Therapeutic uses of autologous endothelial cells for vascular disease

Rajiv GULATI*, Amir LERMAN† and Robert D. SIMARI†‡

*Department of Cardiovascular Medicine, University of Birmingham, Birmingham, U.K., †Division of Cardiovascular Diseases and Internal Medicine, Molecular Medicine Program, Mayo Clinic College of Medicine, Rochester, MN, U.S.A., and ‡Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, U.S.A.

ABSTRACT

Endothelial cells play important structural and functional roles in vascular homoeostasis. Perturbations in endothelial cell number and function are directly involved with the initiation and progression of multiple cardiovascular diseases, including atherosclerosis, hypertension and congestive heart failure. Attempts to modify these disorders have included pharmacological strategies to improve vascular and thus endothelial function. A goal of biological approaches to these disorders is the delivery of endothelial cells that might act to provide beneficial endothelial-derived factors. However, this approach has generally been limited by the lack of readily available autologous endothelial cells for delivery. The isolation of circulation-derived endothelial progenitor cells allows for direct access to autologous endothelial cells for preclinical and clinical studies. Preclinical studies using autologous endothelial cells have demonstrated beneficial effects when delivered in animal models of vascular injury and grafting. These effects are related to the endothelial nature of the cells and may be paracrine in nature. Ongoing studies are aimed at defining the nature of these effects and optimizing delivery strategies cognizant of these mechanisms.

INTRODUCTION

The endothelium plays a privileged role in cardiovascular biology. Previously conceived as a static barrier between tissue and blood, endothelial cells play key regulatory and integrating roles in cardiovascular biology. These roles include the direct regulation of vascular form and function, regulation of transport of solutes to tissue and regulation of an anti-thrombotic interface between tissues and circulating blood. When endothelial number or function is perturbed, there is the potential for significant alterations in vascular and tissue function. In fact, defects in endothelial function or structure are hallmarks of important vascular diseases, including systemic and pulmonary hypertension, atherosclerosis and the exuberant response to vascular injury referred to as restenosis.

Attempts to modulate this wide range of diseases associated with dysfunctional endothelium include the direct delivery of endothelial cells. The goal of such delivery is to provide a cellular source of regulated expression of endothelial-derived factors to modulate the disease process. Attempts to translate this approach have been limited by the lack of suitable autologous sources of endothelial cells. However, new sources of endothelial cells have been recently defined to include circulating mononuclear cells. Thus this concept of cell delivery to modulate vascular disease has witnessed a revival. This review is intended to define opportunities for the therapeutic use of endothelial cell delivery, to

Key words: atherosclerosis, culture-modified mononuclear cell (CMMC), endothelial cell, endothelium, restenosis, vascular disease.

Abbreviations: CHF, congestive heart failure; CMMC, culture-modified mononuclear cell; EPC, endothelial precursor cell; ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; NO, nitric oxide; eNOS, endothelial NO synthase; OEC, outgrowth endothelial cells; PBMC, peripheral blood mononuclear cell; PGI2, prostacyclin; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

Correspondence: Dr Robert D. Simari, Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, U.S.A. (email simari.robert@mayo.edu).
define the potential autologous sources of cells and to describe the translational preclinical studies that have generated interest in this area of research.

**CELLULAR ENDOTHELIAL FUNCTION AND DYSFUNCTION**

Over the last two decades, it has become evident that the vascular endothelium is more than just an anatomical barrier between the bloodstream and the vascular wall [1]. Rather, the single-cell layer of endothelial cells has been recognized as an active paracrine, endocrine and autocrine organ that is indispensable for regulation of vascular tone and maintenance of vascular homoeostasis. Under normal conditions, endothelial production and release of vasodilators, such as NO (nitric oxide), PG12 (prostacyclin) and EDHF (endothelium-derived hyperpolarizing factor), on the one hand and vasoconstrictors, such as ET-1 (endothelin-1), angiotensin II, prostaglandin H2 and thromboxane A2, on the other is well balanced, favouring vasodilation in response to physiological stimuli, including exercise or stress. In contrast, endothelial dysfunction is characterized by disruption of this critical balance with a reduction of the bioavailability of vasodilators, in particular NO, whereas endothelium-derived contracting factors are increased [2]. This imbalance leads to an impairment of endothelium-dependent vasodilation, which represents the functional characteristic of endothelial dysfunction. However, aside from its vasodilatory potential, NO also has antiplatelet, antithrombotic, antiproliferative and anti-inflammatory properties, whereas PG12 is a potent inhibitor of platelet aggregation. Conversely, ET-1 and angiotensin II, the major vasoconstrictor opponents of NO, also exert prothrombotic, mitogenic and pro-inflammatory effects, and thromboxane A2 promotes platelet aggregation aside from its vasoconstricting effect. Thus endothelial dysfunction, aside from denoting impaired endothelium-dependent vasodilation, also comprises a specific state of ‘endothelial activation’, which is characterized by a pro-inflammatory, proliferative and procoagulatory milieu that favours all forms of vascular disease [2]. Given this relationship between endothelial dysfunction and atherosclerosis, it is likely that the status of endothelial function may reflect the propensity of an individual to develop atherosclerotic disease and its sequelae [3].

The traditional view of endothelial dysfunction is that it represents a disease of the vascular wall and its response to physical or chemical injury. Endothelial dysfunction is associated with traditional atherosclerosis risk factors and is associated with vascular injury which creates local endothelial cell activation. However, the systemic nature of endothelial dysfunction and atherosclerosis raises the possibility that endothelial dysfunction may originate not in the vascular wall, but rather in the cross-talk between the vascular wall and the circulating cells.

The vascular wall and the endothelium in particular are undergoing a constant process of injury and repair in response to mechanical and chemical injuries [2]. Emerging evidence suggests that bone-marrow-derived endothelial stem and progenitor cells contribute to the repair of vascular injury and play a role in tissue repair. The bone marrow contains vascular progenitor cells that can mobilize to the injury site and complement repair afforded by pre-existing endothelium [4]. Despite experimental evidence demonstrating the contribution of bone-marrow-derived progenitors to tissue revascularization, the importance of these cells in repairing vascular damage in the clinical setting remains unknown. At the site of vascular injury, endothelial cells can originate from either adjacent pre-existing blood vessels, or recruitment of bone marrow or circulation-derived progenitor cells [4]. Interestingly, normalization of endothelial function follows the demonstration of intact structural re-endothelialization in animal models [5]. Vascular progenitor cells may play multiple roles in this process. A recent study [6] demonstrated that the degree of endothelial dysfunction might be correlated with the number of circulating endothelial progenitors. Thus one of the possible mechanisms for vascular endothelial dysfunction may be a relative deficiency of progenitors for vascular repair. Moreover, the function of these cells, including generation of NO [7] and their ability to participate in the vascular repair following injury, has been shown to be impaired in an animal model of decreased NO activity [8]. It may be speculated that endothelial NO activity, the hallmark of endothelial function, may be reduced at the level of the multi-potential cells of subjects with systemic endothelial dysfunction and atherosclerosis. This concept is supported further by the observations that in Type II diabetes, a condition which is associated with endothelial dysfunction, recruitment of endothelial progenitors to the site of tissue repair is diminished [9] and that statin therapy [10], one of the major pharmacological interventions that is known to improve endothelial function, promotes the mobilization and the function of progenitor cells.

Thus endothelial dysfunction may be regarded as an abnormal repair and systemic response to injury, rather than a local response to the insult that is mediated by chemical and physical injury. These abnormalities may involve each step of the pathway from the genetic origin of the endothelial cells to their functional integration into the vascular wall.

**ENDOTHELIAL DYSFUNCTION AS A HALLMARK OF VASCULAR DISEASE**

**Atherosclerosis**

All of the major risk factors for atherosclerosis are associated with endothelial dysfunction. Endothelial-dependent vascular responses are impaired in hypertension...
to deeper structures is sufficient to generate neointimal formation. In fact, the loss of endothelium alone without injury is a hallmark of the disorder. At a molecular level, reduced expression of eNOS has been demonstrated in the lungs of patients with primary pulmonary hypertension [41]. Furthermore, clinical attempts to enhance NO-dependent cGMP-mediated effects of sildenafil have demonstrated clinical efficacy [42].

The first description of abnormal endothelium-dependent relaxation in hypertensive animal models was by Konishi and Su [43] in 1983. This observation in spontaneously hypertensive rats has been confirmed in multiple models of hypertension [44,45]. In humans with hypertension, the peripheral response to acetylcholine is blunted [46,47]. Thus systemic and pulmonary arterial hypertension are both associated with endothelial dysfunction.

**CHF (congestive heart failure)**

CHF is a syndrome associated with systemic vascular dysfunction [48,49]. It has been shown that indices of coronary and peripheral vasodilation are abnormal in the setting of left ventricular dysfunction [49–51]. These animal studies suggest a mechanistic role for endothelial-derived factors in the development and establishment of CHF.

Furthermore, attempts to increase endothelial cell number and function have been tested in animal models of CHF. Angiogenic gene transfer approaches have been shown to have therapeutic benefit in the canine pacing model, suggesting an angiogenic defect [52,53]. Furthermore, NO donors have been shown to modulate myocardial oxygen consumption in this model [54]. Multiple clinical approaches to target the vasoconstricntial tone of peripheral vessels in CHF include the use of nitrates and angiotensin blockade [55]. Thus endothelial dysfunction remains a central mechanism in the syndrome of CHF as well as a clinical target for therapy.

**DEFINING AUTOLOGOUS CELL SOURCES OF ENDOTHELIAL CELLS (Table 1)**

**Mature endothelial cells**

Inherent to any strategy of autologous cell delivery is the need to identify an easily obtainable autologous source of
endothelial cells. As all tissues contain mature endothelial cells, these may be isolated from tissue biopsy or harvested in an autologous fashion. Surgical harvesting of vascular tissue provides a source of endothelial cells that can be transplanted for therapeutic purposes. An autologous source offers considerable appeal over allogeneic or xenogeneic sources, given the importance of immune compatibility and tolerance. However, limitations of vascular sources include identification of vessels to harvest, a limited number of cells obtainable and the time necessary to isolate and expand cell numbers. Moreover, it is possible that autologous endothelial cells from any vascular source will be dysfunctional, given that systemic atherosclerosis underlies most of the diseases for which cell therapy may be relevant. In addition, structural, functional and biochemical heterogeneity in endothelial cell phenotype and the critical role of cellular microenvironment may contribute further to dysfunctional endothelial cell performance following cell relocation to a different vascular bed [56].

Pioneering studies obtained endothelial cells by mechanically or enzymatically harvesting autologous venous tissue and mixing the cells with whole blood or tissue culture media prior to incubation with synthetic graft material [57–59]. Cell yields from such experiments were often limited [60]. Crude vein homogenates [61–63] have served as alternative sources of mature endothelium as has adipose tissue, which is a source of microvascular endothelial cells [64,65]. Indeed, human liposuction specimens may offer an enticing cell source in this regard [66].

Using artery-derived endothelial cells for relocation to another arterial system might offer advantages over vein-derived cells in view of the sensitization and resistance of arterial endothelial cells to shear stress. However, clinical translation of such an approach would be limited, primarily because complete surgical harvest of a potential arterial conduit might be required.

**Circulation-derived endothelial cells**

The ability to generate cells with an endothelial phenotype from peripheral blood may obviate the need for surgical harvest. Early evidence for the existence of circulating sources of endothelium was provided by Stump and co-workers [67] in 1963. In an animal model, these investigators suspended a small patch of Dacron within the lumen of an aortic interposition graft that was also made of prosthetic material. The Dacron patch, floating midstream, was thus isolated from contact with native vascular tissue. After 7 days, islands of endothelial cells were identified on the patch surface, leading the authors to postulate circulating blood as the endothelial source. More recently these observations have been corroborated using models of bone-marrow and solid-organ transplantation that have allowed the discrimination of host- and donor-derived cells according to genetic markers. These animal and human studies have revealed that bone-marrow-derived circulating progenitors may contribute to both endothelial and intimal smooth muscle cell formation in multiple models of vascular injury (for a review, see Sata and Walsh [68]) as well as following graft implantation [69].

Data from these studies provide indirect evidence for the existence of circulating endothelium. Mature endothelial cells, probably sloughed from resident vasculature, have been directly demonstrated at a single cell level within peripheral blood [70]. The precise identification of immature circulating endothelial precursors however remains an ongoing area of investigation. Using endothelial-favouring conditions, Lin and co-workers [71] cultured peripheral blood mononuclear cells obtained from volunteers who had previously undergone sex-mismatched bone-marrow transplantation. Early in the culture period, they identified endothelial cells of recipient genotype and low proliferative capacity, suggestive of mature (sloughed) endothelium. Following prolonged culture, endothelial colonies were generated, with cells demonstrating a donor genotype. Moreover, these colonies of OEC (outgrowth endothelial cells) exhibited a proliferative capacity far beyond that of mature cells, consistent with an origin from an immature precursor derived from donor bone marrow (see Figure 1).

Asahara and co-workers [72] were perhaps the first to suggest that a CD34+ circulating subset contained

---

**Table 1  Autologous sources of endothelial cells for delivery**

<table>
<thead>
<tr>
<th>Source</th>
<th>Cons</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td>Requires surgical harvesting</td>
<td>[57–59]</td>
</tr>
<tr>
<td></td>
<td>Limited cell number</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May require culture</td>
<td></td>
</tr>
<tr>
<td>Adipose</td>
<td>Does not require vessel harvest</td>
<td>[64–66]</td>
</tr>
<tr>
<td></td>
<td>Microvascular phenotype</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Does require harvest</td>
<td></td>
</tr>
<tr>
<td></td>
<td>May require culture</td>
<td></td>
</tr>
<tr>
<td>Circulation</td>
<td>Easily obtained by blood draw</td>
<td>[69,72]</td>
</tr>
<tr>
<td></td>
<td>Requires culture</td>
<td></td>
</tr>
</tbody>
</table>

---

© 2005 The Biochemical Society
EPCs (endothelial precursor cells). Part of the evidence provided for this was the expression of selected endothelial antigens by the cultured cell population. Notably, the purity of CD34 cells was only 15%. Harraz and co-workers [73] extended these observations and suggested that the majority of cells exhibiting this phenotype were in fact CD34−.

Similarly, a number of studies have suggested that surface expression of haematopoietic stem cell antigens CD34 and CD133, and/or VEGFR2 (VEGF receptor 2), might identify a population of human EPCs [74–77]. However, it should be remembered that, whereas OEC were generated from, for example for CD34+/VEGFR2+ populations, these studies did not examine the remaining antigen-negative cell controls (CD34−/VEGFR2−). This, taken together with the fact that the immunomagnetic cell isolation techniques used do not provide population purity, leaves considerable room for the possibility that a significant proportion of the endothelial precursor population does not express these markers. A recent study demonstrating outgrowth of endothelial cells from CD34− cord blood precursors highlights this issue further [78].

Regardless of the lack of certainty regarding the nature of circulating endothelial precursors, emerging truths are apparent from multiple in vitro studies [7] (Table 2). When PBMCs (peripheral blood mononuclear cells) are cultured in endothelial-favouring conditions for 4–7 days, the population of cells is mixed, consisting predominantly of CD14+ monocytes, some of which express endothelial markers such as CD31 and vWF (von Willebrand factor) and take up acetylated LDL (low-density lipoprotein) and bind BS-lectin. These cells have been referred to as EPCs. At later times under certain culture conditions, rare colonies of OECs arise from precursors within the original mixed population. OECs are homogenous in nature and more endothelial in phenotype compared with EPCs. Both of these cells have been delivered in animal models of vascular disease.

### AUTOLOGOUS ENDOTHELIAL CELL DELIVERY

#### Delivery of vascular-derived endothelial cells

The initial study of autologous endothelial cell delivery to modulate vascular healing was performed in a synthetic vascular graft in 1978 [57]. Endothelial cells were obtained from a segment of autologous canine vein, placed in suspension and used to coat Dacron vascular grafts prior to infrarenal implantation. At 4 weeks following implantation, 76% of seeded grafts were patent, whereas 22% of the unseeded grafts were patent. This strategy of autologous delivery of cells obtained from vascular...
tissue was extended to include sites of direct vascular injury [79–83]. These studies demonstrated the feasibility of harvesting autologous venous endothelial cells, culture modification of these cells and re-implantation at sites of vascular injury. This delivery was associated with enhanced endothelialization and beneficial effects on vascular remodelling. However, one study failed to demonstrate inhibition of neointimal formation in spite of beneficial effects on remodelling [83]. Thus the approach of autologous cell delivery was shown to affect vascular remodelling following direct delivery, albeit limited by the burdens of vascular cell harvesting and culture. Clinical trials using similar approaches for prosthetic infrainguinal grafts have yielded promising initial results, although a wider clinical role may be limited by the delay incurred by culture expansion of input cells [84].

Edelman and co-workers [85,86] have extended the use of endothelial cell implants to include non-autologous and extraluminal delivery of endothelial cells. In a porcine model of vascular injury, porcine or bovine aortic endothelial cells were implanted in Gelfoam matrices in the perivascular space following injury. Cells from both sources inhibited restenosis and thrombosis. However, the bovine cell implants initiated an inflammatory immune response at the site of implantation. The studies suggest that the mechanism of these findings from perivascular implants might be from paracrine and not direct cellular effects.

**Delivery of circulation-derived endothelial cells**

With the re-discovery of the circulation as a source of endothelial cells, multiple studies have been performed highlighting the potential use of bone-marrow-derived cells to enhance vascular healing at sites of vascular injury. The first of these studies was performed in a splenectomized mouse model. Werner et al. [87] determined that, following systemic delivery, cultured spleen-derived mononuclear cells homed to injured arterial segments. Cell administration was also associated with accelerated re-endothelialization and reduced neointimal formation following injury. Without splenectomy, homing of these cells was predominantly to the spleen obviating the vascular effects. In a xenogeneic model, Fujiyama et al. [88] transplanted defined populations of human mononuclear cells into balloon-injured arteries of athymic nude rats. This model system included systemic gene transfer of MCP-1 (monocyte chemo-attractant protein-1) prior to transplantation. The authors concluded that local delivery of monocytes (CD34+/CD14+) was associated with accelerated endothelialization and reduced neointimal formation compared with delivery of CD34+ cells or unselected mononuclear cells. It was suggested that the delivered monocytes were able to ‘transdifferentiate’ into mature endothelial cells in vivo. This was concluded from the co-expression of endothelial antigens and lack of monocytic markers on detected cells that had been pre-labelled prior to delivery. However, the role of MCP-1 in these studies is unclear. Both of these studies were performed in complex model systems with limited relevance to models of clinical injury and delivery.

Using more direct models of vascular injury and autologous cell delivery, multiple groups have demonstrated the potential use of circulation-derived endothelial cells to improve vascular healing [89–92] (Table 2). The results of these studies are highly complementary and suggest innate mechanisms for the potent effects of cell delivery. In our initial experiments [89], we generated cells with a partial endothelial phenotype from peripheral blood following 7 days of culture (similar to EPCs as defined by Asahara et al. [72]) which we referred to as CMMCs (culture-modified mononuclear cells). This population contains both monocyte-derived cells with endothelial features and a minority of precursors to OECs [7]. Balloon injury to an isolated segment of carotid artery was performed, followed by intraluminal administration of autologous CMMCs. Flow was restored 20 min later. Labelled cells were seen 4 weeks following delivery lining the lumen expressing markers of endothelial, but not monocyte/macrophage, lineage. Cells were also detected in deeper layers expressing macrophage, but not endothelial, markers. Thus delivery of these CMMCs appeared to result in both endothelial and macrophage phenotypes in the arterial wall (Figure 2).

We then evaluated the effect of CMMC delivery on the response to vascular injury, comparing local delivery of a saline-cell suspension immediately after balloon injury with saline alone as a control. To do so we examined carotid rings in an organ chamber 4 weeks after balloon injury and cell delivery. CMMC delivery was associated with a marked improvement in endothelial-dependent vasorelaxation compared with saline, although these responses did not achieve those of normal, uninjured vessels. CMMC delivery was associated with a 55 % reduction in neointimal thickening and significantly enhanced arterial re-endothelialization (Figure 3) [89]. However, it should be noted that the labelled cells made up a vast minority of resident cells 4 weeks following injury, suggesting a paracrine role for these cells.

In a study by He and co-workers [92], very similar effects on vascular structure and function were noted, including re-endothelialization and endothelial function. They demonstrated further that these cells express an array of growth factors and cytokines, including a broad range of angiogenic factors such as VEGF and members of the FGF (fibroblast growth factor) family. These factors may be responsible for the paracrine effects noted in both studies.

OECs have also been demonstrated to have vasculoprotective effects. Kaushal et al. [93] demonstrated
that seeding of decellularized vascular grafts with ovine OECs dramatically improved graft patency. We [94] and others [90] have evaluated the effect of local autologous OEC delivery following balloon injury of a native rabbit artery. Unlike CMMCs, OECs are proliferative, uniformly endothelial and express functional endothelial proteins [95]. Thus we hypothesized that their delivery after arterial injury may modify the vascular response in an endothelial-dependent manner. Importantly, we also elected to compare the effect of delivering PBMCs as a control for cultured cell therapy. Our results [94], together with those of Griese et al. [90], indicate that OEC delivery was associated with an overall reduction in neointimal formation compared with delivery of both saline [90,94] and PBMCs [94]. These studies also demonstrated a lack of long-term residence of delivered cells in spite of a potent effect.

No study has directly compared the effects of CMMCs and OECs. In our hands, the functional and structural effects of OEC delivery are almost identical with those of CMMC delivery [89,94]. Acknowledging that this comparison has limitations (it is retrospective), it remains somewhat surprising that OECs, with their more distinct endothelial properties and with their delivery in approx. 10-fold greater numbers than CMMCs, were not clearly

\[\text{Figure 2} \quad \text{Incorporation of administered CMMCs into vessel wall after balloon carotid injury}\]

(A) Carotid section demonstrating labelled CMMCs on the luminal border 4 weeks after local delivery. Colocalization staining of endothelial antigens CD31 (B) and BS-1 lectin (C), but negative staining for macrophage marker RAM-11 (D). (E) Haematoxylin and eosin staining of an adjacent arterial section. (F) Labelled cells also detected in neointima that do not co-stain for CD31 (G) but do stain for RAM-11 (H), consistent with macrophage lineage. (I) Haematoxylin and eosin staining of an adjacent arterial section. Red indicates CM-DiI fluorescence; green, CD31. Arrows indicate the colocalization of neointimal CMMCs with RAM-11 (blue). Magnification \(\times 40\). This Figure was reproduced from Gulati, R., Jevremovic, D., Peterson, T. E. et al., Autologous culture-modified mononuclear cells confer vascular protection after arterial injury, Circulation 108 (12), 1520–1526, with permission from Lippincott, Williams and Wilkins.

\[\text{Figure 3} \quad \text{Acceleration of re-endothelialization following local CMMC delivery after balloon injury}\]

(A) The re-endothelialized area at 4 weeks is significantly greater in carotids from CMMC-treated animals than saline-treated counterparts. *\(P < 0.05\). Representative photographs of exposed carotid lumens 4 weeks after balloon injury and delivery of CMMCs or saline are shown below. Re-endothelialized areas do not stain blue. (B) En face lumen microscopy 4 weeks after injury and delivery of fluorescence-labelled CMMCs. Absence of Evans Blue staining suggests complete re-endothelialization. Multiple colonies of fluorescence-labelled cells were seen on luminal surface, suggesting direct participation in re-endothelialization (representative example of one colony). This Figure was reproduced from Gulati, R., Jevremovic, D., Peterson, T. E. et al., Autologous culture-modified mononuclear cells confer vascular protection after arterial injury, Circulation 108 (12), 1520–1526, with permission from Lippincott, Williams and Wilkins.
superior in their vascular protectivity, as indexed by vasorelaxation and neointimal formation. At first sight it may thus seem that CMMCs, with their apparently more potent effect per cell and relatively shorter culture times than OECs, would be a sensible choice of agent to select for clinical study. However, as the majority of CMMCs are non-proliferative [96], to generate enough cells for the scale up from animal studies to human therapy might be prohibited by the volumes of blood required. Regardless of the potential practical issues, the combined results from these studies confirm potent protective effects of cell delivery following direct vascular injury. Definition of the nature of these effects and clinical translation remain key issues.

The paracrine nature of cell delivery
Although the modification of vascular responses by cell therapy is likely to be, at least in part, related to restoration of endothelial integrity, several lines of evidence suggest that there may be substantial paracrine effects. First, the residence of the cells in the majority of these studies is temporary and limited. For example, Griese and co-workers [90] used retroviral labelling to enable the identification of delivered OECs following balloon injury and demonstrated labelled cells on the luminal surface at 2 weeks after delivery. However, at 4 weeks, no labelled cells were identified in the same location. Despite this apparent loss of delivered cells, overall arterio-endothelialization increased. Conte et al. [82] noted similar loss of genetic label following delivery of cultured venous endothelial cells in a rabbit model. Similarly, we [89] found few CMMC-derived cells to be detectable 4 weeks after balloon injury and cell delivery. Whereas loss of marker may account for some of these findings, it is also likely that a significant element of the benefit seen with cell delivery occurs through paracrine-based mechanisms.

Secondly, as in the studies by Nugent et al. [85], endoluminal delivery is not necessarily required for potent luminal effects. This suggests that the cells may secrete factors that indirectly affect luminal remodelling. Thirdly, these cells are capable of expressing potent endothelial-derived factors capable of such effects. Cultured monocyte-lineage cells have been shown to secrete growth factors and cytokines in vitro [96]. These findings underline the need for additional studies directed towards defining the relative contribution of direct endothelial resurfacing and the nature of paracrine mechanisms involved in cell-therapeutic models of vascular disease.

Endothelial delivery as a means of vascular gene transfer
Delivery of genetically modified autologous endothelial cells would provide for enhancement of defined paracrine effects or establishment of new therapeutic approaches. These approaches might extend far beyond treatment of vascular disease. In 1989, two papers were published in the same issue of Science establishing this concept [97,98]. Nabel and co-workers [97] used ex vivo transduction syngeneic endothelial cells with subsequent luminal delivery to injured arterial segments. This study resulted in the first demonstration of genetic transduction of the vasculature. Additionally, Wilson et al. [98] used a similar approach to a vascular graft. Since then, both vascular and non-vascular uses have been developed.

Dichek and co-workers [99,100] have used a stent-based delivery of transduced endothelium to affect local gene transfer. This approach was limited by the loss of cells caused by the balloon distention necessary for stent delivery. Additionally, the poor rates of proliferation of adult autologous endothelial cells make circulation-derived sources a better choice for transduction. Several groups have transduced vascular progenitors followed by vascular delivery. Cells have been transduced to over-express proteins normally expressed or to express new proteins in endothelial cells to enhance their native effects. Tissue plasminogen activator [101], eNOS and haemoxigenase [91] have been delivered using this approach.

Therapeutic delivery of peptides using genetic modification of vascular progenitors is not limited to vascular disease. Lin and co-workers [102] have demonstrated their ability to deliver physiological levels of factor VIII in immunodeficient mice. Systemic delivery of transduced cells resulted in transgene expression from liver, lung, spleen and bone marrow of targeted animals. Furthermore, we [103] have used transduction of OECs to deliver retroviral producing cells to target tumours.

VIEW TO THE FUTURE
Translational strategies to biologically and genetically modify and deliver autologous endothelial cells for cardiovascular disease have been helped by the identification of circulation-derived endothelial precursors. Prior studies required and were limited by the harvesting of adult vascular endothelial cells. Multiple studies using circulation-derived endothelial cells have confirmed their ability to enhance vascular healing following acute injury. How these studies will be translated to clinical studies and practice remains an open question. Certain challenges must be addressed for translation to early phase clinical trials. These challenges include defining (i) the mechanisms of effects, (ii) the optimal cell for delivery, (iii) isolation or culture conditions consistent with clinical practice, and (iv) the initial clinical scenarios in which to test the strategy. The selection of these initial clinical studies is critical to the long-term success of the strategy. For long-term widespread application of cell delivery to chronic cardiovascular diseases, conditions for simple and repeatable cell isolation and delivery will be required. Much like the field of cardiovascular gene transfer, the...
success of cell-based therapies will be defined by the scientific rigour at each translational step.

ACKNOWLEDGMENTS

We thank Traci Paulson for her technical support, and acknowledge the support of the National Institutes of Health (HL75566 to R.D.S.), the American Heart Association, State of Minnesota and the Mayo Foundation.

REFERENCES

64 Jarrell, B. E., Williams, K. S., Stokes, G. et al. (1986) Use of freshly isolated capillary endothelial cells for the immediate establishment of a monolayer on a vascular graft at surgery. Surgery 100, 392–399
81 Thompson, M., Budd, J., Eady, S. et al. (1994) Platelet deposition after angioplasty is abolished by restoration of the endothelial cell monolayer. J. Vasc. Surg. 19, 478–486
82 Conte, M., Birinyi, L., Miyata, T. et al. (1994) Efficient repopulation of denuded rabbit arteries with autologous genetically modified endothelial cells. Circulation 23, 2161–2169
88 Fujiyama, S., Amano, K., Uchira, K. et al. (2003) Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. Circ. Res. 93, 980–989

Received 4 January 2005/28 February 2005; accepted 8 March 2005
Published on the Internet 23 June 2005, DOI 10.1042/CS20050002

© 2005 The Biochemical Society