PHASES OF DEVELOPMENT OF THE HUMORAL IMMUNE RESPONSE

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The principles of establishment of the clinical and immunological diagnosis, taking account of the phases of development of the humoral immune response have been elaborated and used in previous investigations. The dynamics of formation of immunoglobulins and circulating immune complexes enabled the phases of the immune response to be distinguished.

The order of transition of the phases of development of the immune response required confirmation by dynamic studies. The results of such studies are described in this paper.

EXPERIMENTAL METHOD

Altogether, 57 women with acute exacerbations of chronic diseases of the genitalia, and also with chronic diseases outside the exacerbation phase, were investigated twice or 3 times at intervals of 1.5-2 weeks.

The clinical and functional diagnosis was made by Professor N. V. Anastas'eva (Department of Obstetrics and Gynecology, Novosibirsk Medical Institute). Levels of immunoglobulins M, G, and A were determined by ELISA, and the results were analyzed on a "Titertek Multiscan MCC/340" instrument at a wavelength of 492 nm. The level of circulating immune complexes (CIC) was determined by the precipitation method using 3 and 4% PÉG-600, and the results were measured on a "Specord M-40" spectrophotometer at a wavelength of 450 nm.

Subdivision of the immune response into phases was carried out in accordance with our previous investigations [2]:

the initial phase of the immune response — phase 1: elevation of the IgM level, various states of IgG and IgA, normal CIC level;

phase of the developed immune response — phase 2: high or falling IgM level, high or falling IgG and IgA level, or of one of them, high CIC level;

final phase of the immune response — phase 3: IgM falls below normal, IgG and IgA or one of them increased or depressed below normal, high CIC level;

phase of normalization of the immune response — phase 4: IgM level within normal limits, IgG and IgA levels or one of them within limits of previous phases, or also in phase of normalization of immune response.

Division into phases was based on the content of IgM and CIC [2].

The results were subjected to statistical analysis by programs of an "Olivetti M-290" personal computer.

EXPERIMENTAL RESULTS

The first group of subjects comprised 26 women with acute and exacerbations of chronic inflammatory diseases of the genitalia. By undertaking two or three investigations it was possible to determine the sequence of phases of development of the immune response corresponding to classical ideas: increased immunoglobulin production, peak of production, lowering of production.

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TABLE 1. Statistical Mean Values of Immunological Parameters Obtained during Repeated Investigations of Women

Group, subgroups	1,2 diseases	Immunoglobulins, g/liter			CIC, optical units			Significance of differences
		M	G	A	3%			
I group I – acute, subaculate diseases	$M_1G_1A_1$ C_1	2,4±0,21	17,2±1,5	2,6±0,22	275±26	323±24	M_1—M_2 C_1—C_2	$p<0,0001$ <0,05
	$M_2G_2A_2$	1,31±0,1	12,9±1,34	1,87±0,18	263±23	302±25	A_1—A_2	<0,01
Ia – acute diseases	C_2 »	2,51±0,25	17,1±1,9	2,39±0,27	233±29	271±27	M_1—M_2	<0,0001
	»	1,34±0,1	13,5±1,38	2,08±0,21	256±28	282±35	C_1—C_2 A_1—A_2	>0,05 >0,05
I b – exacerbation of chronic diseases	»	1,28±0,07	12,4±2,34	1,58±0,18	270±38	322±35	C_1—C_2 A_1—A_2	>0,05 <0,05

Legend. Correlations in group I: M_1-M_2 $\rho = 0.45$ ($p < 0.02$), M_1—G_2 $\rho = 0.59$ ($p < 0.003$) G_2-A_2 $\rho = 0.54$ ($p < 0.007$); 3% G_1 — 3% G_2 $\rho = 0.71$ ($p < 0.0003$).

Analysis of individual parameters at the first investigation showed that only in two women were the initial (first) phase of the immune response tested, i.e., elevation of IgM, and various states (normal or elevated levers) of IgG and IgA observed against a background of a normal CIC level. In 24 of the 26 women the second and third phases of the immune response were present: an increased IgM content or a sharp decrease (phase 3), various states of IgG and IgA, but against the background of a high CIC level.

At the second examination, a change from phase 1 to phase 2 was discovered. In phase 2 values of IgM were lowered and various states of IgG and IgA were found. The third phase was followed by the phase of normalization. The generalized data are given in Table 1. They indicate a significant difference between statistical mean values of all three classes of immunoglobulins, M, G, and A, established at the first and second investigations. Correlation analysis was carried out in the group between the following parameters: between IgM at the first and second tests, IgM at the first and IgG at the second test, IgG and IgA at the second test, and the 3% CIC at the first and second tests.

In accordance with the diagnosis, subgroups were formed: a) acute disease, b) exacerbation (Table 1). The results are evidence of a significant decline in IgM in both subgroups at the second examination, and significance of difference of this type also was discovered for IgA in exacerbations of the chronic disease. Thus the data obtained for this group indicate the regular principles of production of immunoglobulins and CIC during development of the immune response in accordance with the phases we have distinguished. However, in 12 women of the second group studied, some particular features were noted: in 11 women with the diagnosis of "exacerbation" the final phase (phase 3) of the immune response was tested, and only in one woman, with acute disease, was the analogous phase detected. In all these women a new rise of IgM was observed, sometimes with involvement of IgG or IgA, i.e., the initial phase (the second wave of exacerbation), developing either in response to activation of persistent infection or to superadded infection by a different agent. These data indicate the late attendance of the women at the clinic. We tested 19 women with chronic diseases of the genitalia, outside the exacerbation phase. In 10 women of this group one or other phase of development of the immune response was established. Repeated investigation of this group enabled phase 4 (normalization) to be discovered in all the women. However, in seven women the diagnosis of secondary immunodeficiency (ID state) was established. In three women development of the immune response was not tested: in six women the parameter (s) of the immunoglobulins was within limits interpreted as the ID state, but the CIC content was within normal limits. As a result, from the total group of 19 women 13 had an ID state affecting one or two immunoglublins.

To confirm the diagnosis of the ID state, the women were re-examined after 20-30 days. The diagnosis was confirmed in all the women tested. The fundamental importance of the third (final) phase of development of the immune response, which we distinguished, must be emphasized. It allows the precise definition of a criterion for diagnosis of an ID state which differs from ID in that not only is the immunoglobulin level lowered, but the CIC level is invariably normal. We could find no similar criteria in the accessible literature.

We have also suggested for the first time that the phases of development of the humoral immune response be evaluated on the basis not only of immunoglobulin production, but also of the level of CIC formation.

To sum up, the following conclusions may be drawn.

1. Development of the humoral immune response in inflammatory diseases of the female genitalia is characterized by successive phases of immunoglobulin production and CIC formation, and this must be taken into account when the clinical-immunological diagnosis is made.

2. By carrying out repeated investigations the sequence of phases of the immune response was confirmed.

3. A phase of the humoral immune response characterized by a below normal level of immunoglobulin(s) and by a high CIC concentration, distinguishing it from an ID state in which the CIC level is within normal limits, is distinguished for the first time.

LITERATURE CITED

1. I. V. Oradovskaya, L. L. Koshel'skaya, et al., Methodology, Organization, and Results of Mass Immunological Surveys [in Russian], Moscow—Angarsk (1987), pp. 146-150.
2. A. N. Trunov and G. T. Sukhikh, Byull. Sib. Otd. Akad. Med. Nauk SSSR, No. 6, 89 (1989).

STATE OF COAGULATION HEMOSTASIS AND FIBRINOLYSIS IN THE RAPIDLY PROGRESSIVE FORM OF BOTULISM

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The pathogenic action of botulinus toxin is known not to be restricted to blockade of acetylcholine release in myoneural synapses and synapses of the autonomic and central nervous system, but it is also accompanied by marked changes in functional activity of the system regulating the aggregation state of the blood (RASB). In particular, marked disturbances of vascularization of different parts of the brain and spinal cord and of the internal organs, manifested as a combination of signs of ischemia and congestion, by the presence of petechial and larger hemorrhages, thrombosis, and stasis in the capillaries against the background of destructive changes affecting the endothelium and walls of the blood vessels, have been described [1-4, 6, 7]. As yet, however, the character and mechanisms of disturbances of the pro- and anticoagulant components of the hemostasis system, and also the state of fibrinolysis in the course of botulinus poisoning, have not been studied. In was accordingly decided to investigate this problem.

EXPERIMENTAL METHOD

Type C botulism was induced by intraperitoneal injections of botulinus toxin in a dose of 0.025 mg/kg body weight (1 MLD for mice is 0.0005 mg of the dry toxin). Noninbred rats weighing 250 g were used. The experiments were carried out 6 h after injection of the toxin, in the absence of any marked clinical signs of poisoning, and also 24 and 48 h later, against a background of the development of generalized pareses and paralyses of the skeletal muscles. The following parameters of the state of the coagulation mechanism of hemostasis were studied at intervals: the blood clotting time in ordinary and siliconized

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