them, were investigated. As an approach for obtaining glial tissue samples, the process of retrograde and transneuronal degeneration of visual neurons in the Lateral Geniculate Nucleus (LGN) of the brain of Rhesus monkeys was used. Immunization of an other group of monkeys with the immunogen containing allogenic tissue of LGN, induced an experimental disease of slow and long-term evolution with manifest hypofunction of the thyroid gland. Quantitative determination of the contents of prealbumin, albumin, transferrin and total proteins was carried out in the liquor samples obtained by suboccipital puncture before and after immunization the animals. In contrast to the concentration of albumin, transferrin and total proteins, it was found that there was a statistically significant increase of the prealbumin content in the liquor, This may point to more than one origin for this protein.

Key words: glial antigens, proteins, brain, liquor

INTRODUCTION

In the last few years there has been an increasing number of papers devoted to investigations of the neurophysiological, biochemical and metabolic role of glial cells of the brain. Despite this fact there are surprisingly few workers regarding the role of glial tissue antigens in the etiopathogenesis of pathophysiological disorders IN VIVO. In this sphere of investigation the morphological, functional and biochemical heterogeneity of the brain tissue of mammals imposes the problem of selection of the brain sample, as well as the technique of obtaining the most adequate sample of glial tissue, so that its immunological effects could be critically estimated.

Today there are several approaches to obtaining glial tissue. Besides glial tissue culture (Levi, 1924; Lumsden and Pomerat, 1954; Fedoroff,

1978; Skarper et al., 1986), glial tissue samples can be obtained by differential centrifugation of brain tissue in discontinuous gradients of sucrose or Ficoll-sucrose (Rose and Sinha, 1969; Poduslo and Norton, 1972; Rose, 1976), or by using the method of microdissection of neurons in Deiters's nucleus of the brain which Deiters applied in 1865 and Hydén improved (1960). One possibility of obtaining gilal tissue is offered by the process of retrograde degeneration of the central neurons, which has been investigated in a variety of experimentally induced conditions. As early as in 1935, Walker, and a few years later, Lashley (1941) pointed out that ablation of particular regions of the brain leads to retrograde degeneration of neurons in the nuclei of the thalamus. However, it has also been found that the degree of loss of neurons varies from one nucleus to another and from one experimental animal to another. Mihailović et al. (1971) reported that, due to the high degree of organization and differentiation of the viusal system of primates, ablation of the optic cortex from the Rhesus monkey brain and sectioning of the optic nerves lead to the almost total disappearance of neurons in the Lateral Geniculate Nucleus, which becomes replaced by glial cells of the brain. A pilot experiment (Čupić et al., 1977) showed that the allogenic glial tissue of the Lateral Geniculate Nucleus obtained through the process of retrograde degeneration of visual neurons and used as an immunogen, induced in monkeys an experimental disease with manifest hypofunction of the thyroid gland.

The aim of this study was to investigate the pathophysiological disorders and eventual change of the content of prealbumin, albumin, transferrin and total proteins in the cerebrospinal fluid of monkeys immunized by antigens of the allogenic tissue of the degenerated Lateral Geniculate Nucleus.

MATERIALS AND METHODS

The experiments were carried out in two successive stages, on monkeys (Macaca mulatta) of the same blood type, of both sexes and body weight 2 to 3,5 kg.

Method for obtaining glial tissue samples

In order to obtain glial tissue samples, nine monkeys were subjected to bilateral occipital lobectomy and sectioning of the optic nerves under Nembutal anaesthesia and under aseptic conditions. Then the animals were looked after under standard laboratory conditions for eight months. At the expiration of eight months the animals were sacrificed by bleeding to death caused by heart puncture. Then the brain from seven monkeys was quickly taken out and Lateral Geniculate Nucleus (LGN) tissue carefully dissected out under aseptic conditions. Thus obtained glial tissue samples of LGN were stored at —68° C, for use as an immunogen in the next stage of the experiment. The two remaining monkeys, after being bled to death, were subjected to perfusion, through the heart, first with physiological saline and then with 10% formalin. The head was fixed in a stereotaxic instrument and the brain cut *in situ* according to the stereotaxic coordi-

nates, in blocks of 10 mm. The blocks were fixed in 40% formalin, subsequently embedded in celloidin and cut at 26 microns. Preparations of the LGN tissue were stained with haematoxylin-eosin and, using the Klüver-Barrera method and coronal sections of LGN microscopicaly examined.

Immunization procedure

Five monkeys were immunized with a homogenate of glial tissue of the degenerated LGN emulsified with complete Freund adjuvant (Difco) and mineral oil Arlacel "A" (Serva), in volume relations of 2:1:1. One injection of immunogen was injected intramuscularly once a week during a month. Then at intervals of 30 days, one injection of 0.2 ml of homogenate of glial tissue of LGN precipitated with 1% $AlK(SO_4)_2$ solution adjusted with 0.5N NaOH to pH 6.0, was administered intravenously. The total immunization period lasted 330 days.

Monkeys were observed during a year in order to detect signs of the disease.

Scintigraphy

Scintigraphs of the thyroid gland were taken before and eight months after the commencement of immunization using I^{131} by means of a rectilinear scanner "Nucleus Chicago".

Withdrawal of cerebrospinal fluid sample and quantitative determination of prealbumin, albumin, transferrin and total proteins in the liquor

Cerebrospinal fluid was taken by suboccipital puncture of unanaesthetized monkeys under sterile conditions before and 330 days after the beginning of the immunization. Prealbumin, albumin and transferrin were quantitatively determined in samples of nonconcentrated CSF by rocket immunoelectrophoresis in agarose gel, according to Laurell (1956) using the slight modification described by Bock (1973). Standard human serum "Behringwerke AG" and four dilutions were applied to each plate. Electrophoresis was performed in an LKB Multiphor Equipment (Model 21/27). Total proteins in CSF were determined according to Lowry et al. (1951). The concentration of the examined proteins were expressed in g per litre.

RESULTS AND DISCUSSION

Results of morphological analysis of intact LGN and degenerated LGN

The morphological changes in Lateral Geniculate Nucleus in the brain of the Rhesus monkey eight months after bilateral occipital lobectomy and transection of the optic nerves are shown in Figure 1.

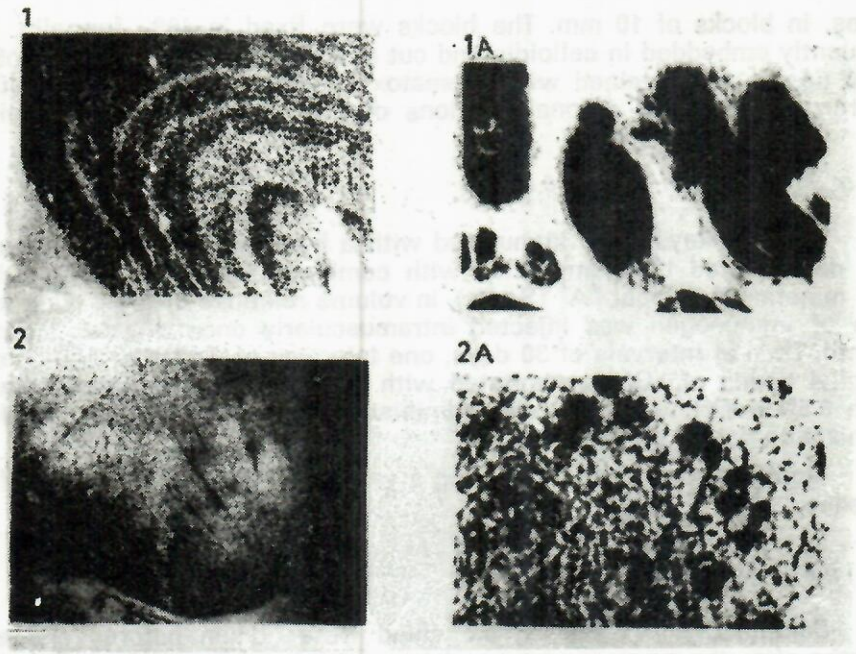

Figure 1. Microscopic appearance of Lateral Geniculate Nucleus of the monkey's brain.
1. LGN — coronal section (Klüver Barrera x 10); 1A — H. E. x 300.
2. LGN 8 months after operatively induced degeneration of neurons — coronal section (Klüver Barrera x 10); 2A — H. E. x 300.

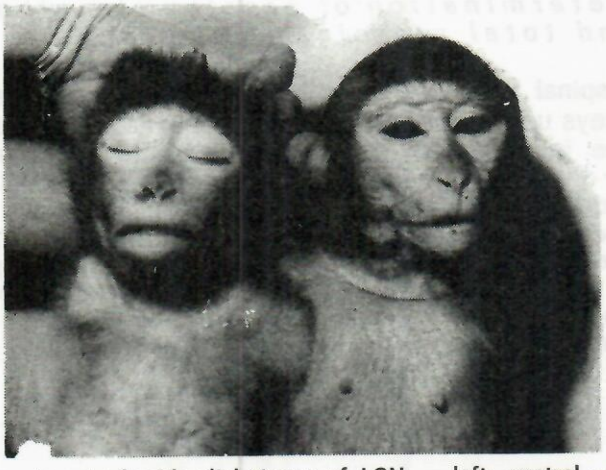

Figure 2. Monkey imunized with glial tissue of LGN — left; control — right.

As Figure 1. illustrates, the LGN of the brain of an intact monkey has a characteristically stratified structure in which six horse-shoe shaped neuronal layers are present. Eight months after the operation the LGN depriving of its afferent optic tract fibres, there was disappearance of its typical

neuronal layers which is shown in Figures 1, 2. A detailed histological analysis of the LGN preparation shows that its nucleus is pratically deprived of neurons and that it contains the main types of glial cells: astrocytes, oligodendrocytes and microglial cells (in Figure 1. compare Figures 1A and 2A). There is the question whether the process of retrograde degeneration of neurons in LGN of the monkey brain offers a qualitative sample of glial tissue or not.

It is known that the main problems of glial tissue culture pertain to the time of maintenance and dedifferentiation of glial cells and these problems are still a focus o interest (Levi, 1934; Chlopin, 1941; Lumsden and Pomerat, 1951; Skarper et al., 1986). In contrast to the forementioned, separation of metabolically active neurons and glia contained in the cerebral cortex of the rat brain by tissue dispersion through a grid system and differential centrifugation in a discontinuous gradient of Ficoll-sucrose (Rose, 1976) has a disadavantage because there is contamination of the glial fraction by neuronal endigs. According to some authors the contamination could be more than 11%. The method of microdissection of the pericaryon contained in Deiters's nucleus which has been improved by Hydén (1960) and his co-workers (Hydén and Lange, 1960; Hydén and Pigon, 1960) cannot provide the necessary quantity of tissue for experimental immunization uder *in vivo* conditions. Besides that, the glial cell sample obtained by this method of microdissection is contaminated by nerves endings of "cutoff" neurons. The contamination, according to the estimations of Hamberger (1963) and Hamberger and Sellström (1976), is about 5% and according to some other authors even more, and above all, the metabolic behaviour of the separated cells is unstable. The results of the first series of our experiments have offered morphological evidence of the disappearance of the typical cytoarchitectonic structure in the Lateral Geniculate nucleus of the operated monkey brain by induction of degeneration of the visual neurons. One should have in mind that the laminar structural organization of the Lateral Geniculate Nucleus is not identical in carnivores and in primates. Thus, the LGN of cat brains has only three neuronal layers, whereas, we can identify six layers in the monkey and in man (Szentagothai, 1973). In previous research carried out in our laboratory, the dynamics of changes in the number of neurons and glial cells was observed in the Lateral Geniculate nucleus of the monkey brain during retrograde cell degeneration (Mihailović et al., 1971). The results of this numerical analysis of cells showed that six months after radical occipital lobectomy the LGN became completely deprived of its neurons and contained 20% more astrocytes and almost double the number of oligodendrocytes and microglial cells with a somewhat higher concentration of capillaries in contrast to the intact LGN of the monkey brain. These investigations also six months after the operation showed that the LGN of the monkey brain can be taken as a tissue sample with stable proportions of the main type of glial cells. Such a sample of glial tissue is suitable for biochemical investigations and it was used for determination of the content of components of the Glutamat GABA system (Čupić et al., 1969), protein (Kržalić et al., 1969) and catecholamine (Nedeljović et al., 1969). Such a sample of glial tissue has been used for immunization experiments.

Clinical signs

Observation of animals with the aim of detecting signs of the disease revelated the all monkeys immunized by allogenic glial tissue LGN developed the same disease of slow and long-term evolution. The first signs of the disease were observed 4 to 6 months after the onset of immunization. The first signs of the disease were difficult breathing, loss of appetite without simultaneous loss of body weight and the appearance of pale periorbital edema. The further development of the disease was dominated by cold and pale edema which gradually spread over the whole face (see Figure 2), then over the dorsal parts of the upper extremities and finally over the lower extremities.
The signs of the disease were identical to those previously reported in the same experimental situation.

Results of the thyroid gland scintigraphy

Scintigraphy of the thyroid gland was done to find out the cause of te prominent sign of the disease, the edema. Thyroid scintigrams of non immunized monkeys and monkeys immunized with glial tissue are shown in Figure 3. In the period of manifested signs of the disease the immunized monkeys had decreased accumulation of I^{131} in the thyroid gland in contrast to the control group (compare thyroid scintigrams A and B).

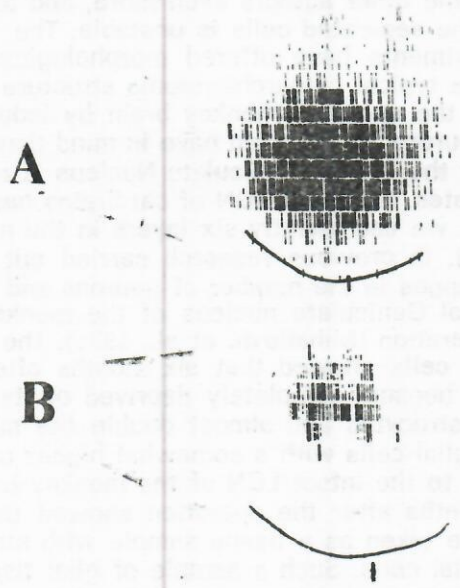

Figure 3. Scintigrams of thyroid gland. A. — thyroid of nonimmunized monkeys; B. — thyroid of monkeys immunized with allogenic tissue of LGN. (For explanation see text).

This scintigraphy analysis confirmed previously obtained radioisotopic determinations of total and free thyroxine, triiodothyronine and thyrotrophin in the serum of immunized monkeys. These results showed that the decrease of the level of thyroxinemia coincided with the onset of the first signs of the disease, so that at te stage of the completely developed clinical picture the level of total thyroxine was 62% lower than the control level at the initial stage (Čupić et al., 1977; 1984).

In a further search for disorders originating in this experimental disease, investigations have been carried out in more than one direction. Thus, in an identical experimental situation, the evolution of changes of visually evoked potential (EP) in LGN was followed during 390 days. It was found that immunization of monkeys with glial tissue induce a slow and gradual decrease in the amplitude, first, of the second and a few months later, of the first component of the visually evoked response in the LGN and in the terminal phase of the experiment also a change in their morphology. The authors consider that the changes of the visually evoked electrical activities in the LGN have a source in the histopathological lesions found in the cerebral cortex of these monkeys (Čupić et al., 1979). On the basis of histological, histochemical and electron microscopic investigations of muscle biopsy specimens of the monkeys immunized with glial tissue of LGN, it has been stated that the changes found in muscles might be induced by hypofunction of the thyroid gland because the muscle biopsy picture corresponds to endocrine myopathy (Čupić and Dožić, 1979). In addition, an investigation has been done on the effect of immunization of monkeys with glial tissue on learning and performance of previously learned tests of visual discrimination (VD). Analysis of the results of these investigations showed that immunization of monkeys with glial tissue, even before the appearance of other signs of disease reduced the retention of previously learned VD test and disturbed the learning of this pattern of behaviour. The authors have come to the conclusion that the changes in animal behaviour represent fine indicators of disfunction between relevant cortical and subcortical structures of the CNS involved in the organization of this type of behaviour (Čupić and Petrović, 1980).

Results of the determination of prealbumin, albumin, transferrin and total proteins in the liquor

In Figures 4 and 5, the results of quantitative determination of some protein components and total proteins contained in liquor samples obtained before and 330 days after the onset of Rhesus monkey immunization are graphically presented.

Analysis of the content of prealbumin in the liquor of intact monkeys showed that its concentration expressed as a mean value was 0.016 ± 0.002 g/l. The quantity of prealbumin expressed as a percentage of total proteins was 1.91%. At the time of the entirely manifested clinical picture, there was an increase of prealbumin content in the liquor of immunized monkeys to 0.053 ± 0.011 g/l, namely 7.04% of the total proteins. The recorded value of prealbumin in the liquor at the stage of evident thy-

roid gland hypofunction induced by glial antigen is statistically significant ($p<0.001$) and exceeds its control values (see Figure 4.).

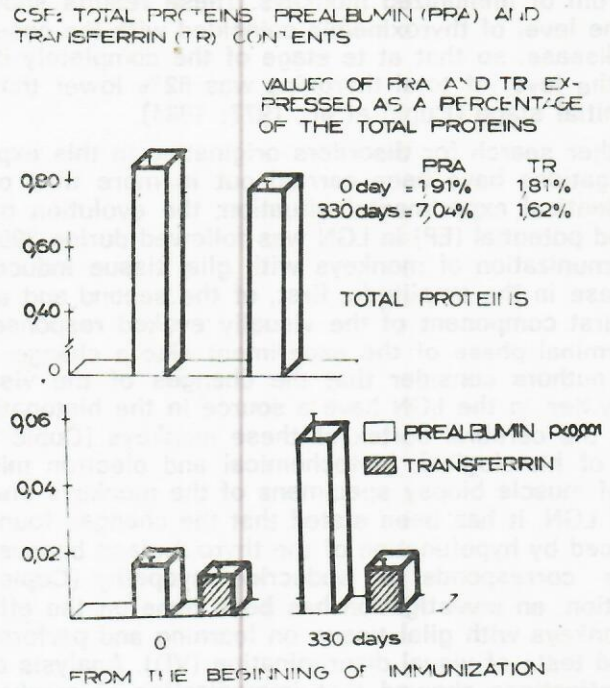

Figure 4. Total proteins, prealbumin (PRA) and transferrin (TR) contents in the CSF of nonimmunized and immunized monkeys.

The average value of transferrin content in the Rhesus monkey liquor obtained before immunization was 0.011 ± 0.004 g/l, whereas the quantity of transferrin of total proteins was 1.81% (Figure 4.). In contrast to prealbumin, the concentration of transferrin in liquor samples obtained from monkeys immunized with glial tissue of LGN was within the control range. Then, the value of CSF transferrin expressed as a percentage of total proteins was 1.61%.

The quantitative determination of albumin content in the liquor of the intact Rhesus monkey ranged from 0.10 to 0.14 g/l, whereas its average value was 0.12 ± 0.14 g/l. The recorded percentage of albumin in total proteins in the liquor was 14.05% Figure 5.). In the liquor samples of monkeys immunized with glial tissue an increase of albumin concentration up to 0.19 ± 0.07 g/l was found. However, the reported increase of albumin content in the CSF was not statistically sigificant. It is important to point out that in contrast to the other investigated liquor protein constituents, the samples of liquor of immunized monkeys showed considerable individual variations of albumin content. As the electrophoregram of albumin illustrates in Figure 6., the increase of albumin content varied from animal to animal, so that the albumin level in the liquor of half of the immunized

monkeys was almost double the initial level. With regard to total proteins, their average concentration in the control liquor was 0.84 ± 0.07 g/l and in liquor from the immunized monkeys the slightly lower value of 0.76 ± 0.09 g/l, was recorded.

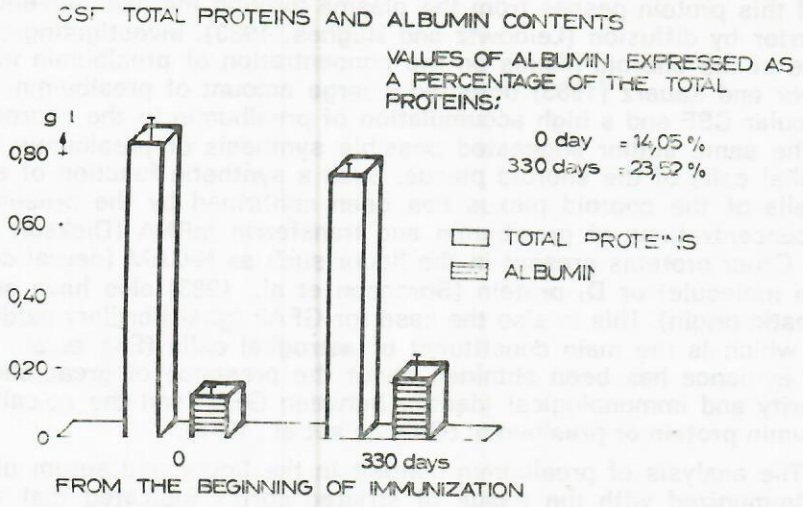

Figure 5. Total proteins and albumin contents in the CSP of nonimmunized and immunized monkeys.

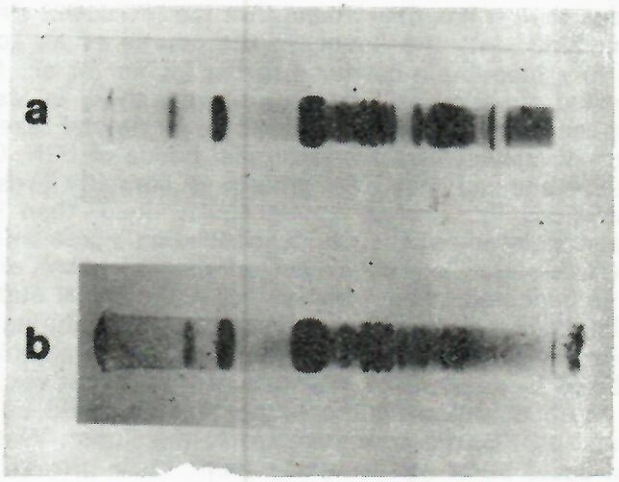

Figure 6. Electrophoregram of albumin in the CSF of nonimmunized (A) and monkeys immunized with the allogenic glial tissue of the LGN (B).

It is known that prealbumin proteins have specific physicochemical characteristics. Considering the role of individual CSF proteins Laurell

(1987) pointed out that prealbumin capacity to bind thyroxine or retinol might be its main role in the liquor, but he also suggested that its other proposed name of "transthyrenine" would be too hasty. Prealbumins represent a smaller fraction of total proteins in serum than in liquor. It is considered that more than one source could be responsible for such a concentration of prealbumin in the CSF in physiological conditions. One portion of this protein passes from the plasma through the haemato-encephalic barrier by diffusion (Leibowtz and Hughes, 1983). Investigating the influence of the choroid plexus on the concentration of prealbumin in CSF, Weisner and Kauerz (1983) detected a large amount of prealbumin in the ventricular CSF and a high accumulation of prealbumin in the choroid plexus. The same author suggested possible synthesis of prealbumin in the epithelial cells of the choroid plexus. Such a synthetic function of epithelial cells of the choroid plexus has been confirmed by the presence of high concentrations of prealbumin and transferrin mRNA (Dickson et al., 1985). Other proteins present in the liquor such as N-CAM (neural cell adhesion molecule) or D_2 protein (Sorensen et al., 1983) also have an non-plasmatic origin). This is also the case for GFAP (glial fibrillary acidic protein), which is the main constituent of astroglial cells (Eng et al., 1971). Much evidence has been obtained about the presence of great chemical similarity and immunological identity between GFAP and the so-called alphalbumin protein or prealbumin (Gheuen set al., 1978).

The analysis of prealbumin content in the liquor and serum of monkeys immunized with the tissue of striated cortex indicated that the increase in the CSF prealbumin content coincided with the decrease of its content in the serum only in the early phase of experimental allergic encephalomyelitis (EAE) but not in the advanced evolution of EAE (Čupić et al, 1986). Besides that, it has been found that the increased sifing of prealbumin from the blood into the liquor, in the course of an acute form of EAE is related to a bloodbrain barrier disorder (Juhler et al., 1984). However, an open-ended question still remains of why there is also increased permeability of the blood-brain barrier during experimetal disease induced by immunization of monkeys by allogenic glial tissue of LGN. On the other hand, Bock (1987) showed that GFA protein is normally present in even higher concentrations in samples of ventricular liquor than in lumbar liquor and she also pointed out that its concentration increases in the majority of degenerative states, and that elevated concentrations of this protein in CSF would be expected to be a marker for pathological states involving astro-glial cells. Our investigations were carried out during the experimental disease which included hypofunction of the thyroid gland and which was induced by antigens of glial tissue of LGN. The results obtained indicated that more than one source of prealbumin could be responsible for the increased prealbumin concentration in the liquor. Whether part of the prealbumin could be of glial origin remains to be proved.

CONCLUSIONS

On the basis of comparative analysis of histological preparations of Lateral Geniculate Nucleus of monkey brains before and eight months after radical occipital lobectomy and sectioning of the optic nerves it was found that this nucleus was practically deprived of neurons and contained the main types of glial cells: astrocytes, oligodendrocytes and microglial cells.

Immunization of monkeys with glial tissue of LGN, obtained through the process of retrograde degeneration of visual neurons, in the sensitized allogenic recipients led to the appearance of an experimental disease of slow and long-term evolution, the dominant signs of which are in keeping with thyroid gland insufficiency.

The clinical manifestations of the disease induced by immunization of monkeys with glial tissue of LGN offered a rough orientation, markedly deviating from the already well-known signs of experimental allergic encephalomyelitis. This suggested a phenomenological difference between these two experimental diseases induced by brain tissue immunogens. On the other hand, this finding has put forward a whole series of questions and assignments to be solved.

In this experimental situation there is an increased concentration of CSF prealbumin, whereas the levels of albumin, transferrin and total proteins varied within control values. It is not excluded that the high content of prealbumin recorded in the liquor of immunized monkeys may have various origins in these experimental conditions.

Finally, in the course of chronic experiments, reported in the present paper a reproducible experimental model has been developed which is suitable for further studies and precise definition of the role of glial antigens in the etiopathogenesis of pathophysiological disorders induced by them.

Acknowledgement

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EKSPERIMENTALNA STUDIJA ANTIGENA GLIJALNOG TKIVA: NJIMA INDUKOVANI PATOFIZIOLOŠKI POREMEĆAJI I PROMENE CSF PROTEINA U MAJMUNA

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SADRŽAJ

U cilju izučavanja uloge glijalnih antigena i nijma indukovanih patofizioloških poremećaja, kao pristup za dobijanje uzorka glijalnog tkiva iskorišćen je operativno indukovan proces retrogradne i transneuronske degeneracije vizuelnih neurona u Nucleus Geniculatum Laterale (LGN) mozga

Rhesus majmuna. Osam meseci po operaciji, tkivo LGN mozga majmuna biva praktično lišeno neurona i ono sadrži samo glavne tipove glijalnih ćelija u ustaljenim proporcijama. Ovako glijalno tkivo poslužilo je kao imunogen korišćen u narednoj seriji ogleda. Imunizacijom druge grupe majmuna glijalnim tkivom LGN izazvano je eksperimentalno obolenje spore i duge evolucije, koje u sebi uključuje i hipofunkciju tireoidne žlezde, koja je objektivizovana nizom rezultata. U toku izvođenja ovih hroničnih ogleda razrađen je eksperimentalni model prikladan za izučavanje i preciznije definisanje uloge glijalnih antigena i njima indukovanih patofizioloških poremećaja. Ispitivanjem pojedinih proteinskih konstituenata i ukupnih proteina u cerebrospinalnoj tečnosti majmuna u periodu manifestne faze obolenja otkriveno je prisustvo povećane koncentracije prealbumina, za koju bi moglo biti odgovorno više od jednog izvora ovog proteina. Za razliku od njega, sadržaj albumina, transferina i ukupnih proteina u likvoru u istoj eksperimenltanoj situaciji kretao se u okviru kontrolnih vrednosti zabeleženih njihovim određivanjem u likvoru neimunizovanih majmuna.