Acta virol. 34 : 554—562, 1990

THE ANALYSIS OF SOME INDICES OF IMMUNE RESPONSE, DNA REPAIR, AND MICRONUCLEI CONTENT IN CELLS FROM TICK-BORNE ENCEPHALITIS PATIENTS

N. N. ILYINSKIKH, E. JU. ZAGROMOV, A. V. LEPEKHIN

Tomsk Medical Institute, 634050 Tomsk, U.S.S.R.

Received November 22, 1988; revised August 28, 1989

Summary. — Patients with tick-borne encephalitis (TBE) had higher counts of red blood cells (RBC) with micronuclei. The majority of patients revealed decreased capacity of blood lymphoid cells for DNA repair except those with a 2-wave pattern of the course of disease; in the latter, the DNA repair was significantly higher than in healthy donors. Patients with TBE revealed lower T-lymphocyte counts due to a decrease in the amount of T-helper cells (the level of T-suppressors was elevated). The intensity of antibody production against TBE virus was significantly enhanced by termination of disease in the majority of patients. The count of natural killer cells was decreased, particularly at the initial stage of disease. At the time of admission to hospital the counts of RBC with micronuclei and of T-helper cells were in reverse proportion. At the terminal stage of disease the same correlation was noted between RBC counts with micronuclei and the antibody level. At the onset of disease a direct correlation was noted between DNA repair and B-lymphocyte and T-helper counts. At the final stage of disease the reverse correlation between the activity of DNA-repair systems and T-suppressor counts was registered. Three months after discharge from hospital, the indices of micronuclear test, natural killer cell activity, and DNA repair returned to normal.

Key words: tick-borne encephalitis; cytogenetic disorders; DNA repair; T- and B-cell immunoreactivity

Introduction

Viruses are known to induce varying cytogenetic disorders in human and animal cells (Buzhievskaya, 1984; Ilyinskikh *et al.*, 1984). Some viruses have been demonstrated to inhibit the system of cell DNA repair thereby leading to cytogenetic aberrations (Zasukhina, 1979). At the same time, the control of genetic homeostasis of the body involves the immune system as well (Burnet, 1971). As known, in many virus-induced infections T-immunosuppression and lowered counts of natural killer cells have been reported (Se-

menov and Gavrilov, 1976; Zhdanov *et al.*, 1986), and it agrees with the elevated rate of cytogenetic disorders in human cells (Ilyinskikh *et al.*, 1986). Previously we described TBE virus-induced cytogenetic disorders in *in vitro* conditions (Ilyinskikh and Ilyinskikh, 1976). In addition, patients with TBE have been reported to have T-immunosuppression (Kvetkova *et al.*, 1978; Larina and Levkovich, 1981; Shmatko, 1982), and the authors have different ideas as to the reasons underlying this phenomenon.

The present report was aimed at complex parallel studies of the status of immune, DNA repair systems and the level of cytogenetic disorders in patients with TBE. A special attention is given to the analysis of relationship between genetic and immunologic changes noted in patients' cells.

Materials and Methods

Twenty-three patients with TBE (21 males and 2 females) have been examined, at the average age of 35 ± 1.2. According to the clinical pattern, the patients were divided as follows: 9 patients developed a mitigated type of disease, 8 had the meningeal type, and 6 had the focal type. Nineteen of the examinees had an one-wave pattern of the disease, and 4 had two-wave type. All the latter developed the meningeal type of the disease. The patients were admitted to hospital on days 1—3 since the onset of disease.

Blood counts were made at the time of admittance to hospital, before discharge (on an average 1 month after the onset of disease) and 3 months after discharge. The diagnosis of TBE in all cases was confirmed by antibody titres elevated more than 4-fold in haemagglutination inhibition test (HIT). The examinees were never vaccinated against TBE, and no cases of tick sucking were recorded. The patients were neither X-rayed nor did they receive chemotherapeutic drugs with a potentially mutagenic effect.

Immunologic examination. In all cases the patients blood was assayed for the activity of natural killer cells (Bunatjan *et al.*, 1985), T- and B-lymphocyte counts (Novikov and Novikova, 1979), theophyline-sensitive and -resistant T-lymphocytes which further on are designated as T-suppressor and T-helper cells, respectively (Lebedjev and Ponyakhina, 1983). The serum antibody levels against TBE virus were determined by HIT (Lennett and Schmidt, 1974).

Cytogenetic and DNA repair analyses. The extent of cytogenetic aberrations in patient cells was determined by micronuclear assay (Ilyinskikh *et al.*, 1986) using peripheral RBC. Ten-twenty thousand RBC from each patient were examined and the number of cells with micronuclei was determined. The smears for micronuclear assay were prepared in a conventional way from a drop of finger blood, fixed in alcohol and stained by Romanovsky-Giemsa. In parallel, some smears were stained with mecaprine or acridine orange and examined in a luminescent microscope. The number of RBC with micronuclei established by the use of both methods proved identical. Under a luminescent microscope the micronuclei looked like green luminescent round-shaped formations. Non-chromatin structures were stained red. For light microscopy, micronuclei were stained with the Romanovsky-Giemsa dye. The 4-nitrochinolyne-1-oxide (4-NCO)- or UV-induced DNA repair was determined by means of scintillation radiometry (Zasukhina *et al.*, 1986) by ^{3}H-thymidine incorporation in a total mass of cells when DNA replication was inhibited by hydroxyurea (10 μmol/ml). As the source of UV light, 2 BUV-15 lamps (254 nm) were used. The dose of UV light was 15 J/m^2, the intensity of radiation being 1.6 J/sec/m^2. To induce repair synthesis, 4-NCO was used at concentration of 2.5×10^{-6} mol/l and 30 min exposure. Isotope (370 kBq/ml) was added to the growth medium immediately after cell treatment with mutagens and incubated for 2 hr. Cells were washed and their fixed number was put on millipore filters (0.3 μm in diameter) in 5% trichloroacetic acid. Radioactivity was measured in a toluene scintillator, Mark III counter. The intensity of repair synthesis was estimated by the index of stimulation, calculated as the ratio of radioactivity (cpm) of mutagen-treated cells to that (cpm) of control cells.

For control 20 healthy donors were examined with the use of the same methods. All the data obtained were processed using the methods of Student and Spierman (Gubler and Genkin, 1973).

Results

The results of cytogenetic and DNA repair analysis indicate that TBE patients with one-wave disease course irrespective of its clinical patternfall into a statistically homogenous group. It was shown (Table 1) that patient with one-wave progression of disease had statistically significantly higher counts of RBC with micronuclei in their peripheral blood at admission to hospital and at discharge than had healthy donors ($1.6 \pm 0.2\%$ at the onset and $0.8 \pm 0.1\%$ at the end of disease, in comparison to $0.3 \pm 0.1\%$ in healthy donors; in both cases p was not lower than 0.05). Before discharge all patients exhibited decreased indices in the micronuclear test as compared to its values at admittance, the counts of RBC with micronuclei returned to normal only 3 months after the discharge of patients from the hospital (Table 1). Patients with two-wave type of TBE progression had similar indices in the micronuclear test.

However, studies of the consequences of UV-stimulated DNA repair revealed that stimulation differed in patients with one- or two-wave pattern of TBE. Thus, in the case of one-wave type the intensity of repair synthesis proved to be 2—3 times as low as that observed in healthy donors, but in the case of the two-wave pattern of TBE this index was significantly higher than in control: 5.7 ± 1.1 following UV-induced stimulation (2.9 ± 0.4 in control) and 7.6 ± 1.8 following 4-NCO stimulation (3.2 ± 0.4 in control).

The analysis of T- and B-systems of immunity demonstrated that in TBE patients a marked T-immunosuppression was observed against the background of B-system stimulation (Table 2). The total counts of T-lymphocytes 1 ml of blood of healthy donors were 1620 ± 61 whereas in patients with TBE at the onset of the disease that index was significantly decreased: 700 ± 58.0 ($p < 0.01$). Before discharge the counts of T-lymphocytes returned to the level observed in controls (Table 2). At the same time it should be noted that this normalisation took place due to a marked increase of the total lymphocyte counts in patients as their relative T-lymphocyte count remained low in all groups of patients examined even 3 months after their discharge from the hospital (Table 2).

Studies of T-helper and T-suppressor counts indicate a significant disbalance of the immunoregulatory cells. The Th/Ts ratio in controls amounts to 2.8 while in patients at the onset of the disease T-helper counts were markedly decreased (the ratio of T_h/T_s was 0.72) while the counts of T-suppressors remained unaltered. After clinical recovery, the number of T-suppressor cells considerably rised in convalescents' blood while T-helper counts did not return to the norm (Table 2) and the ratio of T_h/T_s remained still low at the time of discharge (1.04) or even 3 months later (0.58) (Fig. 1).

Correlation analysis of the data obtained showed that some indices of the body's immune response were closely related to the indices of the micronuclear test and DNA repair synthesis (Table 3). Thus, at the onset of the disease an inverse correlation between the RBC counts with micronuclei and T-lymphocyte number was noted ($r = -0.67$; $p < 0.05$), apparently, it was accounted for mainly by T-helper cells ($r = -0.67$; $p < 0.01$). A similar

Table 1. The number of RBC with micronuclei and DNA repair activity in blood cells of patients with TBE having one- or two-wave progression of the disease

Index	Healthy donors	Patients with TBE				
		one-wave disease progression			two-wave disease progression	
		1	2	3	1	2
The number of RBC with micronuclei, ‰	0.3 ± 0.1	1.6 ± 0.2*	0.8 ± 0.1**	0.2 ± 0.1	2.3 ± 0.1*	1.0 ± 0.3*
The index of DNA repair activity stimulation:						
UV-radiation	2.9 ± 0.4	1.2 ± 0.2*	1.1 ± 0.2*	3.2 ± 2.9	5.7 ± 1.1**	
4-NCO	3.2 ± 0.4	1.0 ± 0.3*			7.6 ± 1.8**	

Footnote. 1 — at admittance, 2 — at discharge, 3 — three months after discharge.
* statistically significant differences from control; $p < 0.01$.
** the same, p being < 0.05.

Table 2. Indices of immunoreactivity in patients with one-wave TBE progression

Index	Healthy donors	Patients with TBE		
		1	2	3
3-lymphocytes				
%	65.8 ± 0.7	40.6 ± 2.6*	58.1 ± 2.7**	57.2 ± 2.2*
total number	1620 ± 61	700 ± 58*	1510 ± 133	1699 ± 135
T-helper cells				
%	48.9 ± 0.6	17.8 ± 4.4*	30.0 ± 1.8*	22.4 ± 1.2*
total number	1203 ± 38	303 ± 63*	772 ± 78*	593 ± 54*
T-suppressor cells				
%	17.1 ± 0.9	24.3 ± 3.5*	28.1 ± 2.8	34.8 ± 2.1*
total number	420 ± 25	417 ± 70	738 ± 95*	1015 ± 108*
T_h/T_s				
B-lymphocytes				
%	17 ± 0.8	22.3 ± 1.7**	30.5 ± 2.2*	23.6 ± 1.6**
total number	411 ± 28	318 ± 39	652 ± 320	632 ± 61
AB		0.83 ± 0.16*	1.74 ± 0.27*	
NK, conventional units	61.2 ± 5.1	42.4 ± 4.9**	54.2 ± 5.2	69.9 ± 2.7

Footnote. AB = antibody level against TBE virus in blood in decimal logarithms.
NK = the activity of natural killer cells in blood. The remaining symbols are the same as in Table 1.

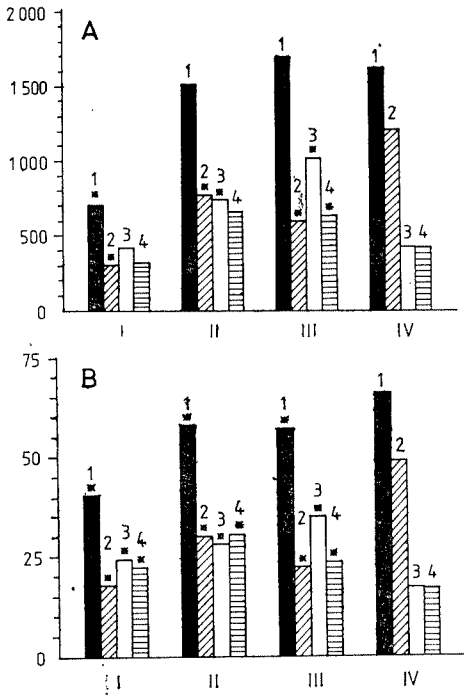

Fig. 1

Number of T and B rosette-forming cells in patients with one-wave course of TBE expressed in absolute (A) and relative (B) indices

I — at the time of admission; II — before discharge from the hospital; III — within 3 months after leaving the hospital; IV — healthy donors (controls).

1 — number of T-rosette forming cells; 2 — T-helpers; 3 — T-suppressors; 4 — T-lymphocytes

Significant differences are indicated by asterisks (< 0.05).

relationship was noted in the case of antibody production intensity as well ($r = -0.56$; $p < 0.05$). The following regulations were observed when DNA repair synthesis indices and those of immune response were compared: at onset of disease, a significant direct correlation between the counts of

Table 3. The correlation coefficients in comparing the indices of genome instability and immunoreactivity in TBE patients with one-wave disease progression

Index	The level of RBC with micronuclei			The index of DNA repair stimulation with UV-radiati'n		
	1	2	3	1	2	3
NK	−0.10	+0.25		−0.17	+0.10	
AB	−0.10	−0.56**		+0.40	−0.33	
T-lymphocytes	−0.57**	−0.26	+0.04	+0.33	−0.22	+0.65*
B-lymphocytes	−0.12	−0.22	+0.22	+0.71*	−0.06	+0.53**
T-suppressor cells	+0.49	−0.08	−0.15	+0.01	−0.62*	+0.52**
T-helper cells	−0.67*	−0.08	+0.34	+0.58**	+0.49	+0.53**
Total leukocyte count	+0.07	+0.34		+0.24	−0.12	

Footnote. The asterisks designate statistically significant values of the correlation coefficient. The remaining symbols are the same as in Tables 1 and 2.

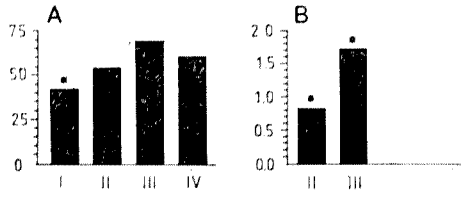

Fig. 2
Activity of natural killer cells (in units — A) and the levels of antibodies (HIT titres — B) to TBE virus (log_{10} values) in patients with one-wave course of their disease
For further explanations see legend to Fig. 1.

T-helpers and B-lymphocytes on the one hand, and the index of repair stimulation on the other hand ($r = +0.58$; $p < 0.05$ and $r = -0.71$, $p < 0.01$) was noted. At the final stage of the disease an inverse correlation was observed between the index of DNA repair stimulation and T-suppressor cell counts ($r = -0.62$, $p < 0.01$).

Three months after discharge of a patient from the hospital a direct correlation was registered between the index of DNA repair stimulation and the T-suppressor cell counts ($r = +0.52$), B-lymphocytes ($r = 0.53$) and peripheral RBC with micronuclei ($p < 0.05$) for all indices. In addition, a directly proportional correlation was noted between the index of repair stimulation on one hand and the T-lymphocyte counts on the other hand ($r = +0.65$; $p < 0.01$).

Discussion

As known, micronuclei are formed mainly as a result of one or several chromosomes lagging behind during the cell division, or in the course of acentric fragment formation (Alov, 1964; Ilyinskikh and Ilyinskikh, 1982). The evidence obtained confirm the results of *in vitro* studies where TBE virus induced increased amounts of aneuploid cells or cells with structural aberrations of chromosomes (Ilyinskikh and Ilyinskikh, 1976). Our results do not contradict the data obtained in cell cultures in relation to the capacity of the TBE virus to inhibit DNA repair (Dubinin and Zasukhina, 1975). However, of interest is the finding that in case of the two-wawe progression of TBE a paradoxical phenomenon was observed: enhanced levels of cells with micronuclei and stimultaneously high values of DNA repair. This finding indicates that these changes do not have much in common. Indeed, there is no significant correlation between the indices of the micronuclear test and DNA repair synthesis ($r = -0.24$ at admittance and $r = +0.49$ at discharge of a patient from hospital; $p > 0.05$ in both cases). Apparently, the changes in the activity of DNA repair system do not affect the appearance of RBC with micronuclei as the latter arise mainly due to disorders in the achromatin system of cell division of bone marrow erythroid series and the index of DNA repair stimulation was registered in nuclear blood cells. Enhanced levels of DNA repair synthesis can be possibly related to higher production of interferon which can markedly stimulate DNA repair processes (Zasukhina, 1979).

We suggested previously that under the influence of the viral infection cytogenetic disorders lead to immunosuppression mainly in T-system immunity (Ilyinskikh, 1986). A number of investigators have reported T-cell

deficiency in TBE (Kvetkova *et al.*, 1978; Larina and Levkovich, 1981; Savchenko *et al.*, 1981; Shmatko, 1982; Smorodintsev and Dubov, 1986), the majority of them agree that TBE virus can replicate in T-lymphocytes and thymus cells leading to their destruction. One may assume that the damage of T-cells is simultaneously accompanied by impairment of their genetic apparatus or that the products formed in the virus-affected cell exert a mutagenic effect (Ilyinskikh *et al.*, 1984).

Indirect evidence has been reported showing that different subpopulations of T-suppressors and T-helpers participate in regulating the production of immunoglobulin subclasses (Ishizaka and Adachi, 1976). Since the antibody level rose in patients in the course of disease it seems likely that the level of T-helpers is quite sufficient for B cell-induced activation of immunoglobulin synthesis, and despite the higher level of T-suppressors, their excess does not inhibit immunoglobulin biosynthesis. It is possible that some T-suppressor cells are genetically deficient and their excess is a result of adaptation of the immune system. In various acute and chronic viral infections (Bastin *et al.*, 1983) the number of T-suppressor cells increases conceivably providing for virus persistence and a long-term pattern of disease. At the same time (Allison, 1977) the T-suppressor inhibit autoimmune responses leading to chromosomal anomalies (Ilyinskikh, 1980), in addition, T-suppressors can violate cell generation (Ataullakhanov *et al.*, 1978). It cannot be ruled out that it leads to decreased killer activity of lymphocytes in the patient blood (Fig. 2).

The results of the correlation analysis prompt that the genetic changes registered in patients with TBE are associated mainly with the activity of humoral immunity since in the case of increased numbers of RBC with micronuclei and decreased capacity of cells to DNA repair T-helper, and in the latter case B-lymphocyte counts as well are decreased. At the termination of the disease higher number of RBC with micronuclei were noted together with a high level of antiviral antibodies. If in the first case the evidence obtained on T-helper or B-lymphocyte suppression can be accounted for by the damage of the genetic apparatus of these cells, there are scarcely any grounds to interpret in the same way the correlation noted between a great number of cells with micronuclei and enhanced antibody formation.

In our opinion, it is more relevant to suggest that in addition to higher amounts of antiviral antibodies, higher production of RBC with micronuclei is mediated via increased levels of antinuclear antibodies with the ability to elevate the number of cytogenetically altered cells (Ilyinskikh, 1986).

References

Allison, A. C. (1977): Mechanism by which autoimmunity can be produced. *Progr. Immunol.* **11**, 512.

Alov, I. A. (1964): Assays on Physiology of Mitotic Cell Division. Medicine, Moscow, 223 p. (in Russian).

Ataullakhanov, R. I., Sidirovich, I. G., and Natmitdinov, A. M. (1978): Suppressor cells of thymic origin (T-suppressor cells). In R. V. Petrov (Ed.): *Immunology* VINITI, Moscow, 282 p. (in Russian).

Bastin, A., Lobley, R. Kh., Tront, R. D., and Geitenby, P. A. (1983): Suppressor T-cells in im-

mune system hemopoiesis and in the conditions of pathology. In R. A. Tomson (Ed.): *Latest Advances in Clinical Immunology*, Medicine, Moscow, 496 p. (in Russian).
Burnet, F. (1971): *Cellular Immunology*. MIR, Moscow, 542 p.
Bunatyan, K. A., Zedgenidze, M. S., Alexeeva, L. V., Rjabova, T. V., Polyanskaya, I. S., Alexeev, L. P., and Fux, B. B. (1985): Studies of natural killer cell activity in children with systemic lupus erythematosus. *Immunologia* **1985**, 5, 62 (in Russian).
Buzhievskaya, T. I. (1984): *Virus-Induced Mutagenesis in Mammalian Cells*, Naukova Dumka, Kiev, 128 p. (in Russian).
Dubinin, N. P., and Zasukhina, G. D. (1975): *Repair Mechanisms of Cells and Viruses*. Nauka, Moscow, 145 p. (in Russian).
Gubler, E. V., and Genkin, A. A. (1973): *The Application of Non-Parameter Criteria of Statistics in Medicobiological Studies*. Medicine, Leningrad, 183 p. (in Russian).
Ilyinskikh, N. N., and Ilyinskikh, I. N. (1976): The effect of tick-borne encephalitis virus on the chromosome apparatus of human cells. *Cytology and Genetics* **10**, 331 p. (in Russian).
Ilyinskikh, N. N. (1980): The level of chromosome aberrations in some diseases with concomitant development of autoimmune processes. *Klin. Med.* **1980**, 5, 44 (in Russian).
Ilyinskikh, N. N. (1986): Genome instability and immunity. *Adv. modern Biol.* **102**, 1 (4), 25 (in Russian).
Ilyinskikh, N. N., and Ilyinskikh, I. N. (1982): Comparative characteristics of various methods of investigating cytogenetic disorderd in bone marrow cells of Rhesus monkey-infected with polyomyelitis virus. *Genetika* **18**, 764 (in Russian).
Ilyinskikh, N. N., Bocharov, E. F., and Ilyinskikh, I. N. (1984): *Infectious Mutagenesis*. Nauka, Novosibirsk, 168 p. (in Russian).
Ilyinskikh, N. N., Ilyinskikh, I. N., and Bocharov, E. F. (1986): *Cytogenetic Homeostasis* and *Immunity*, Nauka, Novosibirsk, 256 p. (in Russian).
Ishizaka, K., and Adachi, T. (1976): Generation of specific helper cells and suppressor cells *in vitro* for the IgE antibody responses. *J. Immunology* **117**, 40.
Kvetkova, E. A., Semenov, B. F., Perekhodova, S. K., and Sokolova, T. F. (1978): Quantitative characteristics of T and B lymphocyte populations in patients with tick-borne encephalitis, pp. 36—43. In *Problems of Immunity and Diagnostics of Natural Focal Diseases*, Medicine Leningrad (in Russian).
Larina, G. I., Levkovich, E. N. (1981): The studies of the mechanism of specific resistance formation mechanism in tick-borne encephalitis at the cellular level, pp. 71—72. In *Viruses and Viral Infections of Man*. Abstracts of the Conference. Moscow, 19021 May (in Russian).
Lebedjev, K. A., and Ponyakina, I. V. (1983): Rosette formation test for the detection of T- and B-immunocompetent cells. Possibilities and limitations. *Immunologia* **1983**, (4), (in Russian).
Lennett, E., and Schmidt, N. (1974): *Laboratory Diagnostics of Viral and Rickettsial Diseases* Medicine, Moscow, 764 p.
Novikov, D. K., and Novikova, V. I. (1979): *Cellular Methods of Immunodiagnosis*. Belarus , Minsk 223 p. (in Russian).
Savchenko, Ju. N., Shmatko, V. G., and Kvetkova, E. A. (1981): Clinical manifestations and immune reactivity in patients with tick-borne encephalitis. *Soviet Medicine* **1981**, 1, 70—71 (in Russian).
Semenov, B. F., and Gavrilov, V. I. (1976): *Immunopathology in Viral Infections*. Medicine, Moscow, 146 p. (in Russian).
Smorodintsev, A. A., and Dubov, A. V. (1986): *Tick-borne Encephalitis and its Vaccine Prevention*. Medicine, Leningrad, 229 p. (in Russian).
Shmatko, V. G. (1982): Clinical picture of acute stage of the fever type of tick-borne encephalitis and certain indices of immunoreactivity, pp. 80—85. In *Native Focal Diseases of Man*. Oms (in Russian).
Zasukhina, G. D. (1979): *Repair Mechanisms of Cells and the Problems of the Environment*. Nauka, Moscow, 183 p. (in Russian).
Zasukhina, G. D., Zharikov, N. M., Lvova, G. N., and Vasiljeva, I. M. (1986): Inhibition of UV- and radiomimetic-induced repair activity in cultured cells of schizophrenia patients. *Radiobiology* **26**, (1), 56—60 (in Russian).
Zhdanov, V. M., Ananjev, V. A., and Stakhanova, V. M. (1986): *Viral Hepatitis*, Moscow, Medicine, 225 p. (in Russian).