PHOSPHOLIPID HAPTENS: CROSS REACTIVITY OF CARDIOLIPIN AND PHOSPHATIDYL-INOSITOL

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In the complement fixation test with specific antisera against the phospholipid haptens cardiolipin and phosphatidyl-inositol cross immunologic reactivity was demonstrated between these haptens.

KEY WORDS: cardiolipin; phosphatidyl-inositol; cross reactions.

Investigation of individual lipid haptens in different systems by various immunochemical methods necessitates the use of antisera with narrow specificity. However, the sera obtained by immunization of animals with a pure lipid hapten in some cases contain antibodies cross-reacting with other haptens structurally similar to the one being studied, and this may interfere with the precise identification of the hapten in a test system.

The object of the present investigation was to obtain antisera against two phospholipid haptens, namely cardiolipin (CL) and phosphatidyl-inositol (PI), and to use them to study cross-reactivity of these haptens.

EXPERIMENTAL METHOD

CL from bovine heart was obtained from Koch-Light Laboratories, bovine brain PI, egg-yolk lecithin, and cholesterol from Sigma Chemical Co.; CL was obtained from the liver of noninbred mice by the method of Faure et al. [4].

The purity of the various preparations was verified by thin-layer chromatography on silica-gel G (Woelm) in two systems of solvents: 1) chloroform–methanol–water (65:25:4) and 2) chloroform–methanol–2N NH_4OH (65:25:2). To detect lipids on the chromatograms a 50% aqueous solution of sulfuric acid or a 5% solution of phosphomolybdic acid in ethanol was used.

Antisera against phospholipid haptens were obtained by two methods. One consisted of immunizing rabbits with CL from bovine heart and PI by the usual method of immunization with lipid haptens [7]. Each hapten was mixed with the auxiliary lipids lecithin and cholesterol in the ratio of 1:10:30, after which the sample was incubated with a 0.1% solution of bovine serum albumin, methylated as described in [13]. After incubation the residue was separated by centrifugation and suspended in 2 ml physiological saline. Rabbits were immunized with the resulting suspension intravenously with a dose of 0.2 ml on alternate days (10 injections). During the whole cycle of immunization each rabbit received 3-5 mg of hapten.

In the second method rabbits were immunized with mouse liver CL. Just as with the first method, the animals received 10 intravenous injections of hapten, and 2 months after the last injection they were given the antigen (hapten+carrier), mixed with Freund's complete adjuvant (1 volume antigen in physiological saline to 1 volume of adjuvant), was injected into a lymph node. One month later the same rabbits received an intramuscular injection of antigen without adjuvant. The total dose of hapten per rabbit was 6-10 mg.

Blood was collected from the immunized rabbits on the 7th-9th day after the last injection. The sera were kept at −20°C.

The complement fixation test (CFT) was carried out by Tarkhanova's method [1]. The antigen was prepared as described in [2]. For this purpose a solution of the lipids in ethanol (hapten–lecithin–cholesterol,

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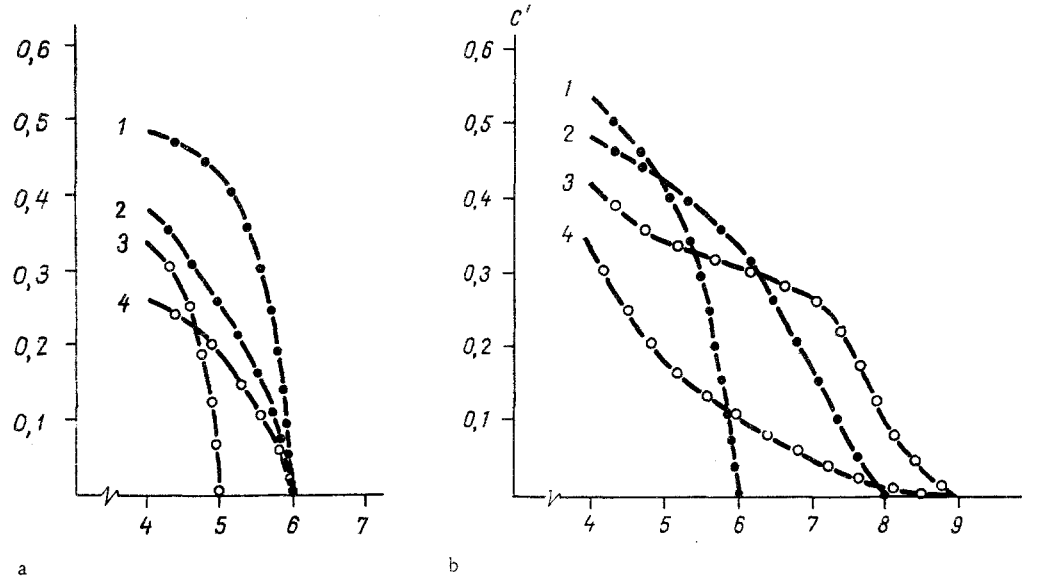

Fig. 1. Curves of interaction between antisera against phosphatidyl-inositol and cardiolipin in the CFT. Here and in Fig. 2: abscissa, logarithms of dilution of antisera to base 2 ($\log_2 P$); ordinate, quantity of fixed complement, expressed in 50% hemolytic units (C'). a) Curves of interaction between antiphosphatidyl-inositol (anti-PI) sera of group 1 with PI (curves 1, 2) and CL (curves 3, 4); b) curves of interaction between anti-PI sera of group 2 with PI (curves 1, 2) and CL (curves 3, 4).

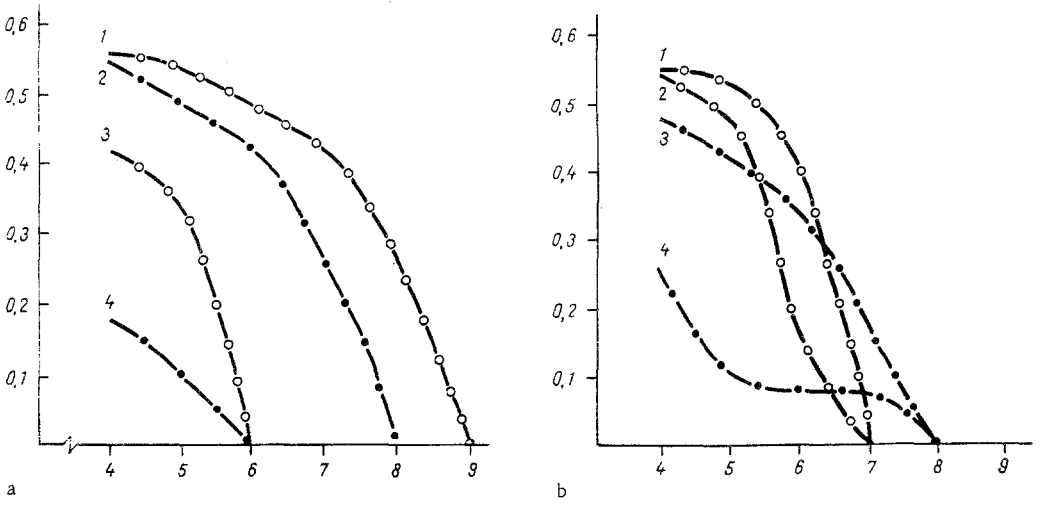

Fig. 2. Curves of interaction between antisera against CL (anti-CL) and PI and CL in CFT. a) Curves of interaction of anti-CL sera of group 1 with CL (curves 1, 2) and PI (curves 3, 4); b) curves of interaction between anti-CL sera of group 2 and CL (curves 1, 2) and PI (curves 3, 4).

0.5 : 0.5 : 9 mg/ml) was diluted before use 100 or 200 times with physiological saline. The quantity of hapten added during the test was constant, namely 5 or 2.5 μg per sample. A standard preparation of dry complement, supplied by the Bacterial Preparations Manufacturing Enterprise of the Moscow Research Institute of Vaccines and Sera was used. To 0.25 ml of the antigen solution 0.1 ml of a solution of complement, diluted 1 : 6 with physiological saline, and 0.25 ml of each dilution of antiserum in a row from 1 : 16 to 1 : 2048 were added. The antigen and antisera were tested for anticomplementary and hemolytic activity.

After incubation for 12-14 h at 4°C the unfixed complement was titrated by means of a hemolytic system consisting of a mixture of sheep's red blood cells and hemolytic serum. Dilutions of antiserum fixing comple-

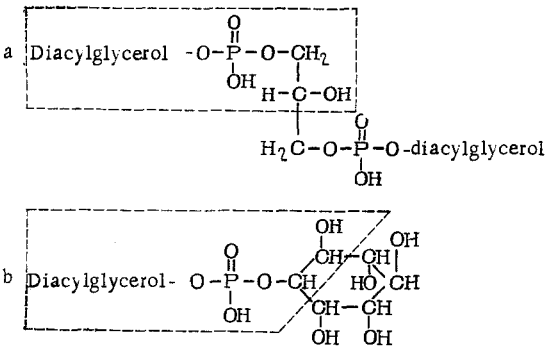

Fig. 3. Diagram of structure of CL (a) and PI (b). Similar antigenic determinants are boxed.

ment were detected on the 50% hemolysis scale. The fixed complement was calculated in 50% hemolytic units by means of the table in [1].

EXPERIMENTAL RESULTS

Altogether eight antisera against PI (anti-PI), seven antisera against bovine heart CL, and two antisera against mouse liver CL (anti-CL) were used. The sera of these rabbits, if taken before immunization, did not react in the CFT with either phospholipid hapten. When assessing cross-reactivity of the haptens, the results obtained in the same experiment with the same antiserum were compared.

All the anti-PI sera reacted in the CFT with both PI and CL from mouse liver or bovine heart. Depending on the type of interaction with these haptens, the sera as a whole could be divided into two groups. Interaction with haptens of the antisera of group 1 is shown graphically in Fig. 1a, and with haptens of the antisera of group 2 in Fig. 1b. As Fig. 1a shows, the curves of interaction with haptens of group 1 antisera have higher values of fixed complement for the homologous than for the heterologous hapten for each point analyzed in the system.

It will be clear from Fig. 1b that anti-PI sera in the zone of low dilutions react more strongly with homologous than with heterologous hapten, and in the zone of high dilutions of antibody against heterologous hapten they still continued to be found although antibodies against the homologous hapten could no longer be tested.

All antisera against CL (bovine heart or mouse liver), regardless of the method of obtaining them, reacted both with CL from bovine heart and from mouse liver and with PI. Just as for the anti-PI sera, two groups of anti-CL sera differing in the character of their interaction with homologous hapten and PI were found. Sera belonging to group 1 gave characteristic curves of interaction with CL and PI as shown in Fig. 2a, from which it can be seen that at all dilutions the anti-CL sera reacted more strongly with the homologous hapten than with PI, thus exhibiting an analogy with the anti-PI sera of group 1.

The curves of interaction of the anti-CL sera of group 2 with homologous and heterologous haptens (Fig. 2b) showed the same regular patterns as were found for the anti-PI sera of group 2.

CL and PI are the phospholipid haptens which have received the most study. Japanese workers, who suggested methods of obtaining antisera against these haptens [7, 11] and who studied the arrangement of the antigenic determinants in the molecules of these haptens, showed that the main antigenic determinants of CL are phosphate groups and the free hydroxyl radical, and also the diglyceride part of the molecule [8, 9], whereas in PI the main determinant is the inositol moiety of the molecule [11]. The use of immunoelectrophoresis and microflocculation revealed no cross-reactions between these haptens [10, 12]. Schiefer [14], who studied active lipids of the mitochondrial membrane immunologically also found no cross-reactions between CL and PI in the CFT. However, Guarniery et al. [6] postulated and later showed [5] the presence of cross-reactions between CL and PI by the microflocculation method and CFT. He explained the cross-reactions by the presence of a common antigenic determinant, including the diglyceride part of the phospholipid molecule with a phosphoric acid residue and hydroxyl group (Fig. 3). Cross-reactions between CL and PI also were found by de Siervo [3].

In the present investigation cross-reactivity between CL and PI in the CFT was found with all anti-PI and anti-CL sera tested.

The discovery of cross-reactions between CL and PI by some workers and their absence in the experiments of others can evidently be explained by the different spectrum of antibodies against the determinants of the lipid hapten in antisera obtained by different research workers. Important factors determining the spectrum of antibodies against a given hapten in each concrete serum may be the nature of the preparation used for immunization, the method of injection of the antigen, the individual differences in the responses of different animals, and the method used to test serum activity.

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STROMAL FIBROBLASTS OF HEMATOPOIETIC ORGANS AND ANTIBODY FORMATION IN CULTURE

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Stromal mechanocytes of thymic, bone-marrow, and splenic origin obtained from monolayer cultures at the 3rd-6th passage, if added to suspension cultures of rabbit spleen cells by the method of Mishell and Dutton, have a significant effect on the accumulation of antibody-forming cells (AFC) by the 4th day in culture. Their action clearly depends on dose. Stromal mechanocytes of bone marrow origin, in doses of 2.1×10^3-6.25×10^5, caused inhibition of AFC formation in culture. Stromal mechanocytes of thymic origin in doses of 2.75×10^3-8×10^5 caused an increase in the number of AFC, whereas mechanocytes of splenic origin in doses of 2.1×10^3-1.3×10^4 had no significant effect, and in doses of 8×10^4-6.25×10^5 inhibited AFC formation. Many of the living cells and AFC were concentrated in the fraction of nonadherent cells.

KEY WORDS: stromal fibroblasts; antibody formation in vitro.

Stromal mechanocytes (fibroblasts) of hematopoietic tissue play an important role in the creation of the microenvironment for lymphocytes in hematopoietic and lymphoid organs [1-6]. This raises the question of the effect of stromal fibroblasts on the development of immunologic reactions in vitro.

In the investigation described below the effect of stromal fibroblasts on antibody formation in culture was studied. The plan of the investigation was to isolate cell lines of stromal fibroblasts from bone marrow,

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