where σ is the standard deviation of the number of cells in the clone; N the number of Ig-positive cells in the colony; $p = n_{mean}/N$ is the probability of expression of the V gene of the particular specificity; n_{mean} is the mean number of cells of that specificity among all colonies tested; $q = 1 - p$.

The following basic assumption was made above: At a certain stage of ontogeny the quantitative ratios between clones of B lymphocytes reflect quantitative ratios between the corresponding V genes in the given population. If this assumption is accepted, the sharply varying quantitative ratios discovered in these experiments between groups of clones and Ig-positive cells in the individual colonies are incompatible with the view that every B cell contains every conceivable V gene of the set, i.e., they are incompatible with the germ-line theory of antibody diversity. The model used is extremely suitable for further detailed quantitative analysis of this problem.

LITERATURE CITED

1. I. V. Khazanova, Byull. Éksp. Biol. Med., No. 10, 83 (1975).
2. B. F. Argyris, J. Exp. Med., 128, 459 (1968).
3. K. A. Ault, E. R. Unanue, D. H. Katz, et al., Proc. Nat. Acad. Sci. (USA), 71, 3111 (1974).
4. B. Benacerraf and B. Levine, J. Exp. Med., 115, 1023 (1962).
5. M. G. Chen and J. C. Schooley, Transplantation, 6, 121 (1968).
6. J. M. Davie and W. E. Paul, J. Exp. Med., 135, 660 (1972).
7. E. A. Doidl and G. W. Siskind, J. Exp. Med., 140, 1285 (1974).
8. P. P. Jones, J. J. Cebra, and L. A. Herzenberg, J. Exp. Med., 139, 581 (1974).
9. D. Osoba, J. Exp. Med., 132, 368 (1970).
10. P. G. Spear and G. M. Edelman, J. Exp. Med., 139, 249 (1974).
11. P. G. Spear, A. Wang, U. Rutishauer, et al., J. Exp. Med., 138, 577 (1973).
12. J. Sterzl and A. M. Silverstein, Adv. Immunol., 6, 337 (1976).
13. G. Sweet and S. L. Welborn, J. Immunol., 106, 1407 (1971).
14. L. L. Yung, T. C. Wyn-Evans, and E. Diener, Europ. J. Immunol., 3, 224 (1973).

ABILITY OF LYMPHOCYTES STIMULATED BY PHYTOHEMAGGLUTININ in vitro TO PARTICIPATE IN THE GRAFT VERSUS HOST REACTION

V. G. Nesterenko and L. V. Koval'chuk

UDC 612.112.94:612.6.02:017.1].014.46:612.111.44

Lymph node cells from CBA mice stimulated for 2 h by phytohemagglutinin were more able, whereas cells cultivated for 44 h with phytohemagglutinin were less able, than intact lymph node cells to participate in the graft versus host reaction when injected into sublethally irradiated (CBA × C57BL/6)F_1 hybrids. Syngeneic lymphocytes and killed allogeneic lymphocytes cultivated in the same way, like phytohemagglutinin itself, had no such action.

KEY WORDS: colony-forming units; phytohemagglutinin; lymphocyte; allogeneic transplant.

The ability of lymphocytes of the peripheral blood and lymphoid organs to proliferate in vitro under the influence of antigen or mitogen has been widely used in experimental and clinical research [7, 8, 10]. The dynamics of many biochemical and morphological processes in stimulated lymphocytes has been studied in

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 579-581, May, 1976. Original article submitted June 25, 1975.

TABLE 1. Cell Composition of Suspensions Injected into Recipients (M ± m)

Cell suspension	Cultivation time (in h)	Small lymphocytes (in %)	Activated lymphocytes (in %)	Blast cells (in %)	Lymphocytes transformed into macrophages	Macrophages (in %)	Mitoses (in %)	Undifferentiated cells (in %)
Lymph node cells of CBA mice	44*	40,2±4,3	32,2±4,1	9,5±2,2	4,7±1,6	7,4±1,9	2,3±0,3	4,7±0,8
	44	84,3±2,6	2,5±0,7	0,75±0,2	3,5±1,3	7±2,1	0	2±0,6
	2*	92,9±0,8	1,3±0,2	0,8±0,1	1,5±0,3	2±0,3	0	1,5±0,3
	2	93±1,1	1,1±0,1	0,6±0,2	1,8±0,2	1,9±0,1	0	1,8±0,4
	0	96,7±0,9	0,8±0,2	0,5±0,3	0,2±0,1	0,1±0,1	0	1,7±0,3

*Cultivation with PHA.

detail [5, 9]. However, comparatively little is known of the ability of lymphocytes activated in vitro to participate in reactions in vivo [6, 11].

The ability of mouse lymphocytes stimulated in vitro by phytohemagglutinin (PHA) to participate in the graft versus host reaction (GVHR) in vivo was investigated.

EXPERIMENTAL METHOD

Male CBA and (CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g were used. Lymph node cells of the mice were cultivated in vitro for 2 or 44 h at 37°C. For cultivation of the cells for 44 h, RPMI-1640 medium (Microbiological Associates Inc.) was used, with the addition of 10% inactivated human blood group IV serum obtained from several donors and 2 mM glutamine. For incubation of the lymphocytes for 2 h medium No. 199, with similar additives, was used. To obtain a cell suspension, the cervical, axillary, and inguinal lymph nodes were removed with sterile precautions, homogenized in a glass homogenizer, and washed twice with centrifugation at 1100 rpm for 10 min each time. The cell suspension was made up with culture medium to a final concentration of $3 \cdot 10^6$ cells/ml and poured into penicillin flasks in volumes of 2 ml. PHA (Difco, P) was added at the beginning of cultivation in a dose of 4 μl/ml. After cultivation the cells were washed once by centrifugation at 1100 rpm for 10 min. Films for cytological examination were prepared from part of the cell suspension and stained with hematoxylin–eosin; the rest of the cells were used for injection into animals. The viability of the cells was estimated by the trypan blue test. Morphological analysis of the cytological specimens was based on Pokrovskaya's classification [2]. To assess functional activity of the stimulated cells, a model of the GVHR based on the ability of lymph node cells of CBA mice to inhibit the formation of endogenous hematopoietic colonies in the spleen of sublethally irradiated (CBA × C57BL/6)F_1 mice was used [4]. The recipient mice were irradiated with ^{137}Ce γ rays on the "Stebel' 3A" apparatus in a dose of 750 R [dose rate 900 R/min; $LD_{100/13}$ for (CBA × C57BL/6)F_1 hybrids 900 R]. One of the following cell suspensions was injected intravenously into the recipients 24 h after irradiation: intact lymph node cells, lymph node cells cultivated for 2 h with or without PHA, and lymph node cells cultivated for 44 h with or without PHA. The dose of viable cells injected varied from $0.5 \cdot 10^6$ to $4 \cdot 10^6$ cells. The control group received no cells. The mice were killed 9 days later and the number of colony-forming units (CFU) in the spleen was counted [12]. The percentage inhibition of CFU was calculated from the results by the formula $(a - b)/a \times 100\%$, where a is the number of CFU in the irradiation control and b the number of CFU in the experiment. The ability of the cells to participate in GVHR was assessed quantitatively by calculating the median active dose (ED_{50}), i.e., the number of donor cells which had to be injected into the recipient to reduce the number of CFU by 50% [1]. The results were subjected to statistical analysis by Lord's method [3].

EXPERIMENTAL RESULTS

Data showing the cytological composition of the different cell suspensions are given in Table 1. Clearly PHA induced blast transformation of the mouse lymphocytes; for instance, the number of blast cells and activated lymphocytes was increased by 13 times but the number of small lymphocytes was reduced by half compared with lymph node cells cultivated without PHA. During long cultivation the number of macrophages and of lymphocytes transformed into macrophages in the cultures also was greater than in the intact lymph node cells and in those cultivated for 2 h. No difference was found in the survival rate or the intensity of blast transformation and the cell composition of the cultivated lymph node cells of the CBA and (CBA × C57BL/6)F_1 mice (the details are not given in Table 1). The results of four experiments carried out to determine the ability of different lymphoid suspensions to participate in the GVHR are summarized in Fig. 1. The intensity of the GVHR as a function of dose of cells is shown in Fig. 2. Cultivation of lymph node cells of CBA mice for 2 h with PHA stimulated their ability to participate in GVHR, whereas cultivation for 44 h depressed it (Figs.

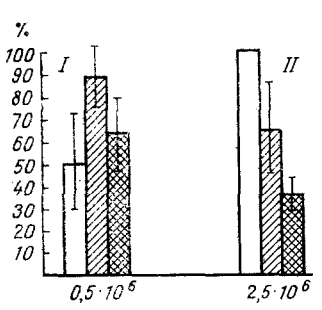

Fig. 1

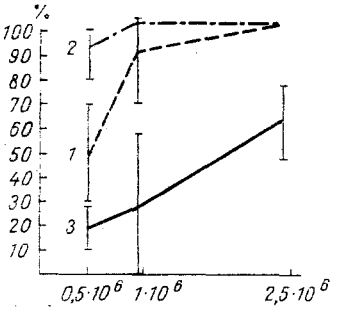

Fig. 2

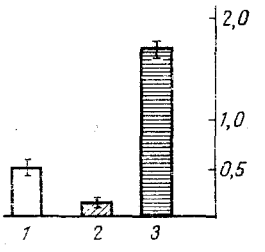

Fig. 3

Fig. 1. Ability of lymph node cells of CBA mice to participate in GVHR: I) cultivation for 2 h, II) for 44 h. Unshaded columns represent intact cells; shaded columns cells cultivated with PHA; cross-hatched columns cells cultivated without PHA. Abscissa, number of cells injected into recipients; ordinate, percentage inhibition of CFU.

Fig. 2. Intensity of GVHR as a function of number of cells injected into recipient: 1) intact cells; 2, 3) cells cultivated for 2 and 44 h respectively with PHA. Abscissa, number of lymph node cells of CBA mice injected; ordinate, percentage inhibition of CFU.

Fig. 3. Determination of ED_{50} for different groups of lymph node cells of CBA mice. Abscissa, different groups of cells: 1) intact cells, 2) cells cultivated for 2 h with PHA, 3) cultivated for 44 h with PHA; ordinate, ED_{50} ($\cdot 10^6$).

1 and 2). Calculation of ED_{50} (Fig. 3) showed that in order to produce GVHR of similar intensity, 3.3 times fewer cells incubated for 2 h and 3.8 times more cells incubated for 44 h with PHA were needed compared with the control (intact lymph node cells). Injection of syngeneic lymphocytes cultivated with PHA or of killed allogeneic (thawed and frozen three times after cultivation) lymphocytes and also of PHA alone (in a dose of 2-12 μl) did not cause inhibition of CFU in the recipients' spleens. This shows that for the GVHR to take place living allogeneic lymphocytes must be present.

It is not yet possible to give a concrete explanation of the differences found in the activity of the different groups of cultivated lymphocytes. However, it can be postulated that the increase in activity of the temporarily stimulated lymph node cells is connected with additional stimulation of the lymphocytes by PHA while their ability to react to foreign antigens was preserved or even enhanced. The decrease in the ability of the donor's lymphocytes to induce a GVHR after cultivation for 44 h with PHA may be connected with the partial loss of their ability to react to foreign antigens during intensive proliferation. The possibility cannot be ruled out that during cultivation for 44 h some of the donor's cells capable of inducing the GVHR may die. To elucidate the causes of the change in activity of the different groups of cultivated lymphocytes further investigations are required.

LITERATURE CITED

1. I. B. Pogozhev, in: Immunology and Prophylaxis of Intestinal Infections [in Russian], Moscow (1962), pp. 191-216.
2. M. P. Pokrovskaya, V. I. Levenson, and N. A. Kraskina, in: Textbook of Microbiology, Clinical Features and Epidemiology of Infectious Diseases in Several Volumes [in Russian], Vol. 3, Moscow (1964), pp. 190-219.
3. G. W. Snedecor, Statistical Methods Applied to Research in Agriculture and Biology [Russian translation], Moscow (1961).
4. R. M. Khaitov, Byull. Éksp. Biol. Med., No. 10, 58 (1972).
5. M. D. Cooper and L. Herbert, Transplant Rev., 11, 3 (1972).
6. J. W. Dyminski and B. F. Argyris, Cell Immunol., 7, 205 (1973).
7. G. Igal, R. K. Gershon, et al., J. Exp. Med., 136, 128 (1972).
8. D. F. Osborne and D. H. Katz, J. Immunol., 111, 1164 (1973).
9. R. M. Gorczynski, J. Immunol., 112, 47 (1974).
10. R. I. Schiff, R. H. Buckley, et al., J. Immunol., 112, 376 (1974).

11. B. T. Rouse and H. Wagner, J. Immunol., 109, 1282 (1972).
12. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).

CORRECTION OF IMMUNE RESPONSE TO SHEEP'S ERYTHROCYTES BY POLYELECTROLYTES IN DIFFERENT STRAINS OF MICE

R. M. Khaitov and A. A. Batyrbekov

UDC 612.017.1.014.46:615.31:547.745

Inbred mice of strain C57BL, A, C57BR, C3H, and CBA, with low, medium, and high reactivity to sheep's red cells (SRBC), were injected with the polymers poly-4-vinylpyridine (P4VP) and polyacrylic acid (PAA) and immunized with SRBC; production of antibody-forming cells (AFC) in their spleen was then determined. Injection of P4VP into C57BL mice was shown to produce a fivefold increase, and injection of PAA a fourfold increase in the immune response. Injection of P4VP and PAA into mice of strain A increased the immune response by 2.5 and 2.8 times respectively. The immune response in C57BR mice was increased fourfold by P4VP and by 4.7 times by PAA. Treatment of C3H mice with P4VP increased the immune response by twice, and treatment with PAA by 2.4 times. Injection of P4VP and PAA into CBA mice did not affect the intensity of the immune response. With an increase in the immunological reactivity toward SRBC in the mice of these strains a decrease in the potentiating effect of polymers on the immune response was thus observed.

KEY WORDS: immune response; polyelectrolytes; genetic differences.

The height and development of the immune response during immunization by various antigens are genetically determined [2, 3]. After many years of study in the writers' laboratory a series of strains of mice with high or low reactivity to a particular antigen has been selected. For example, during immunization with sheep's red cells (SRBC) mice of strain CBA show high reactivity but C57BL mice low reactivity. Mice of strains A, C57BR, and C3H occupy an intermediate position between these two extreme strains [2, 3]. Genetic differences in the height of the immune response are determined at the level of populations of immunocompetent cells [2]. The writers have shown that the accumulation of fewer antibody-forming cells (AFC) in the spleen immunization with SRBC in C57BL mice compared with CBA is largely due to the low intensity of migration and cooperation of T and B lymphocytes in strain C57BL [5].

It is therefore reasonable to suggest that the immune response in C57BL mice, with low reactivity, can be strengthened by methods stimulating migration and cooperation of T and B lymphocytes.

Reports have recently been published that the synthetic compounds poly-4-vinylpyridine (P4VP) and polyacrylic acid (PAA) stimulate migration of B lymphocytes and increase the effectiveness of cooperation between T and B lymphocytes [1].

The object of this investigation was to study the effect of P4VP and PAA on the immune response to SRBC in mice with low, medium, or high levels of reactivity to that antigen.

EXPERIMENTAL METHOD

Mice of strains CBA, C3H, C57BR, A, and C57BL aged 2-3 months and weighing 20-22 g were used. The animals received a single intravenous injection of P4VP or PAA in a dose of 50 mg/kg. The methods of

Institute of Biophysics, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 582-584, May, 1976. Original article submitted September 3, 1975.