in platelet projections (Fig 1A). However, moesin appears to colocalize with only a subset of actin filaments, because scoring of micrographs indicated that the majority (65%, n = 400) of activated platelets (1 minute) had submembranous moesin staining with hollow-appearing unstained centers, whereas 95% had actin staining of the entire cytoplasm. This distinction between moesin and actin filament localization, shown here for suspension-activated platelets, was not noted in a study of glass-adherent platelets.[6]

To explore the association of moesin with actin-based structures, we used selective Triton X-100 solubilization and differential sedimentation to generate operationally defined subcellular platelet fractions.[7] Resting and thrombin-treated platelets were lysed and separated into the cytoskeleton fraction, membrane skeleton fraction, and soluble fraction. Thrombin initiates rapid rearrangements including peripheral actin filament assembly and cross-linking, causing actin binding protein (ABP), talin, myosin, α-actinin, and actin to become incorporated into the cytoskeleton fraction[7,8] (Fig 1B, lower left). Several membrane skeletal proteins, including a subfraction of GPIIb/IIIa, also redistribute to the cytoskeletal fraction[9] (Fig 1B, GPIIb blot). In contrast, moesin, which was found exclusively in the soluble fraction in resting platelets, redistributed to the membrane skeleton fraction, which is known to contain short actin filaments, vinculin, spectrin, and ABP[9] (Fig 1B, moesin blot).

Quantitation showed that moesin redistribution to the membrane skeleton fraction was rapid, increasing dramatically in the first minute after thrombin addition, reaching maximal levels (18% of moesin molecules incorporated) after 2 to 3 minutes, and then decreasing (Fig 1C). Thus, incorporation of moesin molecules into the membrane skeleton coincides with early activation events that include formation of filopodial extensions and onset of platelet aggregation (not shown).

Although their tissue distributions differ, the ERM proteins are approximately 70% identical in sequence, structurally similar, and considered functionally equivalent.[2,3] A recent study of permeabilized cells demonstrated an absolute requirement for moesin (or ezrin or radixin) for actin filament assembly mediated by Rho family GTPases.[10] Another response of platelet moesin to thrombin stimulation involves its phosphorylation, which occurs on threonine-558 near an actin binding site.[6] Moesin phosphorylation is a rapid response, maximal in the first minute and then declining toward basal levels.[6] Because known ERM activation pathways intersect at the stage of conformational unmasking of soluble dormant monomers,[2,3] the timing of these events suggests that the observed dynamic association of moesin with the membrane cytoskeleton and its relocation to transient filopodia and lamellipodia are downstream reactions of moesin phosphorylation.

These findings strongly suggest that moesin is involved in platelet cytoarchitectural rearrangements, filopodia and lamellipodia formation, which are important for the transformation of nonadhesive platelets to the adhesive hemostatically active state. It was suggested for polarized lymphocytes that moesin, through its linkage with the cytoskeleton, promotes cell:cell adhesion by redistributing linked surface membrane adhesion molecules to the uropod.[1] Similarly, platelet moesin, by agonist-induced relocation to filopodial extensions, might redistribute and concentrate linked surface receptors, thus contributing to platelet aggregation.

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Ex Vivo Factors Affecting Contact Phase Activation in Negatively Charged Medical Devices

To The Editor:

We read with interest the article by Scott et al[1] on contact system activation during platelet concentrate filtration. Several groups endeavoring to find a causative role for negatively charged filters in hypotensive/anaphylactoid reactions have reported activation of the coagulation cascade intrinsic pathway (contact phase) by measuring changes in bradykinin and/or kallikrein activity before and after filtration.[2] Al-
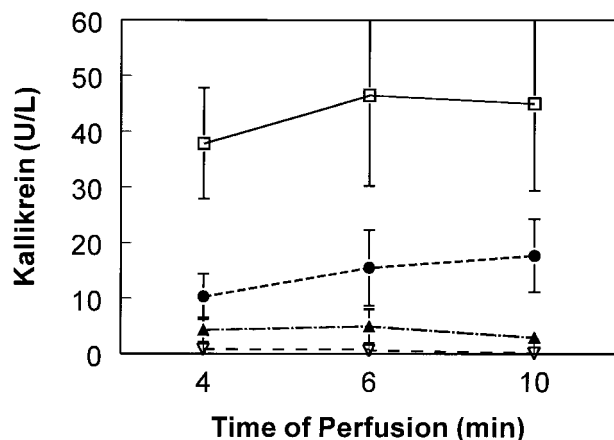
Fig 1. Influence of pH on contact phase activation induced by negatively charged dialysis membrane. Platelet-poor plasma pools were diluted 1:20 in 0.9% saline (5% final plasma content). Pool pHs (measured at 37°C) were adjusted as indicated and perfused (single pass) through mini-hemodialysers constructed of AN69 membrane (250 cm²). At 4, 6, and 10 minutes of perfusion, aliquots of effluent plasma were immediately frozen in methanol/dry ice bath. Plasma kallikrein was determined by chromogenic assay using substrate S2302 (Biogenic, Maurin, France) after modification of a method described by De La Cadena et al.[9] Means and standard deviations (error bars) of six experiments are shown. Kallikrein in the nonperfused pool remained at the baseline of less than 2 U/L over the course of the experiment. Kallikrein for plasma perfused over nonelectronegatively charged membranes (eg, cellulosic) remains at baseline of less than 2 U/L (data not shown). (□) pH 7.1; (●) pH 7.4; (▲) pH 7.6; (▽) pH 7.8.

though a clear cause and effect relationship between clinical reactions and the use of negatively charged bedside filters has not been unequivocally demonstrated, there have been several case reports that support such a hypothesis.[3,4] The observations of Scott et al of minimal high molecular weight kininogen and total kininogen adsorption to negatively charged filters led them to conclude and generalize that there is minimal, if any, contact phase activation during platelet filtration using negatively charged filters. Others that have reported significant activation of the contact system also observed a wide variation in the levels of activation between platelet concentrates.[5] We believe these groups are missing contributions from two critical components of this system: the pH of the platelet or plasma being filtered and enhancement from dilution of the products with crystalloids.

Hypotensive/anaphylactoid type or hypersensitivity reactions (HSR) were reported during hemodialysis in the early 1990s with the use of ACE inhibitor therapy (ACEI) and were found to be more frequent in ACEI-treated patients dialyzed using AN69 (HOSPAL, Lyon, France), a negatively charged dialysis membrane.[6] In response to these reports, our HOSPAL group has been investigating the role of contact phase activation in extracorporeal circuits, including determination of key factors that affect this phenomenon. Potential similarities between HSR in hemodialysis, transfusion medicine, and therapeutic apheresis such as heterogeneity of occurrence, ACEI, and exposure to negatively charged medical devices have been pointed out by Owen and Brecher.[7]

We have seen a significant effect of perfusate pH (eg, platelet concentrate pH) on contact phase activation. For in vitro activation, we see maximum kallikrein and bradykinin activation at or below $pH_{37°C}$ of 7.1 (Fig 1). We believe this pH effect may have significantly affected the results observed by Scott et al. Apheresis platelet concentrates are known to have very large excursions in pH over the course of storage, depending on storage bag type and the number of

platelets in the bag. An initial increase in pH is typically seen during the first 2 days of storage, coinciding with the products tested by Scott et al.

We have also observed a systematic increase in contact phase activation when plasma is diluted with normal saline (0.9% NaCl, pH 5.5). These observations were recently confirmed by Shimizu et al.[8] They reported that bradykinin generation increases 30 times in platelet concentrates diluted 85% with a storage solution (Seto sol; 115 mmol/L NaCl, 4 mmol/L KCl, 3 mmol/L MgCl₂, 10 mmol/L Na₃PO₄, 15 mmol/L acetate, 3 mmol/L Na₃citrate, 10 mmol/L glucose, pH 7.1) or saline.[8] We currently use 5% plasma in saline at a $pH_{37°C}$ of 7.1 to maximize the activation signal as we investigate various devices and components. Tests are conducted at 37°C, and we have found no differences at room temperature.

Blood interaction with an active foreign surface is multifaceted with many interacting variables such as dilution, pH (which may be 6.0 to 7.6 for platelet concentrates), and physical properties of materials. Effects on patients are potentiated not only by the blood/material interactions, but also by predisposing factors in the patient (bradykinin metabolism, acid/base status, and perhaps genetic factors). Therefore, we would encourage investigators in this area to pay special heed to ex vivo factors such as plasma content, pH, and time effects. Understanding these effects may help to clarify a phenomenon that occurs at an inconsistent frequency in the laboratory and an elusive clinical complication rate when using leukocyte reduction filters.

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