with the carbimazole derivative methimazole inhibits IL 2 production. These same factors (thyroid function, anatomical features of the gland, the phase of the disease, the character of treatment) evidently determine the level of IL 2 production in patients with autoimmune thyroiditis.

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INHIBITION OF HETEROPHIL ANTIBODIES TO MYOCARDIAL INTERSTITIAL CONNECTIVE TISSUE ANTIGENS BY α-D-GALACTOSE

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KEY WORDS: heterophil antibodies; myocardium; connective tissue; α-D-galactose.

In previous investigations the writer found heterophil antibodies, reacting with interstitial connective tissue (ICT) cells of bovine myocardium in sera from patients with rheumatic fever and other diseases of the heart and connective tissue. It was shown that the frequency of discovery and the titers of these antibodies were considerably higher in patients with active rheumatic fever than in patients with inactive rheumatic fever and healthy blood donors [2, 4]. The heterophil bovine antigen (HBA) is tissue-specific and is found in all animals in myocardial ICT, in other connective tissue cells of various bovine organs, and also on erythrocytes. It has been shown that reactions of antibodies with bovine myocardial ICT cells are inhibited by D-galactose [3].

Various human diseases are known to be accompanied by the appearance of autoantibodies or heterophil antibodies in the serum. It has been shown recently that the specificity of some of them is linked with certain carbohydrate sequences. These include agglutinins in hemolytic anemia, directed against I- and i-antigens [5], antibodies against T antigen (Thomsen–Fridenreich antigen [8]), whose antigenic specificy is linked with terminal β-D-galactose, heterophil antibodies of the Hanganutsiu–Deicher and Forssman type, etc. [6].

The aim of this investigation was to study the nature of the immunodominant HBA group by inhibiting the reaction of heterophil antibodies with bovine myocardial ICT by various mono- and disaccharides of known chemical structure. Antibodies to HBA also were compared with other antibodies against carbohydrate determinants.

EXPERIMENTAL METHODS

Altogether 23 sera were tested: 10 from patients with rheumatic fever in the active phase, eight from patients with rheumatic fever after artificial heart valve replacement

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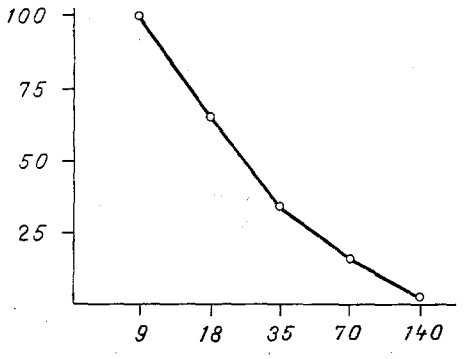

Fig. 1

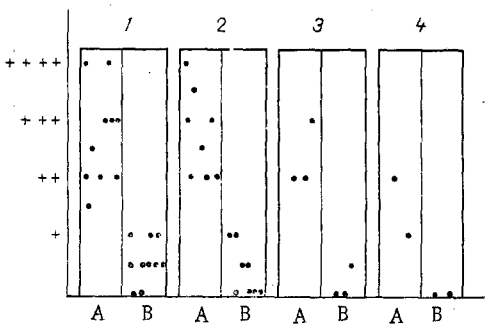

Fig. 2

Fig. 1. Inhibitory action of various concentrations of D-galactose on reaction of serum with bovine myocardial ICT cells. Abscissa, concentration of D-galactose (in mM); ordinate, intensity of luminescence (in % of the initial level).

Fig. 2. Inhibitory action of D-galactose on reaction of sera from patients with various diseases and from healthy blood donors with bovine myocardial ICT cells. 1) Rheumatic fever; 2) rheumatic fever after artificial heart valve replacement operations; 3) myocardial infarction; 4) healthy blood donors. A) Sera before inhibition; B) sera after inhibition. Vertical axis: intensity of reaction with myocardial ICT cells.

operations, three from patients with myocardial infarction, and two from healthy blood donors. All sera reacted strongly with bovine myocardial ICT cells (from ++ to ++++ in a dilution of 1:16) in the indirect immunofluorescence test, conducted by the method described previously [2]. To inhibit the reaction with ICT, mono- and disaccharides were added in a final concentration of 56-84 mM to 0.2 ml of serum in a dilution of 1:8. The following sugars were used: D-glucose D-galactose, D-mannose, L-fucose, N-acetylglucosamine, N-acetylgalactosamine, α- and β-D-methylgalactosides, lactose, melibiose, maltose, cellobiose, and raffinose (from Serva, West Germany, Calbiochem, USA, and Chemapol, Czechloslovakia). The incubation time was 1 h at 37°C and 18 h at 4°C. In some cases the serum was kept with the inhibitor for 1 h at room temperature, after which it was tested on slices of bovine myocardium. Treatment of human group O erythrocytes with neuraminidase (*Vibrio cholerae*, from Koch-Light, England) was carried out with cells in a concentration of $5 \cdot 10^9$ in acetate buffer, pH 5.5, with the addition of 10^{-3} M $CaCl_2$, in the proportion of 100 U of enzyme to 1 ml. The cells were incubated with the enzyme for 70 min and at 37°C, after which the erythrocytes were washed 5 times with buffered physiological saline (BPS), pH 7.2. In the control the erythrocytes were treated with buffer without addition of neuraminidase [7]. The concentration of sialic acids was determined [9] in the supernatant. The erythrocyte residue was added to the serum in the ratio of 1:2 and incubated for 1 h at 37°C and 18 h at 4°C.

RESULTS

D-galactose completely or almost completely inhibited the reaction with bovine myocardial ICT cells. Under these circumstances, reactions with other myocardial structures (disks, sarcoplasm) still took place. Dependence of the degree of inhibition on the dose of monosaccharide is shown in Fig. 1. Addition of 2.5-5 mg of galactose (final concentration 70-140 mM) to 0.2 ml of serum virtually completely inhibited the reaction of the sera with ICT cells. If the dose was reduced, the inhibitory action of galactose was considerably weakened.

Galactose was used in a concentration of 70-84 mM to inhibit reactions of sera taken from patients with various diseases and blood donors (Fig. 2). Marked inhibition of reactions with myocardial ICT cells was observed when sera taken from patients with rheumatic fever, after artificial valve replacement operations, patients with myocardial infarction, and healthy blood donors were used. The other monosaccharides had no inhibitory action.

To investigate whether binding of galactose, present in the serum, takes place with lectins contained in bovine myocardial tissue, slices were treated with a solution of galactose in a concentration of 5-10 mg/ml. The solution was applied to the slices for 45 min

(the usual time of incubation of the serum with myocardial slices), washed with BPS, pH 7.0, after which the serum containing antibodies against bovine heart ICT cells was applied. Fluorescence of the ICT cells was completely preserved under these circumstances.

Inhibition of the reaction with ICT cells by α- and β-methylgalactosides was studied on three sera. The experiments showed that α-methylgalactoside acted more strongly than β-methylgalactoside or D-galactose: The reaction was completely inhibited by the compound in a concentration of 56 mM. Smaller doses of α-methylgalactoside (28 and 14 mM) had no inhibitory activity. β-Methylgalactoside did not stop the reaction or inhibit it partially.

Disaccharides containing α- and β-galactose, the trisaccharide raffinose, containing α-galactose, and as the control, disaccharides containing glucose with α- and β-bonds, were used in the next experiments. Of the five sugars used the disaccharide melibiose (Galα1→6Glc) and the trisaccharide raffinose (Galα1→6Glcα1→2 fructose) had the strongest inhibitory activity. Both sugars contain terminal galactose, connected by an α-bond with glucose. Lactose (Galβ1→4Glc) inhibited the reaction only very weakly, whereas maltose (Glcα1→4Glc) and cellobiose (Glcβ1 → 4Glc) had no inhibitory action.

Further proof that the antigenic specificy of antibodies to HBA is not connected with β-galactose was obtained in experiments in which sera were absorbed with human group O erythrocytes treated with neuraminidase. Removal of sialic acids is known to lead to the appearance of a T antigen on the surface of erythrocytes. Erythrocytes were obtained from three group O donors. After treatment with neuraminidase, the erythrocytes were agglutinated by all three sera, confirming the appearance of a T-determinant on their surface. Control erythrocytes treated with buffer were not agglutinated by the sera. Erythrocytes treated with neuraminidase were used to absorb sera containing antibodies against HBA from three patients (with rheumatic fever, with rheumatic fever after artificial heart valve replacement operations, and with myocardial infarction). In all cases the reaction with ICT cells was completely preserved.

The experiments thus showed that the reaction with bovine myocardial ICT cells is inhibited by α-D-galactose, which evidently is a component of the HBA immunodominant group. Previous observations [1] that HBA differs from antigens of the other heterophil systems were confirmed. We know that the specifity of the Hanganutsiu—Deicher antigen is determined by N-glycolyl-neuraminic acid, and that of the Forssman antigen by N-acetylgalactosamine. HBA also differs from the I- and i-antigens and the T-antigen, whose specificity is determined by β-galactose. The difference between antibodies to HBA and T-antibodies was confirmed by experiments in which the sera were absorbed by erythrocytes treated with neuraminidase.

The ability of erythrocytes of blood group B to inhibit partially the reaction of heterophil antibodies with myocardial ICT cells, discovered by the writer previously [4], can evidently be explained by the presence of a terminal α-galactose residue in the group B antigen [12]. It has also been shown that HBA is regularly detected on rabbit erythrocytes [1]. Tönder et al. [11] described agglutinins to rabbit erythrocytes in human sera and showed that antibody titers are raised in various diseases. The antigenic determinant responsible for the specificity of agglutinins for rabbit erythrocytes has been shown to be pentaglycosyl-ceramide, which contains terminal α-galactose. This component was obtained from bovine erythrocytes and rabbit reticulocytes, and it can therefore be tentatively suggested that identical or similar oligosaccharides are present in the composition of HBA.

Heterophil antibodies to myocardial ICT antigens found in the sera of patients with rheumatic fever and other diseases, and having a tendency to increase their titer during activation of the disease, thus possess specificity due to α-D-galactose. These antibodies and HBA, against which they are directed, evidently constitute a new heterophil system which differs from the heterophil systems described previously.

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EFFECT OF B-ACTIVIN ON HUMAN T SUPPRESSOR CELLS

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A new mediator produced by bone marrow cells, and known as stimulator of antibody producers (SAP), has recently been described. If added to a cell culture or injected into an animal in the productive phase of antibody formation, SAP doubles or trebles the number of antibody producers [3, 4, 7]. Previous investigations on mice showed that one possible mechanism of action of SAP is inhibition of the effect of T suppressor cells by this mediator [1-3]. On the basis of SAP, the immunoregulatory agent B-activin has now been produced [5, 6].

The aim of this investigation was to study the influence of B-activin on the effect of human concanavalin A (con A)-induced T suppressor cells and also on the process of induction of T suppressor cells by con A and stimulation of proliferative activity of lymphocytes by phytohemagglutinin (PHA).

EXPERIMENTAL METHODS

Con A-induced suppression and the effect of B-activin on it were studied in a system in which the test cell culture and the culture for induction of suppressors were prepared simultaneously.

Peripheral blood was obtained from blood donors. Mononuclear cells were isolated on a one-step Ficoll–Urografin density gradient. After isolation the cells were distributed among three penicillin flasks for subsequent culture. The cells, in a concentration of 10^6/ml, were cultured in 3 ml of medium RPMI-1640 (Gibco, USA) with the addition of up to 10% inactivated human group IV (AB) serum, 2 μM glutamine, 10 μM HEPES, 100 U/ml penicillin, and 100 μg/ml streptomycin.

PHA (from Difco, USA), in a dose of 10 μg/ml was added to the first flask (preparation of the test culture), con A (Difco) in a dose of 40 μg/ml was added to the second flask (induction of suppressor cells), and no mitogens were added to the third flask (control of spontaneous proliferation).

After 48 h cells from the first flask were distributed among nine flasks, $3 \cdot 10^5$ cells into each flask. Lymphocytes from the second flask (inhibited with con A) were washed, resuspended in medium RPMI-1640 with the above-mentioned additives, and incubated for 40 min with mitomycin C (40 μg/ml). After incubation the cells were washed twice and transferred into six of the nine flasks containing PHA-stimulated cells, in the ratio of 1:1, and also into three empty flasks, as the control of treatment with mitomycin ($3 \cdot 10^5$ cells

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