3. S. A. Titov and A. A. Kamenskii, Zh. Vyssh. Nerv. Deyat., No. 4, 704 (1980).
4. A. Yu. Shemanov, V. S. Kudrin, and K. S. Raevskii, Neirokhimiya, 3, No. 3, 227 (1984).
5. J. Barchas, E. Erdelyi, and P. Angwin, Anal. Biochem., 50, 1 (1972).
6. D. de Wied, Proc. Soc. Exp. Biol. (New York), 122, 28 (1966).
7. J. Lehmann, R. V. Smith, and S. Z. Langer, Eur. J. Pharmocol., 88, 81 (1983).
8. T. Nagatsu, M. Levitt, and S. Udenfriend, Anal. Biochem., 9, 122 (1964).
9. A. Randrup and J. Nunkvard, Psychopharmacology, 11, 300 (1967).
10. D. H. G. Versteeg, Pharmacol. Ther., 11, 535 (1980).

RED BLOOD CELL TARGETING TO HUMAN AORTIC SMOOTH MUSCLE CELLS

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Targeted transport of drugs and other biologically active substances provides a new approch to the treatment of several diseases [3, 4, 11-13]. Liposomes and red blood cells, filled with the corresponding agent and injected into the blood stream, can be used as containers for transporting drugs to injured or pathologically changed parts of organs and tissues [1, 5]. Targeting is effected by means of vector molecules. Antibodies make good vectors [4, 11], because the antigen–antibody reaction is highly specific.

Many cardiovascular diseases are based on a disturbance of integrity of the endothelial lining of the vessel wall [9, 10]. The damaged endothelium provides access to underlying layers of the wall for substances circulating in the blood which affect metabolism, proliferation, and secretory activity of the cells of the subendothelial layer. Monoclonal antibodies, recognizing surface antigens of subendothelial cells, in conjunction with liposomes or red blood cells, could effect targeted transport and selective action on foci of atherosclerosis. For instance, it was recently reported that monoclonal antibodies, interacting specifically with the surface of rat smooth muscle cells (SMC) can be used to transport immonotoxins to target cells [6]. The writers have obtained monoclonal antibodies interacting with an antigen with mol. wt. of 330,000 daltons, located on the surface of human aortic SMC [2]. Endothelial cells of the aorta and umbilical vein do not contain this antigen.

The aim of this investigation was to study the possibility of using IIG10 monoclonal antibodies as vector for targeted transport of drugs to the subendothelial layer of a damaged region of vessel wall. Experiments were carried out *in vitro* in a model system: SMC, growing on a plastic support, were treated with IIG10 monoclonal antibodies, after which red blood cells, previously conjugated with antibodies to mouse immunoglobulins, were added to them. The results of these tests were analyzed spectrophotometrically and with the scanning electron microscope.

EXPERIMENTAL METHOD

A culture of human aortic SMC was obtained by the method in [8]. Preparation and the properties of IIG10 monoclonal antibodies, and culture of the SMC were described previously [2]. To obtain a mixed culture, endothelial cells and SMC were cocultivated for 3 days. The cells were incubated with monoclonal antibodies (0.002-30 μg/ml) for 30 min at 37°C in an atmosphere of 94% air and 6% CO_2, after which the free antibodies were removed by a triple change of medium.

Rabbit antibodies to mouse immunoglobulin light chains were conjugated with washed human red blood cells with the aid of $CrCl_3$ [7]. About $2 \cdot 10^5$ IgG molecules were attached

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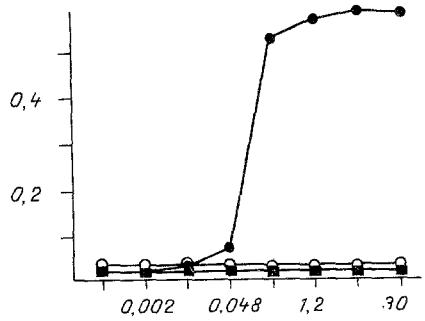

Fig. 1. Binding of red blood cells with surface of smooth-muscle cells treated with IIG10 monoclonal antibodies. Abscissa, concentration of antibodies; ordinate, optical density. Filled circles, SMC incubated with IIG10 monoclonal antibodies; empty circles, incubated with nonimmune mouse immunoglobulins; squares, endothelial cells incubated with IIG10 monoclonal antibodies.

to one red blood cell. To a cell culture preincubated with monoclonal antibodies 0.5-2% (by volume) of a suspension of red cells, conjugated with IgG, was added. To ensure a uniform distribution of red blood cells on the base of the well the culture plates were centrifuged for 3 min at 1000 rpm on a Beckman TJ6 centrifuge, then kept at room temperature for 15 min and recentrifuged, with tilting to an angle of 45° to the horizontal. Optical density was measured on the MR580 Microelisa Autoreader at 405 nm. Samples for scanning electron microscopy were fixed with 2.5% glutaraldehyde, dehydrated in a series of alcohols, and dried at the critical point. The preparations were examined on a Philips PSEM-500 scanning electron microscope.

EXPERIMENTAL RESULTS

To study whether monoclonal antibodies can be used as vector for targeted transport of biologically active substances to the subendothelial layer of the vascular well experiments were carried out in accordance with the following scheme. IIG10 antibodies were added to living cells (smooth-muscle or endothelial), growing in wells of 96-well culture plates on a plastic support, and after incubation and removal of the excess of antibodies, red blood

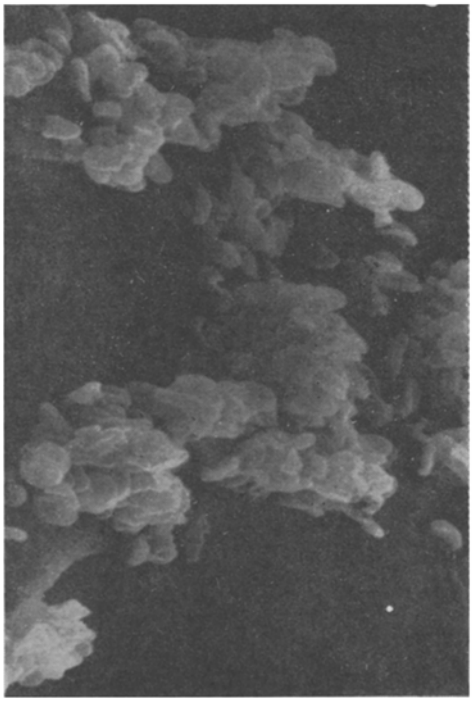

Fig. 2

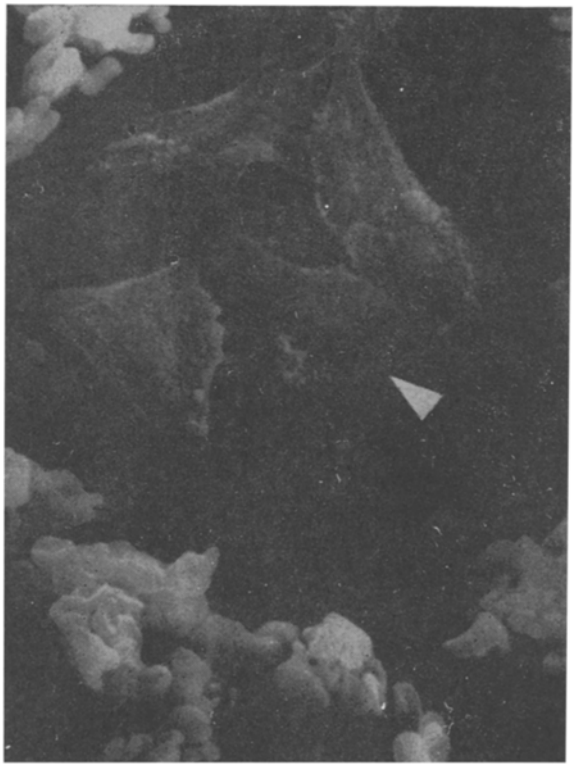

Fig. 3

Fig. 2. Electron photomicrograph of smooth-muscle cell covered with red blood cells. 1700×.

Fig. 3. Electron micrograph of combined culture of smooth-muscle and epithelial cells (arrow). 1900×.

cells conjugated beforehand with rabbit antibodies (IgG) to mouse immunoglobulin light chains were added to the wells. To remove unbound red cells the plates were centrifuged, with tilting to an angle of 45°, then red cells bound with SMC were retained on the cells, whereas those which were not bound moved toward the edge of the well farthest from the axis of rotation, and were concentrated there in the form of a narrow crescent, leaving the base of the well clear. Conversely, the base of the "positive" wells, covered with red cells, appeared red by transmitted light; by measuring optical density at 405 nm it was thus possible to judge whether the red blood cells were bound to the smooth-muscle and endothelial cells.

Dependence of optical density at 405 nm on the concentration of IIG10 antibodies is shown in Fig. 1. The antibodies interacted with SMC to a concentration of 0.024 μg/ml, whereas with endothelial cells no binding was observed even with a very high concentration of antibodies. A single SMC, the surface of which is densely covered with red blood cells, is shown in Fig. 2. Incidentally, the plastic support bound virtually no red blood cells. In a combined culture of SMC and endothelial cells, IIG10 monoclonal antibodies also led to selective binding of the red cells with SMC (Fig. 3). In mixed culture it was easy to distinguish an islet of endothelial cells from SMC on the basis of morphological features (the cells were smaller, polygonal in shape, and gave off only a few processes), and also by the absence of red cells on their surface. Endothelial cells were surrounded on all sides by SMC with red blood cells. The use of IIT10 monoclonal antibodies, distinguishing SMC from endothelial cells, as vector thus enables targeted delivery of red blood cells to SMC to be effected.

It can be concluded from the results of this investigation that it is possible, in principle, to use IIG10 antibodies for targeted transport of erythrocytes to injured regions of blood vessels.

LITERATURE CITED

1. E. I. Chazov, A. V. Alexeev, A. S. Antonov, et al., Proc. Natl. Acad. Sci. USA, 78, 5603 (1981).
2. M. A. Glukhova, A. E. Kabakov, O. I. Ornatskii (O. I. Ornatsky), et al., FEBS Lett., 189, 291 (1985).
3. G. Gregoriadis, Nature, 265, 407 (1977).
4. V. R. Muzykantov, D. V. Sakharov, M. D. Smirnov, et al., FEBS Lett., 182, 62 (1985).
5. M. J. Poznansky and R. L. Juliano, Pharmacol. Rev., 36, 277 (1984).
6. O. Yu. Printseva, A. I. Faerman, A. V. Maksimenko, et al., Experientia, 41, 1342 (1985).
7. S. Romagnan, F. Almerigogna, G. M. Giudzi, and M. Ricci, J. Immunol. Methods, 33, 11 (1980).
8. R. Ross and J. A. Glomset, Science, 180, 1332 (1973).
9. R. Ross, J. A. Glomset, B. Kariya, and L. A. Harker, Proc. Natl. Acad. Sci. USA, 71, 1207 (1974).
10. S. M. Schwartz, Artery, 8, 305 (1980).
11. M. D. Smirnov, G. P. Samokhin, V. R. Muzykantov, et al., Biochem. Biophys. Res. Commun., 116, 99 (1983).
12. V. P. Torchilin and A. L. Klibanov, Enzyme Microb. Technol., 3, 297 (1981).
13. V. P. Torchilin, Targeted Drugs, New York (1983).