

EFFECT OF STIMULATORS OF IMMUNOGLOBULIN BIOSYNTHESIS ON MACROPHAGAL TRANSFORMATION OF LYMPHOCYTES

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The effect of pyrogenal and adjuvant of the Freund type on the transformation of blood lymphocytes into macrophages was studied. Experiments were carried out on rabbits using the method of lymphocyte cultures in vitro. These stimulators of immunogenesis were shown to increase the intensity of transformation of the lymphocytes in culture. The action of pyrogenal and of Freund's complete adjuvant was manifested during the first day after its addition. Correlation is postulated between the ability of lymphocytes to undergo conversion into macrophages and the formation of the immunologic response of the organism.

KEY WORDS: macrophagal transformation of lymphocytes; adjuvants; immunologic response.

Previous communications [1, 10, 11] gave data on the increased ability of blood lymphocytes to be transformed into macrophages after injection of an antigen. The intensity of the antigenic stimulation and the times elapsing after injection of the antigen were found to influence the macrophagal transformation of the lymphocytes. The highest index of macrophagal transformation of the lymphocytes (IMTL) was observed when the immunologic response was complete and antibodies were already circulating in the blood. The data showing the parallel course of these two processes and their interconnection were confirmed by the results of experiments using the immunodepressants ALS and cyclophosphamide, which lowered the IMTL in rabbits, both intact and after preliminary antigenic stimulation.

The object of this investigation was to examine the effect of stimulators of immunogenesis on lymphocyte transformation in culture.

EXPERIMENTAL METHOD

Experiments were carried out on 50 chinchilla rabbits weighing 2.5-3 kg. Peripheral blood leukocytes were grown in culture. The technique of obtaining the blood leukocytes, their preparation and culture in medium No. 199, and also the method of analysis of the results were described previously [2, 9].

Considering the results of experiments in the writers' laboratory [3-7, 14], it was decided to use purified typhoid lipopolysaccharide, in the form of pyrogenal (production batch, N. F. Gamaleya Institute of Epidemiology and Microbiology) and adjuvant mixtures of the Freund type as well studied stimulators of the in vivo immune response. The adjuvant mixtures contained lanolin and mineral oil with or without the addition of killed cells of Mycobacterium smegmae.

EXPERIMENTAL RESULTS

It was shown previously that the intravenous injection of pyrogenal into rabbits in a dose of 100 minimal pyrogenic doses (MPD)/kg body weight is effective, for the body temperature of all the animals was raised by 1-3°C.

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TABLE 1. Dynamics of Macrophagal Transformation of Lymphocytes after Injection of Adjuvant Mixture into Rabbits ($M \pm m$)

Adjuvant	IMTL (%)				
	before stimulation	after stimulation (days)			
		1	3	7	45
Incomplete	10,9 \pm 1,3	11,3 \pm 1,1	12,8 \pm 1,5	14,6 \pm 1,4	12,0 \pm 1,0
Complete	11,0 \pm 1,1	47,6 \pm 2,7	49,6 \pm 2,9	51,6 \pm 2,8	50,2 \pm 2,5

The IMTL of the intact rabbits was $12 \pm 1.3\%$, corresponding to the characteristic level for animals of this species as established previously by a large number of observations ($M \pm m = 11 \pm 1.3\%$). The chosen dose of pyrogenal was then injected into the rabbits, and blood samples were taken 1, 3, 7, and 14 days later for the lymphocyte cultures and to determine IMTL. The value of this index at the above times was 34.4 ± 1.6 , 32.5 ± 1.8 , 37.6 ± 2.0 , and $26.3 \pm 1.5\%$, respectively.

The results show that the pyrogenal increased the ability of the lymphocytes to be transformed into macrophages in culture. It was noted that the effect of pyrogenal was exhibited early. This indicates that it acts, not as an antigen, for according to previous observations [11, 12], antigen increased the ability of lymphocytes to undergo macrophagal transformation not before the third day after their injection.

To confirm this conclusion it was essential to exclude any possible effect of traces of pyrogenal in the culture fluid on the transformation of the lymphocytes taking place in vitro. Before incubation of the cells in medium No. 199 began, pyrogenal was added in doses of 0.5 and 1 MPD/ml. The control cultures were seeded without the stimulator. The presence of pyrogenal in the nutrient medium was found to have no effect on the ability of the lymphocytes to be transformed in macrophages. In the control culture IMTL was $11.2 \pm 1.6\%$, in the presence of 0.5 MPD/ml it was $11.3 \pm 1.2\%$, and after the addition of 1 MPD/ml to the medium it was $12.6 \pm 1.5\%$.

Experiments were carried out with adjuvant mixtures of the Freund type on two groups of rabbits. The complete and incomplete mixtures were injected into the rabbits' paw in a dose of 1.5 ml. Blood for the determination of IMTL was taken 1, 3, 7, and 45 days after injection of the adjuvants. The technique of seeding the cells and the times and method of reading the results were the same as in the experiments with pyrogenal. The experimental results are given in Table 1. They show that the so-called Freund's incomplete mixture, not containing mycobacteria, is a very weak stimulator. The complete adjuvant had a stronger effect. In the animals receiving the complete adjuvant the value of IMTL increased almost five-fold and stayed at that level for 1.5 months.

The difference in the degree of effectiveness of the action of these adjuvant mixtures on the ability of the lymphocytes to undergo transformation accorded well with existing data for their effect on antibody biosynthesis [3, 4]. The complete adjuvant was a much more powerful nonspecific stimulator of immunoglobulin formation than the same mixture of oils not containing mycobacteria, which have a powerful allergizing effect on the organism and reduce the threshold of its sensitivity to antigens. The mechanisms lying at the basis of this property of the mycobacteria and certain chemical derivatives isolated from them are not clear. It has been suggested that injection of stimulators of immunogenesis and, in particular, of Freund's complete adjuvant increases membrane permeability of the macrophagal lysosomes and thereby modifies the functional activity of these cells and of their lysosomal apparatus [13, 15-17].

The results of the present experiments suggest that adjuvants may exert their activity also through lymphocytes, by promoting rapid and intensive transformation of these cells.

The results of the present investigation with three different stimulators of antibody biosynthesis show that their effect on development of the phenomenon of macrophagal transformation of the blood lymphocytes coincides with their adjuvant characteristics. Just as with antibody formation [8], the microbial lipopolysaccharide preparation pyrogenal induces a rapid, moderate, and brief increase in the ability of the lymphocytes to undergo macrophagal transformation. An adjuvant mixture of oils affects this phenomenon even less strongly. The strongest stimulator of the conversion of lymphocytes into macrophages was the complete mixture of Freund type which, as is well known, is an effective adjuvant.

The results of this comparative study point to correlation between the ability of lymphocytes to be converted into macrophages and the formation of the immunologic response.

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