THE PROCOAGULANT ACTIVITY OF HUMAN MONOCYTES. E. Hiller, P. Ostendorf, J. G. Saal.
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Human leukocytes were separated into granulocytes, lymphocytes and monocytes. The procoagulant activity of the cell suspensions was assayed before and after endotoxin stimulation using one-stage methods and a two-stage method specific for tissue factor. The results indicate that the procoagulant activity in mixed leukocytes is almost exclusively derived from monocytes. Also mechanisms other than endotoxin stimulation, e.g., adherence to plastic surfaces, can cause monocytes to release tissue factor. Electron microscopic studies revealed the lysosomes of endotoxin-stimulated monocytes to be of giant size. Activated monocytes may play an important role in mediating the generalized Shwartzman reaction.

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STIMULATION OF MONOCYTE PROCOAGULANT ACTIVITY BY ADHERENCE TO DIFFERENT SURFACES. C.J.W. van Ginkel, J.I.H. Oh, W.G. van Aken and J. Vreeken. Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.

During incubation for several hours human blood leukocytes generate procoagulant activity (PCA). The PCA was identified as tissue thromboplastin-like activity and was not released but remained specifically associated with intact monocytes. We have investigated which stimulus triggers monocytes to generate PCA during incubation when no known stimulating agent is added.

When mononuclear leukocyte suspensions ($5 \cdot 10^9$ cells/l, 25% monocytes) were incubated on glass dishes, $57 \pm 7\%$ of the monocytes were found to adhere to the surface after 3.5 h incubation at 37°C . It was found that the adherent cells shortened the recalcification time from 435 ± 35 to 50 ± 2 sec using normal plasma as substrate. However, when monocyte adherence was prevented by incubating the leukocytes on cuprophane ($3 \pm 1\%$ adherence), much less PCA was detectable (the recalcification time was shortened from 435 ± 35 to 201 ± 19 sec). After exposing to glass the non-adherent monocytes had negligible PCA in comparison to the adherent monocytes. Protein synthesis inhibitors (cycloheximide and actinomycin D) inhibited PCA generation but did not affect monocyte adherence. These data provide the first evidence that adherence of monocytes is a stimulus for the generation of thromboplastin activity.

Furthermore we have shown that the PCA generated by mononuclear leukocytes during incubation on a monolayer of cultured human endothelial cells was only $14 \pm 4\%$ of the PCA generated upon incubation on plastic surface (control).

AN ENZYME IN RABBIT AORTA THAT MAY PLAY A ROLE IN HAEMOSTASIS AND THROMBOSIS. G. E. Lieberman and G. P. Lewis. Royal College of Surgeons of England, London, England.

Platelet interaction with blood vessel walls has been studied with the intention of elucidating regulatory mechanisms involved in haemostasis and thrombosis. Microsomal fractions (Mif) prepared from rabbit aortas have been found to inhibit ADP-induced platelet aggregation when incubated with ADP prior to addition to platelet-rich plasma. Thin layer chromatography of incubation mixtures in which [8- ^{14}C] ADP has been used has proved that the inhibition is due to degradation of ADP. The main degradation product at pH 6.0 is AMP and between the pH optimum of 7.2-8.2, it is adenosine. The rate of ADP breakdown is dependent on the duration, temperature and pH of the incubation, and on the concentrations of ADP and Mif used. Enzymic activity can be destroyed by boiling, and by treatment with perchloric acid. EDTA (Na_2) will remove inhibitory activity but this can be restored by addition of either calcium or magnesium to the incubate. The subcellular localization of the enzyme has been determined by isopycnic centrifugation in a sucrose density gradient, using marker enzymes to establish the distribution of subcellular organelles in the gradient. ADP degrading activity was found to coincide with the peak of 5' nucleotidase (5'NT) activity, the plasma membrane marker.

Localization of the ADP degrading enzyme on the cell membrane means that it is in a position to destroy any ADP which might be released by platelets in their response to damage of the blood vessel wall. Its coincidental location with 5'NT means that any AMP formed will be converted to adenosine which is itself an inhibitor of platelet aggregation. The ADP degrading enzyme could therefore play an important role in limiting the extent of thrombus formation.