MOUSE LYMPHOCYTE IMMUNE RECEPTOR BLOCKADE BY SOME LECTINS

G. V. Burlakov

UDC 612.112.94.017.1.06.08

KEY WORDS: lectins; lectin receptors; immune receptors; luminescent serum against albino mouse globulins.

The effect of different types of lectins on immunologic processes is currently being intensively studied. In particular, they are widely used to study the properties and components of the surface membrane of lymphocytes [6-8]. With their application it has become possible to study not only the distribution of lectin receptors on the surface of lymphocytes, but also to study the mobility of other receptors in the membrane, their redistribution, and the phenomenon of "capping" [9].

The aim of the present investigation was to study the effect of phytohemagglutinin (PHA), concanavalin A (con A), and pea lectin (PL) on immune receptors of mouse lymphocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 180 BALB/c mice weighing 18-20 g. A cell suspension in medium 199 was prepared from the spleens by gentle homogenization. The suspension was filtered through a Kapron filter and layered above a Ficoll—Verografin density gradient by Böyum's method [4]. Lymphocytes were isolated, washed twice in the cold, and diluted with medium 199 so that each 0.2 ml of suspension contained 4 million cells. The viability of the cells was verified (initial control) by the use of 0.1% trypan blue solution. The lymphocytes were tested for viability (subsequent controls) also after their exposure to various dilutions of the best lectins, to the temperature conditions to be used, the luminescent serum, and repeated centrifugation. These controls were set up between 8 and 27 h after isolation of lymphocytes from the spleens.

The lymphocyte suspension was transferred in volumes of 0.2 ml into centrifuge tubes, which contained different dilutions (from 10^{-11} to 10^{-2}) of the test lectins in the same volumes. The lectin dilutions were prepared before use in medium 199. The following types of lectins were used: PHA-P ("Reanal," Hungary), con A (Karl University, Czechoslovakia), and PL (Karl University, Czechoslovakia).

Two controls (C_1 and C_2) were set up simultaneously. C_1) A sample with 0.2 ml of lymphocyte suspension without the addition of test lectins was kept in the refrigerator throughout the experiment. At the end of the experiment (after 8-27 h) lymphocytes from this sample, after addition of luminescent serum and washing 3 times to remove that serum, were tested for viability with 0.1% trypan blue solution. C_2) A sample with 0.2 ml of lymphocyte suspension + 0.2 ml of medium 199. A tube containing this cell suspension was subjected to the same procedures, except the treatment with lectins, as the tubes with the experimental samples (an equal number of times of centrifugation, incubation for the same length of time in a thermostat, at room temperature, and in the cold, treatment with luminescent serum, and so on).

All the experimental tubes, and also C_2, were incubated at 37°C. The incubation time of the lymphocytes with the test lectins varied from 10 to 60 min. The lymphocyte suspension was constantly shaken. After incubation the lymphocytes were washed twice with medium 199 (1:100) in the cold and adjusted to a volume of 0.2 ml. Later, 0.2 ml of luminescent rabbit serum (N. F. Gamaleya Research Institute of Epidemiology and Microbiology) against albino mouse globulins was added to all the tubes, including C_1 and C_2, in a dilution of 1:10. Before the experiment the serum was adsorbed on an acetone powder of BALB/c mouse liver for 1 h at room temperature. It was then centrifuged for 30 min at 8000 rpm.

Department of Pathological Physiology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 8, pp. 227-229, August, 1989. Original article submitted January 19, 1989.

 0007-4888/89/0008-1174$12.50 ©1990 Plenum Publishing Corporation

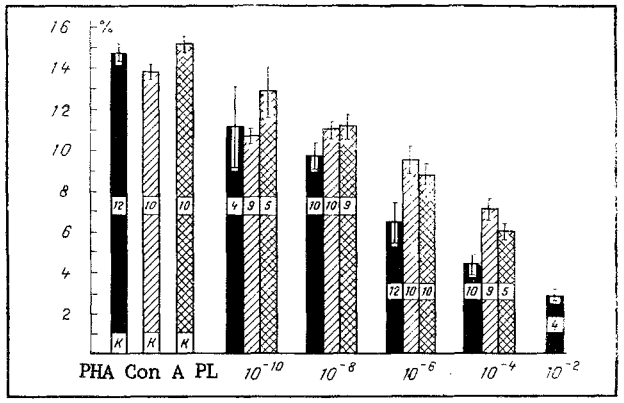

Fig. 1. Changes in percentage of luminescent mouse lymphocytes under the influence of various lectins. At 10^{-10}: PHA) $p < 0.05$; con A) $p < 0.001$; PL) $p < 0.05$ compared with corresponding control; at 10^{-8}-10^{-2}: in all tests $p < 0.001$. Numbers in columns indicate number of experiments. K) Control (C_2). Abscissa, dilution of lectins; ordinate, percentage of luminescent lymphocytes.

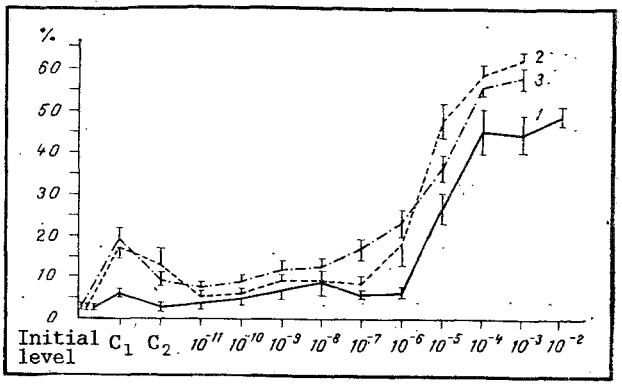

Fig. 2. Effect of PHA (1), con A (2), and PL (3) on viability of lymphocytes in test with 0.1% trypan blue. Test carried out 8-13 h after isolation of lymphocytes from spleens. Duration of incubation with lectins 20 min. Mean values of damaged cells are shown (M ± m). Number of experiments for each case was 10-12; C_1 and C_2) controls: initial level — initial number of damaged cells (immediately after isolation). Abscissa, dilution of lectins; ordinate, percentage of damaged cells.

All tubes with added luminescent serum were kept in the refrigerator for 30 min. The suspension was constantly shaken. The lymphocytes were then washed 3 times with medium 199 (1:100) in the cold to remove the serum and replaced in the refrigerator.

Microscopic examination of the cells was carried out with the "Lyumam I-3" luminescent microscope. Luminescence was excited by blue-violet light, using FS-2, SZS24, and BS8-3 filters. A ZhS18 + ZhZS19-3 "cutoff" filter, 90 × 1.25 objective, and ×10 ocular were used. The strength of the current for microscopy was 4.5 A. Bright green luminescence of the lymphocytes was studied. The number of luminescent cells among 2000 cells was counted. To confirm the results, the cells also were examined by phase-contrast microscopy. The number of "luminescent" lymphocytes was compared before and after treatment with lectins.

The Nfp K_2 binocular light microscope (VEB "Carl Zeiss, Jena") also was used.

EXPERIMENTAL RESULTS

Treatment of lymphocytes from mouse spleens with PHA (the optimal time of incubation with lymphocytes was chosen experimentally and was 15 min) caused a significant decrease in luminescence of the cells. The action was dose-dependent: the higher the concentration of lectin, the greater the decrease in luminescence of the lymphocytes. For instance, after exposure to PHA in concentrations of 10^{-11}-10^{-9}, luminescence of lymphocytes (compared with the control) fell by 29.2-28.9% respectively, with PHA in a concentration of 10^{-8}-10^{-6} it fell by 34.6-56.7%, and with a concentration of 10^{-5}-10^{-2}, it fell by 64.9-81.5%. The dose-dependent decrease in luminescence of the lymphocytes under the influence of the lectins tested is shown in Fig. 1. As a more adequate control for comparison of the results, we used C_2 (not C_1 — see "Experimental Method"). Incidentally, the percentage of luminescence of the lymphocytes in C_1 was 1.5-2.8% higher than in the corresponding C_2. For instance, in the PHA series $C_1 = 17.63 \pm 0.54\%$, whereas $C_2 = 14.83 \pm 0.45\%$; in the con A series the values were 15.40 ± 0.21 and $13.85 \pm 0.41\%$, and in the series PL they were 16.85 ± 0.52 and $15.20 \pm 0.41\%$ respectively.

High concentrations of PHA (10^{-2}-10^{-5}), like the other lectins tested, caused strong agglutination of the lymphocytes and reduced the percentage of viable cells (from an initial 96-98% to 40-50% according to the test with 0.1% trypan blue) (Fig. 2). Changes also were observed in the shape and size of the cells: the lymphocytes frequently were irregular in shape and large, sometimes giant cells appeared. Similar changes were observed during prolonged incubation (more than 30 min) of lymphocytes with lectins. This was not observed in the control tests.

The same general patterns also were found for the action of the other lectins tested. Treatment of lymphocytes with con A led to reduction of their luminescence from 12.9% (at a concentration of 10^{-11}) to 65.7% (at a concentration of 10^{-3}). Under the influence of PL luminescence of the lymphocytes was inhibited from 24.3 to 60.5% (at concentrations of PL of 10^{-11}-10^{-4} respectively). However, PHA had the strongest power of inhibiting luminescence of the cells (Fig. 1).

It will be clear from the results described above that the lectins studied had a marked nonspecific effect on the immune receptors of the lymphocytes. This effect was expressed as blocking of immunoglobulin (evidently Fc) receptors, reacting specifically with antibodies against mouse immunoglobulins. Considering the specificity of action of lectins in relation to lectin receptors (which are carbohydrate in nature and located in the oligosaccharide series of membrane glycoproteins or glycolipids [9]), it can be postulated that this bond (lectin — receptor) already exists and modifies the mutual relations and functional properties of other spatially close molecular units responsible for reaction with various biologically active substances. Evidence of the existence of such a relationship between antigen-binding and mediator receptors of the lymphocyte membrane is given by the work of Ado et al. [1-3]. Our own investigations also indicate that such relations exist also between lectin and immune receptors on the surface membrane of lymphocytes.

LITERATURE CITED

1. A. D. Ado and T. A. Alekseeva, Byull. Éksp. Biol. Med., No. 7, 75 (1983).
2. A. D. Ado, T. A. Alekseeva, and T. A. Avdeeva, Immunologiya, No. 4, 57 (1985).
3. A. D. Ado, T. A. Alekseeva, and V. A. Kamysheva, Byull. Éksp. Biol. Med., No. 8, 231 (1986).
4. A. Böyum, Scand. J. Clin. Lab. Invest., Suppl. 97, 9 (1968).
5. D. A. Hume and M. Y. Weidemann, Mitogenic Lymphocyte Transformation, Amsterdam (1980).
6. G. L. Nicolson, Biochim. Biophys. Acta, 457, 57 (1976).
7. G. L. Nicolson, Biochim. Biophys. Acta, 458, 1 (1976).
8. N. Sharon and H. Lis, Meth. Membr. Biol., 3, 147 (1975).
9. N. Sharon, Adv. Immunol., 34, 213 (1983).