DYNAMICS OF LYMPHOCYTE RECEPTOR AFFINITY FOR HAPTEN DURING THE PRIMARY IMMUNE RESPONSE

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Changes in affinity of antigen-binding receptors of lymphocytes for dinitrophenol (DNP) were studied during the primary immune response in CBA mice. The process was evaluated by the method of inhibition of rosette formation with DNP–ovalbumin–sheep's red cells complex by hapten (DNP–ε-lysine). An increase in affinity of immunoglobulin receptors on the lymphocyte surface for DNA was shown, starting from the 10th day after immunization. The question of the role of affinity and density of receptors on the surface of the immunocyte is discussed.

KEY WORDS: lymphocytes; receptors; hapten; affinity; primary immune response.

One of the important characteristics of an antibody and antibody-like receptor on the lymphocyte surface is its affinity. The affinity of antibodies increases in the course of primary immune response [5]. An increase in affinity of antibodies after immunization is due to proliferation predominantly of precursor cells with high affinity [3].

The investigation of receptors on the surface of the B lymphocyte for dinitrophenol (DNP) conjugated with human γ globulin (HGG) and for NNP* –HGG has shown that their affinity increases during the primary immune response parallel with an increase in affinity of the serum antibodies, whereas the affinity of receptors of the T cells is unchanged [7]. There is evidence [11] to show that the affinity of receptors of B and T cells for the DNP-determinant increases concurrently.

The discovery of receptors for the Fc fragment on the B cell and macrophage raises a question which demands an answer when the affinity of surface immunoglobulins is studied: Are these structure in fact receptors or are they secondarily adsorbed antibodies?

The investigation described below was carried out for two purposes: first, to assess the dynamics of affinity of lymphocyte receptors during the primary response, and second, to discover whether the receptor structures on the immunocyte surface are true receptors or secondarily adsorbed antibody molecules.

EXPERIMENTAL METHOD

CBA mice from the Stolbovaya nursery were used. The animals were immunized in a single session by intraperitoneal injection of 100 μg of DNP_{998}–hemocyanin conjugate. Keyhole limpet hemocyanin (Calbiochem, USA) and ovalbumin united with the DNP group [1] were used. Whooping cough vaccine (10^9 bacterial cells) was used as the adjuvant. The number of DNP groups on the carrier was determined spectrophotometrically in conjunction with determination of nitrogen by the micro-Kjeldahl method.

* NNP – 4-hydroxy-3,5-dinitrophenacetyl.

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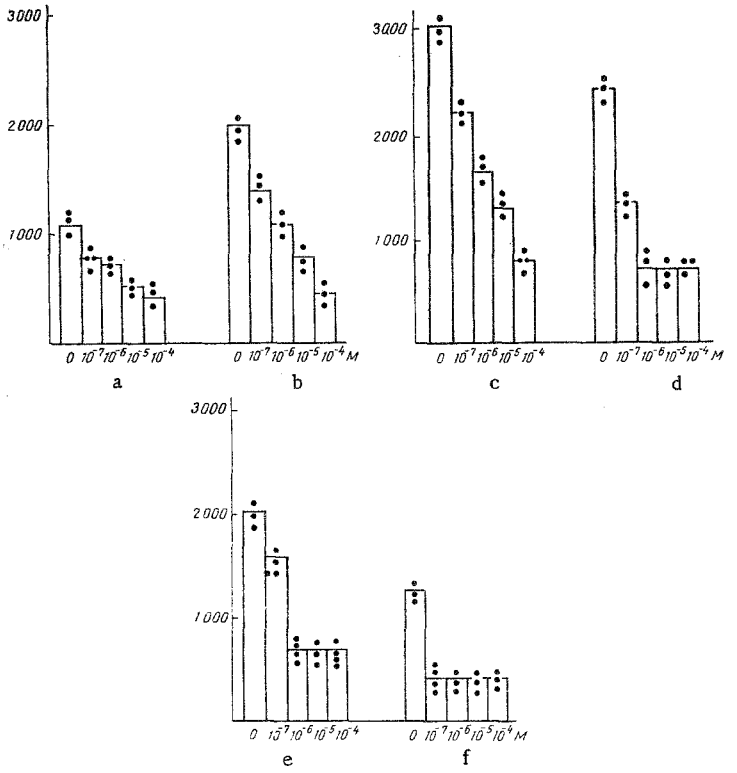

Fig. 1. Number of rosette-forming cells as a function of concentration of inhibitor (DNP–ε-lysine) during investigation at various times after immunization: a) 3rd day, b) 5th day, c) 8th day, d) 10th day, e) 14th day, f) 19th day. Abscissa, concentration of DNP–ε-lysine (M); ordinate, number of rosette-forming cells per 10^6 spleen cells.

The dynamics of the increase in affinity of the lymphocyte receptors was studied by inhibition of rosette formation by various concentrations of DNP–ε-lysine (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}; BDH Chemicals Limited, Poole, England).

Rosettes were obtained by method of [8] modified by the authors. DNP_{23}–ovalbumin was bound to the surface of sheep's red cells with the aid of chromic chloride [9].

The number of rosette-forming cells was determined on the 3rd, 5th, 8th, 10th, 14th, and 19th days after immunization.

To investigate the affinity of the receptors 10^6 spleen cells were incubated with an equal volume of DNP–ε-lysine in different concentrations for 1 h at 4°C, after which sheep's red cells covered with DNP_{23}–ovalbumin were added. Nucleated cells with more than five red cells on their surface were regarded as rosette-forming: To determine the nature of the receptors on the surface of the lymphocytes 0.4 ml of suspension containing $2 \cdot 10^6$ spleen cells were incubated with an equal volume of rabbit antiserum against mouse γ globulin in a dilution of 1 : 10 for 1 h in the cold and then washed with physiological saline together with normal rabbit serum diluted 1 : 250 [10]. As a control, the same cells were incubated with 10% normal rabbit serum.

EXPERIMENTAL RESULTS

The effectiveness of inhibition of rosette formation was increased by increasing the concentration of DNP–ε-lysine. Different concentrations (differing by one order of magnitude) of inhibitor were used and five groups of rosette-forming cells were isolated at different times of the immune response (3rd, 5th, and 8th days; Fig. 1a, b, c). In the later stages after immunization the number of groups was reduced: to 3 on the 10th and 14th days (Fig. 1d, e), and to 2 on the 19th day (Fig. 1f). Antiserum against mouse immunoglobulins inhibited rosette formation considerably (by 60%). Binding of antigen thus took place on account of γ globulin molecules located on the surface of the cell membrane.

Experiments were carried out to rule out the possibility of adsorption of serum antibody molecules onto the lymphocyte surface. On the 5th day after primary immunization, the mice were injected with serum (2 ml) of syngeneic donors immunized 14 days before the experiment; the mice were killed 15 h later and the affinity of the receptors of the spleen cells investigated. The cells of the same mice also were incubated in vitro with 14-day serum for 1 h. The distribution of these cells by affinity was the same in the experiments in vivo and in vitro as on the 5th day after primary immunization.

The experiments thus showed that the ability of splenic lymphocytes to bind red cells covered with hapten changes in the course of the primary response. In the later stages, rosette formation requires lower concentrations of hapten. The rosette-formation response itself (like the change in affinity of the immunoglobulins of the lymphocyte surface during the immune response) is independent of secondary adsorption of serum immunoglobulin molecules. The issue is thus one of a change in hapten-binding capacity of immunoglobulin receptors synthesized by a given lymphocyte and located on its surface. It has been claimed [4, 7] that this phenomenon is completely analogous to the increase in affinity of serum antibody molecules and that it depends on the selective proliferation of cells carrying high-affinity receptors.

The strength of the bond joining the lymphocytes with red cells covered with hapten depends not only on the affinity of the receptor molecules on the surface of the lymphocytes, but also on two other variables: the concentration of hapten molecules on the surface of the red cell and the concentration of immunoglobulin receptor molecules on the surface of the lymphocyte. Assuming that a critical concentration of receptor molecules necessary for binding with the hapten–red cell complex exists on the lymphocyte surface, it must be accepted that the lower the concentration of Ig receptors on the lymphocyte surface, the lower the concentration of competitive inhibitor (in this case DNP–ε-lysine) required to prevent rosette formation.

Other workers [2, 3], using a different method (adsorption of DNP–proteins labeled with I^{125} on lymphocytes, with autoradiographic analysis at the light-optical level) also observed an increase in the affinity of lymphocyte receptors in the course of the immune response. However, these workers found no decrease in concentration of receptors on the surface of the immunocytes during the primary immune response, such as was observed in blast and plasma cells on electron-microscopic analysis [6].

The data obtained by the present workers and published elsewhere [2, 3, 7] can be explained as follows. The relative proportion of blast cells in the population of rosette-forming cells is fairly small. Plasma cells have no Ig receptors whatsoever on their surface. The data now obtained showing an increase in affinity of Ig receptors of rosette-forming cells for DNP-determinants may therefore reflect real changes taking place in the population of the memory cells and, perhaps, of lymphocyte and plasma cells.

However, further investigations are necessary to solve the problem of to what extent the change in binding power of the immunocyte surface during the primary immune response depends on a change in the affinity of the receptors and to what extent on a change in their concentration.

LITERATURE CITED

1. B. Benacerraf and B. B. Levine, J. Exp. Med., 115, 1023 (1962).
2. J. H. Devie and W. E. Paul, J. Exp. Med., 135, 643 (1972).
3. J. H. Devie and W. E. Paul, J. Exp. Med., 135, 660 (1972).
4. J. H. Devie and W. E. Paul, J. Exp. Med., 137, 201 (1973).
5. H. N. Eisen and G. W. Siskind, Biochemistry (Washington), 3, 996 (1964).
6. A. Matter et al., J. Exp. Med., 136, 1008 (1972).
7. E. Möller, W. W. Ballock, and O. Mäkelä, Europ. J. Immunol., 3, 172 (1973).
8. D. Osoba, J. Exp. Med., 132, 368 (1970).
9. G. Sweet and F. L. Welborn, J. Immunol., 106, 1407 (1971).
10. Toshitada et al., J. Exp. Med., 134, 815 (1971).
11. J. Wilson and M. Feldman, Nature New Biol., 245, 177 (1973).