3. I. M. Lyampert, V. Yu. Kolesnikova, E. V. Kochetkova, et al., Immunologiya, No. 4, 86 (1980).
4. I. M. Lyampert, É. I. Drobyshevskaya, A. V. Nekrasov, et al., Immunologiya, No. 1, 64 (1987).
5. S. G. Avrameas, P. Dighiero, P. Lamberi, and J. Gelber, Ann. Immunol. Inst. Pasteur, 134D, 103 (1983).
6. D. G. Braun, B. Kindred, E. B. Jakobson, et al., Eur. J. Immunol., 2, 138 (1972).
7. D. G. Braun, W. Schalek, L. S. Rodkey, and J. K. Wright, in: Pathogenic Streptococci: Streptococcal Disease in Man and Animals, ed. M. T. Parker, Reedbooks Chertsey (1979), pp. 84-85.
8. J. E. Coligan, W. C. Schnute, and T. J. Kindt, J. Immunol., 114, 1154 (1975).
9. J. S. Eisenbarth, Anal. Biochem., 111, 1 (1981).
10. J. Goldstein, B. Halperin, and L. Robert, Nature, 213, 44 (1967).
11. G. Kühler and C. Milstein, Nature, 256, 495 (1975).
12. I. M. Lyampert, L. V. Beletskaya, N. A. Borodiyuk, et al., Immunology, 31, 47 (1976).
13. A. Voller, Enzyme Immunoassay, ed. E. T. Maggio, CRC Press, Boca Raton, Florida (1980), pp. 181-196.
14. E. Whitnack and E. H. Beachey, J. Exp. Med., 162, 1983 (1985).

T_8-POSITIVE LYMPHOCYTES IN INFLAMMATORY INFILTRATES OF THE VESSEL WALL IN NONSPECIFIC AORTO-ARTERITIS

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Nonspecific aorto-arteritis (NAA) is a systemic disease of inflammatory genesis affecting the aorta and main arteries, but intramural vessels are not involved [1]. The disease was described quite a long time ago [11], but its etiology and pathogenesis have not yet been explained. The possibility of the autoimmune, inflammatory-allergic, infectious, and genetic nature of NAA has been discussed [4]. Morphologically, fibrosis of the adventitia, damage to the elastic carcase of the tunica media, local thickening of the intima, and infiltration of the pathological focus chiefly by mononuclear cells are found in the wall of the affected vessels [1, 10]. The morphology of the vessels in NAA has so far been studied by the usual histological methods. Methods of immunomorphology and, in particular, those using monoclonal antibodies (McAb), which enable the types of cells involved in the pathological process to be accurately characterized, have now become widely adopted.

In the investigation described below the walls of blood vessels affected by NAA were studied by means of McAb to functionally different antigens of lymphocytes, and also polyclonal antibodies (PcAb) to basement membrane proteins, the presence of which is characteristic of vascular smooth-muscle cells (SMC).

EXPERIMENTAL METHODS

Segments of vessels removed at operations for NAA from 11 patients were investigated (details of the patients are given in Table 1). Specimens of vessels were frozen and kept in liquid nitrogen. Frozen sections 5 μ thick were dried at 20°C and stored at −20°C. Immediately before staining the sections were fixed in acetone at 20°C for 10 min, washed with phosphate-buffered saline (PBS; pH 7.4) and incubated with a solution of the first antibodies in a working dilution. As the first antibodies we used the following McAb: 1) LT_4 and LT_8

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TABLE 1. Source of Vascular Segments Investigated

Case No.	Sex	Age, years	Sample investigated
1	F	29	Carotid artery
2	F	0	Abdominal aorta
3	F	16	The same
4	F	0	Carotid artery
5	F	29	Abdominal aorta
6	F	33	Carotid artery
7		0	Abdominal aorta
8	F	22	Carotid artery
9	F	36	Abdominal aorta
10	M	36	The same
11	F	30	The same

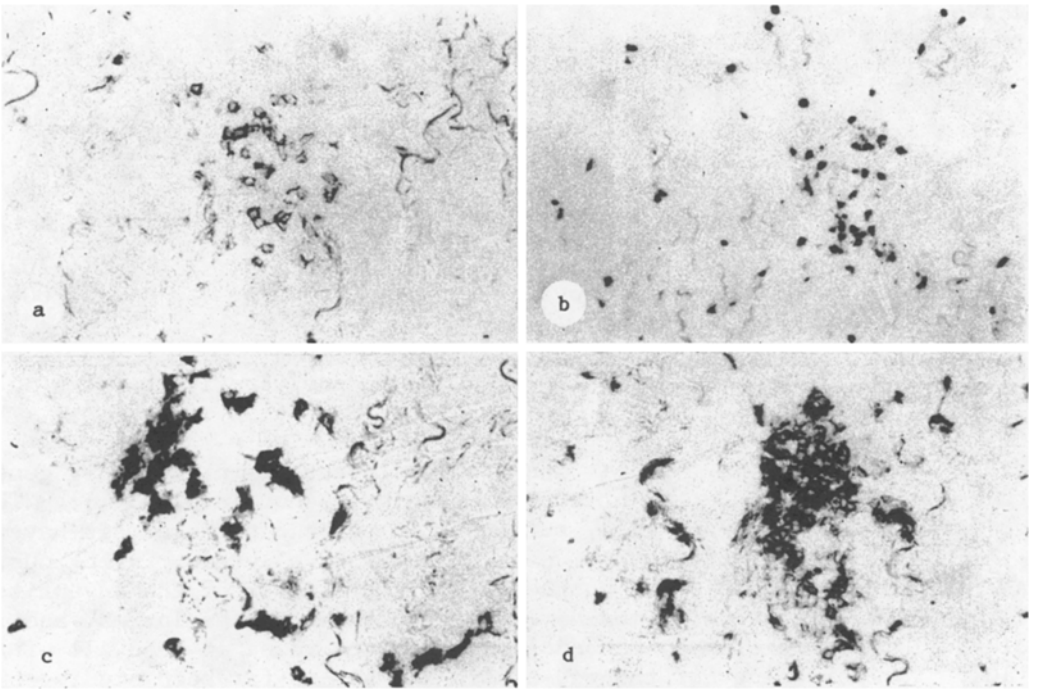
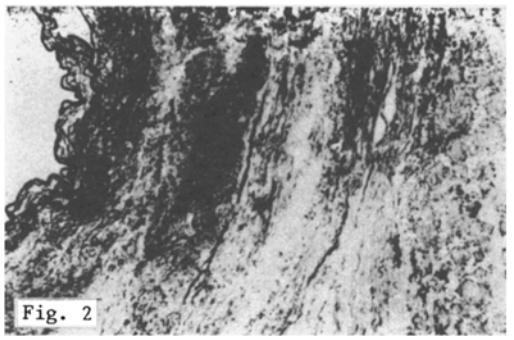


Fig. 1. Detection of lymphocyte antigens in serial sections through vessels taken from NAA patients. a) T_4; b) T_8; c) ICO-11; d) HLA-DR. Case No. 4. PAP method. 190×.

(provided by A. V. Filatov, Institute of Immunology, Ministry of Health of the USSR), specifically recognizing subpopulations of helper cells (inducers and cytotoxic lymphocytes) and suppressors in the T-lymphocyte population, 2) antibodies 100-1, recognizing HLA-DR antigen, and also ICO-11, and McAb to the μ-chain of LFA-1- antigen of lymphocytes (provided by A. Yu. Baryshnikov, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR), and 3) PcAb to type IV collagen (provided by G. L. Idelson, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR) and to laminin (Gibco, USA), of rabbit origin. In the case of McAb the sections were then incubated successively for 30 min with a solution of rabbit antimouse antibodies in a dilution of 1:40 with the addition of 3% normal human serum (NHS) and PAP-complex (peroxidase–antiperoxidase), prepared as described previously [2]. In the case of PcAb, as second antibodies we used goat antirabbit antibodies, conjugated with peroxidase (Miles, USA) in a dilution of 1:200 with the addition of 3% NHS. The reaction was visualized as described previously [2]. T_4-, T_8-, and ICO-11-positive cells were counted by means of an "Olympus" microscope with 20× objective. Neighboring T_4-T_8-ICO-11 sequences were tested in pairs and the mean value of the T_4/T_8 and T_8/ICO-11 ratios determined in the intima and media separately. Serial sections also were stained with hematoxylin and orcein to reveal elastic fibers.

TABLE 2. Relative Numbers of T_4/T_8 and T_8/ICo-11-Positive Lymphocytes in Specimens of Vessels from Patients with NAA (M ± m)

Case No.	T_4/T_3		T_8/ICO-11	
	Media	Intima	Media	Intima
1	0,45±0,02	—	—	—
2	0,26±0,07	0,65±0,07	1,25±0,06	1,38±0,01
3	—	1,62±0,50	—	13,40±8,00
4	0,33±0,02	0,43±0,03	1,14±0,01	1,23±0,30
5	0,30±0,02	0,74±0,09	0,84±0,16	2,83±0,50
7	0,93±0,03	0,29±0,05	1,78±0,35	13,5±2,1
8	0,10±0,01	0,07±0,01	1,72±0,54	10,4±1,1
10	0,20±0,01	—	4,81±0,24	—



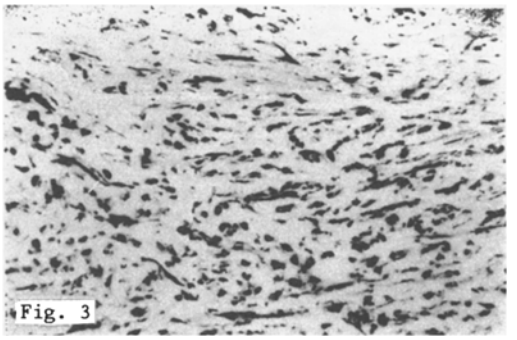


Fig. 2. Case of virtually complete destruction of tunica media. Case No. 9. Lumen of vessel on left. Stained with hematoxylin and orcein. 40×.

Fig. 3. Detection of laminin in intima of vessel affected by NAA. Case No. 9. Indirect immunoperoxidase staining. Nuclei counterstained with hematoxylin. 190×.

EXPERIMENTAL RESULTS

In vessels affected with NAA, T_8-, T_4-, ICO-11- and HLA-DR-positive cells were successfully identified immunohistochemically (Fig. 1). The results of calculation of the ratios of T_4/T_8 and T_8/ICO-11-positive cells of infiltrates in NAA are given in Table 2. It will be noted that in cases 9 and 11 no marked infiltration by mononuclears was found, and they are not therefore included in Table 2. For all samples tested except case 3, the number of T_8-positive cells (cytotoxic and suppressor T lymphocytes) was much greater than the number of T_4-positive cells (helpers and inducers). Thus the T_4/T_8 ratio in the zone of involvement of the vessels with NAA was lower than that in the blood of healthy donors, in which T_4-positive lymphocytes predominate [7]. The result is in agreement with the fall in the T_4/T_8 ratio described in a population of peripheral blood lymphocytes from patients with NAA [5]. LFA-1-antigen (revealed by ICO-11 antibodies) is the adhesion molecule of lymphocytes, and its participation is evidently necessary for the lymphocytes to perform their cytotoxic functions [8]. The ratio of T_8/ICO-11-positive cells in some cases exceeded 1 (Table 2), i.e., not all T_8-positive lymphocytes in vessels affected with NAA can be cytotoxic. Values of the T_8-ICO-11 ratio close to 1 are more characteristic of the media (Table 2), i.e., lymphocytes carrying a cytotoxic function infiltrate mainly the media of the vessel. In other words, it is the media which is the target for cytotoxic cells. This hypothesis is supported by the fact that in cases 9 and 11, when the media was almost completely destroyed (Fig. 2), mononuclear infiltration was not observed.

In support of the view that damage to the vessel wall in NAA is immune in character, a considerable number of HLA-DR-positive cells was found in the zone of injury (Fig. 1d). We know that Ia-antigens and, in particular, HLA-DR, are expressed by macrophages and by

activated T lymphocytes, and their function is evidently linked with the supply of antigens [9].

Cells of the thickened intima have often been described in NAA as fibroblasts. However, we know that the main type of vascular cells, namely smooth muscle cells (SMC), undergo what is called modification of the phenotype under certain conditions, and morphologically they become fibroblast-like [3]. Under these circumstances they preserve some of their type-specific features of SMC, one of which, it is generally agreed, is a basement membrane, which fibroblasts do not possess [6].

Staining sections of vessels affected by NAA with antibodies to basement membrane proteins, laminin and type IV collagen, demonstrated their presence on cells of the intima (Fig. 3). In other words, although in NAA the media undergoes destruction, whereas the intima, on the contrary, undergoes hyperplasia, SMC are the principal cell type in both layers. It can be tentatively suggested that in NAA cytotoxic lymphocytes attack the SMC subpopulation in the media of the vessel.

The results thus showed that T_8-positive lymphocytes predominate in foci of inflammatory infiltration of the vessel wall in NAA, which supports the view that the disease is autoimmune in nature.

LITERATURE CITED

1. A. V. Pokrovskii, Diseases of the Aorta and Its Branches [in Russian], Moscow (1979).
2. A. I. Faerman, A. V. Chervonskii, T. A. Chipysheva, et al., Byull. Éksp. Biol. Med., No. 5, 631 (1987).
3. J. Chamley, G. Campbell, and R. Ross, Physiol. Rev., 59, 1 (1979).
4. P. Gorny and J. N. Fabiani, Inform. Cardiol., 5, 533 (1981).
5. M. Levrero, V. Barnaba, I. Tedeschi, and G. Valesini, Folia Allergol. (Rome), 32, 165 (1985).
6. J. C. F. Poole, S. B. Cromwell, and E. P. Benditt, Am. J. Path., 62, 391 (1971).
7. E. L. Reinherz and S. F. Schlossman, Cell, 19, 821 (1980).
8. F. Sanches-Madrid, A. M. Kremsky, C. F. Ware, et al., Proc. Natl. Acad. Sci. USA, 79, 7489 (1982).
9. J. Schneeberger, Lab. Invest., 55, 123 (1986).

BONE TISSUE FORMATION BY MOUSE BONE MARROW CELL SUSPENSION IN ORGAN CULTURES

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Bone marrow fragments transplanted into a diffusion chamber [4] or beneath the capsule of the kidney [5] invariably form bone tissue. A similar result can be obtained by transplantation of suspension of bone marrow cells into a diffusion chamber [6, 3]. For bone tissue to be formed as a result of transplantation of bone marrow cell suspension into an open system, a definite packing density of the cells must be ensured, and this can be done by using porous frameworks impregnated with cells [8]. The formation of typical bone tissue in organ cultures of fragments of mouse bone marrow has recently been obtained [2, 9].

The aim of this investigation was to study the possibility of bone tissue formation in organ cultures of suspensions of disaggregated bone marrow cells.

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