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TYPE I AND III COLLAGENS AS A POSSIBLE TARGET FOR DRUG DELIVERY TO THE INJURED SITES OF VASCULAR BED

M.D.Smirnov, G.P.Samokhin*, V.R.Muzykantov, G.L.Idelson, S.P.Domogatsky and V.N.Smirnov

Institute of Experimental Cardiology,
USSR Cardiology Research Center,
Academy of Medical Sciences,
3 Cherepkovskaya Str., 15A, 7, Moscow 121552, USSR

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SUMMARY: Interaction of anti-human collagen types I and III antibodies, as well as human red blood cells conjugated with these antibodies, with the surface of denuded intima of human aorta has been studied. Data on the accessibility of antigenic determinants of collagen types I and III for antibodies and red blood cells conjugated with these antibodies have been obtained in ex vivo experiments in an original model. On the basis of the obtained results it is concluded that antigenic determinants of collagen types I and III exposed as a result of blood vessel wall injury can serve as a target for drug delivery to the injured site(s).

According to the current views injury of vascular endothelial lining and subsequent exposure of intimal underlying layers is one of the pathogenetic components of many cardiovascular diseases (1-3). In treatment of these diseases the most promising methods of therapy are those which can provide high local drug concentration at the injured sites. For this purpose methods of drug targeting in containers (liposomes or red blood cells) carrying vectors - molecules with high specific affinity to exposed subendothelial structures - are being developed (4, 5).

According to the data of immunomorphological studies of vessel wall cross-sections, collagen types I and III which normally do not have a direct contact with blood are represen-

* To whom all correspondence should be addressed.

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ted in subendothelial stroma (6). These interstitial collagens, in contrast to basement membrane collagen types IV and V can induce thrombogenesis while exposed into the lumen after the vessel wall injury (2, 3).

Using marked antigenic differences between various collagen types (7), it is possible to use antibodies to interstitial collagens as a vector in drug targeting to the injured site. Recently (5) we have demonstrated *in vitro* selectivity of binding of red blood cells carrying specific antibodies on their surface with the plastic supports coated with collagen of appropriate type.

In the present study we solved two questions related to drug targeting to the injured blood vessel wall. First, it has been found that specific antigenic determinants of interstitial collagens are exposed on the injured intimal surface and accessible for antibody binding like those of collagen adsorbed at plastic surface in radioimmunoassay. Second, significant roughness of subendothelial surface microrelief does not prevent formation of contacts between collagen antigenic determinants and antibodies fixed on red blood cell surface necessary for container attachment.

MATERIALS AND METHODS: Types I and III collagen preparations were purified by differential salt precipitation in neutral and acid solutions from pepsin extract of human placenta (8, 9). Antisera were obtained after repeated immunization of rabbits by collagen solutions emulsified in Freund adjuvant. Antibodies mainly represented by IgG were isolated by affinity chromatography of antisera on immunosorbents obtained by attachment of collagen of the corresponding type to CNBr-activated Sepharose 4B (Pharmacia) (8, 9). Rabbit immunoglobulins were purified from serum of non-immune animals by chromatography on DEAE-cellulose (10). Goat antibody to rabbit IgG kindly given by Dr. V.S.Rukosuev (USSR Cardiology Research Center) was labelled by ^{125}I according to (11) (specific radioactivity was $4 \cdot 10^5$ cpm/μg).

The attachment of immunoglobulins to human red blood cell surface was performed by non-covalent binding through avidin as described earlier (5). Washed red blood cells were treated by biotin N-hydroxysuccinimide ester and then non-bound rea-

gent was washed out. Biotin-modified immunoglobulins were obtained by the same technique. Biotin-carrying red blood cells were incubated with avidin, washed and mixed with immunoglobulins carrying attached biotin. The method provides a stable binding of about 10^5 molecules per one red blood cell.

Vessel segments of thoracic aorta were obtained on section within 2-6 h after death of children aged under 3. Blood clots were washed out by medium 199 (Gibco) and vessels were immediately used in experiments.

Accessibility of subendothelial collagens for antibodies was studied by indirect radioimmunoassay. Vessel segments were cut longitudinally, flattened and endothelial layer was mechanically removed. "Microtest" plate (Falcon) with well bottoms cut out was put on the top. This procedure provided us with the number of identical wells which bottoms represented denuded vessel wall (surface area 0.2 cm^2). In control experiments types I and III collagen were adsorbed on the bottom of "Microtest" plate wells (12).

From 5 to $5 \cdot 10^{-3}$ μg of antibody or non-immune IgG in 50 μl of PBS-BSA (phosphate buffered saline containing 2 mg/ml of bovine serum albumin /Sigma/, pH 7.4) were added into the wells by serial dilution. In control wells only 50 μl of PBS-BSA were added. After incubation for 1 h at 25°C the wells were repeatedly washed by PBS-BSA. Then 0.25 μg of [^{125}I]-labelled goat anti-rabbit IgG antibody in 50 μl PBS-BSA were added into each well. After incubation (1 h, 25°C) the wells were thoroughly washed by PBS-BSA. Bottoms of microplate well or the corresponding vessel sites were cut out and the amount of bound ^{125}I was determined in "Rackgamma" counter (LKB). The background sorption of labelled antibody determined in the control wells was subtracted.

Accessibility of intimal collagens for antibodies attached to red blood cells was also determined by indirect radioimmunoassay in the same way. $2 \cdot 10^6$ antibody- or non-immune IgG-carrying red blood cells were added into the wells in 50 μl PBS-BSA. Then the same procedure as described above was performed.

Red blood cell binding was also studied by the following way: aorta segments 0.3-0.6 cm in diameter, 1.5-2.5 cm in length, thoroughly washed by medium 199 were everted and endothelial layer was mechanically removed. The segments were ligated near the edges so that homogenous central site 1-2 cm in length was formed. Then segments were incubated in PBS-BSA containing antibody-carrying red blood cells ($5 \cdot 10^7$ cells/ml) for 1 h at 25°C in a rotator (15 rpm). Then the vessels were washed by PBS-BSA and placed into solution containing 1 μg of [^{125}I]-labelled goat anti-rabbit IgG antibody. After the second incubation at the same conditions and washing the ligated areas were cut out, the central part was fixed in 0.5% glutaraldehyde and the amount of ^{125}I bound was determined.

RESULTS AND DISCUSSION: Fig. 1 demonstrates typical results of the experiments on antibody binding to collagen-coated surface. Antibodies to collagen types I and III did not significantly differ by the interaction with homologous collagen types. As can be seen from the titration curves, antibody to type III

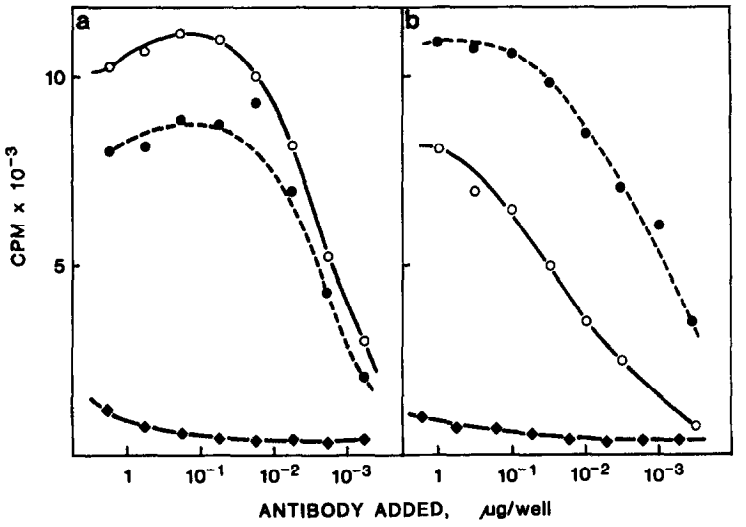

Figure 1: Binding of anti-collagen antibodies to the collagens adsorbed on the plastic surface. a - Antibody to type I collagen; b - antibody to type III collagen. Collagen adsorbed: o - type I; ● - type III. ◆ - binding of non-immune rabbit IgG to both collagen types. Background is subtracted (300 cpm/0.2 cm^2).

collagen weakly binds to the surface coated with type I collagen. In contrast, antibody to type I collagen has the affinity to determinants common for type I and III collagens.

Fig. 2 presents the results of experiments on antibody binding to the injured wall surface. Titration curve for an-

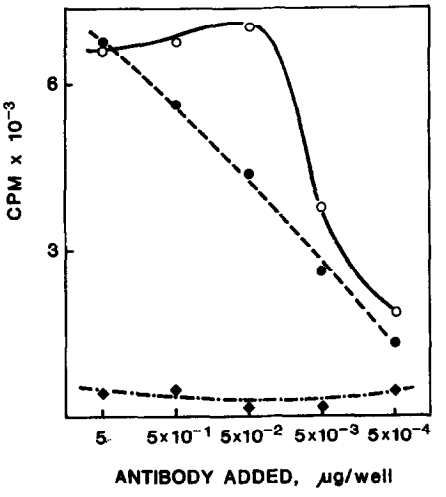

Figure 2: Binding of anti-collagen antibodies to denuded vessel wall. ● - Antibody to type I collagen; o - antibody to type III collagen; ◆ - non-immune rabbit IgG. Background is subtracted (800 cpm/0.2 cm^2).

tibody to type III collagen practically coincides with the curve obtained in the experiments on plastic surface coated with type III collagen (Fig. 1b). This data confirm the accessibility of a significant part of antigenic determinants of type III collagen prevalent in children's vessels (13) for plasma protein after the mechanical injury of vessel wall. In contrast, titration curve for antibody to type I collagen indicates that antigenic determinants of type III collagen common to type I collagen are partially masked, and type I collagen is insignificantly exposed. This agrees with the data on low relative content of type I collagen in intima of children's vessels (13).

Fig. 3 presents diagrams showing the results of experiments on interaction of antibody- or non-immune IgG-carrying red blood cells with injured vessel surface. It is seen that use of antibodies to collagen as a vector increases binding to a vessel by 9-10-fold as compared to non-immune IgG. This result was reproducible in a series of experiments irrespective

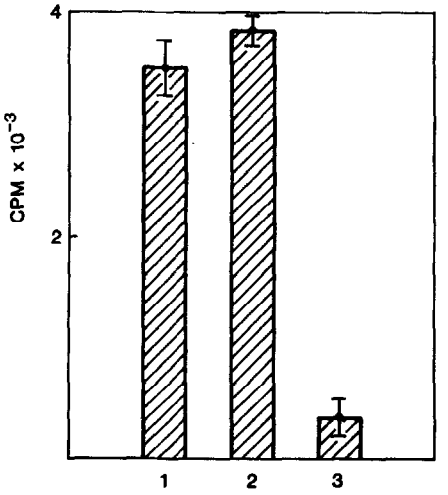

Figure 3: Binding of antibody- or non-immune IgG-carrying red blood cells to denuded vessel wall (experimental model - bottoms of microtitration plate). Proteins attached to the red blood cell surface: 1 - antibody to type I collagen; 2 - antibody to type III collagen; 3 - non-immune rabbit IgG. Background is subtracted (400 cpm/0.2 cm^2).

of the experimental model of injured site (microtitration plate bottom or everted aorta segment). This result is also confirmed by scanning electron microscopy (data not shown).

The obtained data confirm the possibility to use collagen types I and III as a target for delivery of drug entrapped in red blood cell to the site(s) of vessel wall injury. However, for more complex experimental models it is necessary to have more detailed data on distribution of collagen different types in intact and denuded vessels in normal conditions and in different forms of vascular pathology. Possibly, in these studies it is reasonable to use methodological approaches described above together with conventional immunomorphological methods for investigation of interaction of blood components with vessel luminal surface. On the other hand, one must keep in mind possible competition between antibody-carrying red blood cells and human platelets for the collagen exposed at the vessel wall surface. We hope that the knowledge on the exposure of different types of collagen and on the interaction of antibody-carrying red blood cells with this and other proteins at the site of vessel wall injury will stimulate further advance in the field of drug targeting.

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