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Human leukocytes were separated into granulocytes, lymphocytes and monocytes. The pro-
coagulant 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 indi-
cated that procoagulant activity in mixed leukocytes is almost exclusively derived from
monocytes. Also no additional mechanism 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 Schwartzman reaction.

STIMULATION OF MONOCYTE PROCOAGULANT ACTIVITY BY ADHERENCE TO DIFFERENT SURFACES. E.D.W. van
Sinkel, J.J.M. Gm, W.G. van Aken and J. Vreeden. Central Laboratory of The Netherlands Red Cross

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.

Mononuclear leukocyte suspensions (5-10^6 cells/2.5% monocytes) were incubated on glass
dishes coated with 57 ± 7% of the monocytes were found to adhere to the surface after 2.5 h incubation at
37°C. It was found that the adherent cells shortened the recalcification time from 435 ± 35 to
55 ± 8 sec using normal plasma as substrate. However, when monocyte adherence was prevented by
incubating the monocytes on suphurope (3 ± 4% adherence), much less PCA was detectable (the
recalcification time was shortened from 435 ± 35 to 501 ± 19 sec). After exposure 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 thrombopeletic activity.

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

AN ENZYME IN HUMAN ADULT PLATELETS MAY PLAY A ROLE IN HEMOSTASIS AND THROMBOSIS. R. E. Lieberman

Platelet interaction with blood vessel walls has been studied with the intention of elucidating
regulatory mechanisms involved in haemostasis and thrombosis. Microsomal fractions
(Mf) prepared from rabbit aorta 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 [5-14C] 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 at the pH
optimus of 7.0-8.0, 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 Mf used. Respective
activity can be destroyed by boiling and by treatment with peracetic acid. ERDA (Rg), will
remove inhibitory activity but this can be restored by addition of either calcium or magnesium to
the incubate. The subcellular localisation of the enzyme has been determined by isopycnic
centrifugation in a sucrose density gradient, using marker enzymes to establish the distribu-
tion of subcellular organelles in the gradient. ADP-degrading activity was found to coincide
with the peak of 5'-nucleotidase (5'D) activity, the plasma membrane marker.

Localisation 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 coincident location with TRH means that any ADP formed will be con-
verted to adenosine which is itself an inhibitor of platelet aggregation. The ADP-degrading
enzyme could therefore play a significant role in limiting the extent of thrombus formation.