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Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer

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Introduction

Circulating endothelial cells (CECs) were first detected in the 1970s although convenient techniques to isolate them have only recently become available [1]. These cells have now been shown to be present in a variety of vascular disorders but only a few reports have appeared since the mid-1990s. Very recently we were able to demonstrate grossly elevated numbers of CECs as a novel marker of disease activity in patients with ANCA-associated vasculitis [2]. It appears that this approach to vascular injury has not been appreciated so far and that its use should be evaluated in other vascular disorders as well.

Circulating endothelial cells

Early on, CECs were detected in smears of peripheral blood on the basis of their presumed morphology [3]. Endothelial cells were thus shown to be present in peripheral blood from patients with various vascular disorders, such as myocardial infarction. The next achievement in terms of methodology was to employ immunocytochemistry of peripheral blood smears, as demonstrated in patients with sickle-cell anemia [4]. Yet with this technique some endothelial cells and smaller particles may never appear on the slide. Immunomagnetic isolation has recently appeared as a novel, and possibly superior, alternative [1]. Briefly, endothelial cells are isolated from whole blood by virtue of magnetic particles coated with anti-endothelial antibodies. This technique, which may also be employed to isolate microvascular endothelial cells from tissue samples, was first used in myocardial infarction and rickettsiosis [5]. With regard to their phenotype, CECs may differ considerably depending on the type of underlying disorder [1]. For instance, sheets of relatively intact cells have been isolated in patients with acute coronary syndromes whereas severely damaged necrotic cells, membrane fragments and smaller particles were detected in inflammatory disorders. Further analysis of the cell phenotype, such as detection of rickettsial antigens, is cumbersome but certainly feasible [5].

Where studied, numbers of CECs were indicative of, and correlating with, the degree of endothelial injury [4]. We therefore became interested in CECs

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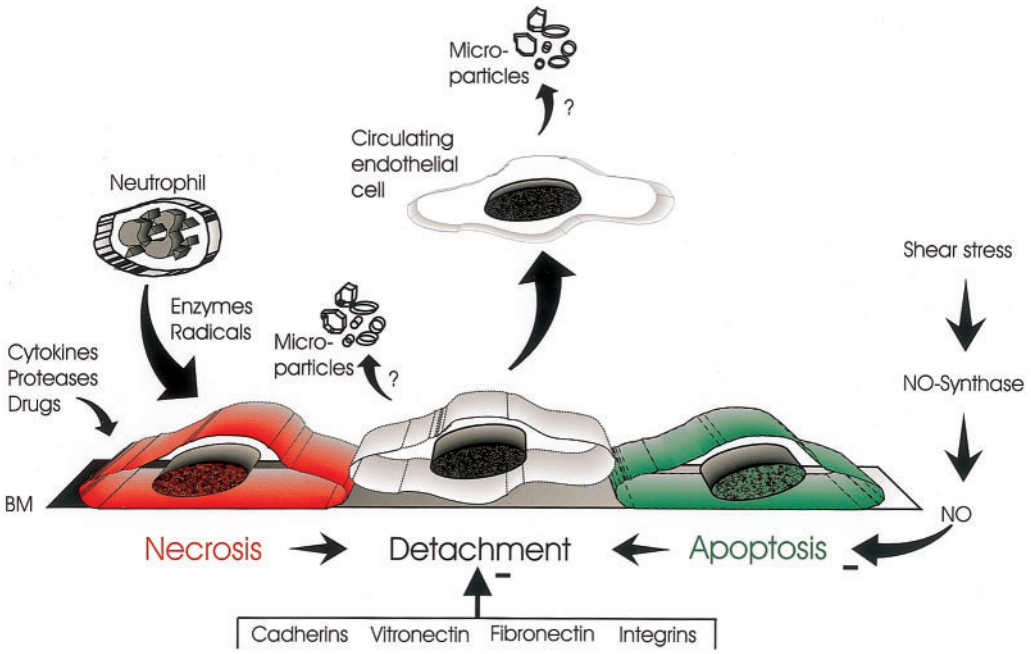

Fig. 1. Endothelial necrosis or apoptosis, and detachment. BM, basement membrane; NO, nitric oxide.

as a possible marker of disease activity in ANCA-associated vasculitis, more so since ANCA titres have performed less well than initially expected. In our study [2], high numbers of CECs (>100 per ml) were detected in patients with active vasculitis; cell numbers declined progressively during the course of successful immunosuppressive treatment. Moderately elevated numbers of CECs were detected in blood obtained from patients in remission. We concluded that the number of CECs is a promising new marker of active ANCA-associated small-vessel vasculitis. Its role in a clinical scenario now remains to be evaluated in further studies.

Life, death and detachment of the endothelial cell layer

Vessels had been regarded as mere tunnels through tissues until Friedrich Daniel von Recklinghausen discovered a cellular lining [6]. These endothelial cells had initially been regarded as a tapestry with few, if any, functional properties. Since then, an ever-increasing array of endothelial cell functions has been reported. Labelling studies estimated turnover times from 47 to over 23 000 days [7] and endothelial proliferation appears to be clustered at sites of vessel branching [8]. In contrast, laminar flow and shear stress suppress endothelial cell apoptosis by virtue of several pathways [9]. On average, 99% of endothelial cells are quiescent and very few CECs are thus detectable in healthy volunteers. Current enumerations are in the range of 5 cells/ml blood while the phenotype of these cells remains unknown.

Mechanisms of endothelial detachment are also poorly understood. In inflammatory disorders various factors such as direct neutrophil attack, cytokines and proteases [10] may play a role. Drugs, such as calcineurin inhibitors [11], are also capable of detaching endothelial cells. In contrast, neighbouring cells and anchorage to extracellular matrix, possibly mediated by vitronectin, fibronectin, cadherins, as well as integrins, are crucial to endothelial cell survival (Fig. 1) [12]. Moreover, one would be curious as to the fate of CECs and would speculate on the possible role of a clearance mechanism, for example in liver or pulmonary capillaries. Finally, one might wonder whether necrotic endothelial cells cause an inflammatory response in their own right, more so after reports that necrotic cells are generally able to interact with various cell subsets [13].

Mechanisms of endothelial repair: progenitor cells for rescue

Local migration and proliferation of endothelial cells adjacent to the site of injury had been regarded as the principal mechanism of endothelial repair until Asahara and colleagues, in 1997, described circulating endothelial progenitor cells (EPCs) [14]. These cells are bone marrow-derived and have the capacity to home in on sites of endothelial injury. Here they incorporate into the endothelium and thereby repair the defects [15]. Transplantation is a promising scenario to study these events since endothelial cells of donor and recipient origin can be distinguished by various techniques. Accordingly, chimerism of the endothelial cell layer has already been demonstrated in renal

transplant recipients with a history of vascular rejection [16]. These findings corroborate the concept that progenitor cells invade the graft as a sequel to widespread endothelial injury. Animal models have also served to elucidate the importance of EPCs in myocardial ischaemia. In this setting increased neovascularization by EPCs improves cardiac function after myocardial infarction [17] and clinical outcomes are closely correlated with the number of mobilized EPCs [18]. Recent work has also elucidated the effects of drugs, particularly statins [19], and a therapeutic use of EPCs is currently under evaluation. An unexpected degree of graft chimerism involving not only endothelial cells was very recently observed in heart transplant recipients [20]. These findings provide compelling evidence that the 'repair squad' concept outlined here applies to a broad variety of tissues.

Perspectives

From a clinician's point of view, CECs are a promising, albeit non-specific, marker of endothelial injury. Further study of the cell phenotype may help to elucidate the pathogenesis of some vascular disorders. Interactions of circulating necrotic endothelial cells with healthy endothelium remain rather speculative at present. EPCs have a pivotal role in response to intimal injury. Their action may be amenable to therapeutic intervention. It is conceivable that concurrent enumeration of CECs and progenitor cells provides a clue to chronicity and extent of vascular injury. Taken together, these new tools provide a fascinating approach to mechanisms of vascular injury and repair.

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