EFFECT OF SPECIFIC ANTIBODIES ON ABILITY OF ANTIGEN-LOADED MACROPHAGES TO INDUCE AN IMMUNOLOGICAL RESPONSE

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Peritoneal macrophages obtained from CBA mice were treated with specific antiserum, loaded with sheep's red cells, and injected into normal recipients. Animals receiving injections of peritoneal macrophages after preliminary treatment with normal mouse serum and animals receiving injections of macrophages loaded with antigen but without preliminary serum treatment were used as the controls. The number of hemolysin-synthesizing cells was determined 4 days later in the recipients' spleen. Preliminary treatment of the macrophages with specific antiserum considerably depressed their immunological response when these cells were injected into normal recipients. The degree of the immunodepressive effect was not reduced if the macrophages were first treated with specific antiserum from which the hemolysins and hemagglutinins had been removed. It is postulated that the immunodepressive effect of the specific antiserum at the macrophage level depends on antibodies with properties resembling those of cytophilic macrophages.

Passive injection of specific antisera during or some time before or after immunization is known to give rise to a marked immunodepressive effect [10-13, 18, 19]. There is as yet no unanimous agreement on the mechanism of the inhibitory action of the antibodies. Some workers state that antibody formation is inhibited by passive antibodies at the lymphocyte level [5, 17]. This depression is the result of composition for the antigen between the globulin-like receptors of antigen-reactive precursor cells and the passively injected antibodies. According to other authorities the inhibitory effect is produced by the passive antibodies at the macrophage level [6, 7, 10, 20]. The need for macrophages to participate in the induction of antibody-formation after injection of several corpuscular and soluble antigens has frequently been demonstrated [1-3, 15-21].

The object of the present investigation was to study the effect of antibodies against sheep's red cells on the formation of the immunological response in a system of syngeneic transfer of macrophages loaded with specific antigen.

EXPERIMENTAL METHODS

A specific serum was obtained by triple immunization of CBA mice with 0.5 ml % of a suspension of sheep's red cells with intervals of 7 days between injections. On the 10 day after the third immunization the serum was obtained and inactivated at 56°C for 30 min. The titer of serum antibodies in the hemagglutination test was 1:2500. Some of the specific serum was exhausted by incubating the antierythrocytic serum with sheep's red cells. The titer of hemagglutinins and hemolysins in the serum after exhaustion was 1:2. To obtain peritoneal macrophages, 1.5 ml of 2% peptone was injected into each mouse. After 3 days the peritoneal cavity of the animals was washed out with Hanks's solution containing heparin (5 units/ml). The cell suspension thus obtained was incubated in Petri dishes at 37°C for 30 min. The cells were

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TABLE 1. Effect of Specific Antiserum on Ability of Macrophages to Induce Antibody Formation in a Syngeneic Transfer System (M ± m)

Series of experiment	With normal serum and sheep's red cells			
	direct	indirect	direct	indirect
	per million nucleated spleen cells		percent of control	
Treatment of peritoneal macrophages: with specific antiserum and sheep's red cells (n = 10)	56,2±2,4	23,0±0,8	29,6	44,7
P	<0,05	<0,05		
with specific antiserum (exhausted) and sheep's red cells (n = 10)	54,9±1,4	21,5±1,6	28,9	41,8
P	<0,05	<0,05		
with normal serum and sheep's red cells (n = 10)	196,0±9,5	53,8±2,7	103,2	104,7
P	>0,05	>0,05		
with sheep's red cells only (n = 10)	190±5,8	51,4±1,9	100,0	100,0

then divided into those adherent and nonadherent to the glass and incubated two more times separately in the manner described above. Of the cells adherent to the glass, 98% were macrophages. The number of viable cells varied from 95 to 98%.

The macrophages thus obtained were incubated with the specific serum, in the native state or adsorbed with antigen, for 1 h at 37°C in the ratio of 20 million cells to 1 ml of serum diluted 1:10. After sensitization with antibodies the macrophages were separated from the serum by centrifuging three times at 1500 rpm at 4°C. The macrophages were then incubated with sheep's red cells (10-15 red cells per macrophage) for 1 h at 37°C. To remove the free red cells the suspension was treated three times with 0.35% NaCl solution. Morphological examination revealed the complete absence of free red cells from the suspension after this treatment. The proportion of viable macrophages was 60-64%.

Macrophages sensitized by antibodies and loaded with the corresponding antigen were injected intraperitoneally into normal syngeneic recipients in a dose of 20 million cells. Animals receiving macrophages treated with normal mouse serum and antigen, and also animals receiving macrophages loaded with antigen only, were used as the controls. Each group consisted of 10 animals. On the 4th day after injection of the macrophages the number of direct and indirect plaque-forming cells was determined in the recipients' spleen by the methods of Jerne and Nordin [8] and Dresser and Wortis [4].

EXPERIMENTAL RESULTS

The experiments showed that peritoneal macrophages loaded with sheep's red cells can induce the formation of a well-marked immunological response when injected into normal recipients (Table 1). The number of direct and indirect plaque-forming cells was 190 ± 5.8 and 51.4 ± 1.9, respectively, per 10^6 nucleated spleen cells. Normal mouse serum had a negligible effect on the ability of macrophages loaded with sheep's red cells to induce an immunological response. In that case the number of direct plaque-forming cells was 196.0 ± 9.5, and the number of indirect 53.8 ± 2.7 per 10^6 nucleated spleen cells ($P > 0.05$). If the macrophages were treated with specific antiserum, marked depression of formation of the immunological response was observed ($P < 0.05$). The numbers of direct and indirect plaque-forming cells were 29.6 and 44.7% of the control.

Treatment of the macrophages with specific antiserum from which hemagglutinating and hemolyzing antibodies were almost totally removed also sharply inhibited their ability to induce antibody formation ($P < 0.05$). The degree of inhibition in this case was the same as after treatment of the macrophages with the original unexhausted specific antiserum. The populations of direct and indirect plaque-forming cells were 28.9 and 41.4%, respectively, of the control.

It can be concluded from these results that specific antibodies can inhibit antibody formation at the macrophage level. Although the corresponding experiments were not carried out, it can be assumed that antibodies inhibiting the immune response at the macrophage level resemble cytophilic antibodies in their properties, for they interact with macrophages in the absence of antigen. This agrees to some extent with the observations of Pierre [14], who found inhibition of the primary immunological response in vitro after

the addition of a population of phagocytic cells, previously loaded with antibodies, to intact mouse spleen lymphocytes. Similar results as regards the ability of cytophilic antibodies to inhibit the immunological response were obtained by Ptak [16], who showed that injection of macrophages, sensitized with cytophilic antibodies and loaded with sheep's red cells, into intact mice caused inhibition of the production of plaque-forming cells in the recipients compared with injection of macrophages not previously sensitized with cytophilic antibodies. Ivanyi [9], who investigated the immunodepressive action of passively injected specific antiserum on the immunological response, found that cytophilic antibodies play an important role in the suppression of antibody formation. Meanwhile, the present experiments revealed certain new regular features of the immunodepressive action of specific antibodies. It is a significant fact that a specific serum from which antierythrocytic antibodies have been removed continues to induce immunodepression of the same degree as the original, unexhausted serum. This result can be explained by at least two circumstances. During repeated adsorption of the immune serum with the specific antigen soluble antigen–antibody complexes may be formed and these may have an immunodepressive action at the macrophage level. However, this assumption is not confirmed by results showing that such a complex can induce immunodepression only if an excess of antibodies is present, and this was completely ruled out by the experimental conditions. At the same time it can be postulated that the cytophilic antibodies, on which the immunodepressive effect depends at the macrophage level, differ considerably in their avidity for the antigen. In the free state these antibodies combine with antigen to only a slight degree, but in the form of complexes with macrophages the level of their interaction with the specific antigen rises significantly [12].

The existence of cytophilic antibodies reacting with the antigen only after interaction with macrophages can thus be postulated. For that reason they persist after repeated adsorption of the serum with the antigen and they continue to exert their immunodepressive effect at the macrophage level. The inhibitory action of the specific antiserum in a system of syngeneic transfer of antigen-loaded macrophages evidently depends on these antibodies.

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