6. I. R. F. Corvalan and I. C. Howard, Eur. J. Immunol., 8, 331 (1978).
7. A. K. Duwe and S. K. Singhal, in: Immune Reactivity of Lymphocytes. Development, Expression and Control, New York (1976), p. 607.
8. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
9. R. V. Petrov and R. M. Khaitov, Cell. Immunol., 28, 298 (1977).
10. S. K. Singhal, S. King, and P. J. Drury, Int. Arch. Allergy, 43, 934 (1972).
11. S. K. Singhal, I. C. Roder, and A. K. Duwe, Fed. Proc., 37, 1245 (1978).

BLAST TRANSFORMATION IN RESPONSE TO PLANT MITOGENS IN WISTAR RATS INFECTED WITH *Mycoplasma arthritidis* OR *Acholeplasma laidlawii*

A. V. Pronin, V. V. Khorobrykh, G. Ya. Kogan, D. R. Kaulen, and A. V. Sanin

UDC 576.858.74.083.35:612.112.94.014.46:615.272:547.962.5

KEY WORDS: blast transformation; mitogens; mycoplasmas.

The action of mycoplasmas on the lymphoid system is known to depend largely on the infecting dose and on ability to utilize arginine [5]. If high concentrations of arginine-utilizing mycoplasmas are added in vitro, marked suppression of the response to plant mitogens is observed and is due to removal of arginine from the culture medium [10]. If the multiplicity of infection is reduced, the inhibitory effect is replaced by a stimulant effect [5]. Mycoplasmas utilizing dextrose in most cases have only a stimulant action regardless of the dose [3, 7, 9]. The results of investigation of blast transformation of lymphocytes taken from infected animals are highly contradictory [3]. In some cases suppression of the response to mitogens after infection with species of arginine-utilizing mycoplasmas could not be found [6], but in other cases it was observed [8]. Infection of animals with dextrose-utilizing mycoplasmas does not affect blast transformation due to plant mitogens [3].

The object of the present investigation was to study the action of phytohemagglutinin (PHA) and concanavalin A (con A) on lymphocytes of rats infected with *Mycoplasma arthritidis* and *Acholeplasma laidlawii*.

EXPERIMENTAL METHOD

The animals were infected with mycoplasmas as described previously [2]. Intact rats and rats into which broth was injected served as the control. The proliferative activity of spleen cells and mesenteric lymph node cells was estimated 7, 14, 28, 33, and 65 days after the beginning of infection. The blast transformation reaction was set up in 3040 Microdisks (Falcon Plastics). Into each well 5×10^5 cells were introduced in a final volume of 0.2 ml medium. The culture medium was RPMI-1640 medium with 10% normal rat serum, 1% 1 M HEPES solution, 1% L-glutamine, 100 units/ml benzylpenicillin, and 100 μg/ml streptomycin-calcium chloride complex. The Microdisks were incubated at 37°C in a humid atmosphere with 5-7% CO_2. Preliminary experiments showed that the optimal concentration of PHA (PHA-P, from Difco) was 0.5 μg/ml and that of con A (from Sigma) was 10 μg/ml. The reaction was read on the 4th day of culture. DNA synthesis was determined by measuring incorporation of thymidine-^{3}H (2.5 μCi/ml, 1 Ci/mmole), added 16 h before the end of the experiment, by the method described previously [1].

EXPERIMENTAL RESULTS

It will be clear from Figs. 1 and 2a that injection of broth or of *A. laidlawii* in vivo caused no appreciable changes in the intensity of thymidine-^{3}H incorporation by the spleen and lymph node cells at all times of the

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 79-81, July, 1980. Original article submitted June 20, 1979.

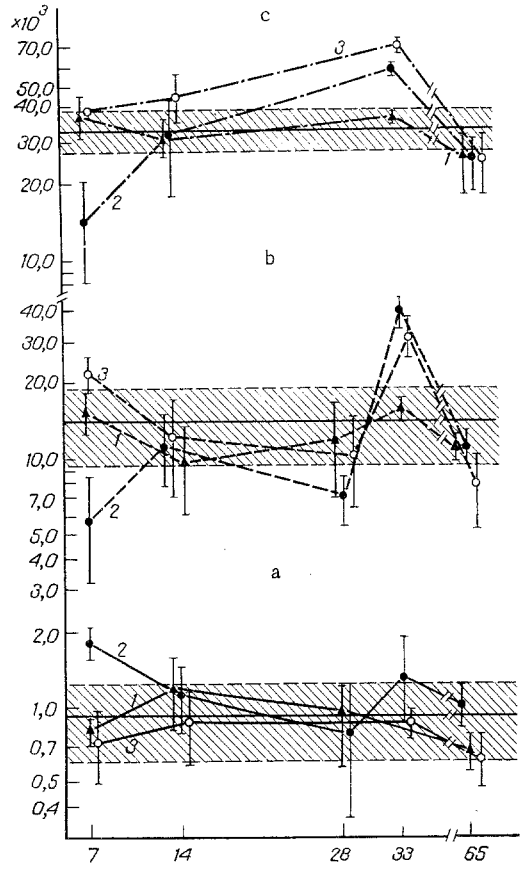

Fig. 1

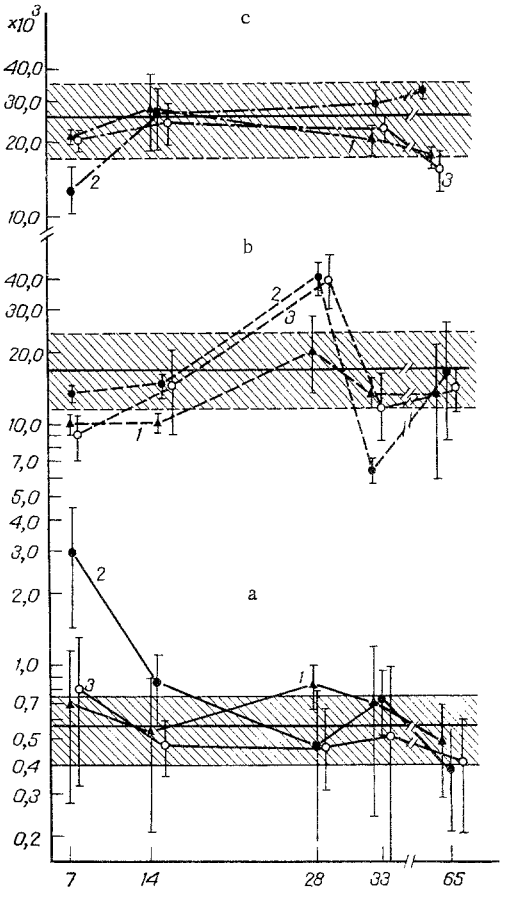

Fig. 2

Fig. 1. DNA synthesis in spleen cells of intact and infected rats at various times after infection without the addition of mitogen (a) and after addition of PHA (b) and con A (c). Abscissa, time after infection (in days); ordinate, incorporation of thymidine-^{3}H (in cpm). 1) Rats inoculated with broth; 2) rats infected with M. arthritidis; 3) rats infected with A. laidlawii. Horizontal lines denote intact rats. Vertical lines and shaded areas denote confidence interval at $P = 0.05$.

Fig. 2. DNA synthesis in lymph node cells from intact and infected rats at different times after infection without addition of mitogen (a) and after addition of PHA (b) and con A (c). Legend as in Fig. 1.

investigation. Conversely, infection with M. arthritidis led to statistically significant stimulation of DNA synthesis 7 days after infection. The effect was more marked in cultures of lymph node cells than of splenocytes, and it persisted until the 14th day.

Even more significant differences between splenic and lymph node lymphocytes were observed after the addition of PHA. The response to the mitogen in spleen cells of animals infected with M. arthritidis was significantly depressed for the first week (Fig. 1b). In about half of the rats it was also depressed toward the end of the second week, whereas in the rest it had regained the normal level by this time. However, complete recovery did not take place, and on the 28th day of infection DNA synthesis was again inhibited. A sharp increase in the intensity of blast transformation in response to PHA compared with that in intact animals or in rats inoculated with broth was observed 33 days after infection, and by the end of the second month the response was back to normal. In cultures of lymph node cells of animals infected with M. arthritidis no decrease in the mitogenic action of PHA was observed in the early period of infection (Fig. 2b), but stimulation occurred a little earlier than in the spleen cells (on the 28th day); however, by the 33rd day the stimulation was replaced by depression.

The course described above also was characteristic of the response of lymph node cells of animals infected with A. laidlawii to PHA (Fig. 2b). The only difference was the absence of depression on the 33rd day. Infection caused by A. laidlawii had a certain stimulant action on the splenocytes, but not statistically signifi-

cant, in the early period after addition of the agent. Later, until the 28th day, proliferative activity induced by PHA was normal, and by the 33rd day it was again stimulated. The level of blast transformation 2 months after infection was close to the control value.

The character of the change in response to con A in the spleen cell cultures differed only a little from the course of the response to PHA (Fig. 1c). Lymph node lymphocytes responded to con A within normal limits at all times of the investigation irrespective of the type of infection. The only exceptions were rats infected with M. arthritidis, in which the response was depressed after 7 days (Fig. 2c).

Depression of the response to PHA and to con A was thus observed in rats infected with M. arthritidis (which utilizes arginine). However, in contrast with investigations by other workers [8] who described only depression of blast transformation under the influence of PHA, reaching a maximum on the 20th-30th day in the case of infection of Sprague Dawley rats with M. arthritidis, the picture in the present experiments was more complex. Strong inhibition on the 7th day was followed by appreciable stimulation toward the 28th-33rd days. Meanwhile synthetic activity of the lymphocytes of the infected rats was stimulated on the 7th day of infection in cultures without the addition of mitogen. Possibly this disagreement may be due to differences in the multiplicity of infection (10^{10} and 2×10^8 CFU/ml) or to strain differences (we used nonarthritogenic strain PG 6, whereas in the experiments of Kaklamanis and Pavlatos the animals were infected with strain PN).

This complex character of the response in the early stages was probably not associated with the action of the arginine-deiminase system only. Lowering of the level of blast transformation in response to mitogens may have been due to a reduction in the number of responding cells on account of their redistribution among the organs, death or blocking of receptors for PHA and con A, or the ability of the mycoplasmas to shift the peak of the response to mitogens. Depression of the mitogenic effect may also have been due to the stimulating action of the mycoplasmas, as a result of which cells which had passed through one cycle of divisions could not respond normally to PHA or con A. However, there is some evidence [5] that in the presence of mycoplasmas, the lymphocytes are even more stable than normal. In addition, facts indicating the absence of blocking of PHA receptors by M. arthritidis have been obtained [11]. Evidence against a redistribution of cells is given by the fact that the response to PHA in lymph nodes of animals infected with M. arthritidis was practically normal in the early stages of infection, although it was higher than in rats inoculated with broth. We consider it more probable that depression of the response to mitogens was connected with the stimulating action of the mycoplasmas. Under these circumstances a subpopulation of cells responding to PHA and con A, and present in large numbers in the spleen, and also some of the lymphocytes not responding to PHA, but sensitive to the action of con A and present in lymph nodes, must be involved.

The second peak of stimulation at the 28th-33rd day, as the data given above show, was nonspecific: infectious induced by both M. arthritidis and A. laidlawii stimulated the response about equally. This peak was perhaps connected with persistence of the microorganisms and with increased production of the factor responsible for stimulation 1 month after infection. It is interesting to note that lymph node cells, consisting as we know to the extent of 73% of $RMTA^+$-lymphocytes, similar in their properties to the T_2 population in mice [4], were activated initially under these circumstances. The activation peak was then shifted to the spleen and involved lymphocytes responding to both PHA and con A. Simultaneously with this, the level of blast transformation in the lymph nodes fell sharply, and this was particularly noticeable in animals infected with M. arthritidis.

The specific character of the effect of infection caused by M. arthritidis and A. laidlawii is thus manifested only in the early stages after infection. Later the changes in the parameter studied were approximately the same in both types of infection.

LITERATURE CITED

1. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., 64, 723 (1977).
2. A. V. Pronin, A. V. Sanin, V. V. Khorobrykh, et al., Zh. Mikrobiol., No. 3, 82 (1979).
3. A. V. Sanin, Biol. Nauki, No. 12, 16 (1978).
4. H. Cantor and R. Asofsky, J. Exp. Med., 135, 764 (1972).
5. B. C. Cole, K. E. Aldridge, and J. R. Ward, Infect. Immun., 18, 393 (1977).
6. B. C. Cole, L. Golightly-Rowland, and J. R. Ward, Ann. Rheum. Dis., 35, 14 (1976).
7. H. Ginsburg and J. Nicolet, Nature New Biol., 246, 143 (1973).
8. E. Kaklamanis and M. Pavlatos, Immunology, 22, 695 (1972).
9. H. Kirchner, H. Brunner, and H. Ruhl, Clin. Exp. Immunol., 29, 176 (1977).

10. M. S. Simberkoff, G. J. Thorbecke, and C. Thomas, J. Exp. Med., 129, 1163 (1969).
11. L. Spitler, K. Cochrum, and M. Fudenberg, Science, 161, 1148 (1968).

EFFECT OF CYCLIC AMP-DEPENDENT PHOSPHORYLATION OF SHEEP'S RED BLOOD CELL MEMBRANES ON THEIR ABILITY TO INDUCE THE HUMORAL IMMUNE RESPONSE

V. M. Novikova and V. A. Kozlov

UDC 612.111.017.1-06: [612.111:612.262:612.398.145.1

KEY WORDS: cyclic AMP; protein kinase; sheep's red blood cells; antibody-forming cells.

Cyclic AMP is known to participate in the regulation of antibody formation in vitro, and in proliferation and differentiation of lymphocytes stimulated by a mitogen or antigen [1]. The function of cyclic AMP as secondary mediator in the transmission of signals is that of activation of a series of protein kinases (PK) phosphorylating different protein substrates [7, 13-15]. Cyclic AMP-dependent phosphorylation of cell membranes of many tissues has been found to be at a low level [14, 15]. The high sensitivity of thymocytes and spleen cells, stimulated by mitogen or antigen, to concentration of exogenous or endogenous cyclic AMP at the beginning of culture [1] suggests that membrane proteins which are antigenic receptors may undergo cyclic AMP-dependent phosphorylation and that cyclic AMP-dependent PK may be located in the immediate vicinity of receptors for the antigen or mitogen. The present writers have also suggested that cyclic AMP-dependent phosphorylation may also influence activity of the antigenic determinants of various cellular antigens on account of changes in the charge and/or conformational changes in their membranes.

The object of the present investigation was to study the possible effect of phosphorylation and of activity of cyclic AMP-dependent and cyclic AMP-independent membrane PK of sheep's red blood cells (SRBC) on the antigenic activity of those membranes in the induction of the humoral immune response in mice.

EXPERIMENTAL METHOD

Experiments were carried out on female (CBA × C57BL/6)F_1 mice obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR.

ATP and (γ-^{32}P)ATP, with a specific activity of 60 Ci/mmole, were obtained from the All-Union Research Institute of Molecular Biology, Head Office of the Microbiological Industry, Academy of Sciences of the USSR. Mice were immunized intravenously with 0.5 ml of a 5% suspension of intact (ISRBC) or phosphorylated SRBC (PSRBC). On the 4th, 5th, and 6th days after immunization the animals were killed and the number of antibody-forming cells (AFC) estimated from the number of plaque-forming cells (PFC) in the spleen by the modified method of local hemolysis in semiliquid medium [3]. The tests for determining the number of PFC were carried out after addition of ISRBC or PSRBC to the reaction medium. The significance of differences was assessed by Student's t-test.

Phosphorylation of SRBC was carried out by the method described in [12], with induction of synthesis by cyclic AMP. The SRBC were washed with medium No. 199 and then with an incubation medium containing 0.03M Tris-HCl buffer, pH 7.5, 0.15M NaCl, 10 mM $MgCl_2$, 10 mM CH_3COONa, 0.3 mM EDTA, 2 mM theophylline, 5 mM NaF, 0.1 mM Na_3PO_4, and 2 μM ATP. The cell residue was resuspended in this same medium to a concentration of 1×10^8 cells/ml. Incubation was carried out at 37°C for 30 min, after which the cells were cooled to 4°C, washed in medium No. 199, and injected as a 5% suspension into mice.

Phosphorylation of SRBC was verified from the incorporation of ^{32}P into cellular material insoluble in TCA [12].

Laboratory of Regulation of Immunopoiesis, Department of Clinical and Experimental Immunology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 82-84, July, 1980. Original article submitted May 14, 1979.