# IMMUNOCHEMICAL IDENTIFICATION AND STUDY OF INTERSPECIFIC THYMUS ANTIGEN (AgT-1)

D. M. Falaleeva, V. V. Kalashnikov,
I. V. Sanina, Yu. A. Baryshkov,
and V. Ya. Arion

UDC 612.438.017.1-08

KEY WORDS: thymus hormones; thymus antigens; thymus gland.

Many investigations of thymus function have shown that several biologically active substances concerned in the regulation of the immune system are synthesized in this gland. At the same time we know that these substances consist of different numbers of molecules or of separate chemical factors, which evidently act either individually (or consecutively), or jointly, helping to restore disturbed immunity [1, 2, 4, 6, 10]. Accordingly the study of the antigenic composition of the thymus, and purification, and elucidation of the physiological role, of the individual proteins will help to give a more precise understanding of the mechanisms of cellular and humoral immunity, and will also help with the obtaining of preparations with a direct action in order to correct immune shifts.

The aim of this investigation was an immunochemical study of one of the interspecific thymus proteins of man and animals identified by the writers (AgT-1).

## EXPERIMENTAL METHOD

Antisera were obtained from rabbits. A conjugated extract of calf thumus mixed with Freund's complete adjuvant served as material for immunization. To enhance the immunogenic properties of low-molecular-weight antigens, 25% glutaraldehyde (from Merck, West Germany) was added drop by drop, with constant shaking, to a mixture of extract of calf thymus with sodium acetate buffer, pH 5.6, taken in the ratio of 10:1, until its final concentration was 0.5%. Formation of a conjugate of high-molecular-weight protein components with low-molecular-weight components was assumed to take place under these circumstances. The resulting conjugate was dialyzed against distilled water for 24 h and then used to immunize animals. The scheme of immunization consisted of four series on injections of the preparation in a dose of 120 mg protein mixed with Freund's complete adjuvant, with intervals of 9 days between injections, which were given alternatively subcutaneously. Blood was taken on the 7th, 9th and 12th days after the last injection from the marginal vein of the ear. Reimmunization was carried out 45-60 days after the last time of taking blood, as a single injection of 100 mg protein subcutaneously. The antisera obtained were exhausted with bovine blood plasma and kidney proteins, at the rate of 20 mg lyophilized plasma or extract to 1 ml of antiserum.

Immunochemical determination of AgT-1 was undertaken by the immunodiffusion method [9] with a standard test system [5], whose sensitivity in these investigations was 3 μg/ml. Electrophoretic mobility was studied by immunoelectrophoresis in 1% agar gel (Difco, USA), made up in Veronal-Medinal buffer, pH 8.6, ionic strength 0.01 [8].

The isoelectric point of the protein was determined by the method of isoelectric focusing [11, 12]. Chromatography was carried out on an anion-exchange cellulose DE-52 (Whatman, USA) in 0.05 M Tris-HCl buffer, pH 7.4. Protein was first eluted with 80 ml of a 2% solution of ampholines, pH 4.0-6.0. Subsequent elution was carried out with 80 ml of a 2% solution of ampholines, pH 2.5-4.0. pH values in the eluted samples were measured on a pH-meter. By superposing the elution profile of AgT-1 on the pH gradient, the isoelectric point of the protein was determined.

Research Institute of Physicochemical Medicine, N. I. Pirogov Second Moscow Medical Institute. N. V. Sklifosovskii Emergency Aid Research Institute, Moscow. (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. 2, pp. 172-174, February, 1985. Original article submitted January 12, 1984.

0007-4888/85/9902-0201$09.50  

TABLE 1. Results of Immunochemical Determination of AgT-1 in Extracts of Human and Bovine Organs and Tissues

| Test object | Man | | Animal | |
|---|---|---|---|---|
| | fetus | adult | fetus | adult |
| Thymus | 12 | — | 48 | — |
| Spleen | 0 | 0 | 48 | 96 |
| Liver | 0 | 0 | 6 | Traces |
| Small intestine | 0 | 12 | 0 | 0 |
| Lung | 0 | 0 | 0 | 6 |
| Others organs and tissues | 0 | 0 | 0 | 0 |

Legend. Results given in micrograms AgT-1/ml tissue extract containing 50 mg protein. Other organs and tissues included brain, heart, kidneys, stomach, large intestine, thyroid glands, adrenals, pancreas, blood vessels, skin, urinary bladder, and blood.

TABLE 2. Physicochemical Properties of AgT-1 from Calf Thymus

| Property | AgT-1 |
|---|---|
| Relative electrophoretic mobility | 0,85±0,03 |
| Relative molecular weight, daltons | 40 000±3 200 |
| Isoelectric point | pH 4,65; pH 4,0 |
| Thermostability | Thermolabile at 80° C for 30 min |
| Precipitation with ammonium sulfate | Precipitated at 50% for saturation |
| Solubility in water | Soluble |

The relative molecular weight was determined by gel filtration on Sephadex G-100 [3].

To determine the biological activity of AgT-1, the method of restoration of sensitivity of spontaneous splenic rosette-forming cells of thymectomized mice (sRFC) to the inhibitory action of azathioprine [7] was used with certain modifications. Essentially these consisted of removing or reducing the restoring ability of a biologically active fraction of thymus (AFT-6) during the action of the latter on sRFC in the presence of azathioprine after preliminary incubation with antibodies against AgT-1 (Ab AgT-1). In control tests AFT-6 was incubated with antibodies against bovine serum albumin (Ab BSA).

Ab AgT-1 and Ab BSA were isolated from the precipitate with the corresponding antigen. For this purpose, the precipitate washed previously with physiological saline was fractionated by gel-filtration on Sephadex G-100, equilibrated with 0.05 M glycine-HCl buffer, pH 2.2. Antibodies eluted in the free volume of the column were neutralized with dry Tris and lyophilized.

## EXPERIMENTAL RESULTS

Immunochemical analysis of the resulting antisera (six series) after absorption with bovine blood plasma revealed the presence of antibodies against 4-6 antigenic components of calf thymus extract. After further exhaustion with bovine kidney extract proteins only two series of antisera continued to detect one antigen in the thymus with electrophoretic mobility of $\alpha_1$-globulins. Testing tissue extracts of different fetal calf organs showed that this antigen is present in thymus, spleen, and liver tissue (Table 1). In cows, AgT-1 was determined in lung extract also.

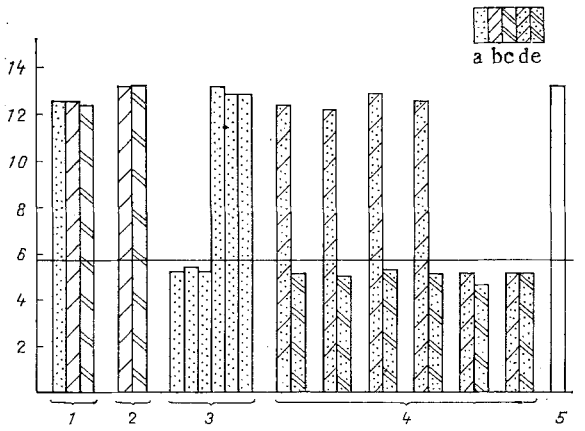


Fig. 1. Restoration of sensitivity of sRFC to inhibitory action of azathioprine. Horizontal axis: 1) control experiment for cytotoxic action of preparations (without azathioprine); 2-4) action on sRFC in presence of azathioprine, preliminary incubation of AFT-6 preparation (20 μg/ml) with antibodies; 5) effect of azathioprine on sRFC. Vertical axis: number of sRFC per $10^4$ cells: a) AFT-6, b) Ab AgT-1, c) Ab BSA, d) AFT-6 + Ab AgT-1, e) AFT-6 + Ab BSA. Columns from left to right: 1) AFT-6, 20 μg/ml, Ab AgT-1, 50 μg/ml, Ab BSA, 50 μg/ml; 2) Ab AgT-1 50 μg/ml, Ab BSA 50 μg/ml; 3) AFT-6 40, 20, 10, 5, 1, and 0.2 μg/ml; 4) Ab AgT-1 100, 50, 40, 30, 20, and 10 μg/ml, Ab BSA in the same concentrations.

Immunochemical comparison of human and animal AgT-1 showed that they are immunochemically identical. AgT-1 was detected in a human fetus in thymus extract only, whereas in the adult it was found only in the small intestine, and in the same concentration, moreover, as in the human fetal thymus.

A study of the physicochemical properties showed that AgT-1 is a microglobulin with molecular weight of 40,000 daltons and with marked intermolecular heterogeneity, as determined by isoelectric focusing (Table 2). AgT-1 is thermolabile. On heating to 80°C it completely loses its immunochemical activity after 30 min.

To study the physiological role of AgT-1, the action of Ab AgT-1 on biological activity of an AFT-6 preparation obtained in the writers' laboratory [1] and used clinically to correct disturbances of the T system of immunity [4] was investigated. The main criterion for preparation and purification of the AFT-6 preparation is the test of recovery of sensitivity of sRFC to the inhibitory action of azathioprine [2]. The AFT-6 preparation in concentrations of 10, 20, and 40 μg/ml was shown to restore sensitivity of sRFC to azathioprine by more than 50% ($ED_{50}$, Fig. 1). Pre-incubation of the AFT-6 preparation in an active concentration of 20 μg/ml with Ab AgT-1 in concentrations of 100, 50, 40, and 30 μg/ml appreciably reduced the restorative action of AFT-6 on sRFC.

Ab AgT-1 in concentrations on 10 and 20 μg/ml, like Ab BSA, had no effect on the action of AFT-6. In control experiments the action of Ab AgT-1 and Ab BSA on sRFC was studied without AFT-6 but in the presence of azathioprine, and a control also was set up to detect the cytotoxic action of these antibodies (without the addition of azathioprine). It will be clear from Fig. 1 that neither Ab AgT-1 nor Ab BSA had any activity in the control tests.

The results thus demonstrate that AgT-1 is a new interspecific antigen, not previously described, with similar immunochemical determinants in the human and bovine thymus. Chromatography with isoelectric focusing revealed heterogeneity. The inhibitory action of Ab AgT-1 on biological activity of the AFT-6 preparation revealed by this investigation is evidence that its chemical activity is partly due to molecules with AgT-1 determinants in their structure. Whether this is connected with the presence of a thermostable form of AgT-1 in the composition of AFT-6, or of low-molecular-weight products of its thermohydrolysis, which may be formed in the stages of AFT-6 purification, still awaits explanation.

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# FcμR ON NEUTROPHILS

S. M. Belotskii, T. I. Snastina, and E. S. Dikovskaya

UDC 616.9-07:616.155.34-097-008.13

KEY WORDS: human and animal neutrophils; FcμR.

Fixation of an antigen on cells involved in protection against infection (and in other immunologic processes) is of decisive importance for the outcome of its interaction with the host. If on contact with a lymphocyte that cell can receive information, which is followed by a phase of specific immune response, it is only close contact between antigen and phagocyte which can lead to ingestion of the former without which its degradation cannot take place.

Micorbial antigens can bind with the surface of a phagocyte through various receptors. An important fraction of these receptors consists of those for Fc-fragments of immunoglobulines (FcR) and for complement (CR), through which the antigen–antibody or antigen–antibody–complement complex (EAC) is attached to the surface of the phagocyte (opsonization of antigen). Although these receptors themselves are without microbial specificity and are directed either against the corresponding class of immunoglobulins or against complement (C), the phase of immune complex formation (i.e., interaction between antigen and antibody) is specific, and this determines the orientation of interaction between immune complex and a phagocyte which has lost the ability to distinguish between "its own" and "another's." The property of the phagocyte of binding immune complexes creates unity of the cellular and humoral factors of antimicrobial defense.

The most active phagocytic cell is the polymorphonuclear neutrophil (PMN). It is this cell which first encounters the microbe in the blood stream and migrates quickly into a focus of inflammation. The presence of FcγR and CR on PMN of man and animals [3, 4, 8] and the absence of FcμR on them [5, 9] are generally accepted. However, it has been shown in a microbial system that IgM can also play the role of opsonins [7, 11, 14], i.e., they can bind antigen with the surface of PMN, and in the absence of C this is possible only through FcμR.

The aim of this investigation was to detect FcμR on PMN of man and animals.

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Laboratory of Experimental Surgery, A. V. Vishnevskii Institute of Surgery, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 2, pp. 174-176, February, 1985. Original article submitted November 21, 1983.

 0007-4888/85/9902-0204$09.50 