state of the satellite myocytes is a reflection of the functional state of the muscle fibers [3], as the present investigation confirmed. The question of the origin of the satellite cells was not specially examined. Some workers [5, 6] who have studied athletes and also conducted experiments on animals after exhausting physical exertion, have observed the formation of satellite cells by separation of nucleo-sarcoplasmic territories from muscle fibers. However, the question of the genesis of satellite myocytes is not yet finally settled [2]. On the basis of the facts described above, relating to the development of destructive changes and of the repair reaction in a single muscle, in the writer's opinion the changes discovered in one section of the muscular apparatus probably reflect to some degree or other a response of the muscular system as a whole to intensified motor activity.

LITERATURE CITED

1. T. P. Beketova and S. M. Sekamova, Byull. Éksp. Biol. Med., No. 7, 107 (1975).
2. A. A. Klishov, Histogenesis and Regeneration of Tissues [in Russian], Leningrad (1984).
3. A. A. Klishov and R. K. Danilov, Arkh. Anat., No. 1, 95 (1981).
4. V. F. Kondalenko, Arkh. Anat., No. 6, 92 (1979).
5. V. F. Kondalenko and Yu. P. Sergeev, Byull. Éksp. Biol. Med., No. 11, 1385 (1976).
6. V. F. Kondalenko and Yu. P. Sergeev, in: Systemic Properties of Tissue Organization [in Russian], Moscow (1977), pp. 115-116.
7. V. F. Kondalenko, Yu. P. Sergeev, and V. V. Ivanitskaya, Arkh. Anat., No. 6, 66 (1981).
8. S. A. Krolenko, The T System of Muscle Fibers [in Russian], Leningrad (1975), p. 125.
9. A. A. Pokrovskii and V. A. Tutel'yan, Lysosomes [in Russian], Moscow (1976), pp. 3-58.
10. A. N. Studitskii, Transplantation of Muscles in Animals [in Russian], Moscow (1977).
11. A. N. Studitskii, The Mechanism of Muscle Contraction [in Russian], Moscow (1979).
12. A. N. Studitskii, Muscles. Movement. Sport. [in Russian], Moscow (1980), p. 63.
13. A. N. Studitskii, The Mechanism of Movement [in Russian], Moscow (1983), p. 63.
14. T. P. Seene, R. A. Masso, and K. P. Alev, Fiziol. Zh. SSSR. No. 3, 354 (1980).
15. M. D. Shmerling, E. E. Filyushina, and I. I. Buzueva, Arkh. Anat., No. 2, 43 (1981).

ULTRASTRUCTURE OF HUMAN BLOOD T LYMPHOCYTES LABELED WITH MONOCLONAL ANTIBODIES*

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The introduction of monoclonal antibodies into immunologic practice has provided new opportunities for the exact detection of subclasses of lymphocytes carrying various membrane antigens and for the study of their submicroscopic organization as well as relations between the structure and function of lymphoid cells. The use of immunoelectronic microscopy, combining specific labeling of certain types of cells with ultrastructural analysis, is the most promising development in this respect. However, investigations of this kind have so far been only sporadic and fragmentary [4, 9].

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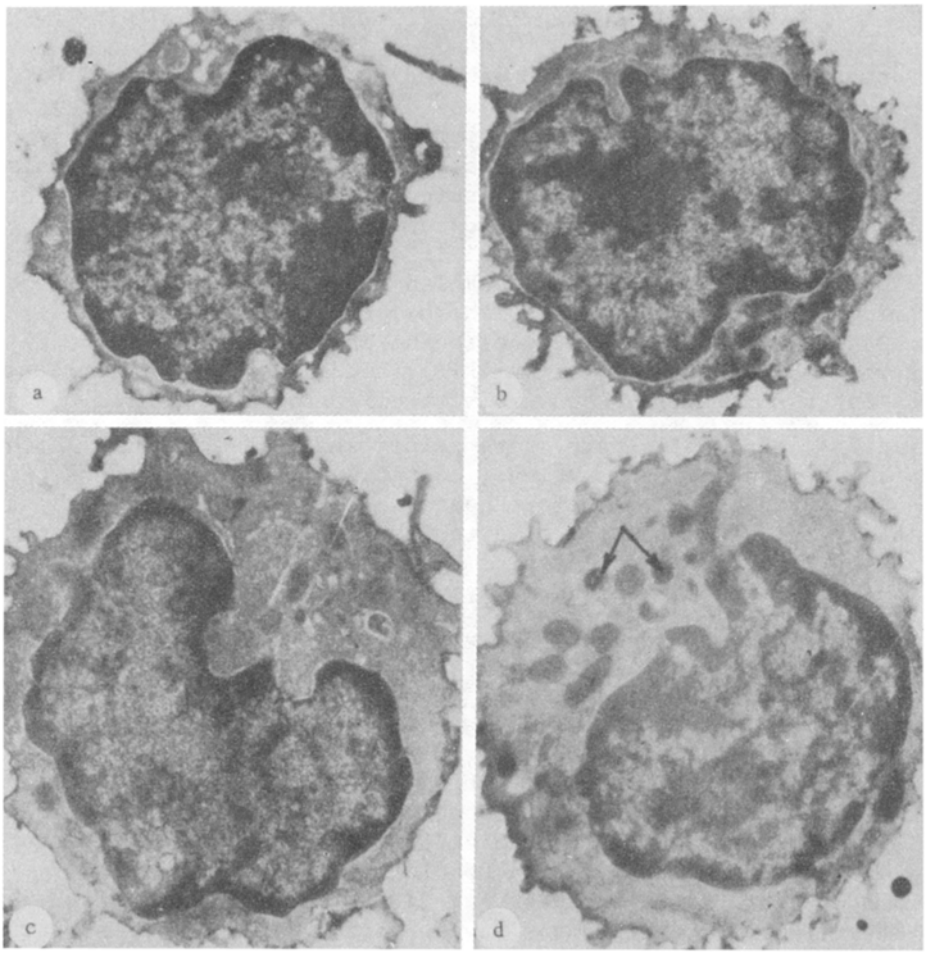

Fig. 1. Ultrastructure of various human blood lymphocytes: a,b) OKT-4^+-lymphocytes, c) OKT-8^+-lymphocyte, d) OKT-8^+-lymphocyte, containing azurophilic granules in cytoplasm (arrows). Magnification 100,000 ×.

The aim of this investigation was to study the ultrastructure of different subpopulations of human blood T lymphocytes identified with the aid of monoclonal antibodies of the "Orthoclone" series (OKT-3, OKT-4, and OKT-8) and a highly sensitive immunocytochemical peroxidase-antiperoxidase (PAP) method [2].

EXPERMENTAL METHOD

Heparinized venous blood from clinically healthy persons (first-time blood donors) was used. Mononuclear cells were isolated by centrifugation in a Ficoll—Hypaque density gradient [5]. Suspensions of the isolated cells were transferred into plastic test tubes ($2 \cdot 10^6$ to $3 \cdot 10^6$ cells per tube) and fixed with 0.6% glutaraldehyde solution for 10 min at 4°C. Monoclonal OKT-3, OKT-4, and OKT-8 antibodies (from Ortho, USA) were used as the primary agent for treating the cells by the PAP method. To each tube was added 5 µl of the corresponding monoclonal antibodies, after which the tubes were incubated for 30 min at 0°C. The cells were then incubated consecutively with rabbit serum against mouse immunoglobulin (in a dilution of 1:20), pig serum against rabbit immunoglobulin (1:40), and peroxidase-antiperoxidase complex (1:50, from Dako, Denmark), or of our own make. The preparations were then fixed a second time with 1.6% glutaraldehyde solution, tested for peroxidase activity [7], postfixed with OsO_4, dehydrated, and embedded in Araldite. Sections cut on an LKB-8800 ultrotome were strained with lead citrate and examined in the JEM-100C electron microscope. In control experiments, either treatment of the cells with monoclonal antibodies was omitted, or instead normal CBA mouse serum was used. The reaction product, irrespective of the type of monoclonal antibodies, was distributed either uniformly over the surface of the cell membrane or in separate areas, most marked in the region of the microvilli. In the control specimens, no specific staining of the cell surface was observed.

EXPERIMENTAL RESULTS

The submicroscopic structure of cells labeled with OKT-3-antibodies (OKT-3^+), and consisting of all of human blood T lymphocytes, was distinguished by its great variability. Among the OKT-3^+ cells there were some which were small (diameter 5-6 μ) lymphocytes with a high nucleo-cytoplasmic ratio and with a large heterochromic nucleus, and also larger cells (diameter 8-10 μ) with a lower nucleo-cytoplasmic ratio, often with a euchromatic nucleus, and whose cytoplasm contained a well-developed lamellar apparatus, many mitochondria, and sometimes a centrosome. Occasionally osmiophilic granules and also multivascular bodies could be seen. Most of the OKT-3^+ cells resembled in structure blood lymphocytes which form rosettes with sheep's erythrocytes (E-RFC), whose morphological spectrum also is wide [1]. Meanwhile, among OCT-3^+ lymphocytes, cells with the characteristic structure of lymphocytes forming rosettes with mouse erythrocytes (EM-RFC), and also found in fractions of B lymphocytes obtained by laser sorting [1, 4], were virtually never discovered.

The OKT-4^+ cells (inducers/helpers) were mainly small (diameter 5-7 μ), with a high nucleo-cytoplasmic ratio and a round nucleus, containing many granules of heterochromatin (Fig. 1a, b). A poorly developed lamellar apparatus, with solitary mitochondria and small vesicles, was located in the narrow rim of cytoplasm. Sometimes one osmiophilic granule or several such granules, grouped in one part of the cyotplasm, were found. In their ultrastructure the OKT-4^+ cells resembled the Tμ-lymphocytes of human blood [6, 8].

The OKT-8^+ cells (suppressors/killers) were mainly larger (diameter 7-10 μ) than the OKT-4^+ cells, and had many microvilli on their surface. These cells were characterized by a relatively low nucleo-cytoplasmic ratio and a bean-shaped nucleus in which granules of heterochromatin were distributed mainly along the nuclear membrane (Fig. 1c, d). The cytoplasm of the OKT-8^+ cells contained a well developed Golgi apparatus and many mitochondria. In some cells electron-dense osmiophilic granules were found (Fig. 1d) and, very rarely, parallel tubular structures. In their ultrastructure these cells were similar to the large granule-containing lymphocytes (GCL), belonging to the subpopulation of natural killer (NK) cells [3]. Some of the OKT-8^+-cells were morphologically identical with T_G-lymphocytes. The reason for this is that the FC-receptor for IgG is expressed on a large part of the GCL (NK cell) [6]. The results of the present investigations of the ultrastructure of OKT-8^+-cells are in good general agreement with those obtained by workers [9] who used colloidal gold to detect labeled lymphocytes. It myst be pointed out that among the OKT-4^+- and OKT-8^+-lymphocytes there were a few cells whose ultrastructure did not fit into the categories described above, and individual cells, with different surface antigens, could have a similar structure. It can be assumed that such lymphocytes posses additional surface determinants or belong to subclasses which have not yet been studied.

The investigations thus show that different subpopulations of human blood T lymphocytes, identified with the aid of monoclonal antibodies of the OKT series with respect to their membrane antigens, are characterized by certain differences in their submicroscopic structure. It may be hoped that as information on the ultrastructure of the different lymphocyte subpopulations accumulates, these data may be used not only to identify the cells, but also to assess changes in their functional state in various diseases.

LITERATURE CITED

1. M. A. Gruzov, B. M. Khomenko, and K. P. Zak, Vrach. Delo, No. 12, 63 (1984).
2. K. P. Zak, S. V. Komissarenko, M. A. Gruzov, et al., Dokl. Akad. Nauk SSSR, 261, No. 1, 229 (1981).
3. K. P. Zak, M. A. Gruzov, B. M. Khomenko, and V. S. Shlyakhovenko, Dokl. Akad. Nauk Ukr. SSR, Ser. B, No. 4, 73 (1983).
4. A. M. Boesen and P. Hokland, Arch. Path. Anat. Abt. B, Zellpathol, 41, 107 (1982).
5. A. Boyum, Scand. J. Immunol., 5, Suppl. 5, 10 (1976).
6. M. Ferrarini, A. Cadoni, A. T. Franzi, et al., in: Thymus, Thymic Hormones and T Lymphocytes, London (1980), p. 39.
7. R. C. Graham and M. J. Karnovsky, J. Histochem. Cytochem., 14, 291 (1966).
8. Y. Kanayama, A. Hiraoka, I. Konishi, et al., Acta Haemat., 70, 220 (1983).
9. E. Matutes and D. Catovsky, Clin. Exp. Immunol., 50, 416 (1982).