Thus the filterability of leukocytes from different donors differs sharply, and together with the number of leukocytes, it determines the filtration properties of whole blood. Meanwhile, erythrocytes had no effect on filtration of leukocytes in whole blood.

LITERATURE CITED

1. M. J. Alderman, A. Ridge, A. A. Morley, et al., J. Clin. Path., **34**, 163 (1981).
2. U. Bagge and P.-J. Branemark, Adv. Microcirc., **7**, 1 (1977).
3. U. Bagge, B. Amundson, and C. Lauritzen, Acta Physiol. Scand., **108**, 159 (1980).
4. E. Branum, L. Cummins, M. Bartilson, et al., Clin. Chem., **34**, 110 (1988).
5. J. E. Carvin, J. Exp. Med., **114**, 51 (1961).
6. P. Gachtgens, K. Ley, A. R. Price, and R. Müller, Proc. Appl. Microcirc., **7**, 15 (1985).
7. P. L. La Celle, Blood Cells, **12**, 179 (1986).
8. G. W. Schmid-Schoenbein, Y. Y. Shih, and S. Chien, Blood, **56**, 866 (1980).
9. G. W. Schmid-Schoenbein, S. Usami, and P. Skalak, Microvasc. Res., **19**, 45 (1980).
10. G. W. Schmid-Schoenbein, K. L. P. Sung, H. Tözeren, et al., Biophys. J., **36**, 243 (1981).
11. G. W. Schmid-Schoenbein, Fed. Proc., **46**, 2397 (1987).

IDENTIFICATION OF A PROTEIN WITH THE PROPERTIES OF TENASCIN IN EMBRYONIC CARTILAGE TISSUE MATRIX AND IN CULTURES OF HUMAN EMBRYONIC FIBROBLASTS

E. E. Safronova

UDC 612.751.2:612.646/.015:577.112/.08

KEY WORDS: tenascin, proteoglycans, cartilage tissue, human fibroblasts.

A leading role of macromolecular components of the extracellular matrix (ECM) in processes of cell differentiation, regulation of cell movement, morphogenesis of organs, and embryonic development, growth, and aging, has been demonstrated during the last 20 years [3, 6]. Although the structure of the main micromolecular components of the ECM of cartilage tissue, namely collagen and proteoglycan aggregates (PGA), has now been characterized, much still remains unexplained in the interaction between these components during organization of the matrix. Participation of a whole range of molecular structures of varied nature has been suggested in these interactions (collagens IX, X, and XI, proteoglycans, chondronectin, and other glycoproteins) [8, 9, 11]. Recently a new protein, tenascin, with high molecular weight (>100 kD) has been described in the ECM of chick embryos and hamsters. It may occupy in the cartilage matrix a position similar to that occupied by laminin in the matrix of basement membranes [11]. Tenascin has also been found in certain human tumors [5].

In the course of a study of structural changes in the proteoglycan component of the cartilage matrix at different stages of embryonic development, we found a protein, and the investigation described below was devoted to a study of its properties.

Laboratory of Biochemistry of Diagnostic Programs, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 276-278, September, 1990. Original article submitted August 8, 1989.

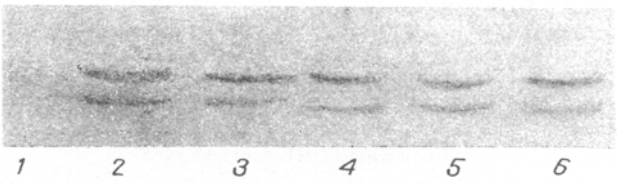

Fig. 1. Immunoblotting of binding proteins with monoclonal antibodies to tenascin (nitrocellulose film stained with 3,3′-diaminobenzidine). Binding proteins from costal cartilage of embryos aged: 1) 7 weeks, 2) 9 weeks, 3) 17-19 weeks, 4) 21 weeks, 5) 24 weeks, 6) 28 weeks.

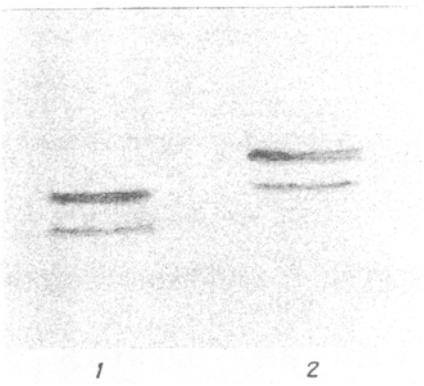

Fig. 2. Immunoblotting of culture of human embryonic fibroblasts with monoclonal antibodies to tenascin (nitrocellulose film stained with 3,3′-diaminobenzidine). 1) Cell residue, cells lysed in 0.1% solution of SDS; 2) culture fluid.

EXPERIMENTAL METHOD

The test material consisted of samples of costal cartilage of human embryos obtained during artificial termination of pregnancy and spontaneous abortions at 6, 9, 17-19, 21, 24, and 28 weeks of embryonic development, and also specimens of costal cartilage obtained at autopsy at the stages of 7 and 12 years of postembryonic development. The medical abortion material was quickly transferred to a vacuum flask with dry ice. The autopsy material, obtained during planned termination of pregnancy, was obtained in the course of 2-3 h, and the postnatal autopsy material not later than 24 h after death. The material was kept at −70°C. All procedures connected with treatment of the tissue and extraction of PGA were conducted at 4°C. The cartilage tissue was freed from perichondrium, cut into small pieces with a microtomy knife, and immersed in 12 volumes of a 4 M solution of guanidine hydrochloride in 0.15 M CH_3COONa (pH 7.0), containing protease inhibitors (0.05 M EDTA, 5 mM phenylmethylsulfonyl fluoride, 5 mM iodoacetamide, and 5 mM aminocaproic acid). PGA were extracted in the course of 24 h with slow shaking. After filtration of the extracts through a layer of glass wool they were dialyzed for 16 h against 20 volumes of 0.15 M CH_3COONa solution (pH 7.0), containing protease inhibitors. The PGA were isolated by centrifugation in a CsCl density gradient in an L8-70 ultracentrifuge (Beckman, Austria) at 160,000g for 50 h at 8°C. The bottom fraction (≈3 ml), containing PGA, was dissociated in a solution of 5.5 M guanidine hydrochloride in 0.15 M CH_3COONa (pH 6.3), with slow shaking for 12 h. The guanidine concentration was adjusted to 4 M and the CsCl concentration to 3 M, after which the components of PGA were separated by gradient ultracentrifugation at 160,000g for 60 h. Electrophoresis of binding proteins (dialyzed and lyophilized top fraction of the gradient, 3 ml) was carried out in a 7-25% polyacrylamide gel (PAG) gradient with sodium dodecylsulfate (SDS) [7]. The protein (≈50 μg) was introduced into wells in the concentrating gel. Immunoblotting was carried out by the method in [10] with monoclonal antibodies to tenascin BC-2, obtained in the laboratory of Professor L. Dzardi (National Cancer Research Center, Genoa, Italy), to whom the author is grateful. The cells were cultured by standard methods [1]. The protein concentration was determined by the method in [4].

EXPERIMENTAL RESULTS

The proteoglycan component of the human embryonic cartilage matrix at different stages of embryogenesis has not previously been studied. On electrophoresis under denaturing conditions for fractions of binding proteins with mol.wt. of 52, 48, 44, and 41.5 kD, described in hyaline cartilage [2], are distinguished. No significant variations were found in the number of protein fractions with mol.wt. of under 52 kD, but in 9-week embryos a protein with mol.wt. of about 200 kD, absent in the fraction of binding proteins in the postnatal period, appeared. This protein was present in all the samples of cartilage studied from the 9th through the 28th week of pregnancy and was represented by two fractions, whose molecular weight, determined by means of a calibration curve, was 205 and 195 kD. In its molecular weight this protein was similar to tenascin, found during PAG electrophoresis under denaturing conditions in ECM of the sternal cartilage of chick embryos and also in certain human tumors [5, 11]. During PAG electrophoresis under reducing conditions the purified fractions of intact tenascin from chick embryonic sternal cartilage were found in the form of two major fractions with mol.wt. of 205 and 195 kD and two minor fractions with mol.wt. of 220 and 170 kD [11].

During immunoblotting we obtained two precipitation bands for tenascin (Fig. 1). Immunoblotting carried out with a culture of human embryonic fibroblasts (cell residue and culture fluid) also revealed a protein with properties of tenascin (Fig. 2). In both the cells and the culture fluid, PAG electrophoresis under denaturing conditions revealed two fractions of a protein, whose molecular weight, determined from a calibration curve, was 195 and 205, and 205 and 220 kD, respectively. In proliferating cells, newly synthesized molecules of the protein evidently were found, whereas in the culture fluid these were molecules transported for the matrix. The results of immunoblotting enabled the protein found in the cartilage matrix and in the culture of human embryonic fibroblasts to be identified with tenascin. Tenascin was found for the first time both in ECM of costal cartilage and in a culture of human embryonic fibroblasts. Because of the hexabrachial structure of tenascin [11] it can be tentatively suggested that it performs the function of intermolecular and cellular-molecular interaction.

LITERATURE CITED

1. V. I. Kukharenko, A. M. Kuliev, K. N. Grinberg, et al., Tsitologiya, No. 10, 1228 (1974).
2. S. P. Feshchenko, K. D. Krasnopol'skaya, S. S. Shishkin, and I. R. Erber, Biokhimiya, **50**, No. 4, 693 (1985).
3. J. D. Aplin and R. C. Hughes, Biochim. Biophys. Acta, **694**, 375 (1982).
4. M. Bradford, Analyt. Biochem., **72**, 248 (1976).
5. H. P. Erickson and H. C. Taylor, J. Cell Biol., **105**, 1387 (1978).
6. E. D. Hay, J. Cell Biol., **91**, 205 (1981).
7. U. K. Laemmli, Nature, **227**, 680 (1970).
8. S. Lilja and H. J. Barrach, Exp. Path., **23**, 173 (1983).
9. A. Oldberg and E. Ruoslahti, J. Biol. Chem., **257**, 4859 (1982).
10. H. Towbin, T. Staehelin, and J. Gordon, Proc. Nat. Acad. Sci. USA, **76**, 4350 (1979).
11. L. Vaughan, S. Huber, M. Chiquet, and K. H. Winterhalter, EMBO J., **6**, 349 (1987).