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To a great co-author,

Best regards,

Roy.

Microtubule Reorganization in Platelets · May Express PF3 Activity

F. K. SCHATTAUER VERLAG · STUTTGART–NEW YORK

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Microtubule Reorganization in Platelets May Express PF3 Activity

25th November, 1974

Dear Madam,

We wish to propose that the changes occurring at or near the human platelet surface (which enhance and accelerate fibrin formation) are a consequence of the activities initiated by the structural elements resembling microtubules or microfibrils which are present within projections from the platelet surface.

Platelet Factor 3 (PF3) is a general term describing the pro-coagulant contribution of platelets to the process of intrinsic blood coagulation (de Gaetano et al. 1972). This PF3 activity is associated primarily with the membrane fraction of homogenized platelets (Marcus et al. 1967) and is a property of the membrane phospholipids (Aggeler et al. 1971). In reality, PF3 designates an activity unique to platelets rather than a biochemical enity which can be isolated and characterized (Marcus 1969). Normal, unactivated platelets in their discoid form do not exhibit PF3 activity, but exposure to mild concentrations of various aggregating agents induce reversible shape changes with the concomitant transient expression or "exposure" of PF3 activity (Born 1972; de Gaetano et al. 1972). This activation of PF3 activity is divorced from the platelet release reaction (Aggeler et al. 1971) and results in a change in the platelet surface leading to a modified affinity or reactivity with plasma clotting factors so that the modified surface acts as a catalytic region on which clotting factors can interreact under optimal conditions (Hardisty and Hutton 1966; Walsh 1974).

The first measurable step in the sequence of events leading to the reversible phase of platelet aggregation and the eventual induction of the release reaction is the rapid isovolumic transformation of the resting discoid platelet to a more spherical shape having numerous surface projections, i.e. the disk-echinocyte transformation (Born 1972; Bull and Zucker 1965; Macmillan and Oliver 1965). Rodman (1971) presented electron micrographs depicting intermediate stages in the contraction and break up of the circumferential band or torus of microtubules which were presumably involved in or responsible for the maintenance of the resting discoid form (White 1972). Microtubular bundles resulting from the break up of this postulated cytoskeleton can be seen in various orientations within the cell, which at this early stage of transformation has small structureless blunt projections (Rodman 1971). At a later stage of this reversible phase, the projections from the platelet surface are longer and narrower and can be seen with the electron microscope to contain structural elements resembling bundles of microtubules or microfilaments which are lying parallel to the long axis of the projection (Rodman 1971; Silver 1965).

Born (1972) measured the volume of plasma trapped between echinocytic platelets packed by centrifugation at 10,000 g for 3 minutes and found that the newly formed protrusions were remarkably rigid and prevented close cell packing. He concluded that this unexpected structural rigidity was due to the presence within the protrusions of bundles of microtubules or microfibrils (possibly derived from the original

microtubular cytoskeleton). During the second (irreversible) phase of platelet aggregation these projections became more deformable, possibly indicating beakdown of those elements responsible for the structural rigidity (Born 1972).

Fig. 1 is an electron micrograph showing a portion of a fibrin strand (F) and part of a platelet which had been partially disrupted with ultrasound (1 MHz continuous

Fig. 1

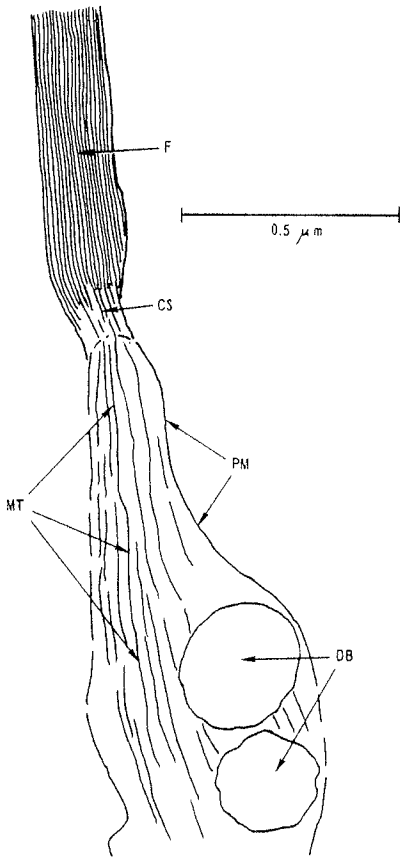

Fig. 1 is an electron micrograph, together with its schematic representation, showing a fibrin strand (F) linked by connecting strands (CS) to filamentous structures (MT) within the projection from a platelet surface. The platelet plasma membrane (PM) has been partially disrupted by ultrasonic irradiation, while large organelles remain intact, e.g. dense bodies (DB).

wave irradiation of fresh citrated human platelet rich plasma (PRP) at an average intensity of the order of 0.5 W/cm² for 5 minutes at 26° C (for details see O'Brien et al. 1974); 30 minutes after treatment the PRP was recalcified, allowed to clot for 15 minutes at 37° C, and the clot fixed in phosphate buffered 2% glutaraldehyde pH 7.4 post fixed in 1% buffered osmium and embedded in Epon. Thin sections were examined with a Siemens 1 A electron microscope). Dense filamentous structures (MT) can be seen running the length of the platelet projection and appear to merge with fibrillar structures associated with the fibrin strand (CS). The membrane in the region of the projection tip is apparently penetrated by these filamentous structures and cannot be visualized clearly (Fig. 1). We have observed similar filamentous structures within projections from control (untreated) cells and platelets subjected to hydrodynamic shear stress in a cone and plate viscometer. In every case where platelets co-existed with fibrin, platelet projections could be seen which contained filo-

mentous structures which appeared to merge in a continnous manner with fibrin strands.

The platelet depicted in Fig. 1 had been exposed before recalcification to ultrasonic irradiation under conditions where one or more forms of cavitational activity had been present. As a consequence, holes are visible in the plasma membrane (PM) and most of the cellular contents excluding the filamentous structures (MT) and dense bodies (DB) have escaped. It should be noted that damaged or exposed portions of extracellular or intracellular membranes have not acted as initiators of fibrin formation (Fig. 1). The same large hydrodynamic shear stresses which produced the holes in the platelet membrane must also have washed off some of the mucopolysaccharide layer which coats the cell. Nevertheless, fibrin is only seen at the tip of the platelet projection. This suggests that expression of PF3 activity is more than the simple exposure of a phospholipid catalytic site on the membrane surface.

It is not likely that the filamentous structures observed within the platelet in Figure 1 could have been produced by the impact and subsequent termination of an elongating fibrin strand with a partially disrupted platelet. The holes seen in the plasma membrane (PM) indicate that at the time of recalcification the intracellular environment of the platelet had essentially the same composition as the surrounding plasma. Consequently, a rapidly elongating fibrin strand would be expected to terminate completely on contact with the platelet boundary or else continue through the platelet body without altering its morphology.

We therefore propose that the bundles of microtubules or microfilaments, which seem to be responsible for the structural rigidity of the platelet projection, induce a change in that portion of the platelet membrane occupying the tip of the projection such that it exhibits the pro-coagulant catalytic properties designated as "PF3 activity." One interpretation of this proposal is to assume that the microtubular structures are secretory in nature, i.e., actively release for example an enzymatic material which digests away portions of the membrane and/or its external mucopolysaccharide coat so as to expose a catalytic phospholipid surface. Similarly, the material transported or secreted in this region might consist of platelet fibrinogen together with all the platelet-associated pro-coagulation factors needed to initiate small-scale fibrin formation within the membrane and its mucopolysaccharide coat. Even minute quantities of fibrin formed within this region would act as a 'nucleus' for subsequent fibrin formation and growth utilizing the plasma coagulation system.

It should be noted that expression of PF3 activity is inhibited in normal human platelets when they are induced to aggregate in the presence of Colchicine (a drug which binds to microtubular structures and causes their de-polymerization) (Gold, et al. 1973).

A. R. Williams,*
S. M. Sykes,
W. D. O'Brien, Jr.
Bureau of Radiological Health,
U. S. Food and Drug Administration,
Rockville, Maryland,
20852, U.S.A.

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* Permanent address: Departments of Anatomy and Medical Biophysics, University of Manchester, Manchester, England.

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