Correspondence

Letters to the Editor will be published, if suitable, as space permits. They should not exceed 1000 words (typed double-spaced) in length and may be subject to editing or abridgment.

Whence Cometh Neointimal Myofibroblasts?
To the Editor:

We greatly enjoyed the elegant and informative study recently published by Shi et colleagues. Using bromodeoxyuridine (BrdU) labeling after severe endoluminal coronary artery injury, the authors demonstrated activation and proliferation of porcine adventitial fibroblasts that initially lacked actin expression. Subsequently, the adventitial fibroblasts acquired actin expression indicative of myofibroblastic differentiation, and these proliferating adventitia-derived myofibroblasts were then shown to translocate the gap between dissected media to contribute to neointima formation. Smaller numbers of similar BrdU-positive nonmuscle cells were demonstrated in the media as well. In their discussion, the authors note that the precise mechanisms regulating fibroblast migration to neointima are unknown.

It has been our experience2–4 and that of others5,6 in human material that endothelium and perivascular adventitial and interstitial stromal dendritic fibroblast-like cells express CD34, a transmembrane glycoprotein known as the hematopoietic progenitor cell antigen.7 It is increasingly thought that these cells are uncommitted stromal reserve cells2–4 that, we feel, constitute the raw material of the microvascular unit for use in repair, stromal remodeling, and homeostasis. CD34 has a glycosylated mucin domain that is a ligand for integrins, and thus CD34 may be involved in regulating proadhesive and antiahesive cellular behavior.8,9 The intracytoplasmic domain of the molecule is probably involved in signal transduction and cellular differentiation10 and therefore might be involved in regulation of phenotypic plasticity of CD34-positive adventitial fibroblasts engaged in myofibroblastic differentiation. We have also shown CD31 positive endothelial differentiation of CD34-positive dendritic cells in cardiac myxomas.11

In addition to being concentrated in the adventitia, CD34-positive fibroblasts are also interspersed among the medial smooth muscle cells in some vessels (personal observations). Thus, the distribution of CD34-positive fibroblasts in human vessels is similar to the distribution of BrdU-labeled adventitial and scattered medial cells in the porcine material of Shi et al. In studies of myofibroblastic differentiation of CD34-positive stromal cells in mammary stromal tumors7 and in fibroma of tendon sheath,2 we noted that increasing actin expression in the tumor cells often correlated with CD34 downregulation. We wonder whether studying CD34 expression in the porcine material of Shi et al might further elucidate the molecular basis of adventitial myofibroblast differentiation and migration in medial injury repair and neointima formation.

We are also interested in the role of dendritic stromal histiocyes that express coagulation FXIIIa, the so-called collagen-associated dendrophages of Nickoloff,3 in matrix remodeling and stromal repair as well as in morphogenesis2 and tumors.2–4 Transglutaminase FXIIIa is a potent and pleiotropic fibroblast growth factor that regulates both fibroblast proliferation and matrix protein synthesis and catalyzes polymerization of matrix proteins including fibrin, fibronectin, and collagens.12,13 FXIIIa coated on plastic causes formation of actin-positive stress fibers in fibroblasts, modulates novel cell spreading and adhesive behavior in fibroblasts and other similarly grown normal and neoplastic cells, and induces phosphorylation of tyrosine residues in 120- and 70-kD proteins in these fibroblasts.14 Most normal connective tissue contains scattered microvascular adventitial FXIIIa-positive dendrophages,3,2 and these cells are often markedly increased in inflammatory and reparative processes.1 FXIIIa-positive dendrophages combine with CD34-positive dendritic cells in a variety of mesenchymal tumors,2–4 including the quasithrombotic neoplasm cardiac myxoma.3 Thus, we suggest that examination of the material of Shi et al from the standpoint of FXIIIa reactivity to detect these important mitogenic microvascular cells might also be informative with regard to the mechanisms of myofibroblast differentiation and matrix remodeling in neointima formation.

In conclusion, we applaud the excellent study of Shi and colleagues and look forward to further molecular investigations into the processes of microvascular myofibroblastic differentiation and migration implicit in their results.

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Evolution of Early TIMI 2 Flow After Thrombolysis for Acute Myocardial Infarction
To the Editor:

The GUSTO-1 Angiographic Investigators conclude in their paper,1 “Because at early angiography (eg, 90 minutes) one cannot predict the natural evolution of a given infarct-related artery with TIMI grade 2 flow (progression to grade 3 or arrest at grade 2) and because improvement in infarct arterial flow from TIMI grade 2 to 3 is associated with a significant improvement in left ventricular function, our observations suggest that perhaps a strategy of rescue angioplasty for vessels demonstrating early, sluggish flow warrants consideration.” Further additional argument supporting this proposal was provided by the GUSTO Investigators in another article,2 “...the surprising finding that TIMI grade 2 flow was associated with a higher mortality during the first 4 hours [and importantly during the first 24 hours as well] than TIMI grade 0 or 1 flow may indicate that partial reperfusion has a detrimental effect on the myocardium.” Specifically, this is the case in the subgroup of patients characterized by the coincidence of presentation with a short delay after onset of symptoms and critically depressed left ventricular function (ie, patients with an extensive area at risk
of ischemia threatening with large potential infarct), who are most likely to benefit from the rescue intervention.

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Extracorporeal Circulation

To the Editor:

The original work of Sanjay Kaul et al, “Inhibition of Acute Stent Thrombosis Under High-Shear Flow Conditions by a Nitric Oxide Donor, DMHD/NO,” is a well-designed and controlled study. When it comes to drawing clinical implications, the authors did not address a major limitation of this study: the thrombogenic effect of extracorporeal circulation (EC).

EC initiates humoral and cellular responses leading to enhanced thrombogenicity through integrated multifactorial pathways. It was recently shown that during EC, there is an activation of the complement system and that the terminal components C5a and C5b-9 directly contribute to platelet and neutrophil activation. In a different study (non-EC), it was shown that C4d fixation to erythrocytes resulted in decreased membrane deformability.

EC induces proteolytic activity, causing pronounced platelet degranulation and erythrocyte membrane damage, leading to formation of echinocytes. A decrease of erythrocyte deformability due to a change in sodium contents as well as decreased 2,3-DPG content was observed. Thus, prolonged artificial circulation provokes structural reorganization in erythrocytes, thereby affecting both blood rheology and gas exchange. Direct shear-induced platelet aggregation, mediated by binding of von Willebrand factor to platelet glycoprotein Ib, which caused degranulation, was also demonstrated.

A very important forgotten concept is the “inversion phenomenon” of blood viscosity in the microcirculation, which takes into consideration the rheology of cell aggregates and single cells. The appearance of sludge-like aggregates in the blood will cause an immediate amplification of the resistance to the point of no flow.

Thus, it is reasonable to assume that the actual viscosity in EC experiments is much higher than that calculated by the flow and radius and has a great impact on flow conditions and thrombus formation. We should bear these facts in mind when we attempt to integrate these EC ex vivo studies into the clinical world.

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connexin43 Gene Mutations and Heterotaxy

To the Editor:

Recently, an interesting observation has been reported by Gebbia et al1 in this journal regarding connexin43 gene mutations in patients with heterotaxy. Previously, another paper was published by Britz-Cunningham et al2 reporting human connexin43 gene mutations in patients with visceral heterotaxy syndrome. Their article was referred to in the McKusick catalogue in the description of Ivemark syndrome (MIM 208530),3 and connexin43 mutations were thought to be the cause of asplenia and polysplenia syndromes with cardiovascular anomalies. Only some hundred such cases have been reported so far in the literature, and most of them were sporadic, although familiar occurrences have also been described, suggesting autosomal recessive inheritance.4 The first evidence for the autosomal recessive inheritance was the identification of connexin43 gene mutations in these patients by Britz-Cunningham et al.2 Two other groups examined connexin43 mutations in patients with heterotaxy syndromes, but neither of them could detect any mutation in the cytoplasmic carboxy terminal region of the gene.5,6 Gebbia and colleagues7 continued the search for mutation in additional patients with sporadic and familial heterotaxy, but they could not detect any connexin43 mutation in any patients with heterotaxy. Because only three groups have reported the investigation of connexin43 mutations so far in heterotaxy syndromes, and because there was a striking difference in their results, we sequenced that critical region of the connexin43 gene. On the basis of the results of Gebbia et al and our observations, we believe that it is more and more likely that the results reported by Britz-Cunningham et al were a laboratory artifact.

We should consider changing the description of Ivemark syndrome in the McKusick catalogue regarding connexin-43 mutations.

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Response
We have read with interest the letter to the Editor of Toth et al describing their inability to detect connexin-43 mutations in 11 cases of heterotaxy. The negative results of Toth et al now brings to 78 the number of reported heterotaxy cases in which no cx43 mutations could be found in the 200 base pairs containing all of the nucleotide changes reported by Britz-Cunningham et al.1–3 Mice either lacking or overexpressing cx43 develop right-heart outflow obstruction, but the complex cardiac and extracardiac malformations typical of heterotaxy have not been observed.4,5 The absence of animal-model data to support a role of cx43 in mammalian left-right axis development as well as the inability of three independent groups to detect cx43 mutations in a large number of heterotaxy cases suggests the possibility of laboratory artifact underlying the original detection of “mutations” reported by Britz-Cunningham et al.

Family studies indicate that heterotaxy is likely to be quite heterogeneous genetically.6 For example, we have identified recently a gene for X-linked defects in transgenic mice overexpressing the Cx43 gap junction gene. Development. 1997;124:1281–1292.

Conversely, Maalej and Folts4 showed that platelet activation was also enhanced by increasing the shear stress and, in these circumstances, the anti-thrombotic effect of aspirin was also overcome. We postulated7 that red cells, leukocytes, and products released after endothelial cell damage could also be involved in platelet activation under the experimental conditions referred to in the Folts model.4

The article by Santos et al2 confirmed our previous finding from ex vivo experiments. In 1988, we suggested8 that the behavior of aspirinated platelets varied with dose and time elapsed between aspirin ingestion and blood collection when platelet-rich plasma was stirred with arachidonic acid plus ADP, with platelet-activating factor, or with collagen: independently of time elapsed since blood collection, a complete synergistic aggregation was obtained after a single or a daily repeated low dose (50 mg) of aspirin, as an effect that is independent of thromboxane A2 formation. When 500 mg aspirin was administered, the response was related to time: 2.5 hours after aspirin intake, synergism was abolished, but full irreversible aggregation was restored in the 24-hour blood samples. This effect was also independent of thromboxane A2 whose concentration in serum was 2% to 8% of the preaspirin values. Nor did a daily repeated 500-mg aspirin produce a cumulative effect; synergism was obtained after aspirin intake for 7 to 10 days. This fact, together with the various origins of ADP in addition to erythrocytes (endothelial cells, platelets), makes the point of cooperation between different cells very complex. On the basis of our published studies, it does not seem to us that the aspirin regimen proposed by Valles et al will be able to modify the response of platelets in the presence of damage to the endothelium, in which many agonists from different cells are released.

Why low-dose aspirin prevented thrombosis and thromboembolism in different clinical trials (secondary myocardial infarction, stroke, and thromboembolic events in patients with cardiac valve prostheses), as higher-dose aspirin did, is an open question that is difficult to answer at present.

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Response

We thank Dr Altman and his colleagues for their interesting and provocative comments concerning the results presented in our recent publication in Circulation and the associated Editorial.1,2 We agree with Dr Altman that different cell types are involved in the pathogenesis of cardiovascular diseases. Our experimental work was specifically oriented to demonstrate that thrombosis is a multicellular process, and we have conducted an extensive series of in vitro studies on platelet responsiveness as modulated by endothelial cells,1–3 neutrophils,4,9 and erythrocytes.5–7 Our studies of the effects of aspirin on platelet–erythrocyte5–7 and platelet–neutrophil interactions10 have demonstrated that aspirin directly reduces the prothrombotic effect of erythrocytes1 and enhances the inhibitory effects of neutrophils on platelet reactivity in vitro.10 This indicates that cell–cell interactions modulate effects of aspirin on platelets.

The conclusion of our article1 was that daily low-dose aspirin administration required intermittent supplementation with a high dose to overcome the prothrombotic effect of erythrocytes, which otherwise negates the protective effects of low-dose aspirin. Our conclusions derive from an experimental system1–3,9,10 devised to more closely approximate participation of multiple cell types in the thrombotic process. Moreover, this system separately measures platelet activation (release reaction) and recruitment (proaggregatory effect of cell-free releasates from combined suspensions of activated platelets and other cells) at an early time point during platelet–erythrocyte interactions. This allows better characterization of the erythrocyte response to aspirin ex vivo. This premise differs from Dr Altman’s approach,12 in which pairs of platelet agonists are directly added to platelet preparations.

The synergistic action of different pairs of agonists as studied by Dr Altman12 reduces the inhibitory effect of aspirin on platelet reactivity. This effect is unrelated to TXA2 synthesis.12 In our report,3 we found that aspirin was ineffective in blocking platelet reactivity in the presence of erythrocytes originating from donors who had not ingested aspirin or from donors who had taken a low dose of aspirin, despite blockade of platelet TXA2 formation. In contrast, when erythrocytes were treated with an adequate dose of aspirin, their prothrombotic activity was inhibited in normal donors. This has led us to propose the following clinical regimen: A 500–mg dose should be dispensed after each 2 weeks of low-dose aspirin. Under these conditions, the daily low dose continuously inhibits platelet TXA2 formation, and the intermittent high dose blocks the prothrombotic activity of erythrocytes, as demonstrated in our system.

Thus, we agree with Dr Altman about the limited protection provided by aspirin due to TXA2–independent mechanisms of platelet activation, blockade of erythrocyte prothrombotic potential with an adequate dose of aspirin may improve the clinical effectiveness of this medication.

The comments of Drs Rocca and FitzGerald in their Editorial2 concerning the appropriateness of additional clinical studies related to the effects of aspirin on erythrocyte prothrombotic activity were of importance. In fact, such an investigation was completed and is in press.13 The overall goal in these studies is to optimize the clinical use of aspirin as an antithrombotic agent.

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The Apollo 15 Space Syndrome

To the Editor:

With the permanent space station planned for the near future and with the recent surge of interest regarding manned missions to Mars, the potential for life-threatening risks looms dead ahead. In 1971 on the Apollo 15 lunar mission, astronauts Irwin and Scott both experienced severe pain and edema of the fingertips.1
addition, the mission was considered an “anomaly” since it was the first during any space mission distinguished by significant arrhythmias, more severe in Irwin’s case with a brief loss of consciousness during an episode of bigeminy after return to the command module.² Twenty-one months later, Irwin experienced a myocardial infarction.² It is conceivable that Irwin’s infarct was not coincidental but was at least partially triggered by endothelial injuries resulting from the Apollo 15 mission.

In the presence of microgravity, there is invariably a shift of fluid to the upper part of the body. But in addition on the Apollo 15 mission, there was a malfunction of the Insuit water devices, resulting in water deprivation, particularly in Irwin’s case during the three extravehicular activities (EVAs) of up to 7 hours each, whereas Scott’s Insuit apparatus functioned partially.² Irwin, sweating profusely and extremely thirsty during the EVAs, lost 5% of his weight compared with his mean preflight weight (from 74.3 to 70.8 kg), whereas Scott lost about half that much (from 81.1 to 78.9 kg).² This dehydration would have intensified the potential for microgravity-related renin-angiotensin elevations² and catecholamine elevations,³ with the latter aggravated by pain-provoked sleep deprivation as well.¹

Before the Apollo 15 mission, there was a predisposition for a significant magnesium ion deficit, which may persist for several months, since training occurred “in intense summer heat.”²,³ This deficit would be compounded by a magnesium deficit secondary to skeletal muscle atrophy resulting from even this brief space mission (12 days).² This conceivably could predispose to the serious arrhythmias and potassium deficits,² potential catecholamine elevations with enhanced thrombus formation, and potential endothelial injuries of both peripheral and coronary vessels.³,⁴

With a water deficit, in addition to compensatory renin-angiotensin elevations,² there is a loss of protection from increased free radicals (superoxide anions),⁵ which inactivate nitric oxide⁶ conducing to endothelial injuries and in turn to vasospasm.³

The Apollo 15 space syndrome, characterized by extremely painful swollen fingertips possibly secondary to peripheral vasospasm and compression by fluid, trapped distally, could serve as a warning that coronary vasospasm (possibly silent)⁶ might also exist, predisposing ultimately to a myocardial infarction with or without an associated atherosclerotic plaque rupture, even without radiation effects playing a role.²,⁷

Finally, this syndrome may be ultimately more common on longer space missions (with invariable angiotensin elevations² and potential imbalance with nitric oxide reductions and more frequent magnesium ion deficits²), portending serious endothelial dysfunction.³ Since young women retain magnesium better on marginal magnesium intakes than young men,² and estrogens have been shown to have several cardiovascular protective effects,¹⁰ it is tempting to speculate that young female astronauts are far less likely to experience this syndrome.

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Whence Cometh Neointimal Myofibroblasts?
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