COMPETITION BETWEEN HETEROLOGOUS ANTIGENS IN A MICROMODIFICATION OF OUCHTERLONY'S GEL DIFFUSION TEST

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When heterologous tissue antigens are placed in neighboring wells the phenomenon of inhibition of the gel diffusion reaction, due to competition between the antigens, is observed.

In a previous paper [2] the phenomenon of inhibition of the gel diffusion reaction, taking place when human erythrocyte and serum antigens are placed in neighboring wells, was described.

An analogous phenomenon for two systems of heterologous antigens has been investigated and the results are described below.

EXPERIMENTAL METHOD

Antigens were prepared as saline extracts [3]. Extracts of human and bovine tissues were lyophilized. The original dilutions of human tissue extracts were obtained by dissolving 50 mg of the lyophilized antigen powder in 1 ml of distilled water, and the original dilutions of bovine tissue extracts by dissolving 100 mg of lyophilized powder in 1 ml distilled water. Extracts of monkeys' tissues were not lyophilized; the protein content in extracts of monkeys' spleens was 6.2-8.2 mg/ml. Exhausted rabbit antisera against the spleen from the cadaver of a person dying from chronic myeloid leukemia [4] were used in the investigation. The antisera were preserved in a lyophilized state. The micromodification of Ouchterlony's gel diffusion test was carried out in 1% agar made up in physiological saline [1], in veronal-medinal buffer, pH 8.6, and ionic strength 0.025, or in the same veronal-medinal buffer with the addition of NaCl up to 0.85% concentration.

EXPERIMENTAL RESULTS

The study of the antigenic structure of leukemic and normal tissues in extracts of human organs, using an antimyeloblastosis serum obtained after the fourth cycle of immunization, revealed a leukocytic antigen which was present in a greatly increased amount in the spleens of persons dying from chronic myeloid leukemia [4]. In this paper for convenience it will be referred to as antigen I, and the extracts from spleens of persons dying from chronic myeloid leukemia as leukemia spleen extracts. It was impossible to detect this antigen in the organs and sera of monkeys (Macaca rhesus), cows, rats, or rabbits, but if extracts of organs of monkeys or cows were poured into wells next to wells containing leukemic spleen extracts, the formation of precipitation lines due to interaction between antigen I and antimyeloblastosis serum was partially or completely suppressed. The tissue extracts of rats and rabbits had no inhibitory action. Inhibition of the reaction was exhibited equally in agar gel made up in physiological saline and in veronal-medinal buffer. If the antimyeloblastosis serum was used in dilutions of 1 : 4 or 1 : 8, antigen I was found in the leukemic spleen extracts up to dilutions of 1 : 40 or 1 : 80. To perform the inhibition tests, a test system consisting of antiserum in a dilution of 1 : 8 and leukemic spleen extract in a dilution of 1 : 40 was used. Under these conditions the monkey spleen extracts had an inhibitory action up to dilutions of 1 : 5-1 : 10, and bovine spleen extracts in the original dilution only. Antigen I could not be detected in ex-

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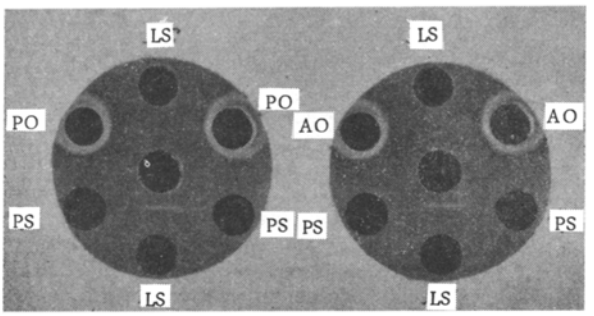

Fig. 1

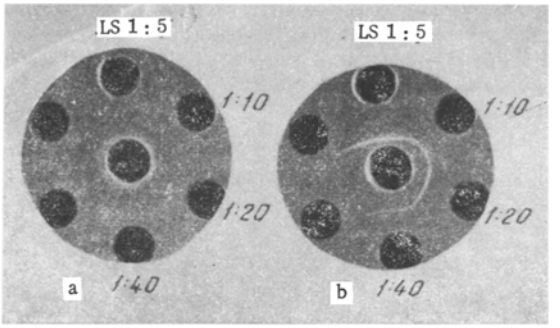

Fig. 2

Fig. 1. Effect of antiserum on inhibitory activity of monkey spleen extract. In central wells: antimyeloblastosis serum in dilution 1 : 8; LS – leukemic spleen extract in dilution 1 : 40; AO – antimyeloblastosis serum in dilution 1 : 4 + monkey spleen extract in original dilution; PO – physiological saline + monkey spleen extract in original dilution; PS – physiological saline.

Fig. 2. Exhaustion of antiserum by monkey spleen extract. In central well: a) antimyeloblastosis serum in dilution 1 : 2 + monkey spleen extract in dilution 1 : 2; b) antimyeloblastosis serum in dilution 1 : 4. In peripheral wells: leukemic spleen extract (LS) in dilutions of 1 : 5, 1 : 10, 1 : 20, and 1 : 40.

tracts of monkey or bovine spleens in any dilution from the original to 1 : 160. Consequently, the possibility that the inhibitory action of these extracts could be due to their high content of antigen I, an excess of which leads to the formation of soluble antigen – antibody complexes, can be ruled out.

It has been suggested that the inhibitor either blocks the active centers of antibodies or acts on the antigen, modifying it so that it can no longer react with antibodies, or splits up this antigen with the formation of fragments of molecules containing antigenic determinants which, like incomplete antigens, block the active centers of the antibodies.

To test these hypotheses an attempt was made to discover any decrease in the inhibitory activity of monkey spleen extracts after the addition of antimyeloblastosis serum to them. Different dilutions of monkey spleen extracts were mixed with different volumes of physiological saline or of antiserum in a dilution of 1 : 4. The mixtures were incubated for 1 h and then poured into wells next to wells containing the test system. The results showed that monkey spleen extracts, after the addition of antimyeloblastosis serum, had a weaker inhibitory action than extracts to which physiological saline had been added (Fig. 1). Consequently, the antiserum inactivates part of the inhibitor, although the possibility cannot be ruled out that the decrease in the inhibitory activity of the monkey spleen extracts is due to nonimmunologic mechanisms.

To determine whether or not the inhibitor is actually bound with antibodies against antigen I, monkey spleen extracts in a dilution of 1 : 2 were added to antimyeloblastosis serum in a dilution of 1 : 2 and to leukemic spleen extract in dilutions of 1 : 5, 1 : 10, and 1 : 20. As a result of the reaction between antiserum exhausted by the monkey spleen extract and the leukemic spleen extract in various dilutions, no precipitation line was formed (Fig. 2). After the addition of monkey spleen extract to the antigen, the reaction was less strongly inhibited and a precipitation line was formed (Fig. 3). The results of these experiments indicated that the inhibitor is bound with antibodies against antigen I. In the case of action of inhibitor on the antigen, the reaction would have been suppressed more strongly after addition of monkey spleen extract to the leukemic spleen extracts.

The dependence of inhibition on the ratio between concentrations of inhibitor and antigen during their reaction with various dilutions of antisera was also studied. To different dilutions of extracts of leukemic human spleen (1 : 5, 1 : 10, and 1 : 20) an equal volume of physiological saline or monkey spleen extract in a dilution of 1 : 2 was added. Extracts of leukemic spleen in dilutions of 1 : 10, 1 : 20, and 1 : 40 or mixtures of extracts were poured into the central wells, and antiserum in dilutions of 1 : 2, 1 : 4, 1 : 8, and 1 : 16 into the peripheral wells. Leukemic spleen extracts mixed with monkey spleen extracts reacted with smaller dilutions of antiserum than leukemic spleen extracts alone in the corresponding dilutions (Fig. 3b, c). Be-

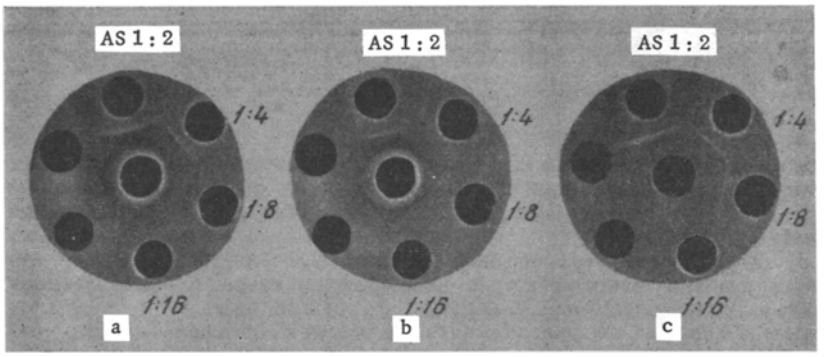

Fig. 3. Dependence of inhibition on ratio between concentrations of antigen and inhibitor in mixture of extracts. In central well: a) leukemic spleen extract in dilution of 1 : 10 + monkey spleen in dilution of 1 : 2; b) leukemic spleen extract in dilution of 1 : 20 + monkey spleen extract in dilution of 1 : 2; c) leukemic spleen extract in dilution of 1 : 40. In peripheral wells: antimyeloblastosis spleen (AS) in dilutions of 1 : 2, 1 : 4, 1 : 8, and 1 : 16.

sides, as the concentration of leukemic spleen extracts in the mixture of extracts fell, a precipitation line was formed near wells with smaller dilutions of antimyeloblastosis serum (Fig. 3a, b). If, however, the inhibitor acted on antigen I, lowering its concentration in the mixture of extracts, with a decrease in the concentration of this antigen it would react with higher dilutions of antiserum. The results can be explained only by competition between inhibitor and antigen for the active centers of antibodies.

Some properties of the inhibitor were studied. Like antigen I, it was found to be inactivated by heating on a water bath at 60° for 30 min, and not to dialyze. The inhibitor is evidently a protein similar to antigen I, and not to incomplete antigen of low molecular weight.

The phenomenon of inhibition of the gel diffusion reaction with antigens are placed in neighboring wells was further studied in another system of heterologous extracts. Previously, an antigen conventionally described as a hemocytoblast antigen [3] was discovered in the blood hemocytoblasts of patients with leukemias and in the normal human thymus, peripheral lymphoid organs, and mucous membrane of the gastrointestinal tract. With the aid of antiserum against human fetal thymus [3] this antigen was found in the organs of monkeys. It could be detected in the organs of cows or calves. If, however, extracts of the spleen, lymph glands, thymus, or liver of calves were poured into wells next to wells with the test system, the formation of a precipitation line corresponding to hemocytoblast antigen was partially or completely suppressed. Experiments analogous to those performed to study inhibition of the reaction between antimyeloblastosis serum and antigen I showed that the phenomenon of inhibition in the system hemocytoblast antigen – antibodies against it – calf tissue extracts is likewise due to competition between antigens, and that the inhibitor, like hemocytoblast antigen [3], does not undergo dialysis.

It is thus established for these two systems of heterologous tissue antigens that the phenomenon of inhibition of the gel diffusion reaction when these antigens are placed in neighboring wells is due to competition between antigens. Antigens giving a visible precipitation line when reacting with antisera are evidently similar to the inhibitors of the reaction; the inhibitors, moreover, are not incomplete antigens of the Landsteiner type.

LITERATURE CITED

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