nucleus cells with a large cytoplasm content was detected as compared with the control, in which only isolated epithelial cells were found in the postoperative period. Fibroblasts in smears of the first two groups were found earlier in the postoperative period (1st-10th days) than in the control group (7th-25th days).

It should also be noted that in the baseline as well as control smears on days 2, 4, 10, and 15 a small coccal and bacillus flora was found. No flora was detected in the first two groups during the entire postoperative period.

The results obtained suggest an activating effect of the natural cytokines complex on the O_2-dependent function of vaginal phagocytes. This cell function is attended by phagocytosis and the generation of active forms of oxygen. The absence of flora observed in the experimental groups is assumed to be due to the bactericidal effect and to the activated phagocytosis. The cytokines complex affects the CL activity of cells and also prolongs the period of high intensity.

From the increase of the epithelium content in smears from animals treated with cytokines, it may be assumed that the cytokines complex has an epithelium-forming effect on the vaginal mucosa. The earlier appearance of fibroblasts in smears from the first two groups also confirms the acceleration of the regeneration process in a wound induced by cytokines.

Thus, we established an activating effect of the natural cytokines complex on phagocytes of rat vaginal mucosa during the postoperative period, as well as a bactericidal and epithelium-forming effect.

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Analysis of Antineuronal Antibodies in Sera of Patients with Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a chronic progressive brain disease causing motoneuron degeneration, which is manifested clinically by spastic paresis and paralysis, dysphasia and dysphagia, as well as respiratory distress in the final stages of disease. Despite the efforts of many years and numerous studies, the etiology and pathogenesis of ALS remain unclear, and there is no medical treatment for the disease. Genetic factors, the effect of heavy metals, disturbances in parathyroid gland metabolism, hypo-

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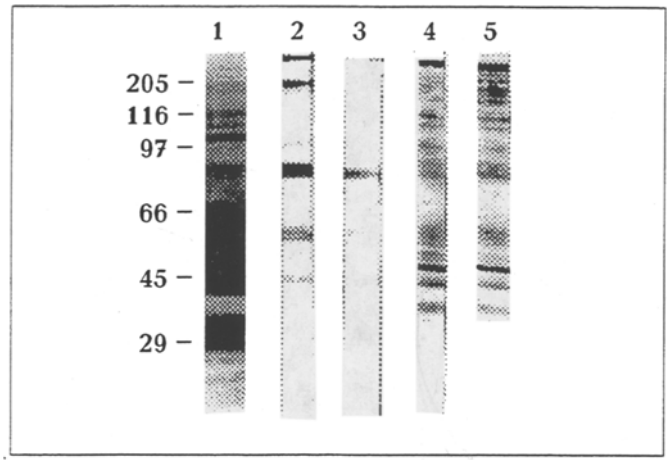

Fig.1. Interaction between serum antibodies and neural tissue proteins; effect of alkaline phosphatase on this process. Separation of mouse brain protein extract in PAGE (1). Corresponding immunoblots developed by serum antibodies: ALS patient (2), the same after preliminary blot treatment with alkaline phosphatase (3); healthy donor (4), the same after preliminary blot treatment with alkaline protease (5). Serum dilution is 1:20. Positions and mol.weights (kD) of protein markers are shown on the left.

glycemia, transmitter effects, viral infection, and changes in immunity are assumed to be possible causes; in addition, it has been suggested that there can be multiple cause's [17]. The results of histological study show the atrophy of large motoneurons followed by their degradation and replacement by glial cells. Structural changes in the cytoskeleton of neurons are of great importance in the degradation process. Neurofilaments (NF) and microtubules (MT) threaded through the cytoplasm of nearly all cells are the main structural components of the neuron cytoskeleton [14]. Unfortunately, only a little information is available on the cellular and molecular aspects of motoneuron degradation in ALS. The structural changes in NF have been studied in more detail. The process of motoneuron degradation in ALS has been shown to be accompanied by the formation of numerous associations of 10 nm NF (globules and spheroids) both in dendrites and in the proximal parts of axons [13]. In motoneurons the formation of such NF clusters has been found to be accompanied by a more intensive and, perhaps, premature phosphorylation of NF proteins [10]. The changes in NF structure and distribution may be associated with disturbances of the axocurrent. The rates of both the fast and slow components of the axocurrent have been shown to change in ALS [3]. In patients with ALS biochemical changes of MT proteins (tubulin and MT-associated proteins) have been detected in axons of peripheral, sensory, and motoneurons prior to their ultrastructural degradation [2]. In ALS, Golgi apparatus fragmentation, analogous to that in cultured cells induced by MT depolymerization with specific reagents [11], has been noted both in neurons with other indications of ultrastructural degradation and in cells without any cytological changes.

Thus, it may be assumed that not only the neurofilaments but also, perhaps, even primarily, the MT are involved in the process of motoneuron degradation in ALS. A more detailed and broader study of the cytoskeletal changes occurring in ALS may assist in understanding the pathogenesis.

In connection with the established presence in the blood serum of ALS patients of antibodies to cytoskeletal neuron proteins, in particular, to NF proteins [1,15,17], one of the methods of studying the state of the neuron cytoskeleton is the testing of autoimmune serum antibodies to neural tissue antigens.

Antibodies to a variety of neural tissue antigen determinants have been found in serum from both ALS patients and patients with other neurological disease as well as healthy donors [4,7,15]. Immunoblotting shows that a set of neural tissue antigens binding serum antibodies may vary considerably in the amount, molecular weight, and the intensity of stained bands both in patients with ALS and in the control group. No antibodies specific for ALS have been found. Data are available only on an extremely high occurrence of antibodies to certain neural tissue determinants in ALS patients in comparison with the control [4,15].

In view of this, we aimed to perform a more detailed examination of the antineuronal antibodies in serum from ALS patients in order to evaluate their functional and diagnostic role.

MATERIAL AND METHODS

Serum from ALS patients as well as healthy donors was used in the experiments. The modified Coons immunofluorescent method [5] was used for initial screening of the serum samples for the presence of antineuronal antibodies. Cryostat sections of mouse spinal cord, cerebral hemispheres, and brainstem were used in setting up the reaction of antibody identification. Antisera against human IgG were used for conjugate with fluorescein-isothiocyanate. The preparations were observed using the luminescence microscopy method (200-fold magn.). The intensity of luminescence was evaluated on a 3-point scale. To produce the protein samples the mouse brain was homogenized and extracted with a buffer containing 50 mM imidazole (pH 7.2), 0.5 mM $MgCl_2$, 0.5 mM EDTA, and 1 mM mercaptoethanol (buffer A), in a 1:1 mass-volume ratio. The homogenate was centrifuged at 150,000 g for 60 min at 4°C. The supernatant was used for the electrophoretic separation of proteins. The MT protein

preparation was isolated from cattle brain by the method of polymerization-depolymerization cycles [14] using buffer A containing 50 mM KCl and 1mM EGTA at pH 6.7. SDS-electrophoresis [9] was carried out in a 6-12% PAGE gradient. The gels were stained with Coomassie R-250. The electrophoretic protein transfer onto nitrocellulose filters, followed by the development of blots by serum antibodies (immunoblotting), was carried out after Towbin et al. [16]. Samples of sera were used in a 1:10 or 1:20 dilution. For the production of various types of serum antibodies (IgG and IgM) the serum samples were separated by affinity chromatography on A-protein Superose using the FPLC system (Pharmacia, Sweden). The treatment of the blots (nitrocellulose filters after protein transfer) with alkaline phosphatase (type VII-G, Sigma, 20 IU/ml) and the treatment of cryostat sections for the Coons reaction were carried out as previously described [8].

RESULTS

Based on the initial screening data, 11 ALS patients and 10 healthy donors (control) were selected for antibody analysis. Five serum samples from ALS patients showed a positive Coons reaction with the maximum luminescence intensity, and 6 samples exhibited the mean intensity. The absence of luminescence or a minimal intensity was detected in the control samples.

Immunoblotting analysis showed that, in spite of the differences in the Coons reaction, the samples of sera from both ALS patients and healthy donors reacted with a variety of neural tissue antigens (Figs. 1 and 2, *4*). High-molecular proteins with molecular weights of 150-200 kD and proteins with average molecular weights of 70 and 50 kD were found to be the major components in the spectrum of antigens binding with serum antibodies from ALS patients. It is noteworthy that antigens with the same molecular weights also bound with antibodies of the control sera; however, these antigens were not always major components in this case. The presence of serum antibodies to these groups of antigens in sera from ALS patients has been previously reported [4,15]. Protein antigens with molecular weights of 200, 150, and 70 kD are assumed to be the NF protein triplet. The presence of serum antibodies to NF antigens has been previously detected [1,4,15]. It should be noted, however, that this has been established with certainty only in the case of high-molecular components, based on immunoblotting data on the reactions of serum antibodies with an NF protein preparation [15]. The identity of the antigen with a molecular weight of 70 kD with the corresponding NF protein may be confirmed only indirectly [4]. No information is available in the literature on the nature of the antigen with a molecular weight of 50 kD. Taking into account that MT containing mainly tubulin with a molecular weight of 50 kD is one of the main components of the neuronal cytoskeleton, in addition to NF, it is reasonable to assume the presence of antitubulin antibodies in the test sera. In the present study this assumption was verified by the experiment. The interaction between antibodies from ALS patients and healthy donors and the MT preparation was examined. The MT preparation used (Fig. 2, *a*) contained tubulin as the main protein component as well as minor quantities of MT-associated proteins. From the data obtained (Fig. 2, *b*) no antitubulin antibodies were found either in the ALS patients or in the control. Note that, due to the MT instability, autoimmune serum antibodies to MT are found rather rarely in contrast to antibodies to more stable NF [12]. Thus, the exact nature of the major antigen with a molecular weight of 50 kD as well as of minor antigens binding to serum antibodies in ALS remains unclear. It is quite likely, however, that there are MT-associated proteins of nervous tissue among these antigens.

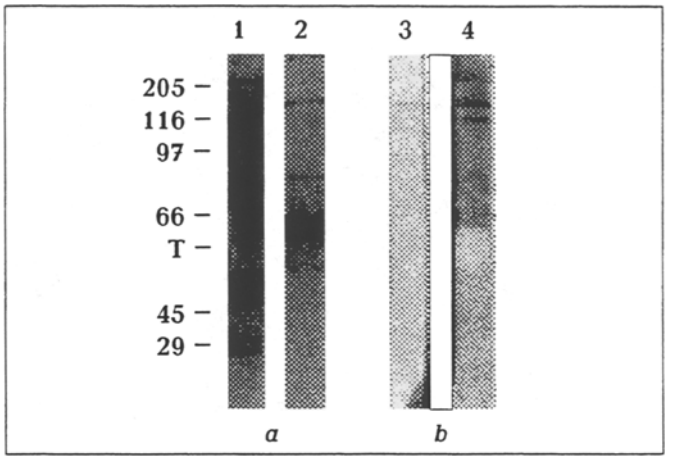

Fig.2. Interaction between serum antibodies and MT proteins. *a*) Separation of protein markers (1) and MT proteins (2) in PAGE; mol.weights (kD) and tubulin position are shown on the left. *b*) corresponding immunoblots developed by serum antibodies: ALS patient (3), healthy donor (4). Serum dilution is 1:10.

The presence of serum antibodies to the antigens described above both in ALS patients and in the control complicates the interpretation of the role of these antigens in ALS diagnostics and pathogenesis. For a more detailed analysis we determined the affiliation of the serum antibodies found to various classes of immunoglobulins. Eight samples of sera from ALS patients as well as 8 control samples were analyzed. Affinity chromatography on A-protein Superose was used to separate the total serum immunoglobulins into 2 main fractions: a purified IgG fraction and an IgM fraction with an admixture of the other serum components. Immunoblotting was

TABLE 1. Affiliation of Serum Antineuronal Antibodies to Various Types of Igs in ALS and in Control

Antigen, mol.weight, kD	ALS		Control	
	IgM	IgG	IgM	IgG
50	6/8	3/8	3/8	8/8
70	7/8	8/8	6/8	8/8
150–200	7/8	8/8	6/8	8/8

then used to examine the interaction on each fraction with neural tissue antigens. The results obtained are presented in Table 1. Antibodies to the 50 kD and 70 kD major antigens from the ALS patients were found to be mainly IgM, whereas antibodies from the control sera were found to be mostly IgG. Antibodies to high-molecular antigens (150-200 kD) belonged to both types of immunoglobulins both in the case of ALS and in the control samples, which is in accordance with published data [15]. Therefore, in the case of ALS the prevalence of IgM was detected among autoimmune serum antibodies in the early stages of the initial immune response.

It has been noted previously, that in ALS patients a premature and more intensive process of NF protein phosphorylation as compared with the norm is followed by the appearance of phosphorylated proteins in the perikaryon as well as their accumulation in the proximal part of the axon [11]. The surplus phosphorylation leads also to the formation of new epitopes, and, perhaps, to the enhancement of protein immunogenity. In this connection, in the present study the chemical nature of the antigenic determinants of nervous tissue binding with serum antibodies from ALS patients as well as from control samples was examined. For the removal phosphate groups from the protein surface the filters were treated with alkaline phosphatase, after which blots were developed by serum antibodies. The results obtained (see Fig. 1, *2* and *3*) suggest that the phosphatase treatment decreases the intensity of the interaction of antibodies with all groups of antigens from sera of ALS patients, but to the greatest extent in the case of the high-molecular components.

In control samples (see Fig. 1, *4* and *5*) no marked effect of alkaline phosphatase on the antibody reaction was detected. The same results were obtained by the immunofluorescence assay. The preliminary treatment of cryostat brain sections with alkaline phosphatase was found to decrease considerably the intensity of the sample luminescence induced by autoimmune antibodies from ALS patients and not to affect the immunofluorescence reaction in the control samples. Consequently, it may be concluded that serum antibodies from ALS patients are directed mainly against phosphoepitopes in protein antigenic determinants of neural tissue, attesting to a possible role of the changes in the phosphorylation process in ALS pathogenesis. It has been shown that restructuring of intermediate filaments (among them NF) is regulated by the process of structural protein phosphorylation due to the decrease in the stability of phosphorylated polypeptide subunits [7]. Phosphorylation of MT-associated proteins leads to a decrease of their MT affinity, followed by destabilization of these cytoskeletal structures [6]. The changes in the phosphorylation process in motoneurons in ALS patients are likely to cause such changes in the structure of the main components of the cytoskeleton culminating in total destruction and eventually death of the neuron.

Thus, the data obtained confirm the prevalence of IgM among serum antibodies from ALS patients, as well as the targeting of these antibodies against phosphoepitopes of neural tissue protein antigenic determinants. It may be concluded that the changes in the protein phosphorylation process play a part in the pathogenesis of ALS.

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