Red blood cell targeting to smooth muscle cells

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Monoclonal antibody discriminating between endothelial and smooth muscle cells is suggested to be used as a vector for directed transport of drugs to injured (denuded) areas of blood vessel wall. An in vitro model system was used in the studies: vascular smooth muscle or endothelial cells grown on plastic surface were treated with specific mouse monoclonal antibody recognizing an antigen localized on the surface of smooth muscle rather than endothelial cells; then erythrocytes coated with secondary (rabbit antimouse) antibodies were added. The results were analyzed spectrophotometrically or with scanning electron microscopy. Under the experimental conditions, erythrocytes, possible 'containers' for carrying the drugs, were found to bind only to smooth muscle cells. The data show that antibody provides absolute discrimination between endothelial and smooth muscle cells and, thus, may be used as a vector for drug targeting.

Drug targeting *Monoclonal antibody* *Erythrocyte* *(Smooth muscle cell)* *Endothelium*

1. INTRODUCTION

Specific cell markers have a wide range of applications in developmental biology, cell biology and physiology. They may be used for tissue typing, propagation of organ cultures, monitoring of cell differentiation in vitro and drug targeting. The targeting of drugs seems to be a promising approach in treatment of many diseases [1–7]. The main principle in this method is as follows: delivery of drug is directed by a vector molecule, antibody or any other specific ligand which is able to interact with a target, that may be, for instance, a certain group of the cells within an organ or tissue. Very often it is important to create and to maintain a high concentration of the drug locally in the area where a pathological process takes place. In such a situation drugs may be delivered in 'containers' – special reservoirs, natural or prepared in vitro, that would serve to carry a portion of drug to a desired organ or tissue and also to protect it. Liposomes and red blood cells or their ghosts are possible candidates for use as containers [8–10]. Both liposomes and erythrocytes may be injected into the bloodstream and then deliver drugs to a target exposed to the circulation system. One of the serious problems involved is to provide specificity of delivering, i.e. a vector for targeting. Monoclonal antibodies are supposed to be good vectors because of the high specificity of their interaction with antigens.

Morphological studies show that endothelial injury is one of the pathogenetic components of many cardiovascular diseases [11–14]. The underlying layers of the vessel wall become exposed to the bloodstream as a result of mechanical disintegration and increased permeability of the endothelium, so that circulating substances that may affect metabolism, proliferation, and contractile and secretory activity of the cells inhabiting subendothelial layers can reach them. The delivery of drugs – low-M_r substances or enzymes – to such a focus of a pathological process seems to be a promising approach in the treatment of atherosclerosis or other diseases that involve the vascular wall. A ligand that would specifically bind to compounds of subendothelial layers could be a vector in this case. Recently, a conjugate of ricin (a

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plant toxin) with a monoclonal antibody to a surface antigen of rat aorta smooth muscle cells was reported to inhibit specifically protein biosynthesis in target smooth muscle cells [15].

We have developed a monoclonal antibody recognizing an antigen of M_r 330000 localized on the surface of smooth muscle cells of human aorta [16]. Endothelial cells from human aorta and umbilical vein do not contain the antigen. This antibody which discriminates between endothelial and smooth muscle cells is a possible vector for the delivery of drugs to the injured (denuded) area of the vessel wall where subendothelial layers are exposed.

2. MATERIALS AND METHODS

A culture of smooth muscle cells from human aorta was prepared according to Ross and Glomset [17]. Cells were used between the 6th and 8th passages. Endothelial cells from human aorta were a generous gift from Dr S. Danilov (USSR Cardiology Research Center, Moscow, USSR). To prepare mixed cultures endothelial cells were seeded (10000 cells per well) in 24-well tissue culture plates (Flow), on the third day of cultivation smooth muscle cells were added at a density of 10000 cells per well and after 2 more days the cultures were used for experiments. Monoclonal antibody IIG10 was prepared as described in [16]. A preparation used here was obtained by precipitation with $(NH_4)_2SO_4$ (50% of saturation) from ascitic fluid. The antibody (0.002–30 μg/ml) was added to living cells in culture medium supplemented with 10% human serum, and after 30 min incubation at 37°C in an atmosphere of 6% CO_2-94% air unbound antibody was removed by washing with culture medium. Red blood cells were separated by centrifugation of heparin-anticoagulated human blood and washed in saline. Affinity-purified rabbit anti-mouse IgG was conjugated to washed erythrocytes by $CrCl_3$ as in [18]. Briefly, equal volumes of 50% (v/v) erythrocyte suspension and antibodies (3 mg/ml) were mixed together and vortex-mixed in $CrCl_3$ prepared on saline for 30 min at 20°C. To prevent aggregation red blood cells were suspended in saline containing 10 mg/ml BSA and then washed by centrifugation. To evaluate the amount of bound antibodies ^{125}I-IgG was added as a tracer before conjugation. A standard preparation contained 2×10^5 molecules of IgG bound per erythrocyte. An erythrocyte suspension (0.5–2%, v/v) was added to the cells grown in tissue culture plates and pretreated with specific antibodies. Centrifugation for 3 min at 1000 rpm in a Beckman TJ 6 centrifuge resulted in even distribution of erythrocytes on the bottom of the well. After 15 min incubation at room temperature the plate was centrifuged again, this time being tilted at 45° to the axis. As a result of this procedure unbound red blood cells moved down to the edges of the wells while specifically attached erythrocytes were retained on the surface of the cultured cells. The bottom of the 'positive' well was coated with erythrocytes, so they appeared red, whereas in the clean 'negative' wells all the erythrocytes were found in a narrow half-moon band at the very edge of the well. The absorbance in the wells of the 96-well plates was determined with a Microelisa MR 580 autoreader (Dynatech) at 405 nm. For scanning electron microscopy the samples prepared in the wells of the 24-well plates were fixed with 2.5% glutaraldehyde overnight at 4°C, then the bottom of each well was cut out, the samples dehydrated in ethanol, dried in a critical point drier (Hitachi, HCP-2) and analyzed with

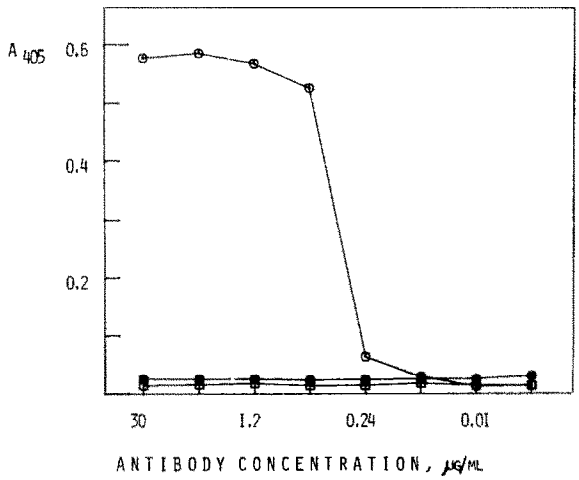

Fig.1. Monoclonal antibody-directed binding of erythrocytes to smooth muscle cells. (Circles) Smooth muscle cells were treated with monoclonal antibody IIG10 (open symbols) or non-immune mouse immunoglobulins (closed symbols) and then erythrocytes coated with secondary antibodies were added. (Squares) Endothelial cells were pretreated with monoclonal antibody followed by erythrocytes.

a scanning electron microscope (Philips, PSEM-500).

3. RESULTS AND DISCUSSION

To demonstrate that monoclonal antibody IIG10 which distinguishes between endothelial and smooth muscle cells may be used as a vector for drug targeting, we have carried out an experiment according to the following scheme: (i) cells (endothelial or vascular smooth muscle) were grown in 96- or 24-well tissue culture plates; (ii) specific antibody (IIG10) was added to the cells; (iii) unbound antibody was removed by washing with culture medium; (iv) erythrocytes precoated with secondary antibody (rabbit antimouse immunoglobulins) were added to the cells; (v) unbound erythrocytes were removed by centrifugation to the edges of the wells; (vi) plates were analyzed spectrophotometrically or by means of scanning electron microscopy.

Fig.1 shows monoclonal antibody IIG10-directed binding of the red blood cells to smooth muscle cells. The antibody was still active at 0.24 μg/ml, while endothelial cells treated with this antibody did not bind any erythrocytes. Smooth muscle cells coated with red blood cells are shown in fig.2. In rather sparse cultures erythrocytes are distributed on the bottom of the well following the

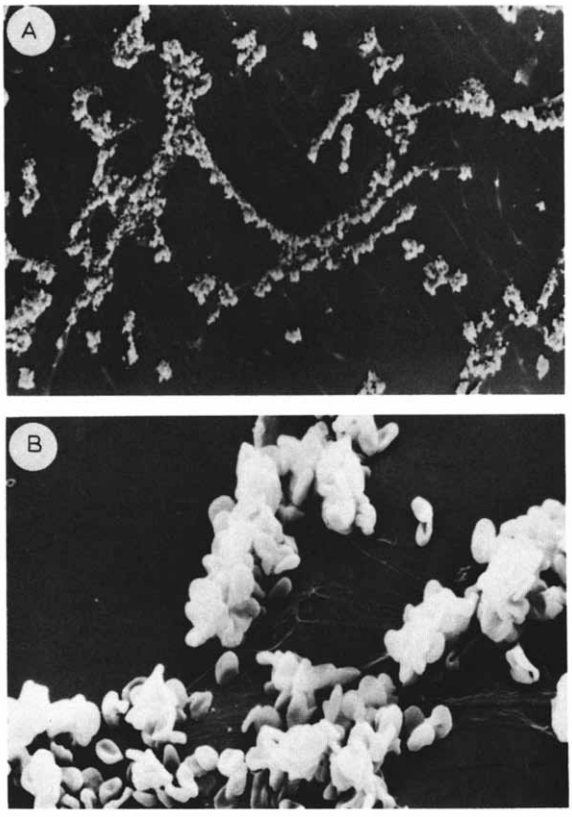

Fig.2. Scanning electron micrographs of smooth muscle cells coated with erythrocytes. Monoclonal antibody-directed binding of red blood cells to smooth muscle cells was performed as described in the text. (A) Smooth muscle cells coated with erythrocytes. General view of the culture. × 260. (B) Higher magnification. × 1350.

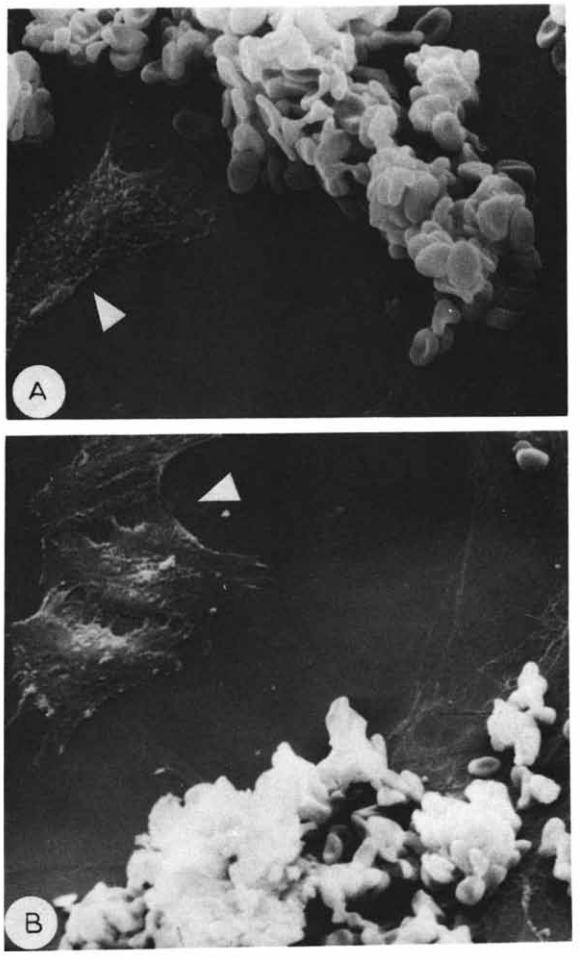

Fig.3. Scanning electron micrograph of co-cultivated smooth muscle and endothelial cells. A cell culture was pretreated with antibody IIG10 and then red blood cells carrying secondary antibody were added. Endothelial cells are denoted by the arrowheads. (A) × 1560. (B) × 1400.

contours of the cells and it can be clearly seen that they do not stick to the plastic surface at all (fig.2A).

To show that the method used here provides really high specificity based on the properties of monoclonal antibody IIG10, we co-cultured smooth muscle and endothelial cells, and then used these mixed cultures for the experiments carried out according to the scheme described above. Morphologically endothelial and smooth muscle cells differ significantly – in general smooth muscle cells are spread more widely and of irregular shape, while endothelial cells form characteristic islets (see fig.3), thus enabling one to distinguish between them on a micrograph. In the mixed cultures red blood cells bound only to smooth muscle cells, the surface of endothelial cells being absolutely clean. Thus, monoclonal antibody IIG10 used as a vector in our studies enables to discriminate between endothelial and smooth muscle cells growing in mixed culture as well.

We conclude that antibody IIG10 providing really high specificity may be used for drug targeting to an injured surface of a blood vessel wall.

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