

Leading article

Platelet dysfunction: a new dimension in inflammatory bowel disease

Until recently, interest in the role of platelets in inflammatory bowel disease (IBD) was confined to an awareness of thrombocytosis as a marker of disease activity,¹ and as a possible predisposing factor to systemic thromboembolism. Two recent conceptual advances suggest that platelets may contribute to the pathogenesis of IBD at the mucosal level. Firstly, it is now recognised that platelets themselves participate in the inflammatory response by acting as a potent source of inflammatory mediators and modulating the activity of other inflammatory cells.² Secondly, multifocal microinfarction in the mesenteric vasculature has been proposed as an early event in the pathogenesis of Crohn's disease³; this hypothesis has drawn attention again to earlier speculation about a vascular origin for ulcerative colitis.^{4,5} We discuss here the evidence for a pathogenic role for platelets in IBD, and possible therapeutic implications of this.

Platelets as inflammatory cells

Platelets are capable of directly eliciting an inflammatory response. Injection of extracts of platelets into the skin of healthy volunteers produces a strong inflammatory reaction – rubor, dolor, calor, and turgor – which persists for several hours.⁶ Extracts of neutrophils and basophils fail to produce this reaction and eosinophils give only an early histamine-like response. Over the last two decades, the proinflammatory properties of platelets have gradually been elucidated²; those which may be important in IBD are described.

RELEASE OF INFLAMMATORY MEDIATORS

Activated platelets release a range of inflammatory mediators including platelet activating factor (PAF), thromboxane (TX), 12-hydroxyicosatetraenoic acid (12-HETE), platelet factor 4 (PF4), serotonin, platelet derived growth factor (PDGF), and transforming growth factor beta (TGF β). Platelets are also capable of producing oxygen free radicals, via IgE receptor mediated activation.⁷ As well as contributing to chemoattraction and activation of other inflammatory cells (see below), some of these platelet derived mediators may increase vascular permeability and modulate vascular tone.^{8,9}

RECRUITMENT AND CHEMOTAXIS OF OTHER INFLAMMATORY CELLS

Activated platelets are capable of promoting recruitment of neutrophils by surface expression of P-selectin, the specific adhesion molecule for neutrophil attachment.¹⁰ Release of PF4, 12-HETE, PDGF, and TGF β causes chemoattractant of neutrophils, monocytes, and eosinophils.¹¹⁻¹⁴ PF4, once discharged from activated platelets, is rapidly taken up by endothelial cells¹¹ and may be important in directing neutrophils to the endothelium prior to

diapedesis. Serotonin promotes neutrophil adhesion to endothelium¹⁵ and activated platelets may themselves directly induce endothelial cell secretion of interleukin-8 which enhances neutrophil diapedesis.¹⁶

MODULATION OF ACTIVITY OF OTHER INFLAMMATORY CELLS

PAF production by neutrophils is greatly enhanced in the presence of platelets.¹⁷ Platelets and neutrophils cooperatively synthesise a range of chemoattractant lipoxigenase products which cannot be made by either cell type alone.¹⁸ Platelet derived 12-HETE enhances monocyte procoagulant activity.¹⁹ PDGF and TGF β promote mitogenesis in fibroblasts^{20,14} and may thereby stimulate local fibrosis and angiogenesis.

Platelets in vascular disease

In view of the compelling evidence that abnormalities within mesenteric blood vessels contribute to the pathogenesis of IBD,³⁻⁵ it is of interest that abnormal platelet behaviour is seen in other disorders in which vascular pathology is a feature.

In ischaemic heart disease, for example, increased platelet aggregation *in vitro*^{21,22} and increased mean platelet volume²³ predict coronary events and mortality. Moreover, antiplatelet therapy is of proved benefit in reducing morbidity and mortality.^{24,25} Platelets have also been implicated in the pathogenesis of vascular disease associated with diabetes mellitus. Increased platelet aggregation *in vitro*, serum β thromboglobulin²⁶ and platelet activation (as indicated by expression of the surface markers P-selectin and GP53)²⁷ have all been reported.

Evidence for abnormal platelet function in IBD

That platelets can behave as inflammatory cells and that mesenteric microinfarction may have a role in the pathogenesis of Crohn's disease provide the basis for the hypothesis that platelets may participate in the inflammatory process in the intestinal mucosa of patients with IBD.

Laboratory assessment of platelet behaviour can be compromised by the susceptibility of platelets to *in vitro* activation. Measurement of platelet size and survival are also subject to artefact and require careful standardisation and interpretation. Recent work using more reliable methods has provided increasing evidence for abnormalities of platelet function and morphology in IBD.

PLATELET AGGREGATION IN VITRO

Traditional Born aggregometry²⁸ allows *in vitro* assessment of platelet response to aggregating agents. Spontaneous platelet aggregation²⁹⁻³³ has been documented in patients with IBD including those in remission,

and increased sensitivity to adenosine diphosphate, arachidonic acid, ristocetin, collagen, and thrombin³²⁻³⁴ has also been described.

PLATELET AGGREGATE FORMATION IN VIVO

Circulating platelet aggregates in venous blood have been identified in active Crohn's disease and ulcerative colitis.³⁴ Histological examination of rectal biopsies reveals intra-capillary platelet aggregates in patients with ulcerative colitis, Crohn's disease and also in those with self-limiting infective colitis³⁵ but not in control (normal) biopsies.

ACTIVATION DEPENDENT PLATELET SURFACE ANTIGENS P-SELECTIN AND GP53

The functional status of circulating platelets *in vivo* can be assessed using single cell flow cytometry. The glycoproteins P-selectin (the neutrophil adhesion molecule) and GP53 (whose function is unknown) are extruded to the platelet surface membrane during activation and can be detected using specific fluorescent antibodies. Increased expression of these markers is seen in peripheral venous blood in Crohn's disease and in ulcerative colitis, independently of disease activity.^{36 37} P-selectin expression is greater in finger-tip capillary blood than in venous blood in normal individuals; this difference is exaggerated in patients with Crohn's disease, implying that their platelets are more susceptible to activation in the microcirculation.³⁸

SERUM PROTEINS DERIVED FROM PLATELET GRANULES

β Thromboglobulin (β TG) and PF4 are discharged from alpha granules at activation and can be measured in serum using radioimmunoassay or enzyme linked immunosorbent assay. Standardised venesection technique and handling of samples is important to avoid falsely raised levels of these proteins due to *in vitro* platelet activation. β TG and PF4 are discharged from platelets at similar rates but *in vivo* PF4 is rapidly taken up by endothelium; artefactually high levels may therefore be identified by examining the β TG/PF4 ratio for each specimen.³⁹ Increased serum concentrations of these two markers individually have been reported in ulcerative colitis and Crohn's disease both in remission and relapse^{33 40}; the results imply either increased platelet activation *in vivo* or a reduced threshold for activation *in vitro*.

PLATELET SIZE

Automated counters routinely produce a value for mean platelet volume. However, time dependent shape, and hence volume, changes occur after venesection and this varies with the anticoagulant used.⁴¹ The mean platelet volume, assessed by validated methods, and the platelet count are normally inversely related⁴²; studies on the mean platelet volume in IBD using various methods have shown smaller mean platelet volume compared with controls,^{43 44} consistent with the increased platelet count seen in IBD subjects.

PLATELET SURVIVAL

Standardisation of the measurement of platelet survival in healthy subjects and in disease states using radiolabelled platelets is controversial. The first report of platelet survival in IBD showed a decreased half-time⁴⁵ and a more recent study showed a similar trend.³³ Since the platelet count is raised in active IBD, these results suggest a greatly increased rate of platelet production.

Why are platelet function and morphology abnormal in IBD?

There are several possible mechanisms for increased platelet activation in IBD. Endothelial cell damage in the mesenteric vasculature exposes basement membrane collagen, to which platelets are exquisitely sensitive, and this could trigger platelet activation. The increase in serum von Willebrand factor (a marker of endothelial cell damage) found in IBD, in Crohn's disease independently of disease activity, is consistent with this possibility.⁴⁶ Release of PAF⁴⁷ and thromboxane A₂³³ from platelets or activated neutrophils into the mesenteric circulation at the site of intestinal disease could perpetuate or amplify platelet activation; monocytes and neutrophils activated by endotoxin⁴⁸ or other absorbed bacterial products could also stimulate platelets.⁴⁹ Virus induced changes in the endothelium could promote platelet adherence and activation *in vitro* before any detectable endothelial cell disruption⁵⁰; this observation is particularly interesting in the light of the recent report of persisting measles infection in foci of granulomatous vasculitis in Crohn's disease tissue.⁵¹

The evidence relating to platelet count, size, and survival is as yet more difficult to explain. Circulating platelet count is the net result of the balance between rates of production and destruction. Factors responsible for stimulating thrombopoiesis include interleukins 3 and 6, and thrombopoietin,⁵² and circulating interleukin 6 at least is raised in active Crohn's disease.⁵³ The published data suggesting that, despite being activated, platelets in IBD are small^{33 43 44} presents a paradox: in ischaemic heart and diabetic vascular disease, for example, platelets are activated but large,^{23 27} consistent with previous evidence that the reactivity of subpopulations of platelets correlates positively with their volume.⁵⁴ Platelet size is probably determined at the time of thrombopoiesis; no significant change takes place during the time the individual platelet spends in the circulation.⁵⁵ Determination of platelet size is complex and is influenced by megakaryocyte volume and ploidy.⁵⁶ Platelet destruction rate, platelet thromboxane production, and the bleeding time contribute to the complex feedback mechanism which determines the properties of megakaryocytes⁵⁶; different profiles of these variables in IBD and ischaemic heart disease may account for the contrasting mean platelet volume in these diseases. Alternatively, reduced mean platelet volume in the peripheral circulation in IBD could be explained by consumption or sequestration of large activated platelets in the intestinal vasculature. Although such a phenomenon would contribute to reduced platelet survival, attempts to image sequestration in the mesenteric vasculature in IBD using radiolabelled platelets have proved inconclusive so far.³³

Pathogenetic consequences of abnormal platelet function in IBD

Activated hyperaggregable platelets may contribute to the pathogenesis of the mucosal lesion in IBD by local release of inflammatory mediators, and chemoattraction and activation of other inflammatory cells, as described earlier. Platelet phospholipid membrane is the primary site for assembly of the coagulation factors essential for the generation of thrombin and consequently fibrin⁵⁷; it is therefore possible that a platelet abnormality underlies the procoagulant state observed in IBD. Expression of P-selectin on the platelet surface membrane promotes neutrophil accumulation and fibrin deposition at the site of vascular injury.⁵⁸ Circulating platelet aggregates could precipitate ischaemic damage by occluding intestinal

microvasculature; platelet derived thromboxane A₂ could exacerbate ischaemia by inducing local vasoconstriction. PDGF and TGFβ may be important in stricture formation, particularly in Crohn's disease, as well as in the physiological repair mechanisms which follow tissue damage. Platelet aggregate derived PDGF in the digital microcirculation has been implicated in the aetiology of finger clubbing in patients with right to left haemodynamic shunts⁵⁹ and could contribute to clubbing in Crohn's disease.⁶⁰

Therapeutic implications of platelet dysfunction in IBD

If platelet dysfunction does play a major role in the pathogenesis of IBD, agents which reduce platelet activity should prove useful in its treatment. Indeed, selective antithromboxane agents (whose effects in vivo include inhibition of platelet aggregation) suppress inflammation associated with the trinitrobenzene-sulphonic acid model of colitis, endotoxin induced intestinal damage, and NSAID induced small bowel ulceration in rats.⁶¹⁻⁶³

Sulphasalazine inhibits platelet aggregation, at least in vitro⁶⁴; however, the high concentration required for this action (IC₅₀ 2 mM) and its many other pharmacological effects,⁶⁵ preclude the conclusion that sulphasalazine works in vivo primarily by this mechanism.

Low dose aspirin also inhibits platelet aggregation by reducing platelet TX synthesis and may be worth assessment in IBD; higher doses, however, as with NSAIDs, may have a detrimental effect by inhibiting prostaglandin synthesis, enhancing leucotriene synthesis, or through other actions.^{66 67} Specific dual TX synthesis inhibitors and receptor antagonists are now available for use in man. Ridoqrel (Janssen), in a preliminary study, seemed to benefit patients with ulcerative colitis⁶⁸ and, like Picotamide (Samil Gruppo), an agent in use in Europe for treatment of arterial vascular disease for some years, is now undergoing clinical trial in IBD.

Conclusion

There is now good evidence that platelet function is abnormal in Crohn's disease and ulcerative colitis, independently of disease activity. Confirmation that increased platelet activity contributes to the pathogenesis of the mucosal lesion in IBD awaits the results of therapeutic trials with specific antiplatelet agents. A successful outcome to such clinical studies would add a new dimension to the treatment of patients with IBD.

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