ANTIHEMAGGLUTINATING ANTIBODY SPECTRUM FOLLOWING EXPERIMENTAL IMMUNIZATION WITH TICK-BORNE ENCEPHALITIS VIRUSES

Translation No 1816

July 1966

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ANTIHEMAGGLUTINATING ANTIBODY SPECTRUM FOLLOWING EXPERIMENTAL IMMUNIZATION WITH TICK-BORNE ENCEPHALITIS VIRUSES

[Following is the translation of an article by K. K. Lvov, V. A. Zaklinskaya, M.P. Chumakov and L. S. Levina, Institute of Poliomyelitis and Viral Encephaliteses, USSR Academy of Medical Sciences, Moscow, published in the Russian-language periodical Voprosy virusologii (Problems of Virology), No 6, 1965, pages 657--663. It was submitted on 9 Jul 1965. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

Among the various approaches to the classification of arbovirus infections the most recognized is the system based on the antigenic properties of viruses, revealed, in particular, in the RPGA [4, 6, 7] [RPGA = passive hemagglutination reaction]. This makes it possible to detect group antigenic properties of viruses. In the separate investigations, devoted to clearing up the nature of the antigenic interdependencies between separate representatives of the tick-borne encephalitis group [1-3, 5, 8], the determination of the regularities of formation of homologous and heterologous antihemagglutinins is far from complete. Also, this problem has great importance in interpreting the results of serological investigations in foci of infection and when resolving a number of problems connected with the immunoprophylaxis of tick-borne encephalitis.

The aim of the present work is a study of the regularities of formation and the dynamics of homologous and heterologous antihemagglutinins, developing after experimental immunization with various viruses of the tick-borne encephalitis group.

Materials and Methods

In the work we used 14 strains of viruses from the group. The tick-borne encephalitis virus was represented by 8 strains: 2 of these were isolated in the Far East (Sofin, Khabarovskiy-17), 2 - in Western Siberia (Bars and Alshevskiy), 1 - in Moscow following laboratory infection (Pan), 1 - in Belorussia (256), 1 - in Czechoslovakia (Khipr), and 1 - in Sweden (20536). Thus, the strains of tick-borne encephalitis used encompass all the main geographical regions in which this virus is distributed. Each of the remaining viruses of the tick-borne encephalitis group was represented by one strain: Scotland encephalomyelitis of sheep (I-40, Scotland), Omsk hemorrhagic fever (Nikitina - Omskaya Oblast, Western Siberia), Langat (TR-21-Malaya), Kyasanur forest disease (W-372 - India), Powassan (Powassan - Canada), and also the Negishi strain (Japan).

White rats were used as the laboratory animals. Immunization was carried out with a 10% brain suspension from suckling rats, infected with the

corresponding strain of the virus. In addition to that, for immunization we used the industrial series of cerebral (Sofin strain) and cultural (Pan strain) vaccines against tick-borne encephalitis. We introduced the brain of noninfected rats (placebo) to the control group of animals in an analogous manner.

Each strain, and also the vaccines, were used to immunize 2 groups of rats each. There were 5 animals in each group. The animals of the first group were immunized one time (intraperitoneally with 1 ml), and the animals of the second group - 3 times (with a 10-day interval between injections). After 2--3 months following the initial course of immunization all the animals were reimmunized one time with the corresponding preparations.

Blood for immunization was taken from the hearts of the rats before immunization, after 10 days, 1, 2 and 3 months following immunization, and following reimmunization -- after 10 days and 4½ months. The blood from all 5 animals of each group was united for investigation in one test. In each period of investigation 2 tests were checked: From hypo- and hyperimmunized animals. All told 185 tests were taken, each of which we investigated with the entire collection of 14 antigens.

The RPGA was set up by the generally accepted method. In the test we used 8 AU of antigens. During the process of work optimum pH values were controlled constantly for each antigen (in the greater majority of cases the optimum was pH 6.4).

Dynamics of antibodies. Prior to switching to an analysis of the cross experiments in the RPGA we will compare the dynamics of the homologous and heterologous antibodies. Since it may be thought that the number of immunizations performed may be reflected in the dynamics of heterologous antibodies, this comparison should be made separately for the groups of animals which received 3 and 1 inoculations.

The dynamics of homologous and heterologous antibodies in animals after a single immunization are presented in figure 1.

For each period of investigation we present the geometric mean titers of antibodies in 13--14 tests in the case of homologous antibodies and in 164--196 tests in the case of heterologous antibodies. The titers of homologous and heterologous antibodies, already appearing in a number of cases by the 10th day after immunization, increase sharply by the end of the 1st month. A further intensive increase in them is observed during the next month. After 10 days following reimmunization a sharp increase of titers is noted. These gradually decrease during the next 4½ months. It is important to note that in all the periods of investigation, including the early ones, a clear parallelism is revealed between the titers of the homologous and heterologous antibodies. A quantitative difference between these two types of antibodies (1.5--2 log) is preserved throughout the entire period of observation -- both after immunization and after reimmunization.

Dynamics of antibodies in animals which received a 3-fold immunization have a certain uniqueness (figure 2).

The greatest titers of antibodies after the initial course of immunization are noted during a one month period of investigation. In this period they exceed by 3--4 log the level of antibodies in hypoimmunized animals. But throughout the course of the following month a noticeable lowering of titers takes place, while in the animals which received a single immunization an increase of antibodies is detected in this period. The lowering of titers continues throughout the third month. As usual, reimmunization leads to a powerful increase in the titers of antibodies, however, throughout the entire period after immunization they had the same values as in the rats which had been reimmunized after a single primary immunization. The differences in the titers of homologous and heterologous antibodies (1.5--2.5 log), just as in the previous case, bear a constant nature and do not change essentially during the various periods of investigation following immunization and reimmunization. Thus, the differences in the dynamics of heterologous and homologous antibodies have a quantitative nature.

Spectrum of antibodies. Since the quantitative difference in the titers of homologous antibodies remains practically constant during the various periods of investigation, then there will be no distortion of results if we combine the data from investigating tests taken during the various periods. Therefore, we did just this in analyzing the spectrum of antibodies after immunization of animals with various strains. In order to exclude accidental results during a single investigation of tests, we calculated the average values of titers of homologous and heterologous antibodies in tests on hypo- and hyperimmunized animals, gathered in various periods after immunization. Each result of cross titration represents the average geometric titers of antihemagglutinins in the individually investigated 8 tests (2 tests for each period of investigation), collected in various periods after the immunization.

The titers of antibodies in the sera of rats immunized with the strains of tick-borned encephalitis (KE), isolated in the Far East (Sofin, Khabarovskiy-17), have the same level in respect to all the strains of this virus (figure 3). Lower titers (by 1--2 log) are obtained when investigating the sera with antigens of Scotland encephalomyelitis of sheep (ShEO), Omsk hemorrhagic fever (OGL), and Negishi. A still sharper lowering is noted when investigating the antigens of Kyasanur forest disease (KLB) and Powassan. Approximately the same relation is expressed when investigating the antisera to the strains from Western Siberia (Bars, Alshevskiy).

The titers of antihemagglutinins in the antiserum to the Pan strain (figure 4) mainly preserve similar relations. The difference increases some-what with the level of heterological antibodies to the remaining viruses of the tick-borne encephalitis group.

No principle differences were observed from the above cited data in the results of investigating the sera against strains of tick-borne encephalitis of western origin (see figure 4).

As is apparent from the results presented, a great similarity is noted in the titers of antibodies to the various strains of the tick-borne encephalitis virus. In exactly the same manner the antisera to various strains of this virus react monotypically when investigated with a collection of various antigens. On the basis of this we united all the results from the investigation of antisera to all the strains of tick-borne encephalitis. The combined data are presented in the table.

The antisera to the virus of Scotland encephalomyelitis of sheep had the highest titers with homologous antigen. The titers with the antigens of Omsk hemorrhagic fever and the western strains of tick-borne encephalitis were lower by 1 log. The titers of antibodies to the eastern strains of tick-borne encephalitis and to the viruses of Langat and Negishi differed by 2--3 log. As before, the greatest differences were noted with the viruses of Kyasanur forest disease (5 log) and Powassan (6 log).

The antiserum to the virus of Omsk hemorrhagic fever had titers of antibodies to all the strains of tick-borne encephalitis and to the Langat virus which were close to the level of homologous antibodies. The titers of antibodies to the viruses of Negishi, Scotland encephalomyelitis of sheep, Kyasanur forest disease and Powassan were lower by 2, 3, 4 and 6 log correspondingly.

The antiserum to the Langat virus had high heterologous titers of antibodies to all the strains of tick-borne encephalitis and to the virus of Omsk hemorrhagic fever. The heterologous antibodies to the viruses of Scotland encephalomyelitis of sheep and Negishi differed by 2--3 log, and to the viruses of Kyasanur forest disease and Powassan - by 3--4 log.

The antiserum to the Negishi virus behaved uniquely with the various antigens. The titers of antihemagglutinins to the western strains of tick-borne encephalitis, and also to the viruses of Scotland encephalomyelitis of sheep and Omsk hemorrhagic fever, differed insignificantly (1--2 log) from homologous. But with all the strains of eastern origin, excluding the Sofin strain, and with the Langat virus the difference in titers reached 3 log. The titers of antibodies with the viruses of Powassan and Kyasanur forest disease were lowered by 4--5 log.

The titers of antihemagglutinins in the antisera to the virus of Kyasanur forest disease were lower to all the antigens in comparison with the homologous ones. The least difference in titer (down to 1 log) was observed when setting up the reaction with the eastern strains of tick-borne encephalitis, and with the viruses of Omsk hemorrhagic fever and Langat. The titers of antibodies with western strains of tick-borne encephalitis and with the viruses of Scotland encephalomyelitis of sheep and Negishi were lower by 2--3 log, and with the virus of Powassan -- by 4 log.

The titers of heterologous antibodies in the antiserum to the Powassan virus were lower by 4--7 log than the level of hemagglutinins with homologous antigen. The greatest difference in the titer of hemagglutinins was observed when using the viruses of Omsk hemorrhagic fever, Negishi, and Kyasanur forest disease.

The sera of vaccinated animals contained antihemagglutinins in respect to all the antigens of tick-borne encephalitis. Revaccination caused the appearance of antibodies to the viruses of Scotland encephalomyelitis of sheep and Negishi, and to animals immunized with cultural vaccine -- also to the viruses of Omsk hemorrhagic fever and Langat.

All the sera collected from animals that had received placebo reacted negatively in the RPGA.

These materials repeat the above considered interrelationships between the tick-borne encephalitis virus and other representatives of this complex. There is a great similarity in the spectra of antihemagglutinins developing after immunization with the viruses of tick-borne encephalitis, Scotland encephalomyelitis of sheep, Omsk hemorrhagic fever, Langat and Negishi. On the other hand, the antigenic properties of the viruses of Kyasanur forest disease and Powassan are characterized by a great uniqueness. In an antigenic respect the virus of Kyasanur forest disease is somewhat closer to the viruses of tick-borne encephalitis, Omsk hemorrhagic fever and Langat. The antigenic ties between the Powassan virus and other representatives of the group are expressed weakly. In this respect our data conforms with the results obtained by Okuno et al. /8/.

Conclusions

1. A study was made of the regularities of formation and the dynamics of homologous and heterologous antihemagglutinins, developing after immunization of animals with the eastern and western strains of tick-borne encephalitis and the viruses of Scotland encephalomyelitis of sheep, Omsk hemorrhagic fever, Kyasanur forest disease, Langat, Powassan and Negishi.

2. The differences in the dynamics of homologous and heterologous antihemagglutinins after hypo- and hyperimmunization and reimmunization bear a quantitative nature. The difference in the titers of homologous and heterologous antibodies essentially does not change in the various periods in which the animals were investigated.

3. Immunization with any strain of tick-borne encephalitis virus leads to the formation of antihemagglutinating antibodies on an equal level to all the remaining strains related to this virus. There is a simultaneous formation of antibodies to all the other representatives of this group, but in lower titers. For the viruses of Omsk hemorrhagic fever, Langat, Scotland encephalomyelitis of sheep and Negishi the difference in titers of antibodies reach large differences, if the antibodies are determined against the viruses of Kyasanur forest disease (3--5 log) and especially Powassan (5--7 log).

4. Immunization with any other virus of the subgroup, except Powassan, also leads to the appearance of antibodies to the remaining representatives of the complex. The titers of antibodies here are lower by 1--3 log than to the homologous virus. In all cases the antibody titers to the viruses of

Kyasanur forest disease and Powassan are significantly lower (by 4--6 log). The heterologous antibody titers after immunization with the 2 latter viruses, especially Powassan, are significantly lower than the homologous ones.

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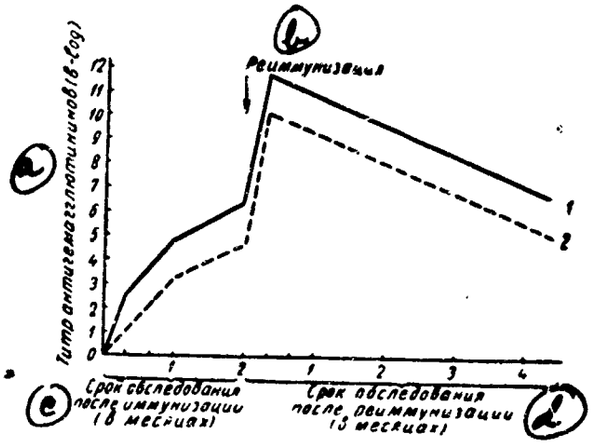

Figure 1. Dynamics of homologous (1) and heterologous (2) antibodies after the one-time primary immunization of animals.

a - titer of antihemagglutinins (in -log); b - reimmunization;
c - period of investigation after immunization (in months);
d - period of investigation after reimmunization (in months).

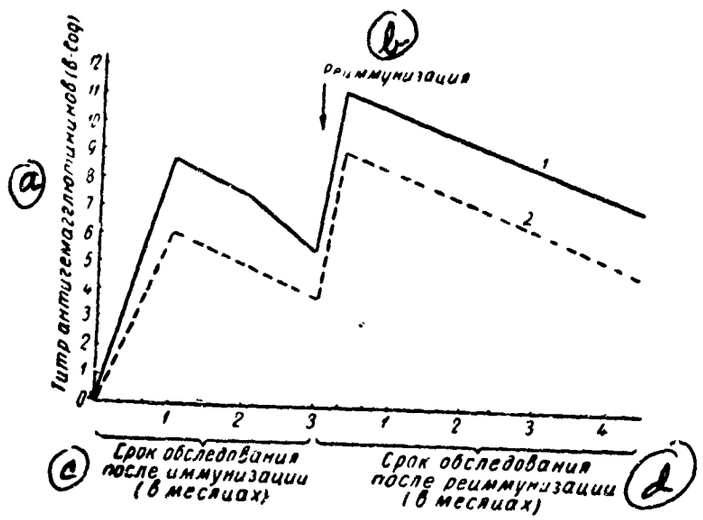

Figure 2. Dynamics of homologous (1) and heterologous (2) antibodies after a 3-fold primary immunization of animals. For each period of investigation the average results are cited for the titration of 13--14 sera in the case of homologous antibodies and 164--196 sera in the case of heterologous antibodies.

a - titer of antihemagglutinins (in -log); b - reimmunization;
c - period of investigation after immunization (in months);
d - period of investigation after reimmunization (in months).

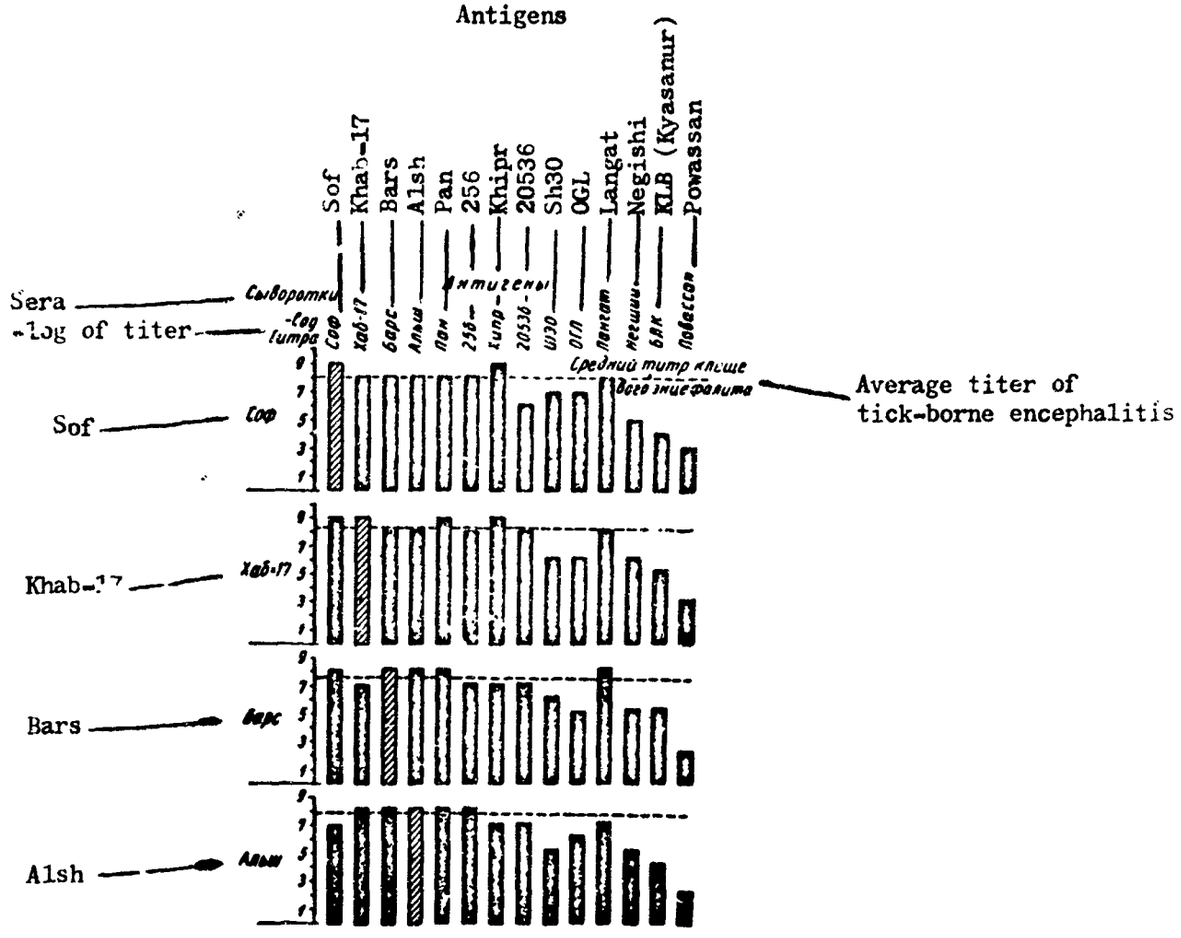

Figure 3. Titers of homologous and heterologous antibodies in animals immunized with strains of tick-borne encephalitis virus of eastern origin.

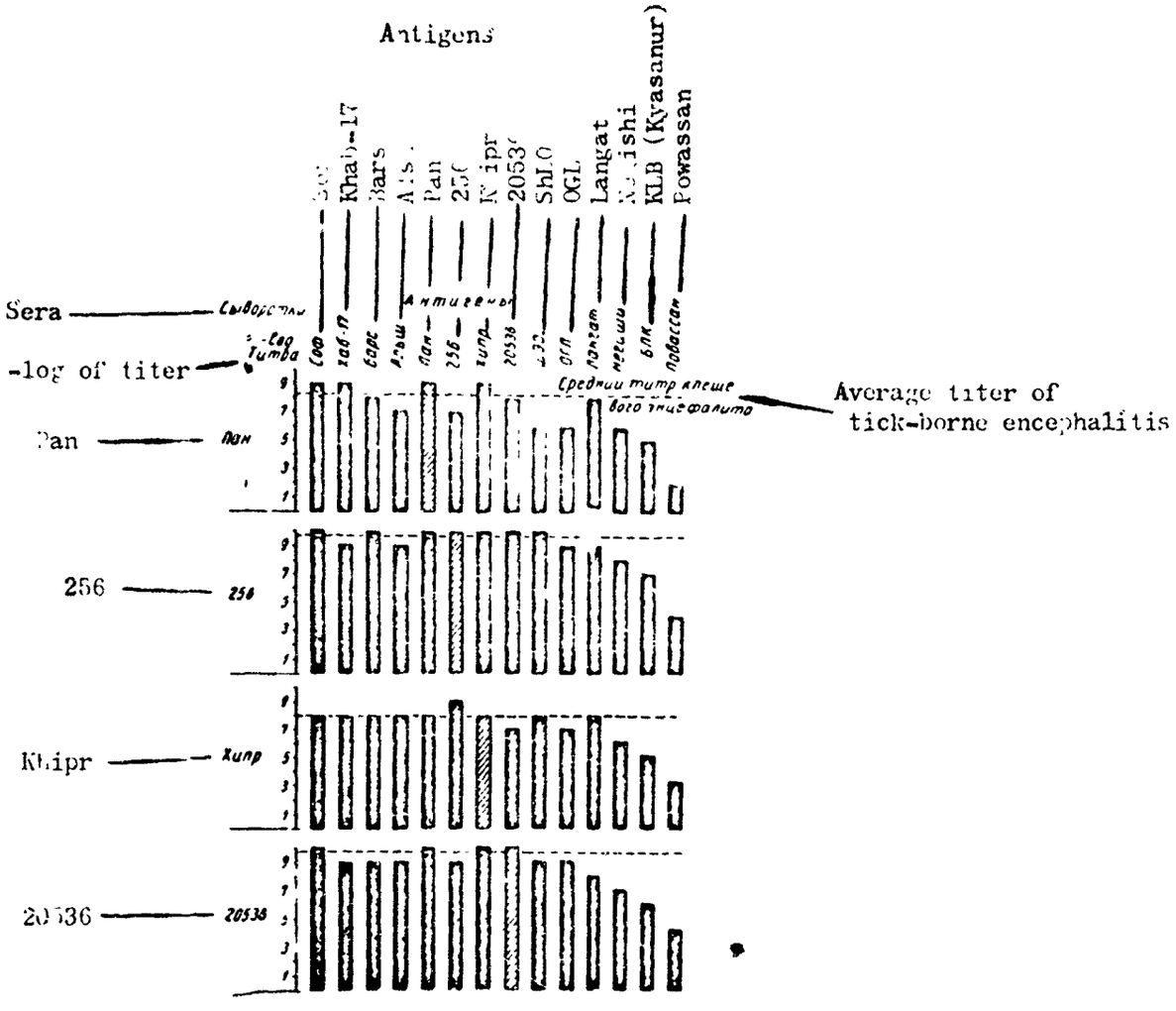

Figure 4. Titers of homologous and heterologous antibodies in animals immunized with strains of tick-borne encephalitis virus of western origin.

Antigenic interrelations within the group of tick-borne encephalitis based on the data of the RPGA

Sera	Antigens						
	KE (8 strains) a	ShEO b	OGL c	Langat	Negishi	KLB d	Powassan
KE (8 strains)	9^1	7^2	7	8^2	6^2	5^2	3^2
ShEO	6^2	8	6	6	5	3	2
OGL	7^2	5	8	7	6	4	2
Langat	7^2	5	6	7	4	4	3
Negishi	5^2	5	6	4	7	2	3
KLB	5^2	4	5	5	3	6	2
Powassan	3^2	4	2	3	2	2	9
Cultural vaccine	2^2	1	1	1	1	0	0
Cerebral vaccine	2^2	1	0	0	1	0	0
Placebo	0^2	0	0	0	0	0	0

1 - Reverse log of the average geometric titers of antibodies in 59 tests, investigated with 8 antigens of tick-borne encephalitis virus (472 separate results); each test represents a mixture of sera from 5 animals.
2 - Same for 59 tests, investigated with the stated antigen (59 separate results).
All the remaining values are the results of average investigations of 8 tests (8 separate results).
a - Tick-borne encephalitis
b - Scotland encephalomyelitis of sheep
c - Omsk hemorrhagic fever
d - Kyasanur forest disease