ing dialysis certain small molecules, previously components of RF, passed into the dialysis fluid, and this led to normalization of these pathological Ig to some degree. It can naturally be assumed that in cases when no such changes took place (Nos. 13, 17, 18), RF nevertheless was a complex of two molecules, but with higher affinity for each other. Incidentally, on the basis of our data it cannot be concluded that the Ig which are components of RF are absolutely identical to normal Ig, for only three of the 10 proteins dialyzed became indistinguishable from normal. We consider that the difference between these Ig ought to exist, and it is manifested as abnormal affinity of Ig-RF toward certain small molecules. After addition of the latter the RF may perhaps become capable of forming complexes with the individual's own IgG.

It can thus be shown by the monolayers method that proteins possessing the properties of RF, isolated from the blood serum of patients with RA, behave differently from normal serum Ig. It follows from the results of the analysis that at least some, and possibly all, RF are Ig modified by small molecules. Carefully conducted control experiments will rule out the possibility of addition of these molecules during isolation of RF.

LITERATURE CITED

1. N. A. Matveeva, L. V. Chasovnikova, and V. V. Lavrent'ev, Biofizika, 28, No. 3, 407 (1983).
2. A. A. Trapeznikov, Zh. Fiz. Khim., 19, No. 4-5, 228 (1945).
3. L. V. Chasovnikova, N. A. Matveeva, and V. V. Lavrent'ev, Biofizika, 27, No. 1, 17 (1982).
4. L. V. Chasovnikova, N. A. Matveeva, and V. V. Lavrent'ev, Biofizika, No. 3, 435 (1982).
5. L. V. Chasovnikova, N. A. Matveeva, D. V. Stefani, and V. V. Lavrent'ev, Mol. Genet., No. 3, 39 (1983).
6. L. V. Chasovnikova, N. A. Matveeva, A. K. Kurek, et al., Mol. Genet., No. 11, 36 (1986).
7. P. Brown, F. A. Nardella, and M. Mannik, Arthr. Rheum., 25, No. 9, 1101 (1982).
8. E. D. Day, Advanced Immunochemistry, Baltimore (1972), p. 108.
9. M. Duc Dodon and G. A. Quash, Immunology, 42, 401 (1981).
10. M. Duc Dodon and G. A. Quash, Rheumatology, 3, 97 (1983).
11. P. Grabar and C. A. Williams, Biochim, Biophys. Acta, 10, 193 (1953).
12. H. G. Kunkel and E. M. Tan, Adv. Immunol., 4, 351 (1964).
13. R. B. Parekh, R. A. Dwek, B. J. Sutton, and D. L. Fernandes, Nature, 316, 452 (1985).
14. J.-L. Preud'homme, F. Duarte, and P. Auconturier, Diagnost. Immunol., 2, 219 (1984).
15. J. Turkova, Meth. Enzymol., 44, 66 (1976).

MITOGENIC ACTION OF *Legionella pneumophila*: RATIO OF PROLIFERATING T AND B CELL CHANGES AFTER IMMUNIZATION

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KEY WORDS: immunization; activity of T and B cells

Despite recent investigations by several groups of workers [7] the mechanisms of development of protective immunity in legionelloses have not been discovered. It is not clear what type of immune response (humoral or cellular) plays the leading role in the conditions of infection associated with legionellas. According to data in the literature, the serum (but not lymphocytes) of guinea pigs immunized with *L. pneumophila* antigens (LPA) can transfer protective immunity in the case of intratracheal infection [4]. On the other hand, there is evidence that after intraperitoneal immunization of guinea pigs with killed *L. pneumophila* cells the animals become resistant to intraperitoneal, but not to aerosol infection [5], although immunization leads to synthesis of antibodies against *L. pneumophilia* in high titers.

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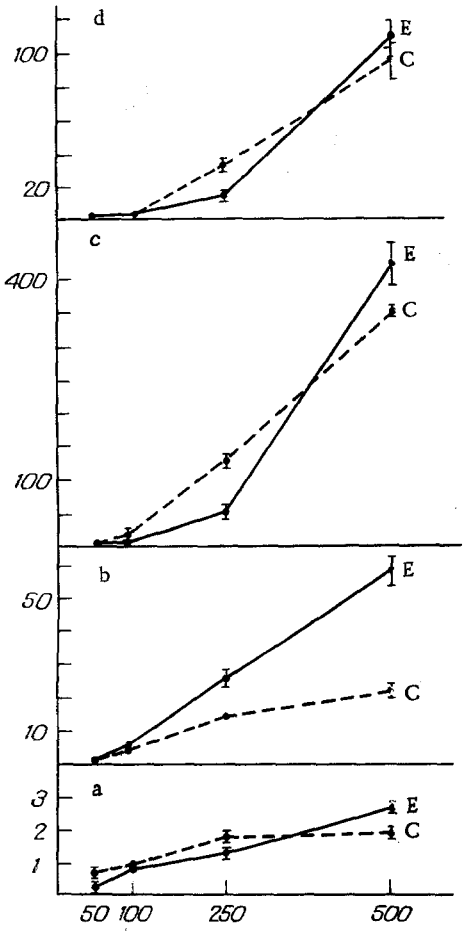

Fig. 1. Dependence of proliferative activity of cells of control and immunized guinea pigs on cell concentration in culture. Abscissa, cell concentration × 10^{-3}; ordinate, incorporation of ^{3}H-thymidine, cpm × 10^{-3}. C) Control; E) experiment. Linear regression coefficient (m) shown in parentheses. Thin vertical lines denote standard error. a) Cultures without mitogen; b, c, d) cultures stimulated by *E. coli* lipopolysaccharide (100 µg/ml); con A (20 µg/ml); and LPA (100 µg/ml) respectively. Total time of culture 65 h.

Since *L. pneumophila* is a facultative intracellular parasite, circulating antibodies alone evidently cannot ensure effective protection against legionellosis. To create protective immunity, the T system of immunity must evidently be involved. Nonspecific activation of antibody synthesis after the infection with *L. pneumophila*, on the other hand, may create favorable conditions for preservation of bacteria in the body [8].

In the investigation described below changes in activity of T and B cells were studied after administration of LPA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred guinea pigs of both sexes weighing up to 200 g obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR.

Strain Phil of *L. pneumophila*, grown on carbon-yeast agar, was used. The antigen (LPA) was isolated by Flesher's method [6]. Guinea pigs were immunized with this antigen subcutaneously in a dose of 1 mg/ml as protein. The blast transformation reaction with spleen cells of immune and control guinea pigs was set up by the method described previously [3]. Splenocytes were fractionated on Nitron, manufactured at the Saratov Chemical Combine by the method developed by Pletneva [2]. The significance of the results was assessed by Student's t test. The coefficients of regression for the cell concentration–effect curves were calculated by the formula:

$$m = \frac{\Sigma xy - \Sigma x \Sigma y}{n\Sigma x^2 - (\Sigma x)^2},$$

where m is the regression coefficient, x denotes the logarithm of cell concentration; y the logarithm of label incorporation, in cpm; n the number of parallel determinations. Data differed by not more than 3σ from the theoretical curve, calculated by the method of least squares [1], were included in regression analysis. The number of cells of different types limiting proliferation was determined by Peterson's method [11].

TABLE 1. Proliferative Activity of Guinea Pig Splenocytes at Different Times after Immunization with LPA (incorporation of ^{3}H-thymidine, cpm, M ± m)

Mitogen	Group of animals	Time after immunization, days		
		7	14	28
—	Control	1 247±79	1,273±107	1 078±77
	Experimental	1 011±48	2 879±297	731±122
Con A	Control	32 526±2 073 (26,0)	46 467±1 137 (38,1)	48 838±11 444 (45,3)
	Experimental	10 577±620 (10,5)	23 063±3 345 (8,0)	15 438±1355 (21,1)
LPS	Control	1 309±82 (1,0)	1 688±126 (1,3)	627±153 (0,6)
	Experimental	690±49 (0,7)	1 810±34 (0,6)	762±228 (1,0)
LPA	Control	7 203±636 (5,8)	6 356±1 029 (5,0)	2 273±609 (2,1)
	Experimental	4 390±484 (4,3)	6 275±332 (2,2)	8 012±870 (10,9)

Legend. Control group consisted of intact guinea pigs, experimental group of guinea pigs immunized with LPA. Index of stimulation given in parentheses. Total time of culture 120 h, cell concentration in culture 250,000 per well.

EXPERIMENTAL RESULTS

In the initial experiments proliferative activity of the splenocytes was investigated in four cell concentrations (Fig. 1).

Spontaneous incorporation of ^{3}H-thymidine in the control and experimental animals 7 days after immunization with LPA did not exceed 3000 cpm with the maximal cell concentration (500,000 per well). Splenocytes proliferated under the influence of the B-cell mitogen — lipopolysaccharide (Fig. 1b). The reaction increased with an increase in cell concentration as a linear function, and the comparatively low regression coefficient (1.410 for control and 1.648 for experimental animals) indicated only weak dependence of the response on cooperation with extraneous cells, i.e., the response depended only on the concentration of LPS-sensitive cells. The response of splenocytes of immunized guinea pigs to LPS was much stronger than that of splenocytes of intact animals (58.518 ± 5186 cpm compared with 22.954 ± 1141 cpm for 500,000 cells per well), which may indicate an increase in the number of B cells and (or) activity of individual B cells after immunization with LPA.

Different results were obtained in cultures stimulated by concanavalin A (con A; Fig. 1c). The linear regression curves characterizing the response of the experimental and control animals to con A were virtually indistinguishable (m was 1.933 and 1.985, respectively), although with a cell concentration of 250,000 per well, reactions of the splenocytes in the experiment were lower (56.922 ± 9555 cpm, compared with 125,473 ± 5302 cpm in the control), whereas with a cell concentration of 500,000 per well, they were rather higher than the control values (425,610 ± 31,912 and 352,008 ± 5575 cpm, respectively).

The response of the spleen cells to LPA was stronger in absolute values than the response to LPS; lymphocytes of intact and immunized animals, moreover, were stimulated to an equal degree, i.e., LPA evidently has a nonspecific mitogenic action on lymphocytes. However, regression analysis showed that a suspension of splenocytes from experimental animals was relatively poorer than that from control guinea pigs for one type of cell participating in the induction of proliferation (m = 2.785 in the experiment and 2.260 in the control).

The specific proliferative response to LPA was thus masked by the strong mitogenic action of this antigen. A mitogenic action of LPA on intact lymphocytes also has been discovered in mice [7, 9, 15] and in man [12, 13]. In mice under these circumstances mainly B cells were stimulated, and the active factor was evidently associated with LPS [7, 15]. According to data obtained by Friedman et al. [7], splenocytes of intact guinea pigs are not stimulated by an extract of a sonicated structure of *L. pneumophila*, whereas immune lymphocytes begin to respond to LPA 3-4 weeks after immunization. The disagreement between data obtained by these workers and our own observations may perhaps be due to differences in the type of LPA and in the times of culture. We know that if the lymphocyte blast transformation reaction is assessed on the 5th day, only the specific response to LPA will remain at that time [7, 15], and for that reason, in the subsequent experiments the duration of culture of the splenocytes with LPA or mitogens was increased to 120 h. As Table 1 shows,

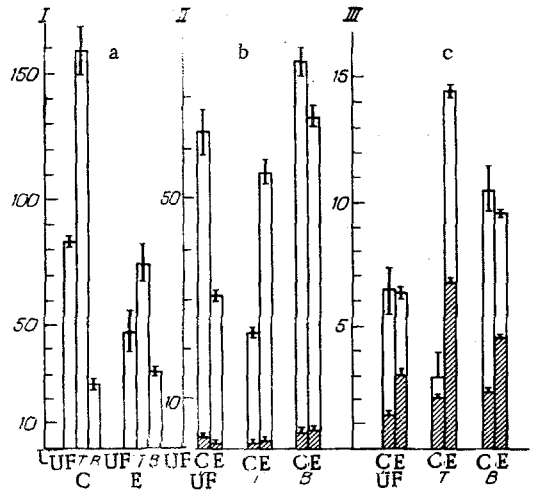

Fig. 2. Stimulating action of LPA on splenocytes fractionated on columns with nylon wool. a) Response of fractionated splenocytes to con A; b, c) incorporation of ^{3}H-thymidine. Abscissa: C) control; E) experiment (splenocytes of immunized guinea pigs 6-14 days after immunization). Total duration of culture 65 (a, b) and 120 (c) h. UF) Unfractionated splenocytes; T) cells nonadherent to nylon wool; B) adherent cells. Shaded part of columns — spontaneous incorporation of label. Short vertical lines indicate standard error. Concentration of cells in culture 150,000 per well.

splenocytes of experimental and control animals were not stimulated by LPS after 5 days in culture. The response to con A in the control also was reduced by 50-75%, and the immune animals responded to con A significantly weaker than the control guinea pigs at all times of the investigation. The nonspecific mitogenic action of LPA after culture of splenocytes for 5 days was very considerably weakened, and on the 28th day after immunization the splenocytes of the experimental animals responded to LPA significantly more strongly than the controls.

Thus on the 5th day of culture the B-cell mitogen LPS does not stimulate lymphocytes, whereas the T-cell mitogen con A, and LPA, continue to act, although less strongly. It is only under these circumstances that the specific response to LPA can be detected on the 28th day after immunization. The nonspecific mitogenic action of LPA may perhaps be connected with activation of B cells, whereas specific blast transformation involves T lymphocytes also.

Specific blast transformation in mice immunized with LPA was discovered on the 7th-15th day in 3-day cultures and until the 35th day after immunization in 5-day cultures [15]. It was not established what cells are specifically stimulated by LPA in mice, but we know that in man, after recovery from Legionnaires' disease, T cells are stimulated much more strongly than B cells [14].

To obtain more detailed characteristics of the lymphocyte subpopulation activated by LPA in guinea pigs, experiments were carried out with fractionation of splenocytes on columns with nylon wool. Three cell populations were obtained: unfractionated cells, cells adherent to nylon wool (B), with a high proportion of B lymphocytes, and cells nonadherent to nylon wool (T), with a high proportion of T lymphocytes. The results of a comparative study of the response to con A in these three populations are given in Fig. 2. Whereas B cells of intact and immune guinea pigs responded virtually equally to LPA, T cells of immune animals were stimulated much less strongly by LPA than in the control. The differences were particularly marked in this respect on the 5th day of culture. On the 3rd day they were less marked because of the strong nonspecific response to LPA. These differences could be associated not only with differences in the activity of the T cells, but also with the fact that A cells, which are necessary for the induction of blast transformation, if obtained from the experimental animals adhered more effectively to the nylon wool than those from the control guinea pigs. To rule out this possibility, in some experiments the fractionated cells were mixed with 10% of unfractionated autologous splenocytes, irradiated in a dose of 3300 rads, to serve as a source of A cells [11]. The nonproliferating A cells potentiated the response of B cells of the experimental animals to LPA to some degree but had no effect on the activity of the other cell populations.

Thus LPA probably has a strong mitogenic action on the B cells of guinea pigs, but after immunization of the animals, T cells also begin to respond to LPA.

LITERATURE CITED

1. G. F. Lakin, Biometrics, 3rd edition [in Russian], Moscow (1980), p. 185.
2. T. P. Pletneva, D. R. Kaulen, and V. V. Khorobrykh, Med. Ref. Zh., III, 14 (1979).
3. A. V. Pronin, V. V.Khorobrykh, G. Ya. Kagan, et al., Byull. Éksp. Biol. Med., No. 7, 79 (1980).

4. M. G. Caparon and W. Johnson, Legionella, Washington (1984), pp. 168-171.
5. T. K. Einstein, R. Tamada, J. Meissler, et al., Infect. Immun., 45, 685 (1984).
6. A. R. Flesher, H. S. Sennings, C. Lugowsky, et al., J. Infect. Dis., 145, 224 (1982).
7. H. Friedman, T. Klein, and R. Widen, Legionella, Washington (1984), pp. 145-152.
8. H. Friedman, R. Widen, and T. Klein, Legionella, Washington (1984), pp. 187-190.
9. H. Friedman, R. Widen, T. Klein, et al., Infect. Immun., 43, 314 (1984).
10. T. Klein, R. Widen, K. Cabrian, et al., Legionella, Washington (1984), pp. 178-180.
11. W. J. Peterson, Cell. Immunol., 70, 260 (1982).
12. J. P. Plouffe and I. M. Baird, J. Clin. Lab. Immunol., 5, 149 (1981).
13. J. P. Plouffe and I. M. Baird, Legionella, Washington (1984), pp. 186-187.
14. J. P. Plouffe and I. M. Baird, Legionella, Washington (1984), pp. 190-191.
15. R. Widen, T. Klein, H. Friedman, et al., Legionella, Washington (1984).

RECOMBINANT env PROTEIN IN DIAGNOSIS OF AIDS

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The acquired immunodeficiency syndrome (AIDS) is a dangerous disease caused by a retrovirus which is spreading rapidly at present in many countries of the world. Test systems based on the ELISA technique are most commonly used for the diagnosis of AIDS. Production of suitable diagnostic kits requires a considerable number of virus antigens, the preparation of which requires the use of large quantities of expensive media and sera to culture the virus, and also special measures of protection of personnel working with the virus. The use of antigens obtained by genetic engineering methods in these test systems is therefore interesting. With test systems of this kind it is possible to detect antibodies to single virus antigens, and they may therefore, be useful to determine the stage of the disease and to predict its outcome [6, 8].

The writers previously described a method of obtaining AIDS virus antigens coded by the env gene in *Escherichia coli* cells [1, 2]. In this paper we describe the results of testing blood sera from patients with AIDS and AIDS-related complex and also of sera from groups at increased risk of developing AIDS, by the use of a diagnostic kit based on recombinant protein.

EXPERIMENTAL METHOD

The lysate of a culture of AIDS virus of antigen-producing strain JM 107/pUCenv1 [2] was obtained by the method of Emtage et al. [4] with certain modifications. The cell residue obtained from 2 liters of induced culture [2] was resuspended in 15 ml of cold buffer containing 25% sucrose, 50 mM Tris-HCl (pH 8.0), 20 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF), after which 5 ml of a solution of lysozyme (initial concentration 10 mg/ml) was added and the mixture was incubated for 5 min at 0°C. Next 2.5 ml of 0.5 M EDTA solution was added and the sample was incubated for a further 5 min at 0°C. The suspension was then treated with 25 ml of lytic buffer (0.1% Triton X-100, 50 mM Tris-HCl, pH 8.0, 50 mM EDTA), and the lysate was treated with ultrasound until disappearance of viscosity, and then centrifuged at 10,000g for 45 min at 4°C. The supernatant was used in the subsequent work.

Blood sera were tested by ELISA, using the complex sandwich technique with competition [9]. For this purpose, 96-well polystyrene panels (Costar, Great Britain) were sensitized

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