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INFLUENCE OF IMMUNOMODULATION ON THE FIRST STAGE OF ANTIGEN SPECIFIC RESPONSE TO HERPES VACCINE IN EXPERIMENT

The influence of immunomodulation on dynamics of early antigen specific response (antigen binding lymphocytes - ABL) was studied in the experiment with rabbits immunization by herpes vaccine. Acceleration of appearance and disappearance of ABL after one-time immunization with herpes vaccine by introduction of licensed preparations of interleukin-1, interleukin-2, polyoxidonium and interferon inductor bacterial was revealed.

MARIA OMAROVA,
BORIS KARALNIK,
TATYANA DENISOVA,
ALTAI KALYMBETOV

Scientific Center of Hygiene and Epidemiology, Kazakhstan

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Introduction

Immune modulation in the treatment of such chronic infection as herpes has a greate importance. More than 7000 studies have been published on this issue. They reflect data on clinical and immunological efficiency of different immunomodulators. However, the effect of immune modulation on the first phase of antigen-specific response - the formation of lymphocytes with receptors for herpes virus (antigen binding lymphocytes - ABL) has not been studied so far. The aim of the work is the analysis of this effect in the experiment.

Materials and methods

10 outbred rabbits weighing 2-2.5 kg were immunized with 0.5 ml herpes inactivated vaccine produced by Research Center for Hygiene and Epidemiology (SCHE, Almaty) subcutaneously ($0.5 \bullet 10^{-5.3}$ CPE_{50}). Simultaneously 8 rabbits received intravenously in 0.5 ml the licensed preparations of immunomodulators - roncoleukin (IL-2) produced by "Biotech Ltd.", St. Petersburg, in dose of 250,000 IU (rabbits number 1 and 2), betaleukin (IL-1) produced by Research Institute of Highly Pure Biopreparations (St-Petersburg) in dose of 0.5 mg (rabbits number 3 and 4), poly-oxidonium produced by Research and Production Association "Petrovax Farm", Moscow, in dose of 3 mg (rabbits number 5 and 6) and interferon inductor bacterial liquid (IIB) produced by the SCHE (rabbis number 7 and 8). Control rabbits (number 9 10) instead of immunomo-dulator received 0.5 ml 0.85 % solution of NaCl.

Blood of each animal was obtained from the boundary ear vein, collected in a solution of heparin, 25 U/ml in the time before and after 2, 4, 7, 14, 21, 28 and 35 days after immunization. Lymphocytes from each individual sample were separated on the gradient of ficoll-verografin with density 1.077. ABL of herpes specificity was determined by indirect rosette formation modified method (Deryabin et al., 1993) in each individual lymphocyte suspension with immune reagents of optimal (to determine total relative content of ABL) and suboptimal sensitivity (to determine ABL of high avidity) (Karalnik et al., 2010). In addition, nonspecific to the herpes virus early response of lymphocytes was determined by the same method using control immune reagent - sorbent not conjugated with herpes virus. In this case lymphocytes with receptors to sorbent were revealed. One antigen occupies only a small part of the sorbent surface (Karalnik and Deryabin, 1999).

It was previously shown that in experiment of simultaneous immunization by several antigens each ABL had receptors to one antigen only (Karalnik and Tsarevskij, 1974).

Therefore, to find out more exact substance of total ABL or ABL of high avidity (HABL), the content of lymphocytes formed rosettes with the control reagent was subtracted from experimental values of ABL. The content of low avidity ABL (LABL) was discovered by subtracting the content of HABL from the total content ABL. Statistical methods of series comparison and the method of integral comparison of the curves were used. Difference between series or curves estimated as proven if the probability of the null hypothesis (P) was no more than 0.05.

Results and discussion

From the data (Figure 1) it is clear that ABL prior immunization of animals were absent. This reflects the specificity of the test of ABL determination. It is revealed that LABL in all the rabbits were found within 2 days after immunization, then their content gradually increased and afterwards these cells quickly disappeared. Deadline of the last detection of LABL depend on the features of used immunomodulator: in control rabbits and in the IL-2 employment they disappear after 28 days, in the IL-1 and polyoxidonium use - after 21 days, in the IIB employment - after 14 days. HABL at using IL-1 or IIB were detected simultaneously with LABL, but in the control animals and in the IL-2 or polyoxidonium use - later. Disappearance of HABL was noted at 7 days earlier than LABL at IL-1 or IL-2 using and in control animals but at polyoxidonium or IIBL employment HABL and LABL disappeared simultaneously.

Comparison of dependence curves of LABL and HABL content from time after immunization revealed not only a significant exceeding of LABL curve over the same curve for HABL, but also significant non-parallelism of these curves regardless of the immunomodulation (Table 1). The absence of parallelism is due as a later appearance of HABL compared to LABL (it was detected in control rabbits and rabbits, which had been introduced IL-2 or polyoxidonium) as the shift of the maximum of HABL content of compared to LABL to later days (that is revealed in the control rabbits and rabbits which had been introduced IL-2 or polyoxidonium). Because of the detected shift of maximum in the rabbits of the last 3 groups, the content of HABL in 21 days after immunization was significantly more than the one of LABL despite a general excess of LABL curve above the HABL curve.

Based on these results it can be assumed that during ABL formation, as well as in antibodies production, the selection of the components with greater avidity develops. For the present it is not clear how this selection in ABL manifests itself: in large number of receptors for the antigen in the membrane ABL, or in increase the affinity of membrane receptors.

Next, there were compared the ABL content curves (both low and high avidity) in the control group and animals which received various immunomodulators (Table 2). Of the 4 used immunomodulators only polyoxidonium provided the significant, 2.5-fold, decrease in total (for the period of experience) content of LABL in comparison with control rabbits. Accordingly, the modulation by polyoxidonium provided the less value of general content of LABL than roncoleukine, 2 times, betaleukin - in 2.2, and IIB - in 1.8 times. The total content of HABL at use of polyoxidonium was also much smaller than the modulation by roncoleukin (2.2-fold), betaleukin and IIB (1.7 times). At the introduction of betaleukin this index in 1.3 and 1.4 times was less than at the modulation by roncoleukin and IIB respectively.

We also calculated the integral content of ABL by trapezoid method of definition of the area limited by terms of experience (the horizontal axis) and the curve of dependence of the ABL content on the time after immunization. These results are given in Table 3. In general, they correspond to data obtained by the method of integral comparison of the curves.

FIGURE 1. INFLUENCE OF IMMUNOMODULATORS ON DYNAMICS OF ABL OF LOW (A) AND HIGH (B) AVIDITY

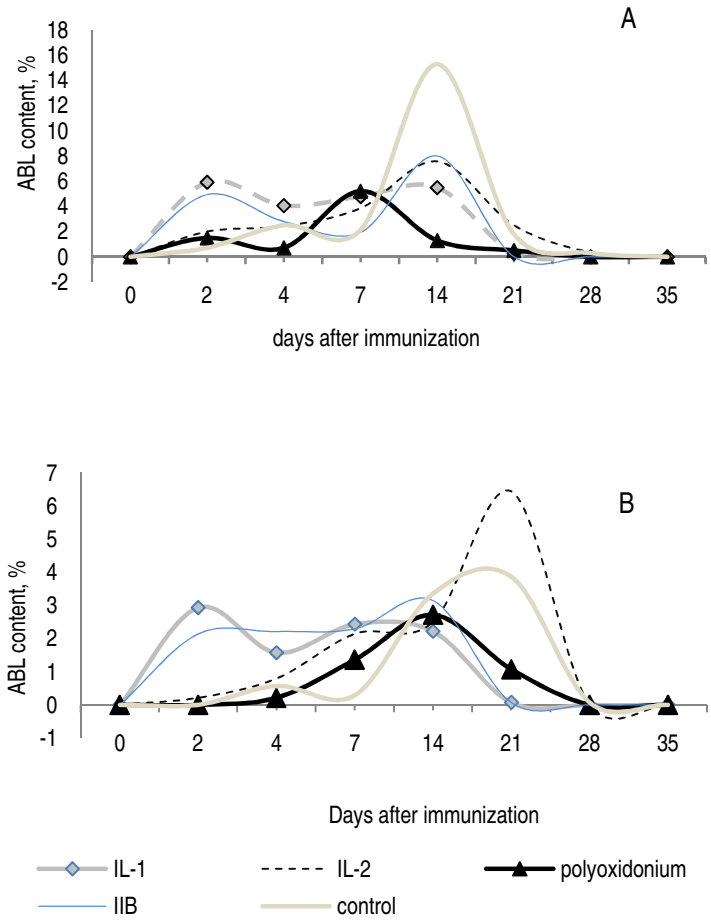

TABLE 1. RESULTS OF THE COMPARISON OF CURVES OF THE RELATIVE CONTENT OF LABL AND HABL DYNAMICS AT DIFFERENT IMMUNOMODULATION

Immunomodulator	Criterions of difference of curves of LABL and HABL dynamics					
	General		Exceeding		Non-parallelism	
	F_1	P	F_2	P	F_3	P
IL-2	10.64	<<0.001	9.38	<<0.001	9.07	<<0.001
IL-1	9.22	<<0.001	37.62	<<0.001	2.95	0.05>P>0.01
Polyoxidonium	10.50	<<0.001	6.10	<<0.001	9.28	<<0.001
IIB	7.37	<<0.001	15.46	<<0.001	4.28	0.05>P>0.01
Control	10.74	<<0.001	11.40	<<0.001	8.46	<<0.001

Notes: F-value of criterion, P - the probability of the null hypothesis.

Using the results of this assessment, obtained by two methods, we calculated portion of HABL in the total content of ABL of any avidity for the period of experience. It turned

out that in the control animals and animals that had been entered betaleukin or IIB it was small and almost identical (0.27, 0.30 and 0.28, respectively). In rabbits, received roncoleukin or polyoxidonium, this index was somewhat higher - 0.40 and 0.41 respectively.

TABLE 2. RESULTS OF THE COMPARISON OF CURVES OF THE ABL CONTENT AT USE OF DIFFERENT IMMUNOMODULATORS

Comparable groups of animals with use	Avidity of ABL	Criterions of curves difference					
		Total		Exceeding		Non-parallelism	
		F_1	P	F_2	P	F_3	P
Roncoleukin and without immunomodulation	Low	4.31	<0.001	1.07	>>0.05	4.13	<0.001
	High	4.86	<<0.001	4.26	0.01>P>0.001	1.67	>0.05
Betaleukin and without immunomodulation	Low	8.79	<<0.001	0.30	>>0.05	8.74	<<0.001
	High	15.14	<<0.001	0.58	>>0.05	15.05	<<0.001
Polyoxidonium and without immunomodulation	Low	14.96	<<0.001	12.94	<<0.001	12.80	<<0.001
	High	5.48	<<0.001	4.41	0.01>P>0.001	4.74	<0.001
IIB and without immunomodulation	Low	4.58	<0.001	2.22	>0.05	4.20	<0.001
	High	11.52	<<0.001	0.26	>>0.05	11.48	<<0.001
Roncoleukin and betaleukin	Low	5.49	<<0.001	0.67	>>0.05	5.38	<<0.001
	High	16.21	<<0.001	2.73	0.01>P>0.001	15.76	<<0.001
Roncoleukin and polyoxidonium	Low	12.43	<<0.001	23.13	<<0.001	8.57	<<0.001
	High	4.31	<0.001	1.07	>>0.05	4.13	<0.001
Roncoleukin and IIB	Low	4.86	<<0.001	4.26	0.01>P>0.001	1.67	>0.05
	High	8.79	<<0.001	0.30	>>0.05	8.74	<<0.001
Betaleukin and polyoxidonium	Low	15.14	<<0.001	0.58	>>0.05	15.05	<<0.001
	High	14.96	<<0.001	12.94	<<0.001	12.80	<<0.001
Betaleukin and IIB	Low	5.48	<<0.001	4.41	0.01>P>0.001	4.74	<0.001
	High	4.58	<0.001	2.22	>0.05	4.20	<0.001
Polyoxidonium and IIB	Low	11.52	<<0.001	0.26	>>0.05	11.48	<<0.001
	High	5.49	<<0.001	0.67	>>0.05	5.38	<<0.001

Notes: F-value of criterion, P - the probability of the null hypothesis.

However, the integral estimation covering the whole period of experiment must be taken into account in analysis of immunomodulation influence on the velocity of ABL selection. Since the process depends on many factors; one from these factors is the possible difference of influence of used modulators on comparative dynamics of LABL and HABL.

The more precise estimation of the possible impact of immunomodulation on velocity of ABL selection can be reached through comparison of the time of HABL appearance and achieving of its maximum content. These data are shown in Table 3. From the data of table is visible that the HABL appear the most early under use of betaleukin and IIB, its content reaches maximum the most early under use of betaleukin. Such data allow us to assume that betaleukin and IIB most actively stimulate ABL selection in the immune response to herpetic inactivated vaccine.

In general, the early stage of nonspecific response can be characterized by following indexes: the days of first discovery of lymphocytes, achievement and value of content maximum, disappearance, duration of revelation and integral (for the period of experiment) content of these lymphocytes. The greater the value of these indexes in the immunized rabbits, the later this phase of the nonspecific response begins and finishes. According to data (Table 4) there were determined the rank distribution of used immunomodulators and control (without immunomodulation) for each of these indexes, and integral rank as well. It was found that each immunomodulator delays the beginning and the completion of nonspecific response. The first position by the value of integral rank of that delay is occupied by roncoleukin, second and third positions are occupied by betaleukin and IIB, and forth position is occupied by polyoxidonium.

TABLE 3. RANK DISTRIBUTION OF INFLUENCE OF VARIOUS IMMUNOMODULATORS ON ABL RESPONSE

Index	Index and rank* at use of immunomodulator				
	Roncoleukin	Betaleukin	Polyoxidonium	IIB	Without immunomodulation (control)
Day of first discovery of LABL	2/3	2/3	2/3	2/3	2/3
Day of first discovery of HABL	4/3.5	2/1.5	7/5	2/1.5	4/3.5
Day of achievement of content maximum of LABL	14/4	2/1	7/2	14/4	14/4
Day of achievement of content maximum of HABL	21/4.5	2/1	14/2.5	14/2.5	21/4.5
Day of disappearance of LABL	between 28 and 35/4.5	between 21 and 28/2.5	between21 and 28/2.5	between 14 and 21/1	between 28 and 35/4.5
Day of disappearance of HABL	between21 and 28/4	between14 and21/1.5	Between 21 and 28/4	between 14 and 21/1.5	between 21 and 28/4
Integral content of LABL for all the time**	102.4/4	86.5/2.5	43.4/1	77.5/2.5	140.0/5
Integral content of HABL for all the time	75.6/5	37.9/3	33.8/2	20.7/1	53.6/4
Period of discovery LABL	26 - 33/4.5	20 - 26/2.5	20 - 26/2.5	13 - 19/1	26 - 33/4.5
Period of discovery of HABL	18 - 25/4.5	13 - 19/1.5	15 - 23/3	13 - 19/1.5	18 - 25/4.5
Value of the content maximum of LABL, %	7,57/3,5	5,07/1,5	5,21/1,5	8,00/3,5	15,29/5
Value of the content of HABL,%	6,43/5	2,93/2	2,71/2	3,14/2	3,86/4
Integral rank of ABL estimation***	4.17+0.21/4.5	1.96+0.23/2	2.62+0.31/3	2.08+0.29/1.5	4.21+0.17

Notes: * - Meaning of index (numerator) and his rank (denominator); ** - the value of the ABL space limited by the horizontal axis and the curve of ABL content; *** - the average rank and its standard error.

TABLE 4. RANK DISTRIBUTION OF INFLUENCE OF IMMUNOMODULATORS ON NONSPECIFIC COMPONENT OF THE EARLY STAGE OF IMMUNE RESPONSE

Index for lymphocytes bounding the sorbent	Index and rank* at use of immunomodulator				
	Roncoleukin	Betaleukin	Polyoxidoniu m	IIB	Without immunomodulation (control)
Day of first discovery	14/4	14/4	2/1	14/4	4/2
Day of achievement of content maximum	14/4	14/4	7/2	14/4	4/1
Value of content maximum	6.71/5	2.21/2	4.36/4	2.43/2	2.36/2
Day of disappearance	between 14 and 21/3.5	between 14 and 21/3.5	between 14 and 21/3.5	between 14 and 21/3.5	between 7 and 14/1
Total content for all the time**	49.9/5	17.9/2	34.1/4	18.8/2	21.2/2
Period of discovery	1-13/4	1-13/4	12-19/1.5	1-13/4	3-11/1.5
Integral rank of its estimation***	4.25+0,25/5	3.25+0.40/3	2.67+0,54/3	3.25+0.40/3	1.58+0.20/1

Notes: * in the numerator - the value of criterion and in the denominator - his rank; ** - the value of the area is calculated by trapezoid method; *** - average rank and its standard error.

But the total content of lymphocytes bounded the sorbent was minimal at use of betaleukin or IIB, and use of roncoleukin and polyoxidonium led to increase of this index. In comparison with control the total content of lymphocytes bounded the sorbent at use betaleukin and IIB was less in 1.2 and 1/1 time accordingly, although at use of roncoleukin and polyoxidonium, on the contrary, was more in 2.4 and 1.6 times accordingly. In general, immunomodulation has a significant stimulating effect on the first phase of antigen specific response, and slows down the non-specific response.

Conclusion

Immunomodulation in the experiment modifies the first phase of antigen specific immune response to herpetic vaccine, detectable by the revelation of antigen binding lymphocytes. Modification appears to acceleration of the emergence and disappearance of antigen binding lymphocytes in rabbits immunized by herpetic inactivated vaccine. This acceleration is strongly manifested at use of betaleukin, polyoxidonium and interferon inducer bacterial.

In the first phase of antigen specific response it was also identified non-specific component of immune response, detected by lymphocytes binding of the sorbent without herpes virus. Immunomodulation of non-specific component in this early phase of immune response is revealed by more slow development of lymphocytes binding the non-specific sorbent.

In the experiment of immunization of rabbits by herpes inactivated vaccine, the opposite effect of immunomodulation on antigen specific (activation) and non-specific (delay) response is found.

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