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Review

Counting the cost: markers of endothelial damage in hematopoietic stem cell transplantation

A Woywodt[1], M Haubitz[1], S Buchholz[2] and B Hertenstein[2]

[1]*Division of Nephrology, Department of Medicine, Hannover Medical School, Hannover, Germany; and* [2]*Division of Haematology/Haemostasis/Oncology, Department of Medicine, Hannover Medical School, Hannover, Germany*

Summary:

During hematopoietic stem cell transplantation (HSCT), endothelial damage is the pathological hallmark of veno-occlusive disease of the liver, thrombotic microangiopathy, capillary leak syndrome and graft-versus-host disease. Events prior to conditioning, the conditioning regimen itself as well as calcineurin inhibitors may all induce endothelial damage. Unfortunately, the relative importance of these factors and their interactions, the time frame of endothelial damage and individual susceptibility remain unknown. Moreover, it is conceivable that conditioning regimens differ markedly in their propensity to initiate endothelial damage. Monitoring endothelial damage and response to treatment is hampered by the current lack of suitable markers. In this regard, an ideal marker should be sensitive and specific and indicate the development of an endothelial disorder prior to the onset of symptoms and organ dysfunction. Soluble markers, such as thrombomodulin, are easily amenable with immunoassays; yet, the interpretation of their levels is hampered by the influence of comorbidity. Evaluation of circulating endothelial cells in HSCT demonstrated a marked and dose-dependent increase in cell numbers after conditioning. The challenge ahead is to establish and evaluate novel markers of endothelial damage to permit early detection of disease, monitor response to treatment and evaluate different conditioning regimens.

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Endothelial damage is a salient feature of atherosclerosis and inflammatory disorders, such as endothelial infection or vasculitis. An ever increasing body of evidence has delineated mechanisms of plaque evolution and rupture, pathways of leukocyte-endothelial interactions and models for the pathogenesis of endothelial injury. However, markers of endothelial damage for use in a clinical scenario are only just becoming available. In this regard, detection of circulating endothelial cells has been a considerable advance.[1] Elegant techniques to isolate these cells, such as immunomagnetic isolation, have recently become available and provide valid data. We have already demonstrated the use of circulating endothelial cells in vasculitis[2] and renal transplant recipients.[3,4] More recently, we were able to demonstrate the presence of elevated numbers of circulating endothelial cells in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT).[5] In this mini-review, we discuss circulating endothelial cells, endothelial microparticles and soluble markers of endothelial damage with an emphasis on HSCT. We discuss our findings in the context of endothelial damage and repair, review current concepts of endothelial damage during HSCT and propose future avenues of research.

Correspondence: Dr A Woywodt, Division of Nephrology, Department of Medicine, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany; E-mail: Woywodt.Alexander@MH-Hannover.de

Biology of healthy endothelium: turnover and repair

Healthy endothelium has a surface of 400 m^2, weighs 1.5 kg and contains some 1.2 trillion endothelial cells. However, the idea that endothelial cells simply provide a tapestry for vessels has been abandoned. Indeed, the endothelium is a highly selective barrier for macromolecules and a non-thrombogenic surface that maintains the fluidity of blood. Endothelial cells control the tone of vascular smooth muscles via nitric oxide, maintain different levels of coagulation depending on functional requirements and exert immune capabilities through interaction with circulating leukocytes.

Little is known about endothelial turnover in healthy subjects. Endothelial proliferation appears to be clustered at sites of vessel branching, while laminar flow has been shown to suppress endothelial apoptosis.[6] On average, 99% of endothelial cells are quiescent within the intima of vessels and very few circulating endothelial cells are detected in healthy volunteers. Enumerations are between 1 and 5 cells per ml while the phenotype of these cells remains ill defined. An apoptotic mode of cell death must be assumed, although mechanisms of endothelial detachment in healthy endothelium remain enigmatic. Factors that propagate endothelial detachment or protect against it are discussed elsewhere.[7]

Table 1 Markers of mature endothelial cells and endothelial progenitor cells

Mature endothelial cells	*Endothelial progenitor cells*
CD146	KDR
von Willebrand factor	TIE-2
CD31	VEGF receptor
Ulex Europaeus lectin 1 binding	AC-133
Bandeirea simplicifolia lectin binding	CD34
Griffonia simplicifolia lectin binding	Uptake of acetylated low-density lipoprotein
CD105	
Vascular endothelial cadherin	
Weibel–Palade bodies	

Much has been learned about the biology of endothelial repair. For long, local proliferation had been regarded as the principal mechanism until in 1997 endothelial progenitor cells (EPCs) were first described. These cells display stem cell and endothelial markers, such as tunica interna endothelial cell kinase 2 (Tie-2), and receptors for the kinase insert domain receptor (KDR) and vascular endothelial growth factor (VEGF). Table 1 summarizes the traditional markers of mature circulating endothelial cells and EPCs. More recently, evidence has emerged to suggest that EPCs are indeed part of the CD14 lineage.[8]

Endothelial progenitor cells must respond to endothelial damage via pathways that have only just emerged. One such mechanism is the release of erythropoietin (EPO), which has been shown to enhance EPCs in animal models and human studies.[9–11] The occurrence of vascular disease is thus believed to be the result of a balance between vascular events and endothelial repair. Accordingly, the inability to mobilize these progenitor cells has been postulated to be a risk factor for cardiovascular disease[12] and treatment with progenitor cells prevents atherosclerosis in a murine model.[13] Transplantation is a particularly promising field to study these events. In particular, demonstration of the Y chromosome in female recipients of renal allograft from male donors has been used to demonstrate endothelial chimerism.[14] These findings indicate that bone marrow-derived EPCs invade the graft in response to local, presumably immune-mediated, injury. Similar results have been reported in heart transplantation[15] although the importance of *trans*-differentiation has been questioned.[16] Interestingly, statins appear to increase EPCs.[17] Preliminary studies in HSCT suggest that EPCs are transplantable.[18,19] Accordingly, endothelial chimerism was demonstrated in an autopsy study of five patients who had received HSCT.[20]

Clinical syndromes of endothelial damage associated with HSCT

The notion that events during HSCT affect endothelial cells is not new.[21] Endothelial disorders associated with HSCT can be divided into acute and chronic syndromes. The former group chiefly encompasses thrombotic microangiopathy,[22] veno-occlusive disease (VOD)[23] and capillary leak syndrome (CLS),[24] while chronic endothelial damage has been implicated in chronic graft-versus-host disease (GVHD).[25]

Transplantation-associated thrombotic microangiopathy is a puzzling disorder that continues to frustrate clinicians ever since its first description some 22 years ago. Many features of the disease, notably microangiopathic hemolytic anemia, are common to all forms of thrombotic microangiopathy.[26] There are, however, some unique features, not least the lack of correlation between laboratory abnormalities and clinical symptoms. Currently, multiple insults to microvascular endothelial cells during the conditioning phase, such as radiation and chemotherapy, are believed to initiate HSCT-associated thrombotic microangiopathy.[21] Severe deficiency of von Willebrand factor cleaving protease can be found in many patients diagnosed with thrombotic thrombocytopenic purpura, but it is rare in patients with HSCT-associated thrombotic microangiopathy. Further uncertainty exists with regard to the management of the disorder. Plasma exchange, a treatment option that works well with many other forms of thrombotic microangiopathy, is often ineffective: large-scale studies are nonexistent and case series have documented less than encouraging results.[21] More recently, defibrotide has been used with the idea of modifying procoagulant surfaces. Protective effects of defibrotide against fludarabine-induced apoptosis of endothelial cell have been demonstrated.[27]

VOD is the second common form of endothelial injury associated with HSCT[23] and liver is most commonly affected. VOD of the liver presents with hepatomegaly, ascites and weight gain. VOD appears to be a disorder of sinusoidal endothelial cells and histology demonstrates sinusoidal fibrosis, necrosis of pericentral hepatocytes and fibrosis of central veins.[28] Early changes of sinusoid endothelial cells have been studied in a toxin-induced model of the disease and include loss of fenestration and the appearance of gaps within the endothelial cell layer. Subsequently, erythrocytes penetrate into the space of Disse and the sinusoidal lining disintegrates with embolization downstream.[29] Various factors, such as aggressive chemotherapy, previous radiation of the liver, pre-existing liver disease and hypercoagulability, have been proposed to damage sinusoid endothelial cells.[28] The incidence of the disease has been reported to decline in recent years and a variety of reasons, most notably the employment of less aggressive conditioning regimens, have been implicated. Again, treatment is an area of uncertainty. Symptomatic measures are often sufficient, whereas defibrotide is used in severe cases.[30] VOD of the lung[31] is rare but feared. The pathologic hallmark of pulmonary VOD is extensive and diffuse occlusion of pulmonary veins by fibrous tissue. Thus, the disorder has previously been described as a venous form of pulmonary hypertension.

CLS is another poorly understood disorder with weight gain, edema, ascites and effusions. CLS occurs in both allogeneic and autologous HSCT and a pivotal role of circulating leukocytes has been proposed.[24] Elevated serum levels of proinflammatory cytokines, such as tumor necrosis factor alpha, have also been reported.[32] As with VOD,

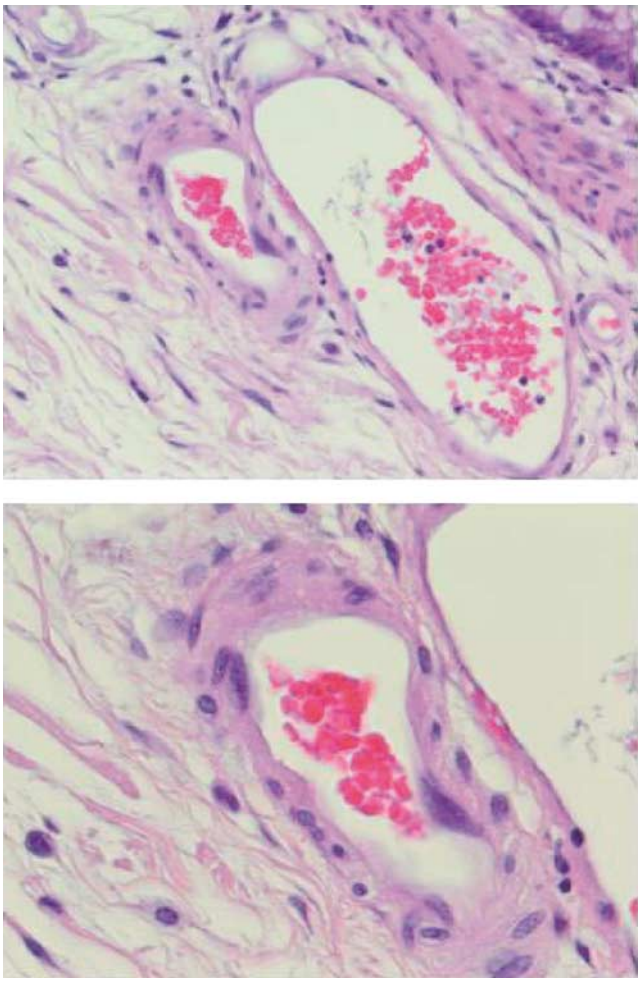

Figure 1 Intestinal venules in a patient with severe endothelial injury during HSCT; note the activated endothelial cells (courtesy of Michael Mengel, MD, Department of Pathology, Hannover Medical School).

high-intensity conditioning is a documented risk factor for CLS.[33]

Finally, chronic GVHD has been linked to endothelial damage in recent studies. Biedermann and colleagues demonstrated that donor cytotoxic T lymphocytes target host endothelial cells. In particular, they demonstrated endothelialitis and loss of dermal microvessels in cutaneous GVDH and found elevated plasma levels of soluble von Willebrand factor.[25] In analogy, Salat *et al*[34] detected elevated plasma levels of thrombomodulin and soluble von Willebrand factor in acute GVHD. In this regard, intestinal GVHD has not been studied to a similar extent. However, the importance to distinguish between intestinal GVHD and intestinal thrombotic microangiopathy has been emphasized very recently.[35]

In summary, clinical syndromes underscore the role of the endothelium as a target organ during HSCT (Figure 1). Chemotherapy, irradiation, graft-versus-host prophylaxis and disease as well as cytokines[36] have all been invoked to explain endothelial damage. Table 2 provides a summary of endothelial syndromes during HSCT.

Table 2 Clinical syndromes of endothelial damage

Syndrome	*Organ(s) involved*	*Signs and symptoms*
Veno-occlusive disease	Liver (less frequently intestine, rarely lung)	Hepatomegaly, weight gain, ascites, deterioration of liver function tests
Thrombotic microangiopathy	Blood, central nervous system, kidney	Seizures, renal failure, hemolytic anemia
Capillary leak syndrome	Entire vasculature	Edema, effusions, weight gain, ascites
GvHD	Skin, intestine	Chronic skin changes, diarrhea

Soluble markers of endothelial damage

A number of proteins are more or less specific to endothelial cells,[37] yet only some are shed from the cell surface. Among these, soluble thrombomodulin, von Willebrand factor and E-selectin are most commonly employed for the evaluation of endothelial damage. Thrombomodulin is expressed by endothelial cells and serves as a receptor for thrombin whereby the thrombin/thrombomodulin complex catalyzes activation of protein C.[38] Circulating levels of thrombomodulin depend on a host of factors, such as liver disease, viral infection, the use of heparin[39] and renal function.[40] These effects render interpretation of thrombomodulin levels exceedingly difficult. Soluble von Willebrand factor has long been proposed as another reliable marker of endothelial damage.[41] The high-molecular-weight procoagulant protein is stored in both intracellular Weibel-Palade bodies and platelet alpha granules and is released upon stimulation by a variety of factors. As with thrombomodulin, several conditions, most notably liver disease, affect plasma levels.[42] E-selectin (CD62E) is specific to endothelial cells and a soluble form has been described. Raised levels have been described in a variety of diseases, such as hypertension[43] and diabetes. However, correlation with other endothelial markers, such as von Willebrand factor, is poor and the value of E-selectin has thus been questioned.[41]

In summary, the use of soluble markers is considerably hampered by a host of factors, such as the influence of renal function. Notably, some degree of renal insufficiency is common during the course of HSCT. Finally, all these markers are, at least to some degree, released during endothelial activation and it is therefore difficult to discriminate activation from damage.[44] Novel markers of endothelial damage are therefore eagerly awaited.

Endothelial microparticles

Endothelial microparticles have recently emerged as markers of activation in eucaryotic cells. They are usually detected by virtue of annexin staining and size. Resulting from exocytic budding, these vesicles consist of cytoplasmic components and phospholipids. It must be emphasized that generation of these particles is not specific to endothelial cells. Instead, microparticles may also derive from platelets, vascular smooth muscle cells, leukocytes and other cell subsets. More recently, attempts have been made to isolate

endothelial microparticles.[45] Endothelial microparticles are capable of inducing a procoagulant state[46] and tissue factor has emerged as an important pathway for these events.[47,48] Interestingly, we have demonstrated tissue factor–positive, circulating endothelial cells in vasculitis.[2] These findings suggest that endothelial tissue factor is a procoagulant mechanism in inflammatory disorders. Furthermore, microparticles are actively involved in inflammatory processes.[49] Taken to the extreme, massive production of microparticles in meningococcal sepsis could well be an important cause of disseminated intravascular coagulation (DIC).[50] Endothelial microparticles have also been detected in patients with acute coronary syndromes. Here, they have been proposed to cause intracoronary thrombi downstream from the vulnerable plaque.[51] Endothelial microparticles may also be involved in target organ damage in severe hypertension,[52] diabetes[53] and the procoagulant state in pregnancy.[54] Microparticles are only just beginning to be evaluated as markers in vasculitis.[55] One study reported an increase of platelet-derived microparticles in patients with chronic GvHD.[56] The evaluation of platelet- and endothelial cell-derived microparticles could, we believe, complement the use of circulating endothelial cells. In particular, kinetics of appearance and clearance from peripheral blood may well differ between entire cells and microparticles.

Circulating endothelial cells: methods of isolation

Circulating endothelial cells were first isolated in the 1970s when detection rested upon the presumed morphology of these cells.[57,58] Immunomagnetic isolation has recently appeared as an alternative. Briefly, endothelial cells are isolated from whole blood with magnetic particles (Dynabeads™) coated with anti-endothelial antibodies, such as anti-CD146 (Figure 2). This antigen is believed to be a specific marker of endothelial cells[59] and the protein is involved in cell–cell cohesion[60] and cytoskeleton rearrangement.[61] Recently, concern has been voiced that EPCs or pericytes may also express CD146.[62] In our experience, however, cells were AC133 and alpha-smooth-muscle-actin negative.[5]

There are, however, pitfalls of the technique. One main problem is nonspecific binding of leukocytes to antibody-coated beads. We evaluated the effects of various changes in the technique by pursuing several approaches while M-450 Pan Mouse IgG Dynabeads™ without anti-CD146 antibody were used as controls. It turned out that several precautions are necessary to eliminate nonspecific binding, which is believed to occur by means of the Fc receptor.[2] Another caveat is the technique of venipuncture, since we were able to provoke elevated cell numbers after 2 min of to-and-fro movement of the butterfly needle. After immunomagnetic isolation, enumeration of isolated cells by acridine staining remains the standard but necessitates considerable experience. A simpler test would therefore be of great utility. Eventually, we devised a simple protocol for immunomagnetic isolation with subsequent UEA-1 lectin stain.[63]

With regard to their phenotype, circulating endothelial cells may differ depending on the type of underlying disorder.[64] Sheets of intact cells have been isolated in patients with acute coronary syndromes, whereas severely damaged and necrotic cells were detected in rickettsial infection and vasculitis. Cells are in the range of 10–50–70 μm but larger aggregates of cells are also seen. Giant cells have been reported in cytomegalovirus infection.[65]

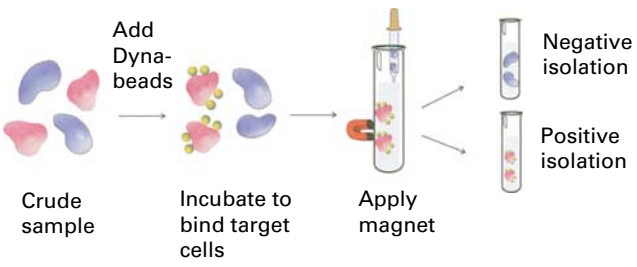

Figure 2 Immunomagnetic isolation.

Circulating endothelial cells in vascular disorders

In an early study, circulating endothelial cells were detected in patients with myocardial infarction and unstable angina.[66] Since then, infectious disorders have been studied as well: In rickettsiosis, *Rickettsia conorii* was demonstrated within cells that had been retrieved by immunomagnetic isolation.[67,68] Likewise, viral antigens were detected on the surface of circulating endothelial cells in CMV infection.[65,69] We became interested in circulating endothelial cells as a possible marker of disease activity in vasculitis, since endothelial damage is the pathological hallmark of these disorders. In our study,[2] high numbers of cells (>100/ml) were detected in patients with active vasculitis; cell numbers declined during the course of treatment (Figure 3).

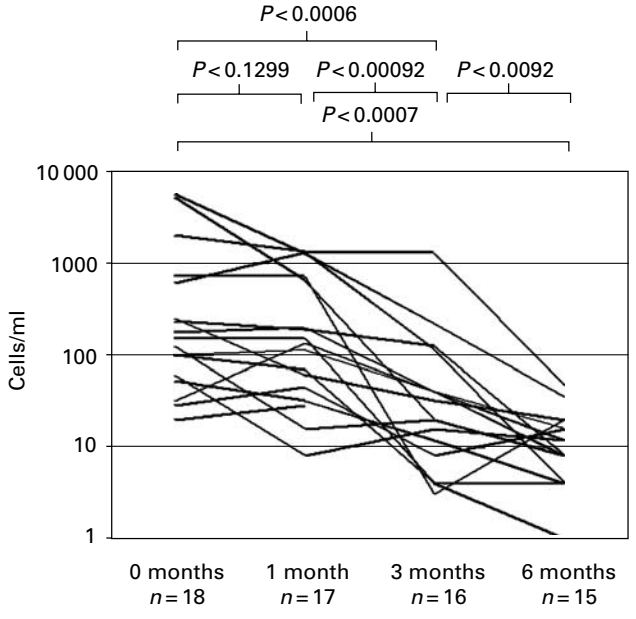

Figure 3 Cell numbers during 6 months of immunosuppressive treatment in patients with small-vessel vasculitis[2] (logarithmic scale).

Controls with infection and nonvasculitic renal disease did not have elevated cell numbers. Interestingly, we demonstrated a necrotic phenotype as evidence of the severity of the inflammatory process. We were interested to demonstrate tissue factor expression by circulating endothelial cells. The implications of our findings in vasculitis are discussed elsewhere.[70] Renal transplantation has provided another exciting opportunity to study vascular disease. Renal transplant recipients display a high incidence of vascular complications. We were able to demonstrate elevated numbers of circulating endothelial cells in renal transplant recipients[71] and noted higher cell numbers with calcineurin inhibitors.[4] These findings lend further support to the hypothesis that calcineurin inhibitors damage microvascular endothelial cells. Studies in patients who receive calcineurin inhibitors for indications other than transplantation as well as monitoring of cell numbers before and after stoppage of such drugs would be worthwhile.

Markers of endothelial damage in bone marrow transplantation

The vast majority of studies into this subject were conducted with soluble markers. Nürnberger and colleagues studied soluble thrombomodulin and plasminogen activator inhibitor type-1 (PAI-1) in relation to transplant-associated complications. In this study, the increase of both markers was correlated with the number of vascular complications (CLS, VOD, sepsis).[72] Along the same line, another study also found increased levels of soluble thrombomodulin in those HSCT patients who developed vascular complications clinically.[73] Luzzatto and co-workers used soluble thrombomodulin and von Willebrand factor to evaluate the effects of HSCT in a pediatric population. In their study, both soluble markers increased after transplantation although the difference to pretransplant values was not significant.[74] Another study evaluated the effects of total body irradiation (TBI) in HSCT and demonstrated that patients who underwent HSCT with TBI as conditioning had higher levels of cGMP as a parameter of endothelial activation than those who received conditioning without TBI. In this study, plasma levels of thrombomodulin were not different between the two groups, leading the authors to assume endothelial activation without frank injury or damage.[75] Catani *et al*[76]also studied thrombomodulin and P-selectin in patients undergoing HSCT. Both markers were normal in all but the one patient who developed severe VOD. Collins *et al*[77] compared soluble von Willebrand factor in patients with autografts and allografts and demonstrated higher levels in allogeneic transplantation. Another study evaluated thrombomodulin and von Willebrand factor in various forms of thrombotic microangiopathy and sought to account for the renal excretion of thrombomodulin by using thrombomodulin/creatinine ratios. In this study, elevated plasma levels of both proteins were documented in classic and HSCT-associated thrombotic microangiopathy. In the latter, plasma levels correlated with severity of the disease.[78]

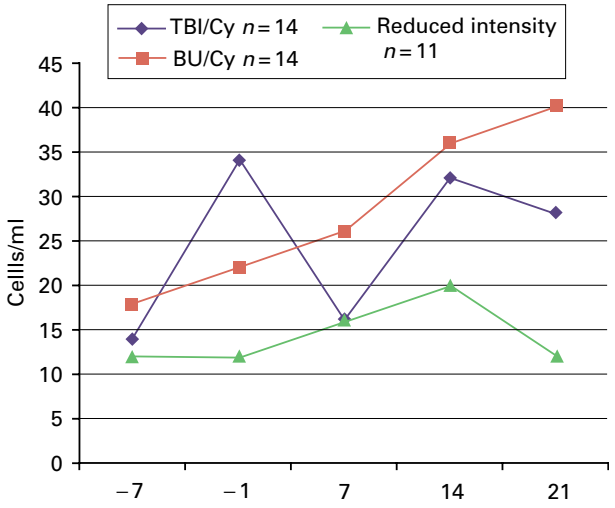

Figure 4 Numbers of circulating endothelial cells during allogeneic HSCT according to conditioning regimens;[5] TBI denotes total body irradiation. BU/CY denotes busulfan/cyclophosphamide.

We have, for the first time, demonstrated elevated numbers of circulating endothelial cells in allogeneic HSCT.[5] After conditioning, cell numbers were significantly elevated (median 44 cells/ml) compared to baseline (median 16 cells/ml) and controls (median 8 cells/ml). Patients with reduced intensity conditioning had significantly lower cell numbers (median 24 cells/ml) than those who received standard conditioning. Patients who received TBI had a brisk rise in cell numbers, while patients who received chemotherapy had more protein elevation (Figure 4). These findings, we believe, are well in line with current concepts of radiation-induced endothelial damage.[79]

Interestingly, cell numbers prior to conditioning were not normal but slightly elevated when compared to healthy controls. In this regard, modestly elevated numbers of circulating endothelial cells in patients with various tumors have been documented[80] and may reflect endothelial apoptosis during tumor angiogenesis.[79] Alternatively, elevated cell numbers prior to conditioning could reflect endothelial damage accrued by previous radiation or chemotherapy.

Moreover, we were interested to note a high variability of cell numbers. We assume that patients display different degrees of vulnerability for the effects of irradiation and chemotherapy. It is conceivable that pre-existing atherosclerosis and age play a role. Moreover, endothelial apoptosis during irradiation depends on a variety of factors such as the nutritional factors and hypoxia. Finally, a genetic background of vulnerability has been suggested.[79]

The effects of radiation on endothelial cells are well documented. In a murine model of endothelial damage in the central nervous system, endothelial apoptosis started after 4 h and peaked after 12 h.[81] In addition, endothelial apoptosis was demonstrated as the pivotal lesion of radiation-induced intestinal damage.[82] This finding was of particular interest, since radiation-induced enteritis had previously been attributed to damaged intestinal stem cells within the crypts of Lieberkuehn.

The effects of cytotoxic chemotherapy on endothelial cells are well documented. Doxorubicin, for example, induces endothelial apoptosis *in vitro*, a process that is prevented by the administration of thalidomide.[83] Earlier studies have demonstrated that induction of apoptosis by doxorubicin is mediated by caspase activation but is Fas/FasL signal pathway independent.[84] Thus, an apoptotic phenotype of circulating endothelial cells must be assumed although this assumption remains unproven at present. Newer therapeutic options may also have profound effects on endothelial cells. For example, use of arsenic compounds has been advocated in acute promyelocytic leukemia.[85] Interestingly, Roboz *et al*[86] have demonstrated a dose- and time-dependent induction of apoptosis in endothelial cells by arsenic trioxide.

Pathophysiology of circulating endothelial cells

One enigma about circulating endothelial cells is their way of detachment from the basement membrane. Factors that may induce detachment as well as protective factors are discussed in detail elsewhere.[7] Another more hypothetical question is whether circulating endothelial cells might be capable of causing an inflammatory response in their own right. Release of substances by circulating necrotic endothelial cells is one pathway by which these cells may gain further importance. High-mobility group 1 (HMGB1) protein is a protein that is released from necrotic cells. After release, HMGB1 binds to RAGE (the receptor for advanced glycation end products) and acts as a potent mediator of inflammation.[87] Other proteins, such as cytochromes, are also released, although their significance remains unclear.[88] Plasma DNA released from necrotic endothelial cells[89] may also cause secondary phenomena. Finally, release of heat shock proteins from necrotic cells has been shown to deliver a maturation signal to dendritic cells.[90] All these proteins may be released from necrotic circulating endothelial cells with a whole array of pathophysiologic consequences. It must be emphasized, however, that such an assumption is entirely hypothetic at present.

There is another pathway by which necrotic circulating endothelial cells could gain pathophysiologic significance. It has been shown previously that cells, such as fibroblasts, are able to sense the presence of necrotic cellular debris in their vicinity.[91] This study demonstrated, for the first time, that necrotic but not apoptotic cells initiate a Toll-like-receptor-2/NFκB-dependent reaction in monocytes and fibroblasts. Moreover, uptake of necrotic cellular material activates macrophages.[92] It is conceivable that healthy endothelial cells or circulating leukocytes react to the presence of necrotic endothelial debris in a similar manner. Internalization of necrotic material by macrophages is also well documented.[93] Recent evidence suggests that mannose-binding lectin[94] and surface phosphatidylserine[95] are involved in this process. Presumably, necrotic endothelial debris undergoes similar mechanisms, which may, in turn, induce other inflammatory signals. Figure 5 provides a summary of possible interactions of circulating endothelial cells with other cell subsets.

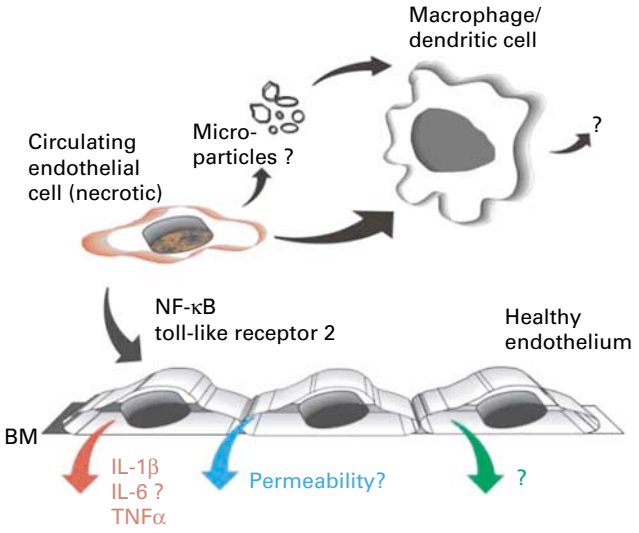

Figure 5 Interactions of circulating endothelial cells with other cell subsets (hypothetic).

Conclusions and avenues for further research

Research into endothelial damage during HSCT has posed more questions than it has answered. Various markers have been employed to study endothelial activation and damage. Soluble markers, such as thrombomodulin, are easily amenable with immunoassays. However, the interpretation of plasma levels is hampered by the influence of comorbidity and the marker may not distinguish between endothelial activation and frank damage. In contrast, detection of circulating endothelial cells implies severe damage. Evaluation of these cells in HSCT demonstrated a marked and dose-dependent increase in cell numbers after conditioning. It is conceivable that higher cell numbers during conditioning predict the subsequent development of endothelial disorders or GVHD. However, our study was neither designed nor powered to answer this question. It must thus be emphasized that the clinical utility of this marker is still under evaluation.

Mechanisms of detachment from the basement membrane also remain enigmatic. Do cells disintegrate into smaller particles *in situ* or after they have been detached from the basement membrane? One would also be curious as to the fate of circulating endothelial cells: Is there a clearance mechanism, for example, in liver, spleen or pulmonary capillaries? Sophisticated studies would be necessary to elucidate such mechanisms. One approach in vasculitis could be selective sampling of venous blood from affected tissues and comparison of cell numbers with mixed venous blood and blood from unaffected tissues.

Moreover, the phenotype of circulating endothelial cells during HSCT is unknown. In vasculitis, cells have been shown to be necrotic in preliminary studies. In HSCT, both necrotic and apoptotic phenotypes are conceivable. Finally, interactions of circulating endothelial cells with healthy endothelial cells, thrombocytes and leukocytes need to be defined.

Table 3 Future avenues of research

Demonstration that elevated numbers of circulating endothelial cells predict subsequent vascular disease or GvHD
Research into the fate of circulating endothelial cells after detachment
Delineation of the interactions of circulating endothelial cells with other cell subsets
Evaluation of the phenotype of circulating endothelial cells during HSCT (apoptotic *vs* necrotic, tissue factor expression, etc)
Evaluation of circulating endothelial cells with the use of novel conditioning regimens
Enumeration of circulating endothelial cells in radiation and chemotherapy (with different drugs)

In spite of all uncertainties, our findings may have therapeutic implications in the future: For example, it is conceivable to screen patients for vascular damage prior to HSCT and offer reduced-intensity conditioning to those who have elevated numbers of circulating endothelial cells. Novel conditioning regimens could be evaluated with respect to their propensity to induce endothelial damage. For example, we are currently evaluating renal transplant recipients with hypertension and vascular damage on renal biopsy; we switch these patients to rapamycin, a drug with supposedly less propensity to cause endothelial damage, and compare numbers of circulating endothelial cells before and after the drug switch. This study is still ongoing. Similar strategies for HSCT recipients with vascular disease and elevated numbers of circulating endothelial cells are conceivable. Other interventions, such as statin treatment,[96] could be evaluated as well with this marker of endothelial damage. Table 3 provides a summary of further avenues of research.

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