

Some Immunological Characteristics of Tick-Borne Encephalitis in Perm Region

Liliya P. Bykova, Anatoliy P. Godovalov, and Olga V. Lebedinskaya

Abstract—It is shown that the relationship of tick-borne encephalitis virus with the human body comes in two ways, the development of acute infection with the outcome in convalescence and long stay by the virus in the body, its persistence in the nervous tissue with periodic reactivation and prolonged circulating immunoglobulin M. In spite of the fact that tick-borne encephalitis virus has a tropism for nerve tissue, involvement in the process of blood cells is an integral component of the infection. Comprehensive study of the relation of factors of innate and adaptive immunity in the tick-borne encephalitis providing insight into the features of chronic disease.

Keywords—Tick-borne encephalitis, phagocytic activity, a progressive.

I. INTRODUCTION

EPIDEMIOLOGICAL situation on viral tick-borne encephalitis disease (TBE) incidence in Perm region remains strained. In 2011, the incidence rate per 100 thousand of population was 9.9, which is 4.3 times higher than the rate in the Russian Federation. Tick-borne encephalitis is characterized by the wide range of onsets with dominated feverish and meningeal forms of the disease. In 1-3% of cases, TBE gets chronic, progressive course disabling patients and shorting their life. It's often difficult to identify the course of infection in way of synchronization. A detailed study of chronic process mainly belongs to experimental studies which have shown that the long-term persistence of the virus plays a significant role in the pathogenesis of chronic disease. Detection of the virus, forming the latent infection, in patients with the chronic phase of the disease was infrequent due to the lack of sensitivity and complexity of laboratory procedures. Nowadays, the definition of virus RNA is made possible by molecular genetic methods. Due to this fact, a new impetus appeared for the study of the innate and acquired immunity in tick-borne encephalitis and their dependence on the circulation of the virus. It is believed that the form variations and severity of encephalitis depend on the status of individual protection, which is primarily determined by a set of protective mechanisms of innate immunity [1]. Previously it was thought that the polymorphonuclear leukocytes, which are

weak in phagocytosis of viral elements, do not play a significant role in the defense against viral infections. More significant role belongs to macrophages and monocytes. It is shown that macrophages can phagocytize virus-infected cells [2]. However, the status of absorbed virus particles and the phagocytic reactivity is unclear. The involvement of macrophages in the pathogenesis of acute and chronic forms of tick-borne encephalitis and symptoms of the humoral response in the next virus attack are of interest.

II. OBJECTIVE

To study the characteristics of the innate and acquired immunity in people infected with tick-borne encephalitis virus in different forms of the disease.

III. MATERIALS AND METHODS

48 patients with tick-borne encephalitis and 30 healthy donors were examined. The diagnosis of encephalitis was confirmed by immunoenzyme and molecular genetic methods. Samples of heparinized blood, cerebrospinal fluid and blood serum were the material. Serum and cerebrospinal fluid of patients were examined by EIA for the determination of immunoglobulin M (IgM) and G (IgG) with test systems Vector-Best CJSC. RNA of TBE virus was determined in the virology laboratory FHSI "Center of Hygiene and Epidemiology in Perm region" with test system "Vecto-VKE-RNK-ampli100". The detection of the amplified DNA after PCR was performed by gel electrophoresis in 8-10% polyacrylamide gel.

Assessment of the leukocytes phagocytic activity (LPA) of patients was performed by a modified method, using as targets of phagocytosis of erythrocyte immunoglobulin sorbent, additionally loaded by TBE virus antigen. Activity of leukocytes adhering to the glass in the presence of TBE virus antigen and unrelated antigens was studied by the method proposed by Holt PG [4].

Statistical analysis of the results was performed using Student *t*-test. Differences were considered statistically significant at $p < 0.05$.

IV. RESULTS AND DISCUSSION

Study of the involvement of phagocytic cells in the pathological process in the chronic form of TBE was started with estimates of the leukocyte cells number. It was found that in chronic TBE against leukocytosis the relative number of neutrophils and eosinophils increased, the number of monocytes and lymphocytes decreased. The absolute

L.P. Bykova is with the Acad. E.A. Wagner Perm State Medical Academy, 26, Petropavlovskaya str., Perm, Russia (e-mail: bylylychka@mail.ru).

A.P. Godovalov is with the Acad. E.A. Wagner Perm State Medical Academy, 26, Petropavlovskaya str., Perm, Russia (e-mail: AGodovalov@gmail.com).

O.V. Lebedinskaya is with the Acad. E.A. Wagner Perm State Medical Academy, 61-74, P.Osipenko str., Perm, Russia (e-mail: lebedinska@mail.ru).

quantitative characteristics had the same tendency but the number of monocytes was similar in compared groups. Leukocytic intoxication index, which reflects the relation of humoral and cellular level, in patients with chronic form was 2.8 ± 1.5 c.u., and in healthy persons – 0.3 ± 0.1 .

It is known that an increase in the index indicates a high activation of endogenous intoxication and histolysis activation [3], which confirms the presence of the cyto-destructive effect of encephalitis virus at chronic stages. While analyzing the ratio of non-specific and specific defense cells we found the reduction of specific protection cell. It means the virus cyto-destructive effect orientation on the cells of monocytic series. Our investigations revealed that the rate of leukocyte adhesion to glass in the presence of TBE antigen in acute TBE was 12.6 ± 3.4 c.u. Which was significantly lower than in healthy donors (22.2 ± 3.1 ; $p < 0.05$). Patients in the late convalescence phase had adhesion index 10.6 ± 2.2 (compared to healthy $p < 0.05$). In chronic tick-borne encephalitis, this index was 12.5 ± 1.3 (compared to healthy $p < 0.05$). Adhesive ability of leukocytes in the presence of an unrelated antigen in patients with acute TBE was 21.0 ± 3.3 , under late convalescence – 19.9 ± 1.9 ; in chronic TBE – 20.6 ± 2.0 c.u. in healthy donors 6.3 ± 1.2 ($p < 0.05$). Since macrophage barrier to viral infections is not always effective, granulocyte response is of interest. One of the types of granulocytes - neutrophils - can make the phagocytic function. Phagocytic activity in the presence of virus TBE antigen in patients with the acute form of the disease was $56.4 \pm 3.5\%$, in late convalescence – $53.7 \pm 3.9\%$, in healthy donors – $53.4 \pm 3.4\%$; $p > 0.05$). Phagocytic activity of neutrophils in chronic TBE was $58.6 \pm 0.3\%$ and was significantly higher than in healthy donors ($33.8 \pm 2.9\%$; $p < 0.05$). Study of phagocytic reaction in the presence of specific antigen showed insignificant increase of the phagocytes activity ($p > 0.05$), as a result of which, according to modern concepts of cell cooperation in the immune response, there is a presentation of antigens and implementation of cellular and humoral immune response.

The study of specific humoral response showed that patients in the onset phase have IgM in titre of 8.2 ± 1.0 , and IgG in titre of 1.3 ± 1.9 in their serum. In patients with residual symptoms of the acute period, conventionally indicated as transition, IgM was detected in titer 4.1 ± 2.1 ($p > 0.05$), IgG was detected in titer 2.0 ± 2.0 ($p > 0.05$). In patients with chronic TBE geometric mean of IgM titer in serum was only 0.7 ± 0.7 ($p > 0.05$), and of IgG titer – 5.7 ± 1.6 ($p > 0.05$). For maximum exclusion of seronegative results and assessment of the nervous system involvement in maintaining the virus we additionally conducted a study on the presence of antigen not only in serum, but in cerebrospinal fluid. In assessing cerebrospinal fluid in patients with the acute form of the disease the geometric mean of specific immunoglobulin M titer was 7.4 ± 0.3 . The geometric mean of immunoglobulin M titer in serum was 6.3 ± 1.1 , and of immunoglobulin G – 6.7 ± 1.4 . The relation between the level of specific immunoglobulin M in serum and cerebrospinal fluid ($r = +0.82$) was detected. These results may indicate the preferential

localization of the TBE virus in the tissues of the nervous system and correspond to the classical concepts of formation of humoral immune response in tick-borne encephalitis. To determine the relation of immune response realization with virus circulation we conducted a molecular genetic test of blood samples of patients for the presence of RNA of TBE virus. The result was positive in all patients with the acute form of the disease. Patients with a prolonged period of recovery had RNA in blood samples in 25% of cases. In chronic course of the disease during the activation, RNA was found in 60% of cases. The dependence between the circulation of the virus and the formation of antigen was analyzed. Co-circulation of TBE virus and IgM was detected in the acute form of TBE. It's necessary to consider that the serological method identifies only the number of antibodies not connected in immune complexes. In 22% of the patients the humoral response was weak in the presence of circulating virus in the blood. The absence of IgG against the survey can indicate the realization of the primary immune response and start of immune complexes circulating. In the late recover the virus in the blood of most patients (75%) did not show up on a background of circulating antibodies of M and G classes. In 25% of cases in the absence of free antibodies, the virus was detected in the blood, which may be indicative of its close connection with blood cells. The virus was not detected in the blood of 40% of patients with chronic form of TBE, and the IgG titer was high. 60% of cases had the opposite situation – the virus circulated in blood, and IgG titer was low, which indicates its output into the blood stream and the next stage of viraemia.

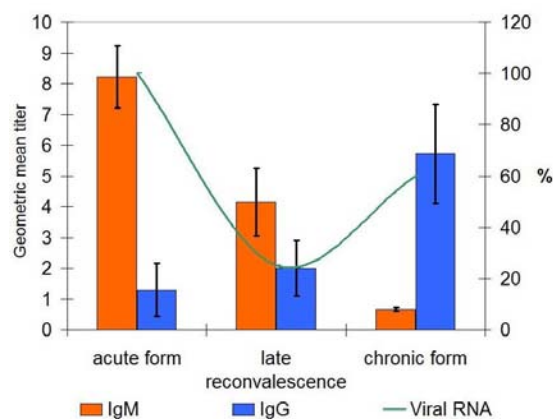


Fig. 1 The amount of specific immunoglobulins M and G to the virus (geometric titer) and the number of tick-borne encephalitis patients in whom viral RNA was found (%)

The studies have shown that the relation of the TBE virus with the human body goes two ways - the development of acute infection with the outcome of recovery and the long-term stay of the virus in the body, its persistence in the nerve tissue with periodic reactivation and prolonged circulation of immunoglobulin M. In spite of the fact that TBE virus has a tropism for neural tissue the involvement of blood cells in the

process is an essential component of the infection. There was a change in the functional activity of leukocytes in acute and chronic forms of tick-borne encephalitis. In the acute form of the disease it was shown by the leukocyte adherence inhibition, enhanced phagocytic response during infection onset and slight decrease in its late period of recovery.

In the chronic form of the disease a significant activation of these cells under the influence of a specific antigenic stimulus was not revealed. Such changes may indicate a lack of effectiveness of the monocyte-macrophage system barrier function in the chronic form of TBE. Viruses are inside leukocytes, that gives them certain invulnerability for particular antibodies in the body. Maintenance of viral replication in the cells of the immune system creates favorable conditions for the reduction of specific defense mechanisms. It makes the disease become chronic. Integrated study of the relation of factors of the innate and acquired immunity in tick-borne encephalitis will make possible to understand the characteristics of chronic disease.

ACKNOWLEDGMENT

The work was supported by RFBR grant 11-04-96037r_ural_a and Perm Region Administrative Body.

REFERENCES

- [1] V.G. Ivanov, G.G. Ivanov, A.K. Barsukov, A.I. Kuznetsova "Relationships between the content of circulating immune complexes, the concentration of serum immunoglobulins and the titers of specific antibodies in patients with tick-borne encephalitis", *Zh. Mikrobiol. Epidemiol. Immunobiol.* vol. (3), 2004, pp. 93-95.
- [2] G.N. Leonova, O.S. Maistrovskaia, V.B. Borisevich "Antigenemia in people infected with tick-borne encephalitis virus", *Vopr. Virusol.* vol. 41(6), 1996, pp. 260-263.
- [3] Zh.G. Mustafina, Iu.S. Kramorenko, V.Iu. Kobtseva "Integral hematological indices in assessing body immunological reactivity in patients with ophthalmic pathology", *Klin. Lab. Diagn.*, vol. 5, 1999, pp. 47-49.
- [4] P.G. Holt, L.M. Roberts, P.J. Fimmel, D. Keast "The L.A.I. microtest: a rapid and sensitive procedure for the demonstration of cell-mediated immunity in vitro", *J. Immunol. Methods*, vol. 8(3), 1975, pp. 277-288.