and pyrogenal if added simultaneously with the stimulator. However, they cannot block liberation of pyrogen by exudate cells which have already been activated. This is evidence that inhibitors of protein synthesis inhibit the activation process, during which the new cell proteins are evidently synthesized, but they do not inhibit the process of liberation of pyrogen.

LITERATURE CITED

1. O. A. Bogomolets, in: Anticytotoxic Serum in Internal Medicine [in Russian], Kiev (1967), p. 15.
2. A. V. Sorokin, Pyrogens [in Russian], Leningrad (1965).
3. A. V. Sorokin and O. M. Efremov, in: Proceedings of the Third Scientific Conference of Pathophysiologists of the North Caucasus [in Russian], Rostov-on-Don (1969), p. 238.
4. A. V. Sorokin, O. M. Efremov, and O. A. Él'kina, Byull. Éksp. Biol. Med., No. 10, 77 (1973).
5. E. Atkins and P. T. Bodel, in: Pyrogens and Fever, Edinburgh (1971), p. 81.
6. P. T. Bodel, Yale J. Biol. Med., 43, 145 (1970).
7. C. G. Cox and G. W. Rafter, Biochem. Med., 5, 227 (1971).
8. M. K. Fleetwood, G. W. Gander, and F. Goodale, Proc. Soc. Exp. Biol. (New York), 149, 336 (1975).
9. H. K. Kaiser and W. B. Wood, J. Exp. Med., 115, 27 (1962).
10. D. M. Moore, S. F. Cheuk, J. D. Morton, et al., J. Exp. Med., 131, 179 (1970).
11. E. S. Shell and E. Atkins, Am. J. Physiol., 212, 1103 (1967).
12. I. Taborsky, Acta Virol., 16, 376 (1972).
13. W. B. Wood, Lancet, 2, 53 (1958).

COMMON ANTIGENS OF STABLE L-FORMS OF GROUP A STREPTOCOCCUS AND HUMAN MYOCARDIAL MUSCLE FIBERS

L. V. Beletskaya, Yu. V. Vul'fovich, É. V. Gnezditskaya, S. A. Goncharova, G. A. Ugryumova, and M. Ya. Korn

UDC 576.851.214.097.2:616.127-018.6

Common antigenic features of stable streptococcal L-forms and of the cytoplasmic membrane of muscle fibers of the human myocardium were demonstrated by an immunofluorescence method. The common antigen is a component of the surface membrane of the muscle cell, adjacent to the sarcolemmal sheath, and of the membranes of the transverse tubules of the macroplasmic reticulum, which pass through the sarcomeres of the muscle fiber in the zone of the L-disks. The reaction was completely prevented by exhaustion of the antiserum against antigen of L-forms by means of a human myocardial tissue homogenate or a suspension of cultures of L-forms grown on a meat or casein medium. Exhaustion with a tissue homogenate of other organs (liver) or with concentrated nutrient medium had virtually no effect on the intensity of the reaction. In the authors' view, the presence of common antigens in cultures of stable L-forms of group A streptococcus and the myocardium may be one cause of the long persistence of L-forms in the human and animal body. KEY WORDS: stable streptococcal L-forms; myocardial muscle fibers; common antigens.

One of the conditions for long persistence of bacteria in the human or animal body is mimicry due to common antigenic components of the microorganism and tissues of the host. It is well known, for example, that hyaluronic acid in the capsule of microorganisms, including the streptococcus, is identical with the hyaluronic acid of the connective tissue of man and animals [11]. Similarity has been demonstrated between the polysaccharide of group A streptococci, which is a component of the cell wall, and the antigens of certain mam-

Laboratory of Streptococcal Infections and Laboratory of Mycoplasmas, Department of General Microbiology, and Laboratory of Opticophysical Methods of Investigation, N. F. Gamaleya Institute of Epidmiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Aacdemician of the Academy of Medical Sciences of the USSR V. D. Timakov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 8, pp. 207-209, August, 1978. Original article submitted October 14, 1977.

0007-4888/78/8608-1064$07.50

malian tissues [1, 4]. Antigens common to the bacterial form of streptococcus and mammalian heart tissues have been found in the cytoplasmic membrane of streptococci and the sarcolemma of mammalian striated muscles [13]. The streptococcal protoplast has also been used as a test object for studies of this kind. No similar investigations have yet been carried out on stable streptococcal L-forms.

Streptococcal L-forms are known to persist for a long time in mammals [2, 3], but the mechanisms of this persistence have received very little study and the factors responsible for their mimicry are not clear.

In the present investigation the reaction of antibodies against a stable culture of group A streptococcal L-forms with components of human myocardial muscle fibers was studied by an immunofluorescence method.

EXPERIMENTAL METHOD

Experiments were carried out on heart tissues of persons of blood group O dying from acute trauma. Liver tissues were used as the control. Sections of organs 5 μ thick were cut in a cryostat from tissue frozen to $-76°C$ and treated by the method of Zabriskie et al. [14], which consists of consecutive dehydration of the tissue section in vacuo (18 h at room temperature) and in cold acetone (2 min at 4°C). A simplified modification of this method, developed by the present writers, also was used. In this case the sections were kept at 4°C for 48 h and then dried for 2 h at room temperature under a fan. Serum against antigens of streptococcal L-forms was then obtained by immunizing rabbits in accordance with the scheme described in [6]. These sera reacted with a culture of L-forms in the immunofluorescence tests in dilutions of 1:20 to 1:80 and in the passive hemagglutination test with antigen of L-forms in a dilution of 1:100,000. The culture of streptococcal L-forms (strain L-406) was grown on medium consisting basically of a tryptic digest of bovine heart muscle or casein hydrolysate with the addition of 10% horse serum, 1000 units/ml penicillin, and 2.6% NaCl for osmotic stabilization. The preparation of antibodies against streptococcal L-forms obtained by absorption of the antiserum with a suspension of the culture of L-forms followed by elution of the antibodies with glycine-hydrochloride buffer (pH 2.6). Luminescent antibodies against rabbit immunoglobulins were prepared by the method described previously [4]. Sections were treated with antiserum in dilutions of 1:20 to 1:80 or with eluate of antibodies against streptococcal L-forms (900 μg protein/ml) for 45 min in a moist chamber, washed in 0.85% NaCl solution with phosphate buffer (pH 7.2), treated with luminescent antibodies, again washed, and mounted in 60% neutral glycerol. Observations were made in the blue-violet region of the spectrum by means of the ML-2 microscope with phase-contrast attachment, and photographs were taken on RF-3 film (magnification: objective 40×, water immersion, ocular homal 3×).

EXPERIMENTAL RESULTS

After treatment of sections of the human myocardium with serum or eluate of antibodies against antigens of group A streptococcal L-forms and subsequent application of luminescent antibodies against rabbit immunoglobulin, a reaction with components of muscle fibers in the zone of the macrolemma was observed (Fig. 1a). After similar treatment of liver sections no reaction was found with the structural components of the parenchyma of the organ. Exhaustion of the serum with myocardial tissue homogenate or with a suspension of the culture of L-forms grown either on meat or on casein medium prevented the reaction. Meanwhile, addition of an equal volume of the medium in which the culture was grown (either in the native form or after the same treatment as the culture of the L-forms received during preparation of the antigen) to the serum had no significant effect on the character of the reaction. Only some degree of weakening of the intensity of fluorescence was observed. A similar result was obtained after exhaustion of the serum by liver tissue homogenate.

Often when myocardial sections were treated with antibodies against L-forms a reaction also was observed in the zone of the very narrow disks of the muscle fiber (Fig. 1b). With the simultaneous use of the phase-contrast attachment it was found that the zone of fluorescence was located in the middle of the I-disks, i.e., it corresponded to the localization of the Z-disk (Fig. 1d). Sometimes the reaction also spread at the same time to the zone of the sarcolemma and the initial part of the Z-disk, adjacent to the sarcolemma (Fig. 1c).

In sections of both the myocardium and the liver, weak fluorescence of tubular structures resembling the basement membrane of small blood vessels was observed. The sera of unimmunized rabbits reacted weakly with the components of the sarcolemma and Z-disks of the myocardial muscle fiber in three of 10 cases investigated. The reaction in these cases was visible only in very low dilutions of serum—not exceeding 1:10.

The sarcolemma of the striated muscle fiber is known to consist of two layers: the sarcolemma proper and the cytoplasmic membrane of the muscle cells which is in close contact with it [12]. At the level of the L-disks the membrane sinks into the cytoplasm of the fiber, to form a network of tubules of the sarcoplasmic

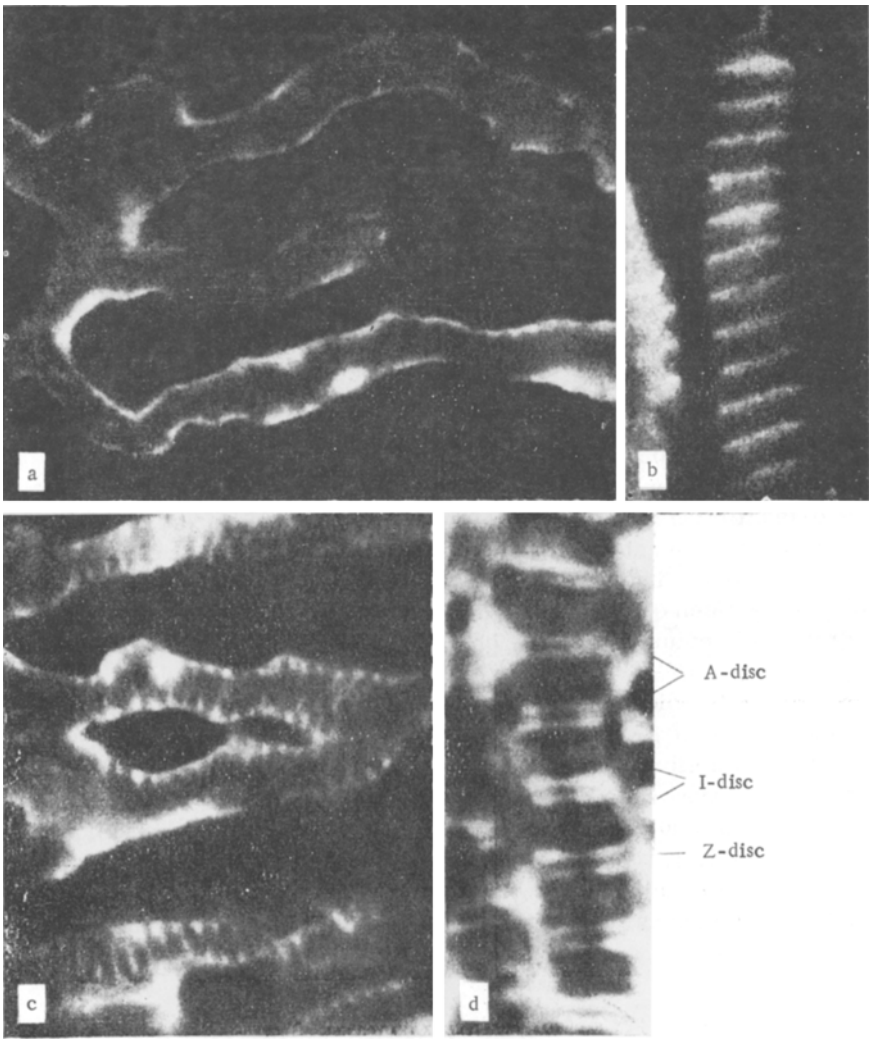

Fig. 1. Sections of human myocardium: a-c) sections treated with serum of rabbit immunized with antigen of stable group A streptococcal L-forms and antibodies against rabbit immunoglobulins, labeled with fluorescein isothiocyanate. Objective 40× (water immersion), ocular, homal 3×. Positive reaction of immunofluorescence in various structures of myocardial muscle fiber: a) sarcolemma; b) Z-disks; c) initial segments of Z-disk (zone of subsarcolemma); d) structure of myocardial muscle fiber. Phase-contrast, objective 40× (water immersion).

reticulum, which is responsible for the transport of metabolic products [10]. These results suggest that the reaction of immunofluorescence with antiserum against antigens of streptococcal L-forms observed in the sections of myocardium takes place on account of components located in the membrane, whether on the surface of the muscle cell or forming the transverse tubules of the cytoplasmic reticulum. The different picture of immunofluorescence in different parts of the same section can evidently be explained by the different stages of contraction of the muscle fibers and the different degree of denaturation of the components of the fiber as a result of drying the section. The reaction between antiserum and myocardial muscle fiber could have appeared as a result of antibodies against components of the meat nutrient medium used to culture the microorganisms [8]. Accordingly some of the cultures of streptococcal L-forms, as was pointed out above, were grown on casein medium. The additon of meat nutrient medium in a native or concentrated form to the test serum in the control experiments somewhat weakened the intensity of the reaction but did not prevent it. Meanwhile, the addition of a suspension of a culture of L-forms grown on casein medium to the serum completely inhibited the reaction. The reaction of myocardial muscle fibers with certain sera of unimmunized rabbits can be explained by the presence of a definite quantity of autoantibodies in the animal's blood against components of the cytoplasmic membrane of the muscle cells.

The results are evidence of the similarity or identity of antigens specific for stable L-forms of group A streptococci and antigens composing the cytoplasmic, including sarcoplasmic, membrane of myocardial muscle fibers. This phenomenon may partly explain why the body does not recognize L-forms as foreign, and this may be one of the conditions responsible for their long persistence.

LITERATURE CITED

1. L. V. Beletskaya, É. V. Gnezditskaya, I. M. Lyampert, et al., Byull. Éksp. Biol. Med., No. 2, 212 (1976).
2. Yu. V. Vul'fovich, "Persistence and harmful action of L-forms of group A hemolytic streptococcus under long-term experimental conditions," Author's Abstract of Candidate's Dissertation, Moscow (1974).
3. G. Ya. Kagan, Yu. V. Vul'fovich, V. S. Gusman, et al., in: Spheroplasts, Protoplasts, and L-forms of Bacteria. Colloquium (ed. by J. Reux), Paris (1977), pp. 247-258.
4. J. M. Lampert et al. (I. M. Lyampert, L. V. Beletskaya, N. A. Borodiyuk, et al.), Scand. J. Immunol. Soc., 4, 409 (1975).
5. J. M. Lampert et al. (I. M. Lyampert and T. A. Danilova), Prog. Allergy, 18, 423 (1975).
6. N. V. Chumachenko and T. D. Smirnova, Lab. Delo, No. 1, 39 (1974).
7. E. H. Freimer and J. B. Zabriskie, in: Current Research on Group A Streptococcus (ed. by R. Caravano), Amsterdam (1968), pp. 145-159.
8. M. H. Kaplan, J. Immunol., 80, 254 (1958).
9. M. H. Kaplan, in: Current Research on Group A Streptococcus (ed. by R. Caravano), Amsterdam (1968).
10. D. A. Nelson and E. S. Benson, J. Cell Biol., 16, 297 (1963).
11. R. W. Quinn and K. P. Singh, Proc. Soc. Exp. Biol. (New York), 95, 290 (1957).
12. D. Spiro and E. H. Sonnenblick, Circulat. Res., 15, 14 (1964).
13. J. B. Zabriskie and E. H. Freimer, J. Exp. Med., 124, 661 (1966).
14. J. B. Zabriskie, K. C. Hsu, and B. C. Seegal, Clin. Exp. Immunol., 7, 147 (1970).

TISSUE-SPECIFIC ANTIGEN IN THE CAUDAL LOBE OF THE CHICK ADENOHYPOPHYSIS

V. M. Barabanov and D. B. Nikolova-Kitova

UDC 591.147.4:616.097

A tissue-specific water-soluble antigen was discovered in the chick adenohypophysis by methods of immunochemical analysis. The content of this antigen was found to be highest in the caudal lobe of the adenohypophysis. During embryonic development the antigen was detected by immunoelectrophoresis and immunofluorescence after the 13th day. Two forms of the antigen were found in chick adenohypophysis – one with high and one with low electrophoretic mobility. It is concluded that cells of the adenohypophysis differ in their level of differentiation. KEY WORDS: chick adenohypophysis; caudal lobe; tissue-specific antigen.

The adenohypophysis of the chick embryo is widely used for the comparative study of the principles of morphogenesis and development of the functions of this organ in the individual development of vertebrates [3-7, 9-11]. Yet there have been few studies of the immunochemistry of development of the chick adenohypophysis [2, 8]. Together with the study of the dynamics of appearance and cellular localization of hormones for the analysis of differentiation of this adenohypophysis, the investigation of its tissue antigens may also yield important results.

The object of the present investigation was to study the tissue antigens of the chick adenohypophysis with the object of discovering specific marker antigens of differentiation.

Laboratory of Immunoembryology, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 8, pp. 209-211, August, 1978. Original article submitted November 30, 1977.