genesis also is present in the cortex only, and not present in the white matter of the brain. It is antigenically similar to polypeptide from the thymus and differs from polypeptide from the white matter of the brain. This suggests that the cerebral cortex contains polypeptide of thymarin type, or that biologically active substances of the thymus and brain are similar to or identical with θ-antigen. The proof of this hypothesis, as well as the solution to the problem of direct participation of the active brain substance in immunogenesis, require a more penetrating and detailed investigation.

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A STUDY OF Fc-RECEPTORS OF HEART VALVE FIBROBLASTS AND THE SEARCH FOR SIMILAR RECEPTORS IN OTHER TISSUES

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All normal human and rabbit sera and also the IgG isolated from them have been shown to react with human and bovine heart valve fibroblasts. These reactions are evidently due to the presence of Fc-receptors on the membranes of heart valve fibroblasts [1]. According to observations by other workers [9], Fc-receptors appeared in cultures of human skin fibroblasts only after infection with cytomegalovirus. The problem of the presence of Fc-receptors on fibroblasts of the interstitial connective tissue (ICT) of the heart and tissues of the joints has not been studied. By now Fc-receptors have been found on the surface of various cells (on lymphocytes, macrophages, and liver, kidney, and placental cells [5, 7, 10, 11]). These receptors have been shown to differ: Some react with immunoglobulins in the form of a complex with antigen, some with monomeric immunoglobulins, some with different classes of immunoglobulins [5, 8, 13, 14, 15].

The object of this investigation was to study some special features of Fc-receptors found on heart valve fibroblasts and also to search for similar receptors on fibroblasts of the ICT of the myocardium and joints and in cultures of mouse fibroblasts (L-cells).

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TABLE 1. The Study of Normal Human and Rabbit Sera, Preparations of IgG, $F(ab')_2$-, and Fc-Fragments on Sections of Heart Valve, Myocardial, and Joint Tissues and Cultures of L-cells

Immunoglobulins	Heart valve fibroblasts		Myocardial ICT cells		Fibroblasts of connective tissue of human fetal joint	L-cells	
	human	bovine	human	bovine		fixed monolayer	suspension
Human:							
Normal sera	+ (31)	+ (43)	— (68)	10 % (68)	+ (23)	+ (3)	+ (3)
IgG	+	+	—	±	+	+	+
Fc-fragments	+	+	—	—	+	+	Not studied
$F(ab')_2$-fragments	+	+	—	—	+	+	" "
IgG_1	+	+	Not studied	—	+	±	" "
Rabbit							
Normal sera	+ (12)	+ (12)	3,6 % (111)	— (11)	+ (10)	+ (9)	+ (3)
IgG	+	+	—	Not studied	+	+	+
$F(ab')_2$-fragments	+	+	—	" "	+	+	Not studied
soluble complex EA + antibodies against EA	±	±	—	—	±	Not studied	" "

Legend. Numbers in parentheses give number of sera tested, numbers outside parentheses give percentage of positive reactions. In valve and joint tissue sections normal sera reacted with fibroblasts in 100% of cases; +) positive reaction, −) negative reaction, ±) weakly positive reaction.

EXPERIMENTAL METHOD

Experiments were carried out by the indirect immunofluorescence method, using pure antibodies against human and rabbit IgG prepared with the aid of an immunosorbent [4], and labeled with fluorescein isothiocyanate. The method of preparation and labeling of the antibodies was described previously [2]. Judging by the gel precipitation test, the antibody preparation contained antibodies against Fab- and Fc-regions of the IgG molecule. Sera from healthy persons, from children aged under 2 years, and sera from unimmunized rabbits in dilutions of 1:16-1:64 were studied.

IgG were isolated from human and rabbit sera on DEAE-cellulose. Aggregated human γ-globulin was prepared from measles γ-globulin by heating for 20 min at 63°C [12]. The resulting preparation was centrifuged at 105,000g for 1.5 h and then separated into three layers. The rabbit IgG was subjected to ultracentrifugation without preliminary heating.

To prepare the soluble complex of egg albumin (EA) + antibodies against EA, with the aid of an immunosorbent (EA, conjugated with Sepharose 4B) antibodies were isolated from an immune rabbit serum by elution with 0.2 M glycine-HCl buffer, pH 2.4. The aggregates were removed from the antibody preparations on a column with Sephadex G-200. The antibody content was determined by Heidelberger's method [6]. The equivalence zone was determined by the quantitative precipitation method. The soluble complex was obtained in an excess of antigens by mixing 0.2 ml of antibodies (2 mg/ml) and 0.05 ml of antigen (20 mg/ml). The complex was kept for 1 h at room temperature, centrifuged at 15,000 rpm, and the supernatant was tested on tissue sections.

IgG of three subclasses (IgG_1, IgG_2, IgG_3) was isolated from sera of patients with G-myeloma [3].

The tests were carried out on tissue sections from bovine and human myocardium and heart valves, human fetal joint tissues, and cultures of mouse L-cells. Sections 4 μ thick were cut in a cryostat from tissue frozen to −70°C and were processed unfixed [1, 2]. The L-cells were cultured in medium No. 199 with the addition of 20% bovine serum. Most of the experiments were carried out on a 24-h culture which was fixed on the coverslip with cold acetone for 10 min and processed in the same way as the tissue sections. To carry out the test in a suspension of living cells the culture was detached with trypsin, washed with medium, and transferred to test tubes, each of which contained $2 \cdot 10^6$ cells. The cell residue was treated with 0.15 ml of serum or IgG (5-10 mg/ml). The mixture was incubated for 20 min at 37°C, the cells were washed 5 times with medium, 0.15 ml of labeled antibodies against human or rabbit IgG was added, the cells were again washed with medium, the suspension was applied to a slide, and after drying, it was mounted in glycerol (pH 7.0). The reaction was read by means of the ML-2 luminescence microscope in blue-violet light and the LYUMAM I-2 microscope in UV-light. Photographs were taken on RF-3 film.

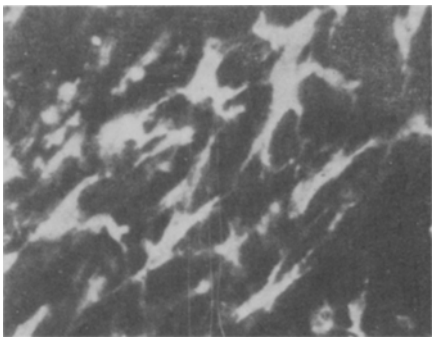

Fig. 1. Reaction of Fc-fragments of human IgG with bovine heart valve fibroblasts. Objective 40×, homal 3×.

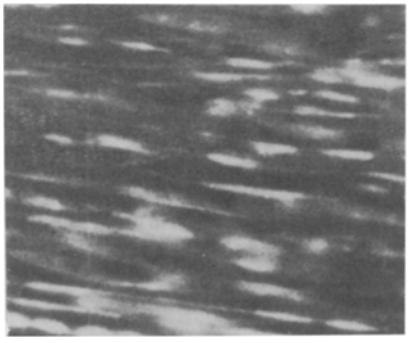

Fig. 2

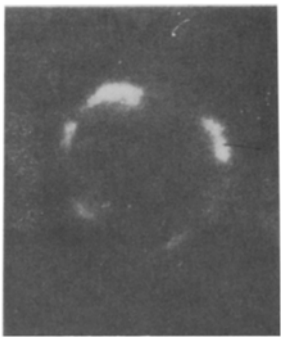

Fig. 3

Fig. 2. Reaction of normal rabbit IgG with fibroblasts of dense connective tissue of human fetal joint. Objective 40×, homal 3×.

Fig. 3. Reaction of normal human IgG with L-cells in suspension. Objective 90×, homal 3×.

EXPERIMENTAL RESULTS

When normal human and rabbit sera were tested positive results were obtained in 100% of cases on sections of human and bovine heart valves, on sections of joint tissues, and on L-cells (Table 1). As a rule, diffuse fluorescence of fibroblasts was observed on sections through the valves and joints, and in some cases the reaction was clearly localized in the region of the cell membrane (Figs. 1 and 2). Fluorescence on a monolayer of L-cells consisted of points of different sizes on the territory of the cell, and diffuse fluorescence was observed less frequently. When the suspension of living cells was stained the reaction was localized in the region of the membrane in the form of points and "caps," which were much larger when human sera and IgG were used (Fig. 3). Donors' sera did not react with ICT from human myocardium, and in 10% of cases they gave positive reactions with ICT cells from bovine myocardium. Normal rabbit sera virtually did not react with myocardial ICT cells (Table 1). Preparations of IgG isolated from normal human and rabbit sera reacted with fibroblasts of heart valves and joints and with L-cells but did not react with human myocardial fibroblasts. Weak reactions were observed with bovine myocardial fibroblasts. On testing of Fc-fragments of human IgG (three series) intensive positive reactions were obtained with two series on human and bovine heart valve fibroblasts, and one series gave a weak reaction. On testing with one of the actively reacting series positive results were obtained on joint fibroblasts and on the monolayer of L-cells. $F(ab')_2$-fragments from normal human and rabbit IgG reacted with valve and joint fibroblasts and with L-cells. Fc- and $F(ab')_2$-fragments did not react with myocardial fibroblasts. When monoclonal immunoglobulins of the three subclasses were tested on sections of valves, positive reactions with fibroblasts (++) were obtained with IgG_1, and very weak reactions with IgG_2 and IgG_3. When joint sections and the monolayer of L-cells were treated, fluorescence of the cell membrane was observed only with IgG_1. Monoclonal IgG did not react with bovine myocardial fibroblasts.

When aggregated human γ-globulin was tested on sections of human and bovine heart valves, reactions with fibroblasts were somewhat weaker (++) than those of the original γ-globulin (from +++ to ++++). The surface layer, practically free from aggregates, reacted with fibroblasts with the same intensity as the lower layers, containing much smaller amounts of aggregates. The same results were obtained during the study of rabbit IgG after ultracentrifugation.

The reaction of antibodies against EA with valve fibroblasts varied from +++ to ++++. The complex prepared in an excess of antigen reacted with most of the cells much less strongly, except for small groups of cells located near capillaries. When antibodies against EA were tested on joint sections, they also reacted intensively with fibroblasts, whereas the complex reacted much less strongly with the cells. Soluble complexes did not react with myocardial fibroblasts.

This investigation thus confirmed data showing the presence of Fc-receptors on heart valve fibroblasts [1] and showed that similar receptors are present on fibroblasts of articular connective tissue and on L-cells. Evidence of this is given by the positive reactions obtained with Fc-fragments of IgG. The receptors react mainly with monomeric immunoglobulins. The reactions were much weaker with soluble complexes obtained in an excess of antigen and also with aggregated immunoglobulins. In the experiments with immunoglobulins of the different subclasses, mainly IgG_1 was found to take part in the reaction. Clear differences were observed between heart valve fibroblasts and myocardial fibroblasts; the latter, like other cells of the myocardial ICT, do not contain Fc-receptors. Positive reactions of $F(ab')_2$-fragments with valve and joint fibroblasts and with L-cells could be due to the presence of small amounts of unsplit IgG as an impurity, which could not be detected by immunodiffusion methods [1]. Another possibility is that positive reactions of $F(ab')_2$-fragments were due to the small part of the size of the IgG molecule which they account for. A similar hypothesis has been expressed on the basis of a study of the Fc-receptor on lymphocytes and other cells [10].

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