8. J. J. Grantham, C. E. Ganote, M. B. Burg, et al., J. Cell Biol., 41, 562 (1969).
9. M. Imai and J. P. Kokko, J. Clin. Invest., 53, 393 (1974).
10. J. P. Kokko and C. C. Tisher, Kidney Int., 10, 64 (1976).
11. H. Levintin, A. Goodman, G. Pigeon, et al., J. Clin. Invest., 41, 1145 (1962).
12. T. Morgan and R. W. Berliner, Am. J. Physiol., 215, 108 (1968).
13. L. Osvaldo and H. Latta, J. Ultrastruct. Res., 15, 144 (1966).
14. G. Siebert, in: The Cell Nucleus (Proceedings of an International Symposium on the Structure and Function of the Cell Nucleus) [in Russian], Moscow (1972, p. 219.
15. C. C. Tisher, R. E. Bulger, and H. Valtin, Am. J. Physiol., 220, 87 (1971).

COMPARATIVE INDIRECT IMMUNOFLUORESCENCE STUDY OF MYOID CELLS OF THE EMBRYONIC AND ADULT HUMAN THYMUS

É. V. Gnezditskaya and L. V. Beletskaya

UDC 612.438+612.64'438].017.1

It was shown by the indirect immunofluorescence method that the lower content of muscle antigens in the myoid cells of the embryonic than in the adult human thymus is due to the higher secretory activity of these cells in the early stages of embryogenesis. Because of the increased secretory activity of the myoid cells the internal medium of the embryonic thymus contains more common antigens with muscle tissue than the adult human thymus. The fact that the functional activity of the myoid cells correlates during individual development with the rate of formation of lymphoid tissue confirms the view that heteroorganic antigens provide the lymphocytes of the thymus with information of the structure of autoantigens necessary for the formation of natural immunologic tolerance to them.
KEY WORDS: *embryo; thymus; indirect immunofluorescence.*

The presence of cells morphologically similar to muscle cells in the epithelial tissue of the human and animal thymus has frequently been described by histologists [6, 7]. Modern methods of investigation have shown that this similarity is not limited to morphological likeness. For instance, myofibrils have been found in the cytoplasm of the myoid cells by electron microscopy [15]. The antigenic similarity of the components of the cytoplasm of myoid cells and the components of muscle fibers of skeletal muscle and myocardium have been demonstrated by the indirect immunofluorescence method [4, 12]. The same method also has revealed other heteroorganic antigens in the epithelial tissue of the thymus, namely antigens common to various epithelial tissues of man and animals [1, 3, 8, 9, 14]. As regards the functional role of these heteroorganic structures of the thymus epithelium it has been suggested that they serve as the source of information on the structure of autoantigens that is required for the formation of natural immunologic tolerance to the body's own antigens in the process of differentiation of the lymphoid cells of the thymus [1, 5].

The object of the present investigation was to study the immunomorphological features of the myoid cells in the embryo, i.e., during the period of active differentiation of thymocytes and the formation of the T system of the body, and to prepare them with the corresponding properties of myoid cells of the adult human thymus, during the period of decline of the function of the organ immediately before its involution.

EXPERIMENTAL METHOD

Experiments were carried out by the indirect immunofluorescence method using pure antibodies against human immunoglobulins labeled with fluorescein isothiocyanate, [10]. Sera of patients with myasthenia, reacting with skeletal muscle and myocardial antigens in dilutions

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 11, pp. 616-620, November, 1978. Original article submitted April 17, 1978.

 0007-4888/78/8611-1540$07.50

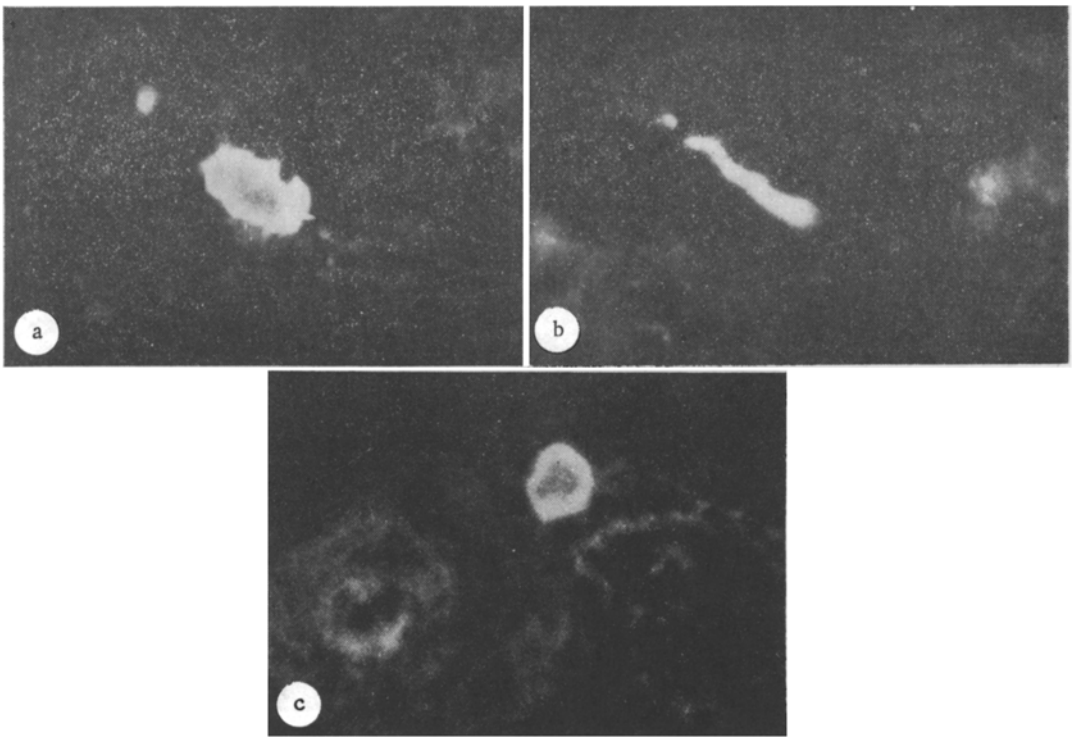

Fig. 1. Myoid cells in adult human thymus: a) oval myoid cell; surface irregularity corresponding to separated granule of myoid antigens, located at some distance from myoid cell, can be seen; b) long myoid cell with separated granules of myoid antigens; c) attachment of myoid cell to Hassall's corpuscle in adult human thymus.

of 1:64-1:128 and 1:1000-1:5000 and not containing antibodies against other heteroorganic structures of the thymus epithelium, were used as the source of antibodies against the antigens of myoid cells. Myoid cells from the thymus of persons dying from acute trauma at the ages of 20-23 years (20 cases) and the thymus from human fetuses (25-28 cm in length, 10 cases) were studied. Thymus slices were incubated with the patient's serum (dilution 1:20 or 1:200) for 18 h at 4°C, washed for 20 min in running 0.85% NaCl solution (pH 7.0), and then treated for 45 min with labeled antibodies against human immunoglobulins. The specificity of the reaction of the serum with antigens of myoid cells was tested in experiments in which the serum was adsorbed with tissue homogenate from human and rabbit myocardial and skeletal muscle and also with homogenates of liver, kidney, and brain tissue and a suspension of epidermal cells and red blood cells from a group AB donor. The serum in the working dilution was mixed with the tissue homogenate in the ratio of 1:2 and incubated for 1 h at 37°C and for 18 h at 4°C.

EXPERIMENTAL RESULTS

Investigation of the adult human thymus by the immunofluorescence method revealed a few diffusely fluorescent cells of an irregular oval shape, either elongated or rounded (Fig. 1a). Comparatively infrequently, elongated myoid cells resembling the muscle fiber of the myocardium or skeletal muscles also were found in the human thymus (Fig. 1b). Besides diffuse fluorescence, the cytoplasm of these cells sometimes showed cross-striation, emphasizing their similarity with the muscle fiber particularly clearly. The myoid cells of the adult human thymus did not exceed 20 μ in length of their axis, but the elongated form could attain a length of 30 μ. As observed previously [4], myoid cells in the human thymus, as in the animal thymus, are located in the subcortical zone of the lobules. In the medullary zone myoid cells are found close to the thymic corpuscles and are sometimes joined to these multicellular lamellar structures (Fig. 1c). On treatment of slices of human thymus with highly active sera, besides an increase in the intensity of fluorescence of the cytoplasm of the myoid cells, granules measuring up to 1 μ were observed around some of them (Fig. 1a, b). The intensity of fluorescence of the granules was rather weaker than that of the cytoplasm of the myoid cells; for that reason, on titration of the serum the reaction with granules

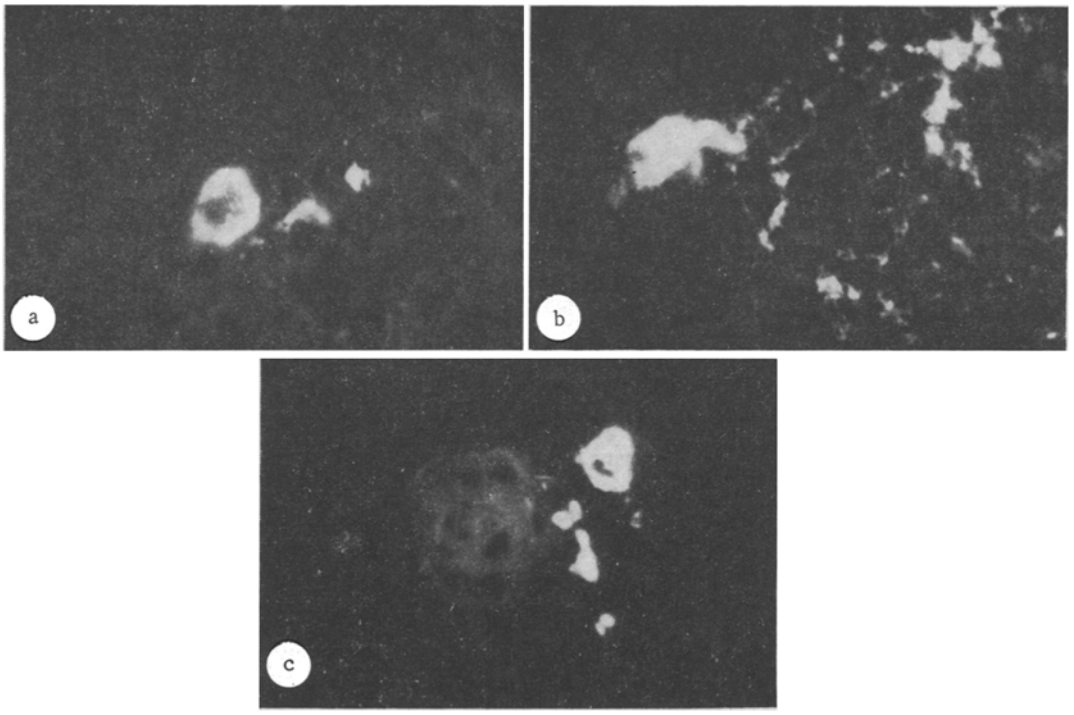

Fig. 2. Myoid cells in embryonic human thymus: a,b) myoid cells secreting myoid antigens; c) attachment of myoid cells to Hassall's corpuscle in embryonic human thymus.

disappeared sooner than that with the cytoplasm of the myoid cells from which they were separated. On the whole, the process of separation of the granules from the cytoplasm of the myoid cells can be represented by saying that, like the attachment of sarcolytes to the thymic corpuscles, granule formation in the adult human thymus is observed relatively rarely and shows considerable individual variation.

In the description of the morphological features of the myoid cells of the embryonic human thymus the first point to notice is that they can be detected only by highly active sera. The use of such sera revealed many round cells in the subcortical and medullary zone of the lobules of the thymus (Fig. 2a, b). Oval and long cells were rare in the embryonic thymus. The myoid cells of the human fetal thymus do not exceed 15 μ in length. According to the results of titration of highly active sera on slices of the embryonic and adult human thymus, they react with myocytes of the embryonic cells in a lower dilution than with the myoid cells of the adult human thymus. During the study of the embryonic myoid cells the fact was noted that both the above phenomena — separation of granules of the cytoplasm and attachment of the myoid cells to Hassall's corpuscles — were a much more conspicuous feature of the embryonic than of the adult human thymus. The process of granule formation in the embryonic thymus was unusually intensive, as a result of which numerous granules of different sizes appeared around the majority of myoid cells (Fig. 2a, b) and at a considerable distance from them in the internal medium of the organ. Attachment of the myoid cells to Hassall's corpuscles was observed equally frequently, and as a result myoid cells could be seen in the embryonic thymus in the area of most of the thymic corpuscles, and several myoid cells were attached simultaneously to many of them (Fig. 2c).

Preliminary adsorption of the sera with human and rabbit muscle tissue homogenate prevented fluorescence of the cytoplasm of the myoid cells and granules detectable in the embryonic and adult human thymus. Adsorption of the serum with tissue homogenate from several other organs (liver, kidney, spleen, brain, suspension of group AB red blood cells and of keratinizing epidermal cells) had no effect on the intensity of fluorescence of the myoid cells and of the granules of cytoplasm separating from them.

The results of this investigation thus indicate considerable differences between the myoid cells of the embryonic and adult human thymus. First, myoid cells of the adult human thymus can be detected by sera of comparatively low activity (antibody titer against muscle tissue antigens not over 1:64-1:128), whereas this antibody level is insufficient to detect myoid cells in the embryonic thymus, and these cells are revealed only by means of highly

active sera (1:1000 and more). It was also shown that highly active sera react with embryonic myoid cells in lower dilutions than with myoid cells of the adult human thymus. These features of the detection of embryonic myoid cells indicate that their cytoplasm contains fewer muscle tissue antigens than myoid cells of the adult human thymus. It also follows from the results of these experiments that the lower content of muscle antigens in the embryonic than in the adult human thymus is evidently due to their high secretory activity, which is responsible for the increased concentration of myoid antigens in the internal medium of the thymus. This process is manifested morphologically as separation of areas of cytoplasm from the surface of the myoid cells, as a result of which numerous granules appear around most of the myoid cells of the embryonic thymus, whereas this is a comparatively rare feature of the adult human thymus.

According to the results of adsorption of the sera by human and rabbit muscle tissue homogenate, antigens detectable by means of the sera used are present in both embryonic and adult human myoid cells and are tissue-specific. The possibility cannot be ruled out that besides antigens common to embryonic and adult human muscle tissue, the myoid cells may also contain antigens of muscle tissue corresponding to a definite stage of its ontogeny, and that the antigenic composition of the myoid cells may change during individual development in accordance with the changes in the antigens of muscle tissue. This problem, because of its fundamental importance to the understanding of the mechanisms of formation of natural immunologic tolerance, requires further investigation.

Many investigators are of the opinion that one function of the thymic corpuscles is that they are the site of completion of the degeneration and necrosis of epithelial cells and lymphocytes of the thymus [13]. This suggestion is based on the fact that Hassall's corpuscles contain many leukocytes and macrophages with high phagocytic activity and many necrotic cells and much nuclear debris in the lumen of their chambers [1, 13]. It has been shown by indirect immunofluorescence that cells containing antigens common to the epidermis of the skin degenerate in the corpuscles of the human thymus [8]. The results of the present investigation indicate that the life cycle of yet another heteroorganic structure of the thymus — the myoid cells — ends in the Hassall's corpuscles. This process takes place particularly intensively in the embryonic thymus, which indicates increased metabolism and wear and tear of its myoid cells compared with those of the adult human thymus.

LITERATURE CITED

1. L. V. Beletskaya and É. V. Gnezditskaya, Byull. Éksp. Biol. Med., No. 6, 87 (1974).
2. L. V. Beletskaya and É. V. Gnezditskaya, Usp. Sovrem. Biol., No. 1, 128 (1975).
3. L. V. Beletskaya, É. V. Gnezditskaya, N. A. Borodiyuk, et al., Byull. Éksp. Biol. Med., No. 2, 212 (1977).
4. L. V. Beletskaya and É. V. Gnezditskaya, Arkh. Anat., No. 2, 5 (1977).
5. F. M. Burnet, Cellular Immunology, Cambridge University Press, New York (1969).
6. A. M. Vasyutochkin, Dokl. Akad. Nauk SSSR, 15, 565 (1937).
7. Sh. D. Galustyan, Structure of the Thymus in the Light of Experimental Analysis [in Russian], Moscow (1949).
8. É. V. Genzditskaya and L. V. Beletskaya, Byull. Éksp. Biol. Med., No. 4, 89 (1974).
9. É. V. Gnezditskaya, L. V. Beletskaya, and D. I. Shagal, Byull. Éksp. Biol. Med., No. 1, 60 (1976).
10. T. A. Danilova and I. M. Lyampert, Byull. Éksp. Biol. Med., No. 3, 68 (1972).
11. J. N. Blau, Nature, 208, 564 (1965).
12. H. W. Van der Geld, T. E. Feltkamp, and H. I. Oosterhuis, Proc. Soc. Exp. Biol. (New York), 115, 781 (1964).
13. K. Konvalainen, Ann. Med. Exp. Fenn., 42, 177 (1964).
14. I. M. Lyampert, L. V. Beletskaya, N. A. Borodiyuk, et al., Scand. J. Immunol., 4, 409 (1975).
15. E. Raviola and G. Raviola, Am. J. Anat., 121, 623 (1967).