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Directed Targeting of Immunoerythrocytes Provides Local Protection of Endothelial Cells From Damage by Hydrogen Peroxide

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Red blood cells bearing anti-mouse IgG antibody on their surface (immunoerythrocytes) may provide for local protection of endothelial cells from the action of hydrogen peroxide. Subconfluent cultures of human umbilical vein endothelial cells responded sharply to increasing concentrations of hydrogen peroxide. Permeabilization of cellular membrane occurred at doses of hydrogen peroxide of from 1 to 3 mM, and was assured by incorporation of trypan blue stain immediately after treatment. Latent damage of cells produced by much lower doses of hydrogen peroxide (0.2–0.4 mM) could be observed after 24-hour incubation of treated cells in the normal culture medium with no hydrogen peroxide. The apparently dead cells differed from intact cells in morphology, were poorly attached to the substrate, and were readily incorporated by trypan blue, thus permitting easy visualization. Immunoerythrocytes bound to the antigen-coated surface enzymatically decreased the concentration of hydrogen peroxide in their microenvironment at least fivefold with respect to the total hydrogen peroxide concentration. Erythrocytes deposited on a part of the endothelial monolayer locally protected it from the damage at hydrogen peroxide concentrations ranging from 0.4 to 1.2 mM. Localization of protected zones corresponded precisely to the geometry of the erythrocyte coating. Immunoerythrocytes targeted to the endothelial cells by means of mouse anti-endothelial antiserum did not impair their viability and protected the endothelium from being killed at 0.3–1.2 mM hydrogen peroxide. This approach might be useful for a cell selection in mixed cell populations. The problem of local protection of cells involved in the inflammation focus are discussed. (Am J Pathol 1987, 128:276–285)

OXYGEN metabolites released by activated phagocytes are cytotoxic agents directed against bacteria,[1] malignant cells,[2] or host cells located in areas of inflammation.[3] Endothelial cells delineating blood vessels are the easy and frequent target for phagocytic attacks.[4,5] Because of its pathogenetic significance, the process of endothelial injury induced by oxygen metabolites has been extensively studied.[6–12] Various substances, such as catalase, superoxide dismutase, and glutathione, have been proposed to protect the endothelium from oxygen metabolities.[13–15]

It may be desirable for a protective substance to be concentrated in areas of inflammation. The drug itself or drug-loaded vehicles could be conjugated to molecules with specific affinity to the zone of injury (a "drug targeting" concept[16]). We have previously shown that antibody-bearing red blood cells (immunoerythrocytes) could be effectively targeted to surfaces containing exposed antigen.[17] Erythrocytes *per se* contain a set of oxygen metabolite scavengers; they could preserve cells from damage with hydrogen peroxide being admixed with the cells.[3,18–20] Immunoerythrocyte targeting might therefore be an effective method for local protection of cells from hydrogen peroxide. We evaluate this approach in a simplified model utilizing a monolayer culture of human umbilical vein endothelial cells. We have demonstrated that targeted immunoerythrocytes locally decrease the concentration of hydrogen peroxide and protect the underlying endothelial cells from its cytotoxic action.

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Materials and Methods

The following reagents were used in this work: collagenase Type I (Worthington), cell culture medium M199 (GIBCO Laboratories UK), chromium chloride (Merck), heparin, trypsin, bovine serum albumin (BSA) (all reagents from Sigma), luminol and HRPO (Reanal, Hungary). Cell culture flasks (Falcon Plastics) and microtiter culture plates (Microtest, Flow Laboratories) were used for cell culturing. Human erythrocytes were prepared from fresh human citrate-anticoagulated blood by four sequential centrifugation/resuspension steps in phosphate-buffered saline (PBS). Affinity-purified rabbit antibodies against mouse immunoglobulins and mouse antiserum against human endothelial cells were kindly provided by Dr. A. V. Roodin (National Cardiology Research Center, USSR).

Culture of Human Endothelial Cells (ECs)

ECs were produced by collagenase treatment from umbilical veins obtained within 24 hours after delivery as previously described.[21] Isolated ECs were cultured in medium M199 supplemented with 20% heat-inactivated pooled human serum with additions of 0.2 mg/ml endothelial growth supplement,[22] 0.1 mg/ml heparin after,[23] 0.1 mg/ml lincomycin, and 0.2 mg/ml fungizone. The cells were seeded in flasks coated with 0.2% gelatin solution in PBS by air-drying. The cells were detached by 1 : 1 mixture of 0.05% trypsin solution and 0.02% EDTA in PBS and passaged by split ratio 1 : 5–1 : 10. The cultures were verified by morphologic criteria, by expression of Factor VIII-related antigen, and by the activity of angiotensin-converting enzyme.

Immunoerythrocyte Preparation

Immunoerythrocytes were produced by coupling rabbit antibodies against mouse immunoglobulins to the surface of freshly washed red blood cells with chromium chloride.[24] Briefly, 0.1 ml of 1 mg/ml $CrCl_3$ in saline was added dropwise to 0.1 ml of densely packed red blood cells, followed by 0.5 mg of the antibodies in 0.1 ml of saline. After 40 minutes of stirring at room temperature, an excess of BSA was added to prevent aggregation, and 5 minutes later immunoerythrocytes were washed by five consecutive centrifugations in PBS. To evaluate the amount of bound antibodies ^{125}I-labeled rabbit IgG was added as a tracer before conjugation. After conjugation, the radioactivity bound to washed immunoerythrocytes was determined with the LKB Rackgamma counter. To determine specificity of immunoerythrocytes binding to the antigen, Multiwell (Flow) plates wells were coated with mouse IgG, BSA, of collagen as described previously.[17] When the unbound protein had been washed out, 0.5 ml 0.5% suspension of immunoerythrocytes or erythrocytes bearing nonimmune rabbit IgG was added into the wells. After 1-hour vortexing in an orbital shaker all nonbound cells were sucked from the wells, and 0.2 ml of distilled water was added to lyse the bound erythrocytes. The amount of bound cells was evaluated according to absorbance of hemoglobin at 405 nm in lysates.

Determination of H_2O_2 Concentration

Samples were mixed with the substrate solution (0.1 mg O-phenilenediamine and 20 μg peroxidase in 1 ml PBS) and incubated, and optical density was read at 490 nm. To determine low concentrations of H_2O_2, samples were mixed with a solution containing luminol and peroxidase,[25] and peaks of luminescence were recorded on an LKB Luminometer 1250. In both cases, concentrations of H_2O_2 were determined by comparison with calibration curves obtained with serial dilutions of standard H_2O_2.

Cytotoxic Action of H_2O_2 on Endothelial Monolayer

Confluent cultures of ECs after one to seven passages were used, approximately 1.5×10^4 cells per well. Hydrogen peroxide was added to the wells to achieve concentrations ranging from 0.05 to 10 mM. The plates were kept in a CO_2 incubator, then washed with fresh culture medium; cultivation was continued in sterile conditions with antibiotics in the medium. The cells were washed again after 24 hours and stained with trypan blue in serum-free M199. Dead (stained) cells were counted in a Nikon microscope.

Decomposition of H_2O_2 by Immunoerythrocytes

Mouse immunoglobulins were absorbed on the surface of the wells of cultural microtiter plates by 2-hour incubation of 20 μg/ml IgG solution in PBS at room temperature, followed by 1-hour incubation of 2 mg/ml BSA in PBS to block free adhesive sites on the plastic. Concomitantly, a row of wells was treated in a similar way, but immunoglobulins were omitted. The wells were washed with PBS and filled with 0.3 ml of H_2O_2 in saline. Then 0.5 μl of 10% immunoerythrocyte suspension was carefully injected by microsyringe into each IgG-coated well to produce an area of approximately 3 sq mm covered with immunoerythrocytes (total surface of the well bottom

is approximately 40 sq mm). For more rapid attachment, the cells were sedimented by centrifugation of plates for 5 minutes at 150g in the bucket rotor TH-4 of the TJ-6B centrifuge (Beckman). After centrifugation in the wells coated with albumin only, 0.5 μl 10% immunoerythrocyte suspension was injected quickly and suspended evenly in the well volume. The concentration of H_2O_2 in the wells was determined as described above after selected intervals of incubation time at room temperature.

Local Protection of Endothelial Cells by Erythrocytes From H_2O_2-Induced Damage

Definite zones of the endothelial monolayer in cultural plates were rapidly coated with native erythrocytes by centrifugation by techniques described above in the presence of media containing H_2O_2 (5×10^5 erythrocytes per well were added). After 75 minutes of incubation, fresh sterile medium without H_2O_2 was placed into thoroughly washed wells (erythrocytes were easily removed from the monolayer by this washing). ECs were cultured for 24 hours under sterile conditions in a CO_2 incubator and then stained with trypan blue in the serum-free medium.

Targeting of Immunoerythrocytes to Endothelial Cells

Mouse antiserum against ECs or nonimmune mouse serum was added to the cultured endothelium in dilution 1 : 1000 and incubated for 1 hour at 37 C in a CO_2 incubator. After thorough washing, 25 μl of 2% suspension of immunoerythrocytes was introduced into each well of the microtiter plate and sedimented as described above. After 15 minutes' incubation at room temperature, nonbound erythrocytes were washed out. For quantitation of the bound erythrocytes, they were lysed by addition of distilled water, and absorbance of hemoglobin was read at 405 nm in an MR 580 Microelisa Auto Reader (Dynatech). The duplicate wells, after washing, were filled with sterile medium containing antibiotics, and plates were kept in a CO_2 incubator for 24 hours. After this incubation immunoerythrocytes remained tightly bound to the EC monolayer. ECs were detached with a standard Trypsin–EDTA mixture and reseeded into 35-mm culture dishes. Four hours later, the attached cells were counted, and their viability was checked by trypan blue staining.

Local Protection of Endothelial Cells by Immunoerythrocytes From H_2O_2-Induced Damage

A monolayer of endothelial cells was treated with mouse anti-EC antiserum. After thorough washing, an immunoerythrocyte-coated zone comprising approximately 3 sq mm was obtained in the wells by the centrifugation technique described above. Most of the surface—about 40 sq mm—remained free from immunoerythrocytes. After standard incubation (75 minutes, 37 C, 5% CO_2) with various concentrations of H_2O_2, the wells prepared in this way were thoroughly washed and refilled with fresh sterile culture media without H_2O_2. The islet of immunoerythrocytes remained tightly bound to endothelial cells after these manipulations. Then ECs were cultured for 24 hours and thoroughly washed again. The cells that remained firmly bound to the bottom were harvested with Trypsin-EDTA and reseeded into 35-sq mm plastic culture dishes for estimation of their viability by trypan blue staining. The last step was necessary to remove adherent immunoerythrocytes that otherwise obscured direct observation.

Results

Cytotoxic Action of H_2O_2 on the Endothelial Cells

Figure 1 shows the dose dependence of cytotoxic action of various concentrations of H_2O_2 on the monolayer of ECs revealed by staining with trypan blue. The curve appears to be very steep: the presence of the effect depends on a threefold change of critical concentration. There is a drastic difference in staining with trypan blue immediately after treatment with H_2O_2 or 24 hours later. In the former case the same treatment produces 50% stained cells at 1–3 mM H_2O_2, whereas LD_{50} corresponds to 0.15–0.20 mM at

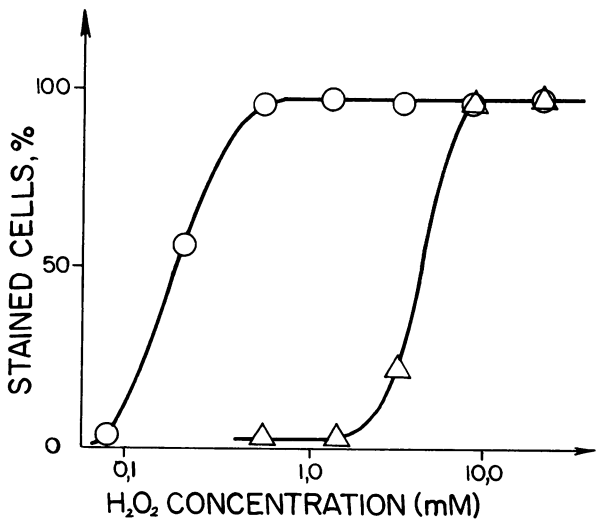

Figure 1—Permeabilization of endothelial cells by exposure to hydrogen peroxide. The percentage of damaged cells was calculated by counting them after staining with trypan blue. The cells were incubated for 24 hours (⊙) or 10 minutes (△) after the withdrawal of hydrogen peroxide. Mean of three experiments.

24 hours. At longer time intervals, the results do not change.

The time dependence of cytotoxic action (presented as the H_2O_2 concentration producing 50% stained with trypan blue 24 hours after treatment) is shown on Figure 2. A high concentration of H_2O_2 (up to 10 mM) is needed to produce the effect at time intervals of 15–30 minutes; 0.2 mM H_2O_2 becomes lethal to ECs after 80 minutes without much change with further treatment. The absence of any detectable cytotoxicity at low doses of H_2O_2 could be due to decomposition of the H_2O_2 by ECs themselves.[26] Under our experimental conditions, however (1.4×10^4 ECs per well), endothelial cells *per se* decreased H_2O_2 concentration moderately, less than 25–35% after 1 hour at 37 C (H_2O_2 concentration range, 0.3–3.0 mM; well volume, 200 μl). This result corresponds reasonably well to data of the other group,[27] though exact values for the rate of H_2O_2 decomposition could vary, depending on conditions of cell cultivation.

The 24-hour cultivation of ECs after injury treatment revealed a number of morphologic signs of cell damage (Figure 3). The cells lost their polygonal form and spread "pseudopods." The nuceli were swollen. The monolayer was no longer confluent, and cells were detached from the bottom with vigorous washing. In contrast, untreated cells remained firmly attached to the substrate.

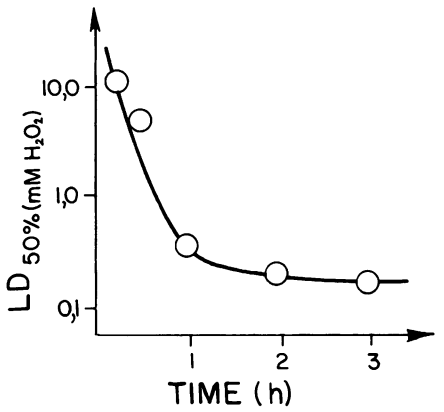

Figure 2—Effect of exposure time with hydrogen peroxide for its cytotoxic action on endothelial cells. Concentrations of hydrogen peroxide needed to kill 50% of the cells at indicated times are plotted. The percentage of dead cells was calculated as above after 24-hour culturing without hydrogen peroxide. Mean of three experiments.

Characteristics of Immunoerythrocytes and Their Specific Binding to Antigens

The method described allowed us to coat red blood cells with rabbit antibodies against mouse immunoglobulin that was tightly bound to the cell surface.[24] Approximately 2×10^5 molecules of IgG could be bound per cell with efficiency of coupling of about 15-20%. Antibodies (IgG) did not leak from the im-

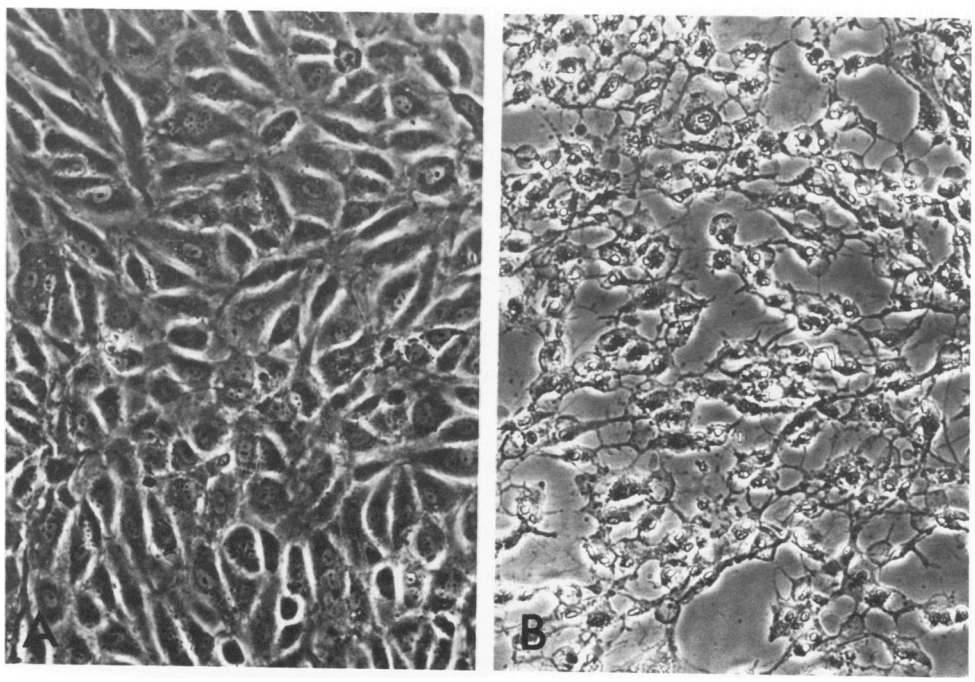

Figure 3—Morphology of the endothelial monolayer after treatment with 0.3 mM hydrogen peroxide followed by incubation for 24 hours in culture. **A**—The untreated control monolayer. **B**—Hydrogen peroxide-treated monolayer. (Phase contrast, ×100)

munoerythrocytes upon storage for 1 day in PBS or after additional washing.

The antioxidant activity of immunoerythrocytes determined as the rate of H_2O_2 decomposition per cell did not change from that of native erythrocytes and corresponded to $0.5–0.8 \times 10^{-6}$ catalase units per erythrocyte.

As can be seen from the data presented in Table 1, the immunoerythrocytes bound specifically to mouse IgG-coated surfaces but not to BSA- or collagen-coated surfaces. Erythrocytes bearing nonimmune rabbit IgG did not bind to any of these surfaces.

Kinetics of H_2O_2 Decomposition by Immunoerythrocytes

To evaluate the effectiveness of the local reduction of H_2O_2 concentration in close proximity to immunoerythrocytes, the kinetics of H_2O_2 decomposition by attached or evenly suspended immunoerythrocytes were compared. As illustrated in Figure 4, decay of H_2O_2 in the former case is five times slower than in the latter. Thus, the initial concentration of hydrogen peroxide and the amounts of introduced erythrocytes were identical in both cases; the rate of H_2O_2 decay was mainly dependent on its concentration in the local space proximate to the attached scavenger. Simple theoretic considerations help in interpreting this data as a local fivefold reduction of H_2O_2 concentration near attached immunoerythrocytes resulting from diffusional limitations.

Local Protection of Endothelial Cells by Normal Erythrocytes From H_2O_2-Induced Damage

These experiments illustrated EC protection when cells were coated with erythrocytes. A sharp zone about 3 sq mm was coated with intact erythrocytes over the endothelial monolayer, whereas the rest of endothelial cells remained free from erythrocytes (Figure 5). After the incubation with H_2O_2 (75 minutes in a CO_2 incubator), intact erythrocytes were easily washed out, and damaged endothelial cells were visually revealed by staining with trypan blue. Figure 6 is a micrograph of the borderline between "protected" and "nonprotected" zones. Both morphologic criteria and trypan blue staining show a great abundance of dead cells in the unprotected zone and prevalence of living cells in the protected zone. All damaged cells could be washed out by vigorous rinsing to leave an islet of viable cells exactly corresponding to the erythrocyte-coated zone (Figure 7). This local protection was observed at H_2O_2 concentrations ranging from 0.46 mM to 1.6 mM. All the cells were killed at higher doses, and all the cells survived at lower doses of hydrogen peroxide.

Table 1—Binding of Immunoerythrocytes to Mouse IgG-, Collagen-, or BSA- Coated Surfaces

	Mouse IgG	Collagen	BSA
Immunoerythrocytes	2.6	<0.008	<0.008
Nonimmune IgG-bearing erythrocytes	<0.008	<0.008	<0.008

The data represent 10^6 erythrocytes per square centimeter.

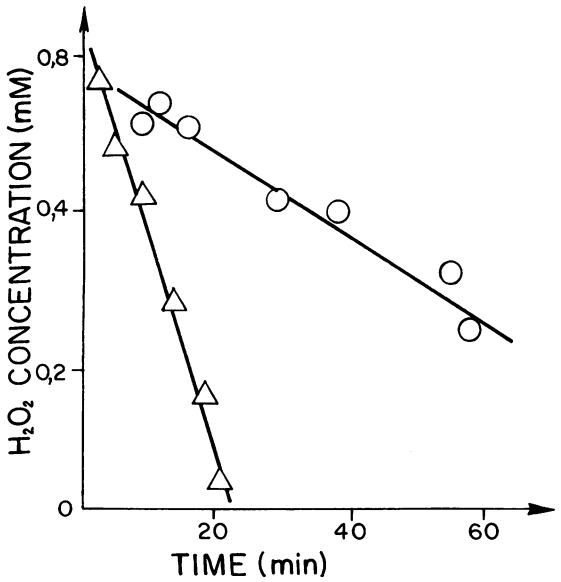

Figure 4—Kinetics of hydrogen peroxide decomposition by immunoerythrocytes. O, antigen-bound; Δ, evenly suspended. Mean of three experiments.

Immunoerythrocyte Targeting: Influence on the Viability of Endothelial Cells

Figure 8 illustrates the targeting of erythrocytes bearing rabbit anti-mouse IgG antibodies (immunoerythrocytes) to the endothelial monolayer treated with either antiendothelial antiserum or nonimmune mouse serum. Immunoerythrocytes specifically bind to the ECs. The efficiency of targeting was proportional to the concentration of the antiserum. In control experiments the same manipulations were performed with erythrocytes bearing nonimmune rabbit IgG instead of immunoerythrocytes. Erythrocytes bearing nonimmune rabbit IgG did not bind to cultured endothelial cells either untreated or treated with a mouse antiserum against human endothelial cells. Thus, specificity of binding of immunoerythrocytes to the target cells was dependent on the specificity of attached rabbit antibodies and the specificity of "primary" mouse antibodies bound to cell antigens. This

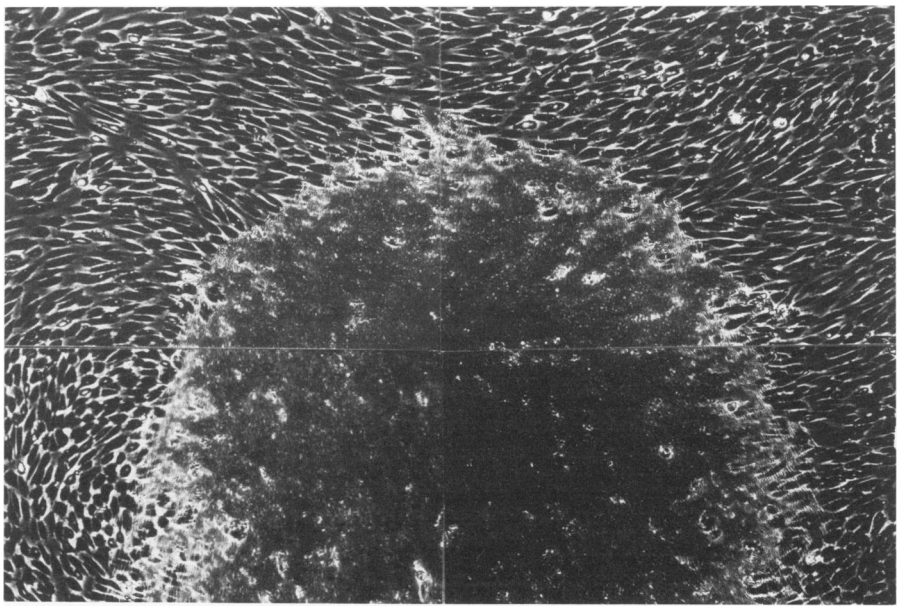

Figure 5—The islet of erythrocytes on EC monolayer. (×40)

is completely consistent with our data obtained previously with the targeting of immunoerythrocytes to smooth muscle cells in the mixed culture of the endothelial and smooth muscle cells using specific monoclonal antibodies against smooth muscle cells.[28]

To determine the influence of targeted immunoerythrocytes on the ECs' viability after the 24 hours in culture, we reseeded cells into Petri dishes and stained them with trypan blue. About 80% of the attached cells appeared to be alive, which was quite similar to the viability of intact endothelial cells incubated without immunoerythrocytes and reseeded under similar conditions. This result enables us to conclude that under the described conditions the targeting of immunoerythrocytes is not harmful to ECs.

Local Protection of Endothelial Cells by Immunoerythrocytes From H_2O_2-Induced Damage

Finally, we have demonstrated protection of a defined zone of the endothelial monolayer, treated with antiendothelial antiserum, by targeting of immun-

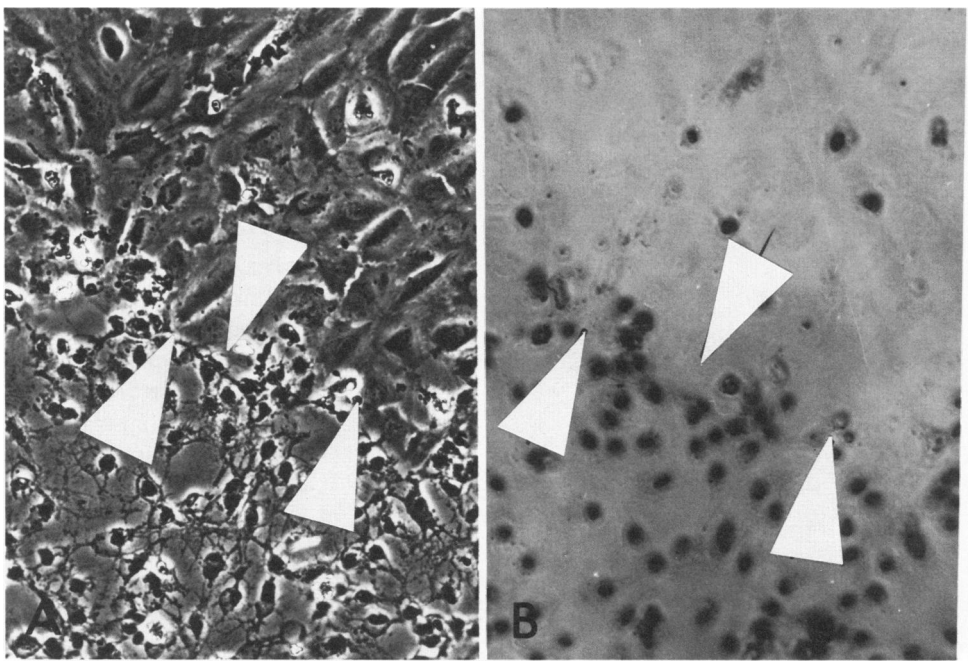

Figure 6–The border line between protected and unprotected endothelium after treatment with hydrogen peroxide, 24-hour culturing, and trypan blue staining. **A**—Phase contrast. **B**—Transmission after trypan staining. (×100). Upper parts correspond to the erythrocyte-protected endothelium.

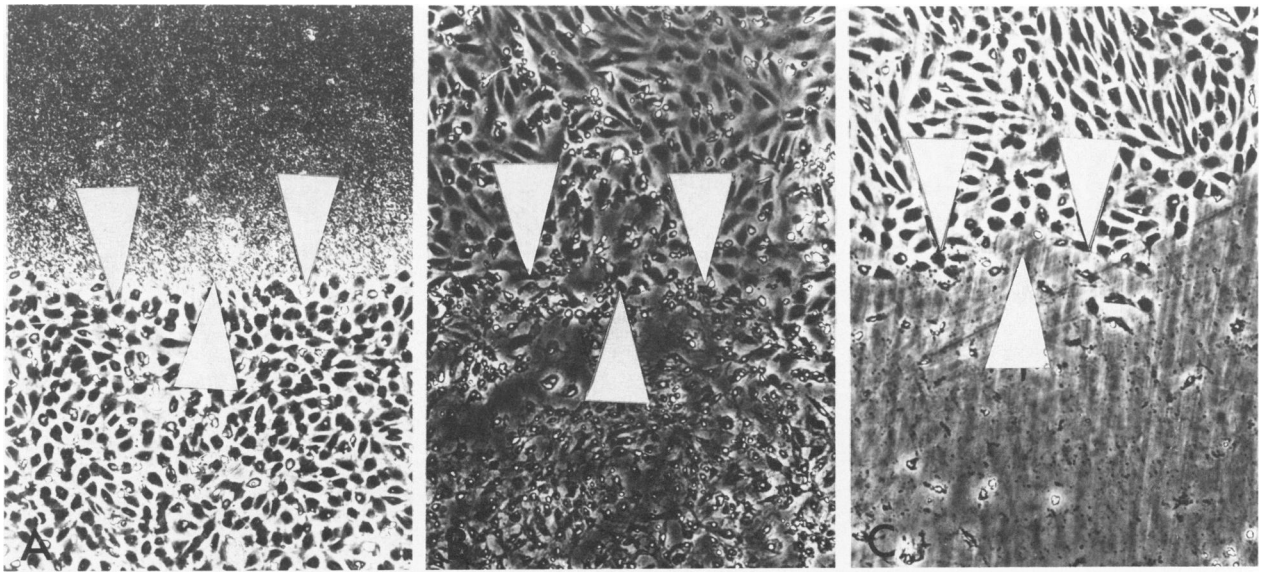

Figure 7—Desquamation of hydrogen peroxide-treated endothelium after 24-hour culturing. *Arrow* shows borderline between protected (upper parts) and nonprotected (bottom parts) zones of the endothelial monolayer. The erythrocyte-protected endothelium remains firmly attached to the substrate. **A**—During incubation with hydrogen peroxide. **B**—After washing and 24-hour incubation under sterile conditions. **C**—After washing, 24-hour incubation under sterile conditions, and repeated vigorous washings.

oerythrocytes to this zone. After incubation with H_2O_2, washing, and 24-hour incubation, the wells were intensively rinsed so that all immunoerythrocyte-free cells in the well were detached completely from the bottom except ECs protected by tightly bound erythrocytes. This discrimination was observed for H_2O_2 concentrations ranging from 0.3 to 1.2 mM. So as immunoerythrocytes obscured direct visualization of trypan blue-stained cells, the cells were first reseeded into sterile Petri dishes. Their viability was evaluated by the ability to attach firmly to substrate and by trypan blue staining that was changed only slightly with respect to control untreated cells (65–75% of alive cells vs 75–85% in control). Therefore, targeted immunoerythrocytes provide effective local protection from H_2O_2.

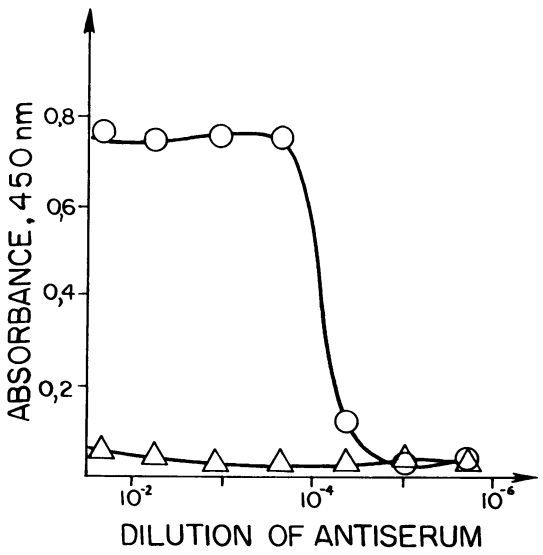

Figure 8—Binding of immunoerythrocytes with the endothelium monolayer preincubated either with mouse anti-endothelial antiserum (○) or with normal mouse serum (△). Mean of three experiments.

Discussion

The effect of H_2O_2 on endothelial cells becomes a matter of interest for many investigators because this influence may occur in areas of inflammation *in vivo*. Thus, the damage of endothelium by H_2O_2 might have pathologic significance.[4,5,29,30] In early works, the extent of injury of ECs was estimated by release of radioactive chromium or by staining with trypan blue.[6,7] These criteria reflected a rough irreversible damage of cellular membrane. Recently, it was shown that nontoxic concentrations of H_2O_2 could dramatically affect more delicate parameters of cellular homeostasis such as adenosine triphosphate levels,[8] synthesis and release of prostacycline,[9,10] and transendothelial transport of albumin.[11] In addition, the time necessary to reveal these indications was significantly shorter than the time of evident cell membrane damage. Cell membrane breakage identified by chromium release occurs sometime after the complete withdrawal of H_2O_2.[31] So cytotoxic action of H_2O_2 on endothelial cells might not be revealed immediately, which was in fact proved by staining the

cells with trypan blue (Figure 1). Cell injury induced by low doses of H_2O_2 (Figure 2) takes a long time to develop but is made obvious by the morphology of the cellular monolayers, the reduced attachment of the dead cells to the substrate, and trypan blue staining. Thus, experimental conditions were chosen that were appropriate for the study of the protection of ECs from H_2O_2. The trypan blue staining enabled us to visualize zones of local protection.

Protecting various cells in the organism from injury induced by oxygen metabolites seems desirable. Various scavengers of reactive oxygen species have been suggested, both enzymatic and nonenzymatic. It was shown that systemic administration of catalase and superoxide dismutase or PEG derivatives of these enzymes substantially reduced the decay of kidneys at glomerulonephritis,[29] infarct size due to ischemia,[32] and lung injury due to systemic activation of complement.[33] Native human erythrocytes, because they possess high intrinsic antioxidant activity, could protect target cells from hydrogen peroxide-induced damage both *in vitro*[3,18,19] and *in vivo* in the case of hyperoxygenation injury of lung.[19,20] Entrapment of protective substances inside liposomes could also be advantageous for intracellular delivery of scavengers.[14,15] To achieve local protection, a more effective method would be to attempt targeting with enzyme scavengers, a potentially valuable approach to reduce therapeutic doses and avoid adverse side effects.[16,34] The great specificity of immunoliposome uptake may be useful for targeted intracellular administration of drugs.[35]

Other biocompatible drug carriers, such as red blood cells, could also serve to deliver inhibitors of H_2O_2 toxicity. We reported earlier that immunoerythrocytes bear both enzymes and antibodies on their surfaces.[17] In addition, the large inner space of erythrocytes can be used to transport therapeutic agents.[34] Native erythrocytes have been used for protection from injury in some specific cases after their intratracheal administration[19,20] because of their high "antioxidant" activity. So application of immunoerythrocytes *in vivo* has some attractive prospects. A number of questions remained to be addressed. Would immunoerythrocytes retain their antioxidant activity? How would binding of immunoerythrocytes and their prolonged contact with endothelial cells affect the ECs' viability? What range of locality would the protection cover? In general, would targeted immunoerythrocytes be suitable as effective protectors of living target cells, or would they be injuring agents? Our experiments performed with antigen-coated surfaces demonstrated a fivefold local decrease of H_2O_2 concentration by targeted immunoerythrocytes (Figure 4). Usage of intact red blood cells helped to visualize the drastic protective effect of this locally reduced access of hydrogen peroxide to the ECs (Figure 6). Sharp dependence of the cell viability on H_2O_2 concentration (Figure 1) helped to demonstrate the effects.

Prolonged incubation of ECs with the surface-bound targeted immunoerythrocytes did not reveal any influence on the ECs' viability. Moreover, these cells were effectively protected from H_2O_2-induced killing. This effect may be applied for cell fractionation in cell culture. It would be "positive" immunoselection, as contrasted to the action of immunotoxins. Antibody against desired cell surface antigen would be sufficient to protect these cells, whereas the whole diversity of contaminant cells would be eliminated. Advantages of this approach were previously discussed,[36] where catalase–anti-catalase antibody complex binding to cells was done to protect them from hydrogen peroxide. Second, it may be interesting to consider our results as an approach for protection of endothelium (as well as other cell types) *in vivo* by means of the targeting of scavengers (immunoerythrocytes, for example) to the regions of inflammation. To evaluate the validity of the latter suggestion, it is necessary to solve some important problems. First, we must learn to recognize those particular regions of vascular endothelium which are involved in the inflammation and really need protection. The targeting of active oxygen metabolite scavengers (in immunoerythrocytes, immunoliposomes or direct antibody-enzyme conjugate forms) to the inflammation zone must be achieved by means of highly specific monoclonal antibodies. At least, white blood cells can identify abnormal endothelium and bind specifically to the surface of ECs.[37–39] Endothelial cells, infected by certain viruses, are recognized by phagocytes as a target to adhere and attack.[40,41] Similar features of endothelium could serve as a target for immunoerythrocytes, immunoliposomes, or immunoenzyme conjugates able to deliver catalase and superoxide dismutase. Next, endothelial cells in different organs could be distinguished by their specific immunologic markers, which may be used for organ-specific targeting of scavengers and drugs. Protection of target cells from damage induced by activated leukocytes in some cases may be inefficient if a tight contact exists between the leukocyte and the attacked surface.[42] Leukocyte-generated oxidants compartmentalized between the two surfaces would be inaccessible to exogeneous scavengers. On the other hand, in some cases activated leukocytes can damage target cells by oxidant-independent means.[43] Therefore, one must thoroughly examine whether the antibody-con-

jugated scavengers (such as immunoerythrocytes) would protect target cells against damage induced by stimulated leukocytes as efficiently as against the damage caused by reagent H_2O_2. Perhaps immunoerythrocytes will be ineffective in the bloodstream by themselves, but scavenger enzymes may be used to increase their efficiency by incorporating them into erythrocyte ghosts or by chemical attachment to the immunoerythrocyte surface. Finally, application of immunoerythrocytes is possible in other tissue compartments such as joints, peritoneal and plural cavities, and the intratracheal spaces.[20]

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