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HETEROPHILIC ANTIBODIES AGAINST ANTIGENS OF MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE AND ERYTHROCYTES OF ANIMALS OF DIFFERENT SPECIES

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UDC 616.153.962.4-097-02: 616-018.2]-078.73

KEY WORDS: heterophilic antibodies; connective tissue; myocardium; erythrocytes.

Heterophilic antibodies, i.e., antibodies reacting with antigens of other species, are found in the sera of patients with various diseases. In most cases these antibodies are discovered in tests with heterologous erythrocytes. For instance, in infectious mononucleosis antibodies are found against erythrocytes of animals of various species. Determination of heterophilic agglutinins against sheep's erythrocytes is used for the diagnosis of this disease [8]. Antibodies of the H–D (Hanganutziu–Deutscher) type, active against a ganglioside located on the ovine and bovine erythrocyte membrane, were first described in serum sickness [7]. These antibodies have also been found in diseases of the liver, kidneys, and intestine [9]. Heterophilic reactions playing a role in kidney transplantation in man have been described. Elevation of the antibody titer against rat erythrocytes, carrying the so-called heterophil transplantation antigen (HTA), frequently correlates in patients with acute graft rejection [10, 11, 14].

In previous investigations the writers found heterophilic antibodies reacting with heterophilic antibodies reacting with cells of the interstitial connective tissue (ICT) of bovine myocardium in the sera of patients with rheumatic fever and other diseases (myocarditis, myocardial infarction, diseases of connective tissue). The frequency of their discovery and the intensity of the reaction were shown to increase considerably in patients with rheumatic fever in the active phase of the disease [2, 5]. Antibody titers were particularly high in patients after heart valve replacement [1]. The corresponding heterophilic antigen has been shown to be a tissue-specific bovine antigen which is found in all animals in cardiac ICT and on erythrocytes. With the aid of antibodies isolated from the sera of patients on erythrocyte stroma and immunosorbent prepared from antigens of bovine connective tissue, the antigen was shown to be located in the cytoplasm and cytoplasmic membrane of connective-tissue cells of various bovine organs. Reactions of the antibodies with myocardial ICT cells were shown to be inhibited by D-galactose [3].

Heterophilic antibodies against rat myocardial ICT antigens have also been described [13] in various heart diseases, but the authors cited did not undertake a detailed study of these antibodies.

The aim of this investigation was to compare reactions of heterophilic antibodies with bovine myocardial ICT with reactions to heart tissues of animals of other species. The dis-

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 0007-4888/85/9901-0100$09.50

TABLE 1. Results of Absorption of Sera Containing Heterophilic Antibodies by Erythrocytes of Different Species of Animals

Species of animal on whose myocardium test was carried out	Erythrocytes used for absorption				
	ox	sheep	rabbit	rat	guinea pig
Ox	—	+	—	—	±
Sheep	—	+	Not tested	Not tested	Not tested
Rabbit	—	Not tested	—	» »	» »
Rat	—	» »	Not tested	—	» »
Guinea pig	Not tested	» »	» »	Not tested	—

Legend. —) Reaction with ICT completely abolished, +) reaction with ICT preserved, ±) reaction partly preserved.

tribution of the corresponding heterophilic antigens on animals' erythrocytes also was studied and heterophilic reactions discovered were compared with other known heterophilic systems.

EXPERIMENTAL METHOD

Altogether 60 sera were tested: 32 from patients with rheumatic fever in the active phase, 18 from patients with rheumatic fever after heat valve replacement, and 10 sera from normal blood donors. The sera were kept at —20°C and studied in dilutions of 1:8-1:16 or higher. Ox, pig, sheep, rabbit, guinea pig, rat and mouse heart tissues were used. From three to ten samples of myocardium of each species were tested. Pieces of tissue were taken from the wall of the left ventricle and frozen at —70°C. The experiments were carried out on unfixed sections cut in a cryostat. The indirect immunofluorescence test with pure antibodies against human IgG, by the technique described previously [2], was used. Ox, sheep, rabbit, guinea pig, and rat erythrocytes were used in the absorption experiments. Erythrocyte residue was added to serum in the ratio of 1:2 and the mixture was incubated for 1 h at 37°C and for 18 h at 4°C.

EXPERIMENTAL RESULTS

All sera were first tested on sections of bovine myocardium. Of 60 sera 38 reacted with ICT cells with an intensity which varied from 1+ to 4+, and 22 gave negative results. Sera containing and not containing antibodies against antigens of bovine heart ICT were tested on the myocardium of other animals.

Positive reactions with ICT were given by the myocardium of animals of all species studied. Reactions were observed only with sera containing antibodies against bovine heart ICT. Sera not containing these antibodies did not react with myocardial ICT from other animals (except one serum with rat heart). Reactions to ox, pig, sheep, and rabbit myocardium agreed completely as regards both the character of the fluorescent cells and the intensity of fluorescence. Fluorescence of spindle-shaped fibroblasts and also of capillary cells was noted (Fig. 1). Different results were obtained on testing sera on rat, mouse, and guinea pig myocardium. Only some sera containing antibodies against bovine heart ICT reacted with myocardial ICT antigens of these species; the intensity of the reactions, moreover, was much weaker than on bovine heart. Reactions also were found not on all specimens of rat and guinea pig myocardium.

In absorption experiments with erythrocytes, sera containing heterophilic antibodies were subjected to parallel absorption by bovine erythrocytes and erythrocytes from another species, after which they were tested on myocardium of both species (Table 1). Sheep's erythrocytes did not abolish fluorescence of ox and sheep heart ICT cells, whereas bovine erythrocytes completely inhibited the reaction of the sera with sheep ICT. Rabbit erythrocytes inhibited the reaction of antibodies with ox and rabbit heart ICT, and bovine erythrocytes also inhibited the reaction with rabbit myocardium ICT. Similar results were obtained on absorption of the sera with rat erythrocytes. Guinea pig erythrocytes completely abolished the reaction to guinea pig myocardium but only partially to bovine myocardium.

Heterophilic antigen of bovine heart ICT (BHA) is thus found regularly in pig, sheep, and rabbit myocardial ICT (Table 2). This antigen was not found in rat and guinea pig ICT in all animals of the corresponding species. BHA also was found on ox, pig, rabbit, and rat erythrocytes and also, to a lesser degree, on guinea pig erythrocytes, which inhibited the

TABLE 2. Presence of BHA in Myocardial ICT and on Erythrocytes of Different Species of Animals

Species of animals	Myocardial ICT	Erythrocytes
Ox	+	+
Pig	+	+
Sheep	+	−
Rabbit	+	+
Rat	±	+
Guinea pig	±	±

Legend. +) Antigen present in all animals tested, −) antigen absent, ±) antigen present in not all animals or in a minority.

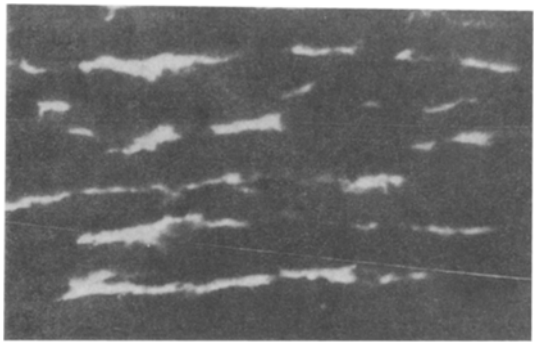

Fig. 1. Tissue section from rabbit heart: fluorescence of interstitial connective tissue cells. Objective 40 ×, Homal 3 ×.

the reaction of the sera with bovine heart ICT only partially. Sheep's erythrocytes did not contain BHA. BHA thus differs from antigens of the heterophilic systems, namely Horssman, Paul–Bunnell, and Hanganutziu, for it is not found on sheep's erythrocytes. BHA is closest in its properties to HTA, which is found on rat, mouse, bovine, and rabbit erythrocytes but is absent on sheep and guinea pig erythrocytes [14]. However, BHA is evidently different from HTA. First, reactions of sera with bovine heart ICT are always inhibited by bovine erythrocytes. Antibodies against HTA, in the contrary, are not always inhibited by bovine erythrocytes [14]. Second, the writers' preliminary data obtained by the heterophilic hemagglutination method showed that sera of patients with high titers of antibodies against bovine ICT (1:128) contain only low titers of agglutinins against rat erythrocytes. At the same time, according to Western workers, titers of anti-HTA-antibodies against rat erythrocytes exceed 1:80 [10, 11].

The causes of appearance of heterophilic antibodies in human sera are unknown. In our present investigations we could find no BHA in ICT of human myocardium or in erythrocytes of blood groups O, A, and AB. Partial inhibition of heterophilic antibodies was observed only after absorption of the sera with group B erythrocytes [5]. The discovery of heterophilic antibodies (anti-HTA) after transplantation of the kidney is evidence that heterophilic antigens may be human alloantigens, cross-reacting with antigens of animals tissues [12, 13]. McDonald et al. [11] found HTA on human B lymphocytes and on cells of B-lymphoid lines. They suggest that HTA is linked with HLA-DR histocompatibility antigens. In addition, an antigen cross-reacting with HTA has been found in many enterobacteria, which may cause the appearance of antibodies against HTA in individuals not possessing it in their tissues [10].

There is also another point of view, according to which heterophilic antibodies may appear in a patient during a disease against antigens which, in the normal person, are "latent" [2, 9, 10]. According to Feizi [6], the appearance of heterophilic antibodies is evidence of pathological changes in the tissues.

Rheumatic fever is a systematic disease of connective tissue. In this disease, and also during operations on heart valves, "latent" antigens capable of inducing an immune response may pass into the blood stream. Meanwhile, during immunization of rabbits with cross-reacting

streptococcal antigens, heterophilic antibodies against human myocardial ICT antigens can be obtained, whereas antibodies against rabbit heart ICT are not formed. Yet bound immunoglobulins are found in ICT of immunized rabbits, which may be evidence of the presence of a "latent" antigen in ICT [4]. The problem of the mechanisms of formation of heterophilic antibodies against myocardial ICT in human sera is still unexplained and requires further study.

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EFFECT OF ADRENALECTOMY ON RECIPIENTS OF ALLOGENEIC LYMPHOCYTES ON INACTIVATION OF ENDOGENOUS COLONY-FORMING CELLS IN MICE

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UDC 612.6.02.017.1.014

KEY WORDS: adrenalectomy, lymphocytes, endogenous colony-forming cells.

T lymphocytes are the principal cells inducing delayed-type hypersensitivity reactions, and on interaction with allogeneic stem cells, they can inactivate them [5, 6]. It has been shown that positive correlation exists between inactivation of endogenous colony formation and transplantation immunity reactions [7, 8]. In endogenous hydrocorticism induced by bilateral adrenalectomy manifestations of transplantation immunity reactions are intensified [3], the number of endogenous colony-forming cells in the spleen is increased [4], with a shift of their differentiation toward erythropoiesis [2], and it is consequently interesting to study the killer activity of lymphocytes toward endogenous colony-forming cells in adrenalectomized recipients.

The aim of this investigation was to study the killer functions of lymph node cells directed against endogenous colony-forming cells in adrenalectomized recipients in a genetic system with one-way incompatibility: parental line — F_1 hybrid.

EXPERIMENTAL METHOD

The donors of lymph node cells were C57BL/6 mice, the recipients (CBA × C57BL/6)F_1 mice. Mice aged 3-4 months were obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR. The adrenals were removed through a midline skin incision in the lumbar region, with division of muscles in the right and left hypochondria and with approach to the upper poles of the kidneys in order to remove the glands. The mock operation consisted of the manipulations mentioned above with the exception of removal of the adrenals. After the operation

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(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 84-85, January, 1985. Original article submitted November 20, 1983.

0007-4888-85/9901-0103$09.50