IMMUNOMORPHOLOGIC FEATURES OF MYOID CELLS IN THE THYMUS OF RHEUMATIC FEVER PATIENTS

É. V. Gnezditskaya, L. V. Beletskaya, G. A. Kosach, and L. A. Malkina

UDC 616-002.77-07:616.438-018-097.2-07

KEY WORDS: thymus; myoid cells; rheumatic fever; autoimmune diseases.

The discovery of antigens in the cytoplasm of myoid cells of the thymus common with antigens of muscle tissues provided a basis for the hypothesis that antigens of myoid cells, like the other thymus antigens derived from other organs, are the source of information on autoantigen structure during the formation of an organism's tolerance to its own antigens [3-5]. There is evidently a profound functional connection between the myoid cells and lymphoid tissue of the thymus. For instance, in embryogenesis during the period of active formation of the T system, the number of myoid cells and their secretory activity are sharply increased. In the postnatal period the number and functional activity of the myoid cells decline and remain low until involution of the organ [6]. It has also been shown that in diseases of muscle tissue, for example, in an autoimmune disease such as myasthenia gravis, besides a high level of autoantibodies against antigens of myoid cells, common with antigens of skeletal muscle [9], severe damage also is observed to cells of the thymus, with the appearance of bound immunoglobulins and complement in their cytoplasm [7].

In rheumatic fever the antibody level against antigens of myoid cells common with the myocardium is raised [2, 10]. It has been suggested that these antibodies may, under certain conditions, have a harmful action on myoid cells and contribute toward the development of autoimmune thymitis [2, 10].

Since one sign of damage to myoid cells is a change in their immunomorphologic properties, in the investigation described below this muscular structure in the thymus of patients with rheumatic fever was studied by the immunofluorescence method.

EXPERIMENTAL METHOD

The immunomorphology of the myoid cells was studied and their number counted in the thymus of rheumatic fever patients undergoing valve implantation operations at the age of 4-18 years (24 cases). As the control the thymus taken from children undergoing operations for congenital heart disease at the age of 5-15 years (eight cases) and the thymus of persons dying from acute trauma at the age of 8-22 years (23 cases) was used. Frozen sections of the thymus were fixed for 10 min in cold acetone and treated for 2 h with serum of a myasthenia patient (dilution 1:200), containing antibodies against antigens of myoid cells common with muscle tissue in a high titer (1:1000 or above), and not reacting with other thymus tissue antigens. At the end of incubation the thymus sections were washed for 20 min in running 0.85% NaCl solution (pH 7.2) and incubated with antibodies against human IgG labeled with fluorescein isothiocyanate (FITC). Antibodies were isolated from rabbit antiserum by means of an immunosorbent with human IgG, treated with glutaraldehyde. The number of myoid cells was counted by means of the index K = A/X, where A is the number of myoid cells found in X fields of vision (usually 300). To detect bound immunoglobulins in the cytoplasm of the myoid cells, sections through the thymus of rheumatic fever patients were treated for 18 h at 4°C with FITC-labeled rabbit immunoglobulin fraction against human IgA, IgM, and IgG (N. F. Gamaleya Institute of Epidemiology and Microbiology) and against the C3 component of complement (from Hyland, USA). To remove any possible contamination by heterophilic antibodies, these preparations were absorbed beforehand (1 h at 37°C and 18 h at 4°C) with a homog-

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 65-68, March, 1982. Original article submitted April 28, 1981.

 0007-4888/82/9303-0306$07.50

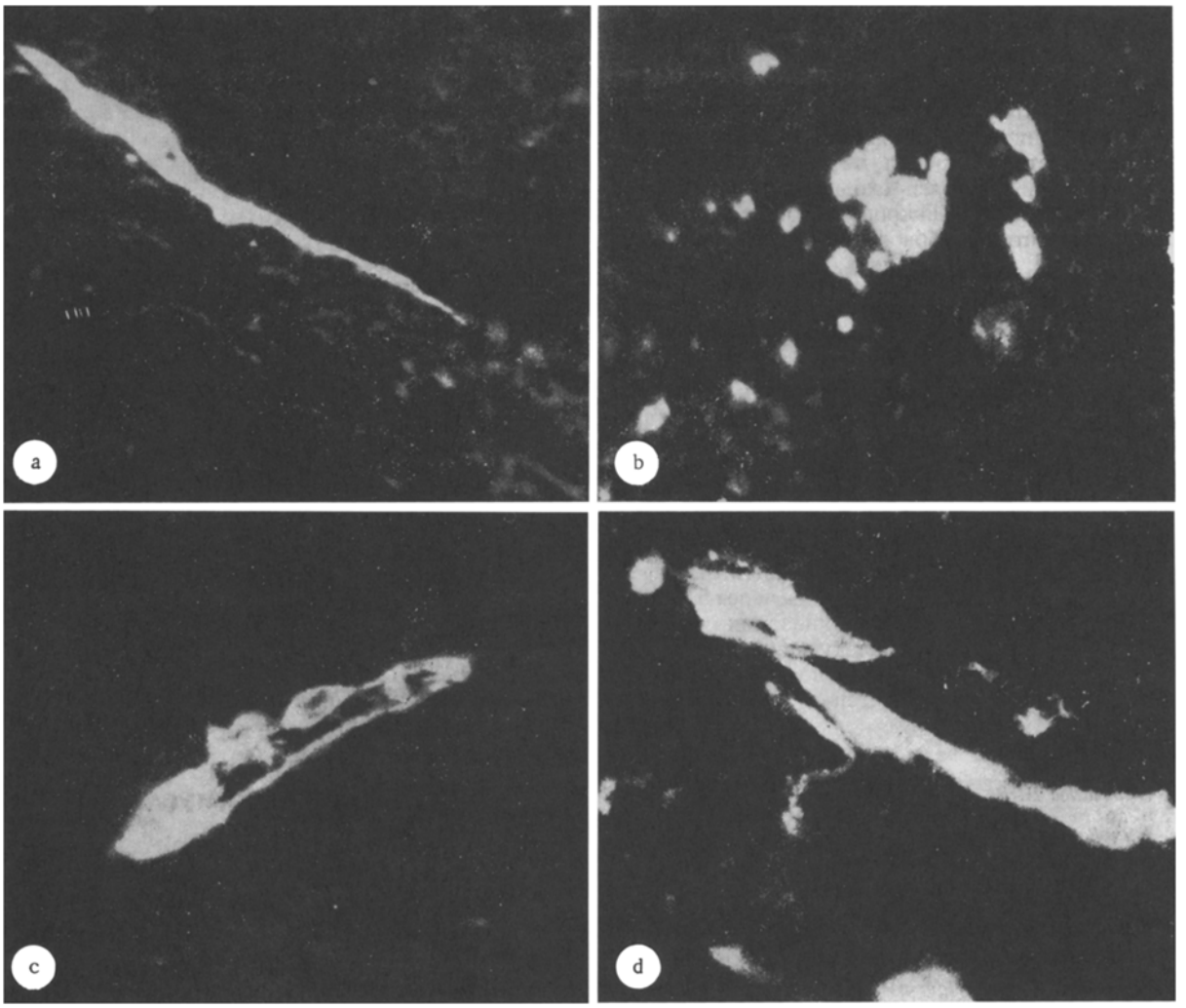

Fig. 1. Changes in myoid cells in thymus of rheumatic fever patients: a) elongated myoid cell located in outer undifferentiated layer of cortical epithelium; b) myoid cells during secretion of granules containing myoid antigens; c) division of long myoid cell by formation of constriction bands into smaller round cells. Indirect immunofluorescence method. d) Degenerating myoid cells, containing fixed IgG in their cytoplasm, located in an area of sclerosed interlobular connective tissue. Direct immunofluorescence method. Magnification: objective 40 (water immersion), ocular homal 3.

enate of human thymus and myocardial tissue. Frozen sections through the thymus were fixed for 10 min in 96° ethanol and stained with hematoxylin and eosin for the histological control.

EXPERIMENTAL RESULTS

Single diffusely fluorescent oval and round myoid cells measuring 15-20 μ were found in thymus sections from children with congenital heart disease and also in the thymus of persons dying from acute trauma, in the medullary zone of the lobules. Under these circumstances the myoid cells had low secretory activity and were rarely attached to Hassall's corpuscles. The results of counting showed that there were equal numbers of myoid cells in the thymus of the two control groups, namely 0.4 ± 0.038 per field of vision. The number of myoid cells in the thymus of patients with rheumatic fever was almost 3 times as great, with an average value of 1.15 ± 0.66 per field of vision. The myoid cells in the thymus of rheumatic fever patients were often arranged in groups and could be seen not only in the medullary zone, as normally, but also in the cortical zone, and sometimes they were located in the outermost and most undifferentiated layers of the cortical epithelium (Fig. 1).

Besides an increase in their number and a change in their localization, most myoid cells in the thymus of rheumatic fever patients were characterized by an increased content of myoid antigens. This was shown by the fact that myoid cells in rheumatic fever have much more intense fluorescence of their cytoplasm than the control, and also that the serum of a patient with rheumatic fever or myasthenia reacts with them in higher dilutions than with elements of the control thymus.

When the morphological features of the myoid cells are assessed it must be pointed out that in rheumatic fever many long cells appear in the thymus (Fig. 1a, c, d). Under normal conditions cells of this shape are found extremely rarely. Myoid cells in rheumatic fever also are characterized by considerable variation in size, as a result of which the round cells may vary in diameter from 10 to 60 μ and the long cells may attain a length of 80-100 μ (Fig. 1a, c, d). Depending on the functional state of the gland, two groups of myoid cells could be distinguished in the thymus of rheumatic fever patients, linked by intermediate forms. The first and more numerous group is composed of comparatively (10-30 μ) round or oblong cells with a bright green fluorescence of their cytoplasm, an increased content of myoid antigens and, because of this, indistinct outlines. These cells have high secretory activity, manifested as the separation of numerous granules containing myoid antigens from their surface (Fig. 1b). The same granules also were found at a considerable distance from them, in the internal medium of the thymus. Myoid cells of this group can proliferate by budding or by means of constriction bands (Fig. 1c). Hypertrophied cells, gradually undergoing degeneration, constitute another group. The dimensions of these cells varied from 30 to 70 μ. They had no secretory activity and were characterized by a sharply increased content of myoid antigens in the cytoplasm. Because of the marked yellowish fluorescence of their cytoplasm the cells sometimes resembled hyaline-like formations contained in the interior of the thymic corpuscles. As these features grew in intensity the myoid cells underwent degeneration, terminating in destruction or their attachment to the Hassall's corpuscles. Bound IgM, IgG, and the C3 component of complement were detected in the cytoplasm of some degenerating cells by direct immunofluorescence. The results of the histological investigations show that the thymus of rheumatic fever patients in many cases exhibits proliferation of interlobular connective tissue and a reduction in size of the lobules. Under these circumstances myoid cells are found not only in the parenchyma, but also on the territory of highly sclerosed interlobular septa. Most myoid cells in this situation underwent degeneration and destruction. Bound IgM or IgG and the C3 component of complement were discovered in the cytoplasm of some of them.

The results thus indicate that myoid cells of the thymus in rheumatic fever undergo profound changes which, in most cases, are expressed as an increase in their number, changes in their size, shape, and localization, an increase in the content of myoid antigens in the cytoplasm, and an increase in secretory activity. At the same time, there are some cells in the population with well-marked signs of degeneration, in whose cytoplasm fixed immunoglobulin and complement can be found. The increase in the number and activity of myoid cells in the thymus of rheumatic fever patients in many cases takes place against the background of atrophy of the thymus parenchyma and marked interstitial sclerosis of the gland. Injury to the epithelial cells and sclerosis of the interlobular connective tissue of the thymus in rheumatic fever have been observed by other workers [1, 8]. The discovery of myoid cells in an area of sclerosed interstitial connective tissue is evidence that it apparently proliferates in an area of death of the parenchyma of the gland, especially of its cortical cells. The character of the change in the myoid cells and, in particular, their functional activation, are evidence that the pathological process affecting the parenchyma of the thymus in rheumatic fever, in the first stages, has little effect on the myoid cells. The reason for this independence of the myoid anlage of the state of the remaining parenchyma of the gland is not yet clear. All that can be postulated is that it is due to the constant excess of myoid antigens in the internal medium of the thymus, observed in the absolute majority of cases of patients with rheumatic fever; in turn, this may contribute to the development of autoimmune reactions to antigens of myoid cells common with the myocardium in rheumatic fever.

LITERATURE CITED

1. M. M. Bagomedov, M. A. Shakhnazarov, and L. G. Chernyakovskaya, in: Current Problems in Immunology and Allergology [in Russian], Vol. 1, Makhachkala (1970), pp. 60-61.
2. L. V. Beletskaya, T. A. Danilova, and D. I. Shagal, Byull. Eksp. Biol. Med., No. 2. 73 (1972).

3. L. V. Beletskaya and E. V. Gnezditskaya, Usp. Sovrem. Biol., 79, No. 1, 128 (1975).
4. L. V. Beletskaya and E. V. Gnezditskaya, Immunologiya, No. 4, 89 (1980).
5. F. M. Burnet, Cellular Immunology, Cambridge University Press (1969).
6. E. V. Gnezditskaya and L. V. Beletskaya, Byull. Eksp. Biol. Med., No. 11, 616 (1978).
7. E. V. Gnezditskaya, L. V. Beletskaya, I. Kh. Ippolitov, et al., Byull. Eksp. Biol. Med., No. 2, 197 (1981).
8. V. G. Papkov, Vopr. Revm., No. 2, 45 (1978).
9. A. J. Strauss, H. W. van der Geld, P. G. Kemp, et al., Ann. N.Y. Acad. Sci., 124, 744 (1965).
10. J. R. Tagg and A. R. McGiven, Lancet, 2, 686 (1972).

DISTRIBUTION OF AMNIOTIC α_1-GLOBULIN IN HUMAN FETAL TISSUES

V. V. Kalashnikov, D. M. Falaleeva, and Yu. S. Tatarinov

UDC 612.647.015.348:547.962.4+618.33-008.939.626.16-04+06.882.04-008.939.624]-074

KEY WORDS: amniotic fluid; amniotic α_1-globulin; antigens of mesenchyme; myeloid series of blood cells; chorionepithelioma of the uterus.

An immonochemical study of human amniotic fluid revealed a protein with the electrophoretic mobility of α_1-globulins that differed from embryonic antigens hitherto known [1]. This protein was not found in the serum of blood donors. Blood serum from pregnant women gave a marked effect of inhibition of the precipitation arc of a standard test system. Considering the electrophoretic mobility of this protein and its discovery in amniotic fluid, it was called amniotic α_1-globulin (α_1-G).

This paper describes the results of an immunofluorescence study of the distribution of α_1-G in fetal tissues and also in some human trophoblastic tumors.

EXPERIMENTAL METHOD

Antisera were obtained by immunization of rabbits with a semipurified preparation of α_1-G isolated from amniotic fluid of human fetuses at the 20th week of intrauterine development [1]. Antibodies against α_1-G were isolated from monospecific antisera with the aid of an immunosorbent prepared on the basis of Ultrogel AcA-34, activated by glutaraldehyde [4], on which the purified preparation of α_1-G was immobilized.

The indirect method of immunofluorescence analysis [2] was used for the immunohistochemical study of α_1-G. Tissues from various organs of 6-10- and 23-24-week fetuses, tissue of a chorionepithelioma of the uterus after fixation with ethanol and acetic acid [5] and embedding in paraffin wax [6], and a suspension of lymphocytes from the blood and various organs of a 23-24-week human fetus, obtained in a Ficoll density gradient (1.073 g/ml) were studied [3]. The resulting suspension contained a small number of cells of the myeloid series. Films were made from the cell suspension, fixed in methanol for 3 min, and then subjected to immunofluorescence analysis.

EXPERIMENTAL RESULTS

The results of immunofluorescence analysis of α_1-G (Table 1) showed the maximal intensity of fluorescence of structures containing this antigen was present in sections of the placenta, where it was located in the syncytio- and cytotrophoblastic cells of the chorion (Fig. 1a). It will be noted that cytotrophoblastic cells exhibited much stronger specific fluorescence than the syncytiotrophoblast. Fluorescence also was observed inside the villi, in cells of the extraembryonic mesenchyme, in Hofbauer–Kashchenko cells and fibroblast-like cells, and

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 3, pp. 68-70, March, 1982. Original article submitted May 14, 1981.

0007-4888/82/9303-0309$07.50