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# PARTICIPATION OF RETICULOENDOTHELIAL SYSTEM IN THE PATHOGENESIS OF EXPERIMENTAL AMYOTROPHIC LEUKOSPONGIOSIS

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*Summary.* — Spleen and peripheral blood lymphocytes were found to play an important role in the pathogenesis of amyotrophic leukospongiosis (AL). At early stage of AL they are the sites of reproduction and accumulation of an unconventional virus; they also provide its dissemination in the body of infected animals. In lymph nodes and visceral organs the AL pathogen was detected in much lower quantities and only in the period of clinically manifest disease. The level of the complement was significantly decreased in the serum of animals with experimental AL. This decrease correlated with the development of clinico-morphological lesions and reached its maximum at the terminal stage of disease.

*Key words: amyotrophic leukospongiosis; slow infection of the CNS; pathogenesis, spleen; lymphocytes of peripheral blood*

## *Introduction*

Amyotrophic leukospongiosis (AL) is a slow infection caused by an unconventional virus. The possibility to elicit experimental AL in squirrel monkeys, guinea pigs and other laboratory animals allowed to study this new nosologic form of subacute transmissive spongiform encephalopathy (Votyakov *et al.*, 1984; Kolomiets *et al.*, 1986).

It was believed until recently that only CNS was involved in the pathogenesis of slow infections caused by unconventional viruses (Erman *et al.*, 1984). It has been shown, however, that some components of reticuloendothelial system, e.g. spleen and lymphocytes participate in the persistence of unconventional viruses in a susceptible organism (Timakov and Zuev, 1977; Brown, 1984; Gajdusek, 1985). The purpose of the present paper has been to study the probable contribution of reticuloendothelial system to the pathogenesis of experimental AL in guinea pigs.

Repeated passages of the AL pathogen in the same animal species as well as retrobulbar injection of infectious material led to a considerable shortening of the incubation period of experimental AL. This allowed us

to produce a suitable laboratory model of the disease characterized by a short incubation period (1 to 2 months) and rapid development of typical clinico-morphological signs (Kolomiets *et al.*, 1985; Kolomiets *et al.*, 1986).

## *Materials and Methods*

Studies on experimental AL were carried out in 30 guinea pigs weighing 250 to 300 g. They were infected by retrobulbar route with 10% brain suspension from the patient D. who died of AL. The brain, visceral organs (spleen, lungs, liver, kidneys and lymph nodes) and whole blood collected from the heart were taken on days 4–5 and 6–7 post-infection and then weekly from 3 animals and used for virological and histological examinations (for details see Kolomiets *et al.*, 1988)*.

## *Results*

Virological findings showed that the unconventional agent of AL has accumulated at early stages (days 4 or 5 p.i.) in high titres in the spleen (Table 1). Histological examination of all organs of infected animals sacrificed on days 4 to 7 p.i. by bleeding failed to detect any noticeable patho-

**Table 1. Infectivity of tissues of different organs in guinea pigs with experimental AL at different intervals post-infection**

| Organ | Incubation period of AL in animals infected with 10% suspensions of the organs of guinea pigs scarificed at different intervals | | | | |
|---|---|---|---|---|---|
| | 4–5* | 6–7* | 13–14* | 21–22* | 30–34* |
| Brain | 102–122 (11/12) | 101–113 (5/6) | 78–80 (6/6) | 65–71 (6/6) | 38 (6/6) |
| Spinal cord | 101–109 (12/15) | 113–128 (5/8) | 74–82 (8/9) | 67–78 (9/9) | 32 (9/9) |
| Spleen | 84 (5/5) | 108 (3/3) | 70 (3/3) | 41 (3/3) | 40 (3/3) |
| Peripheral blood lymphocytes | 119 (1/3) | 101 (2/3) | – (0/3) | 156 (2/3) | 48 (3/3) |
| Lymp nodes | – (0/5) | – (0/3) | – (0/3) | 130 (2/3) | 106 (3/3) |
| Visceral organs (lung, liver, kidney) | – (0/15) | – (0/9) | – (0/9) | 128–147 (7/9) | 84–97 (6/6)** |

One asterisk – days at which the infected animals were sacrificed; two asterisks – not tested. In parentheses – the number of animals with morphologically confirmed AL diagnosis versus the number of infected animals.

Note. Titration of unconventional AL virus showed a linear relationship between virus titre and duration of incubation period: 30 days correspond to 7.5 log $LD_{50}$, 52 days – 6.5 log $LD_{50}$, 75 days – 5.5 log $LD_{50}$, 97 days – 4.5 log $LD_{50}$, 120 days – 3.5 log $LD_{50}$, 142 days – 2.5 log $LD_{50}$, 165 days – 1.5 log $LD_{50}$.

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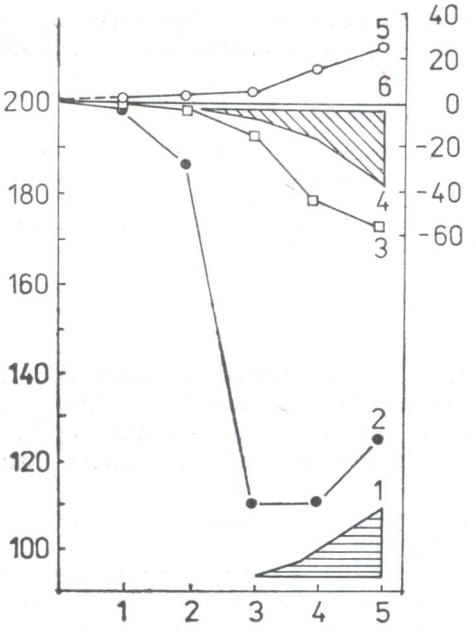


**Fig. 1**
Time course of clinico-morphological changes and complement level in the blood of guinea pigs with experimental AL infection.
1 — development of clinical signs, 2 — complement level in the blood serum of experimental animals, 3 — body weight variations in experimental animals, 4 — development of morphological signs of the disease, 5 — body weight variations in controls, 6 — complement level in the blood serum of control animals.
Abscissa — weeks, ordinate — in the right: complement (units), in the left: body weight (g).

logical changes. In the optic nerve of 1 animal out of 3 only, weak proliferation of glial cells was observed on day 7 p.i. It can be seen from the data presented in the Table that the concentration of the AL pathogen in the splenic tissue decreased slightly on days 6—7 p.i. and later increased gradually reaching maximum at the time when characteristic clinical signs developed (on days 30 to 34 p.i.). It should be noted, however, that at the time of marked clinical signs and during the terminal stage of AL, the titres of the agent in the CNS tissue (first of all in the spinal cord) appeared to be higher than in the spleen. In the CNS characteristic pathologic lesions of varying degree occurred in different regions: they were more marked in the spinal cord than in the brain, while in the spleen only insignificant follicular hyperplasia was seen.

At early stages post-infection the AL pathogen was detected not only in the spleen and CNS but also in the lymphocytes of peripheral blood, which confirms the importance of haematogenic dissemination of the unconventional virus in the body of the infected animal. During the assessment of infectivity of peripheral blood lymphocytes, however, the time course of virus accumulation was found to be somewhat different from that in splenic tissue. After insignificant rise in virus concentration in the blood at the end of week 1 p.i., by week 2 it was undetectable in the lymphocytes and was again observed by week 3. It should be noted that throughout the period of observation the animals showed neither changes in the B : T

lymphocyte ratio of peripheral blood nor immunological abnormalities (as revealed by blast-transformation-test). No specific antibodies reacting with antigens prepared from brain tissue of animal with experimental AL were found in the serum. At the same time, starting from week 2 of the experiment, a significant decrease of complement concentration was registered in the serum of infected animals. It correlated with the development of clinico-morphological pattern of experimental AL and reached a maximum at the terminal stage of the disease (Fig. 1). The role of decreased complement level in AL pathogenesis still remains unknown and needs further investigation.

## *Discussion*

Infectivity assay of lymph node tissue of infected animals have shown the concentration of unconventional AL agent was relatively low in both the lymph nodes and visceral organs at the period of clinical manifestation of the disease. The presence of the AL pathogen in the tissue of essentially all organs at the terminal stage of the disease seems to be determined by its haematogenic dissemination over the organism from the spleen. This suggestion was supported by relatively low concentration of the pathogen in the tissues of these organs in contrast with its high current level in the spleen and confirmed by the absence of any pathologic changes in visceral organs as evident from light microscopic findings.

A similar time course of Creutzfeldt-Jacob disease (CJD) agent dissemination was found in mice (Kuroda *et al.*, 1984). These authors have shown that after intracerebral infection of mice the titre of CJD agent was higher in the spleen than in the CNS as early as on day 7 p.i. As soon as the CJD agent reached its maximal titres in the spleen it was also detected in the peripheral blood and then in other visceral organs.

Our data are in agreement with the results of infectivity assay in the spleen during the incubation period of scrapie. It has been found that the scrapie pathogen accumulates in the spleen where it can persist for a long time without invading or damaging the CNS; the disease is clinically inapparent throughout this period.

Our results suggest that spleen and lymphocytes of peripheral blood play an important role in pathogenesis of AL and also participate in dissemination of the pathogen over the body. The present investigations may open new outlooks for the diagnostic and thearpy of amyotrophic leukospongiosis.

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