ROLE OF VASCULAR ENDOTHELIAL CELLS IN THE ALLOGRAFT RESPONSE

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SUMMARY

Following transplantation endothelial cells lining an allograft come into contact with immune cells of the recipient. Activation of an immune response, by graft endothelial or other cells, will lead to local increases in cytokine production and cell-mediated lysis. Inflammatory cytokines have been shown, mainly *in vitro*, to have marked effects on endothelial function and act to produce a pro-thrombotic, pro-adhesive and pro-mitogenic phenotype. These data are reviewed and ways in which these changes could lead to rejection due to graft lysis or vascular occlusion are discussed.

Transplantation of tissue from one genetically disparate animal to another initiates a series of responses that can ultimately result in tissue destruction and graft rejection. The principal response of the recipient is activation of the immune system towards graft cells. The graft is not a passive target for these cells but itself responds by actively recruiting recipient leucocytes into the transplanted tissue where they can destroy graft cells. Host leucocytes can also directly target cells of the graft vasculature. The lytic response to a graft usually results in acute rejection. Thrombus formation within graft vasculature commonly accompanies the lytic response and this can contribute to acute rejection by occluding perfusion, leading to graft cell necrosis. Another important response to an allograft can be intimal thickening in blood vessels of the graft. This fibroproliferative response significantly decreases vessel lumen diameter which, together with the often attendant thrombi, can result in vascular occlusion and graft necrosis due to insufficient perfusion. This response underlies chronic rejection of transplanted organs. The endothelial cells lining graft vasculature play a critical role in the allograft response. These cells are in direct contact with circulating host immune cells and therefore are at the front line of interaction between host and graft. It is the graft endothelium that promotes infiltration of the transplanted tissue by host leucocytes. It is the endothelium too that, under the influence of the immune response, actually encourages vascular occlusion by favouring thrombus formation and, most likely, graft arteriosclerosis.

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THE VASCULAR ENDOTHELIUM AND COAGULATION

A common finding in the vasculature of grafted tissue in acute and chronic rejection is thrombus formation. Before outlining involvement of the endothelium in the allograft response, therefore, it may be useful to review briefly the role that endothelial cells play in maintaining vessel patency. Heparan sulphate proteoglycans localised at the luminal surface of the endothelium serve to bind the anti-coagulant protein antithrombin III[1] thus providing an anti-thrombotic interface between blood and vessel wall. In addition the endothelium also produces a range of molecules which actively regulate thrombus formation and lysis. These include the 74 kDa protein thrombomodulin which acts to modulate the activity of thrombin and inhibit coagulation.[2,3] Prostacyclin, also called prostaglandin I_2, and endothelial derived relaxing factor are both produced by the endothelium and act as potent vasodilators as well as inhibitors of platelet aggregation.[4–6] In situations such as vessel injury the endothelium can act in a pro-thrombotic fashion. Thromboplastin, also called tissue factor, and factor V are both produced by endothelial cells[2,7] and act to promote coagulation.[8,9] Platelet adhesion is enhanced by von Willebrand factor, again synthesised and secreted by a pro-thrombotic endothelium.[10] If a thrombus forms its degradation is mediated by plasminogen activators and inhibited by plasminogen activator inhibitors.[11] Both of these classes of protein are produced by endothelial

cells[12,13] and it is the balance which dictates fibrinolytic state at the vascular wall.

INVOLVEMENT OF THE ENDOTHELIUM IN THE LYTIC RESPONSE TO AN ALLOGRAFT

The lytic response to an allograft can result in hyperacute rejection or acute rejection. Hyperacute rejection is characterised by accumulation of polymorphonuclear leucocytes in graft capillaries, vascular damage, thrombosis and necrosis of the graft.[14] Vascular changes in hyperacute response are seen within minutes of connection of graft vasculature to that of the recipient and occur where the recipient already possesses antibodies against graft cells.[14] Binding of these antibodies to antigens on graft endothelial cells will lead to activation of complement and endothelial cell lysis. The widespread thrombosis seen under these circumstances is likely to arise as a result of loss of the anti-thrombotic endothelial lining. In addition, exposure of underlying basement membrane will promote platelet aggregation. The observation that antibody binding to endothelium stimulates release of anti-thrombotic heparan sulphate proteoglycans from the cell surface[15] and increases thromboplastin production[16] suggests that thrombosis may even occur before widespread endothelial death.

In acute rejection the lytic response develops over a period of weeks with cells of the vasculature as well as graft parenchyma subject to immune-mediated lysis. Grafts typically exhibit accumulation of infiltrating lymphocytes and macrophages, necrosis of small blood vessels and thrombosis.[17,18] The initiating factor in this response is activation of the recipient immune system against the graft. Helper T lymphocytes of the host can be activated by interaction with foreign antigen bound to self HLA class II molecules. It is not clear exactly which cells present antigen to recipient T cells, although antigen presenting cells of the recipient, dendritic cells and residual donor mononuclear cells could all have a role. In this regard an important observation has been that human endothelial cells express HLA class II molecules constitutively.[19,20] Further, HLA II expression is induced on endothelium activated *in vitro* by interferon-γ (IFNγ)[21] and in inflammation *in vivo*.[20] Since recipient T lymphocytes may perceive donor HLA II molecules in the graft as foreign antigen plus self HLA II,[22] the endothelial HLA may be able to activate recipient immune cells directly. In the presence of activating antigen and co-stimulator, such as interleukin-1 (IL-1), helper T cells produce cytokines including IL-2, IFNγ and tumour necrosis factor-alpha (TNFα). Activated T cells and released cytokines lead to clonal proliferation of helper and cytolytic T cells. Endothelial cells secrete IL-1[23] and so are capable of providing both activating antigen and co-stimulator for T cell activation. Local concentrations of TNF and IL-1 are likely to be elevated at this time due to the presence of leucocytes and IL-1 production by endothelial cells activated by leucocyte-derived cytokines[24] and autoactivated by IL-1.[25]

In addition to a role in activation of the afferent arm of the immune response the endothelium is almost certainly involved in the leucocyte infiltration and thrombosis characteristic of the lytic response to an allograft. Much of what is known about leucocyte interactions with the vascular wall comes from studies with neutrophils. The same sequence of events, however, appears to pertain to other leucocytes. An initial event in movement of leucocytes from the blood to sites within the graft is margination or tethering of these cells to the activated endothelium. Initial tethering of leucocytes to inflamed endothelium is mediated by P-selectin (previously called PADGEM or GMP-140) and E-selectin (previously called ELAM-1) on the surface of the endothelium. P-selectin is rapidly released from Weibel–Palade bodies on stimulation of the endothelium by histamine or thrombin[26] and its expression peaks at about 30 minutes after stimulation. E-selectin expression occurs at about 4–6 hours following stimulation of the cells by inflammatory cytokines IL-1 or TNFα.[27] These cytokines act to increase E-selectin mRNA levels.[27]

Although these selectins are important in initial adhesion of leucocytes to the endothelium they cannot mediate the firm adherence required for transmigration through the endothelium. This firm adherence involves adhesion molecules of the immunoglobulin superfamily on endothelial cells,[28] including intercellular adhesion molecule 1 (ICAM-1), ICAM-2 and vascular cell adhesion molecule-1 (VCAM-1), previously called inducible cell adhesion molecule 110 (INCAM-110). These molecules interact with members of the integrin family of adhesion molecules present on leucocytes, LFA-1 and Mac-1 on polymorphonuclear leucocytes, and LFA-1 and VLA-4 on lymphocytes.[28] ICAM-2 is constitutively expressed on endothelium;[29] however, ICAM-1[30] and VCAM-1[31] are strongly induced by inflammatory cytokines. ICAM-1 expression is increased in endothelial cells of grafts during rejection,[32] and endothelial VCAM-1 has been shown to be dramatically upregulated during inflammation.[33]

Initially the leucocyte integrins bind with only low avidity to their ligands; however, following activation they switch to a high-avidity interaction and firm binding can occur.[34] Platelet activating factor (PAF) and interleukin-8 (IL-8) are produced by endothelium[35,36] and can activate leucocyte integrins[36,37] leading to firm adhesion at the endothelial surface. E-selectin may also be able to induce firm adhesion

via integrin activation.[38] Thus leucocytes can roll along the surface of the endothelium tethered via selectins; on encountering upregulated ICAM or VCAM and activation of the leucocyte integrins the leucocyte will firmly adhere. Transmigration of the leucocyte between endothelial cells probably occurs via LFA-1/ICAM-1 interaction.[39] Movement of the cells is directed by a chemotactic gradient. The identities of the chemotactic agents have yet to be clearly established but are likely to include IL-8.[36]

The cytokines which modulate endothelial–leucocyte interactions leading to infiltration of the graft, also have marked effects on coagulant and fibrinolytic properties of the endothelium. It is likely, therefore, that endothelial cells under the influence of these agents actively participate in formation of thrombi seen during the acute response to an allograft. As in the case of the hyperacute response, where endothelial cells are destroyed, perhaps by cytolytic T cells, there will be exposure of underlying basement membrane and loss of anti-thrombotic surface in the vessel. This will predispose to platelet aggregation and thrombus formation. Experiments with endothelial cells *in vitro* have demonstrated marked effects of inflammatory cytokines on the coagulant and fibrinolytic properties of the cells, suggesting a possible mechanism for thrombus formation in areas of undamaged endothelium in the graft. Endothelial cell expression of pro-thrombotic thromboplastin is enhanced by IL-1 and TNF,[40,41] whereas these cytokines suppress thrombomodulin activity.[42] IL-1 also increases prostacyclin release by endothelium.[43] Plasminogen activator inhibitor production is enhanced by IL-1 and TNF and tissue plasminogen activator is unaltered or decreased.[44] Thus, if these effects occur *in vivo*, it is likely that the inflammatory cytokines close to the vascular wall will shift the balance of the endothelium to a pro-thrombotic/anti-fibrinolytic phenotype.

INVOLVEMENT OF THE ENDOTHELIUM IN THE FIBROPROLIFERATIVE RESPONSE TO AN ALLOGRAFT

Chronic rejection resulting from the fibroproliferative response is the major obstacle to long-term survival of an allograft. This response is characterised by intimal thickening of graft arteries and arterioles occurring within months to a few years of transplantation and has been observed in heart, kidney, liver and lung[45] transplants. Increased fibrosis and necrosis of the graft is seen in parallel with the vascular changes and is likely to result from lack of tissue perfusion following vessel occlusion due to the decrease in lumen size and often associated thrombus formation. The end stage of these changes is chronic rejection of the graft. Intimal thickening is a major cause of graft failure in renal transplants[46] and affects the majority of long-term transplanted hearts (5 years and later).[47,48]

Intimal thickening associated with chronic rejection is diffusely distributed throughout medium and small arteries and arterioles of the graft, differing from the focal thickening seen in more conventional arteriosclerosis.[49] Other changes to the vessels include thinning of the medial layer and focal disruption of the internal elastic lamina.[50,51] The major cell type found in the intima at chronic rejection is smooth muscle cells with some, fewer, lymphocytes and macrophages.[51] There is usually an intact endothelium, although some areas of endothelial denudation coincident with thrombi have been observed.[51] In contrast to the eccentric intimal hyperplasia usually observed in conventional arteriosclerosis, in the allograft thickening of the intima is most commonly seen uniformly around the lumen.[49] This suggests a more diffuse/general activation of intimal thickening in the graft. Interestingly, the vasculature of the recipient is not affected,[49] indicating that the stimulus may be localised in or intrinsic to the graft. Intimal thickening results from migration of smooth muscle cells from the medial layer and proliferation. Studies with an animal model of rejection have demonstrated active smooth muscle cell proliferation in the developing intima.[52] Although the fibroproliferative response is not normally associated with the level of lymphocyte infiltration seen in the lytic response, low-level inflammation at the vascular wall is suggested by the presence of a few infiltrating leucocytes and increased levels of IL-1.[51,52] Elevated concentrations of TNF, derived from the leucocytes, and IL-1, derived from endothelium activated by TNF and subsequently by IL-1 in an autocrine manner, are likely to prevail at the vessel wall.

Growth factors released locally in or at the vascular wall are likely to be the principal stimulus activating migration and proliferation of smooth muscle cells. Indeed, in a rat model of chronic rejection Hayry *et al.*[50] report a tenfold increase in levels of platelet-derived growth factor (PDGF) and insulin-like growth factor-I (IGF-I) and three- and sixfold increases, respectively, in mRNA for these growth factors in the walls of vessels exhibiting the fibroproliferative response compared with control vessels. An important source for these mitogens is likely to be the endothelium. Human endothelial cells express both PDGF[53] and IGF-I.[54] TNFα has been shown to increase markedly release of PDGF,[55] and IL-1 and TNF increase IGF-I expression (N. P. J. Brindle and H. Glazebrook, unpublished data) in human endothelial cells. Endothelial IL-1 could also stimulate smooth muscle cell proliferation indirectly by activating PDGF production by the cells.[56] Endothelial cells also produce the smooth muscle

cell mitogens acidic and basic fibroblast growth factors (aFGF, bFGF).[57,58] These growth factors are not secreted via usual routes from cells as they lack a secretory peptide sequence and the major route of release is likely to be through disruption of the cell membrane during cell damage or death.[59,60] Where endothelial cell death does occur, perhaps resulting from immune cell attack or antibody-mediated lysis, fibroblast growth factor may be released to act on underlying smooth muscle cells thereby contributing to the mitogenic stimulus. In addition to acting directly as a source of smooth muscle cell mitogens the endothelium is responsible for recruitment of leucocytes, as described above, accounting for the macrophages seen in the vessel wall in chronic rejection. Macrophages produce bFGF, IGF-1, PDGF[61–63] and are thus also likely to contribute to the developing mitogenic milieu in the inflamed vascular wall.

CONCLUSION

The known functions of vascular endothelial cells and data from *in vitro* experiments on modulation of endothelial function by inflammatory cytokines suggest strongly that these cells have a central role in both the lytic and fibroproliferative response to an allograft. One hypothesis is that HLA II expressed on venular endothelium of the graft vasculature, in the presence of IL-1 from the endothelium, triggers T cell proliferation. This response may also be induced by resident antigen presenting cells (APCs) in the graft or host APC processing of graft cell antigens. IFNγ produced by lymphocytes upregulates HLA II expression on endothelium thereby reinforcing the immune response, and induces HLA II on adjacent arterial endothelium. Large increases in local cytokine levels will occur due to release of IFNγ and TNFα by activated lymphocytes and IL-1 may also be produced by endothelial cells activated during inflammation. Cell adhesion molecules of the selectin and immunoglobulin family are induced on endothelium by IL-1, TNF and IFNγ, leading to margination and adhesion of lymphocytes and macrophages from the host circulation. Firm adhesion and migration of these leucocytes to infiltrate the graft occurs, probably under the influence of PAF and IL-8 derived from the endothelium. Graft cell lysis, and endothelial lysis, then ensue. The presence of high local concentrations of inflammatory cytokines would favour a pro-thrombotic/anti-fibrinolytic balance at the endothelium pre-disposing to thrombosis. Antibody production against endothelial antigens and B cell activation will augment these responses by promoting endothelial destruction and increasing B cell derived IL-1 levels in the locality. Together these responses would lead to the graft cell lysis and vascular damage seen in acute rejection.

Where the lytic response is low grade or contained, perivascular inflammation may dominate. Here the cytokines IL-1 and TNF could promote a pro-mitogenic phenotype in the endothelium leading to local release of PDGF, IGF-1 and IL-1. These mitogens act to stimulate migration of smooth muscle cells from the media into the intima, where they proliferate leading to intimal thickening. FGF released from damaged endothelium and mitogens released from infiltrating macrophages contribute to this response. Again a pro-thrombotic/anti-fibrinolytic balance at the endothelial surface will result under the influence of inflammatory cytokines. These responses then lead to chronic rejection.

In the scheme outlined here the endothelium plays a critical role in the lytic and fibroproliferative responses seen in graft transplantation. This scheme is based on changes known to occur in the allograft and observations on cytokine modulation of endothelial function seen *in vitro*. Direct testing of components of this hypothesis, especially those derived from *in vitro* studies, may now be useful in deepening our understanding of the mechanisms for allograft rejection, and thereby possibly developing strategies to improve long-term patency of transplanted organs.

Key words: Adhesion, Arteriosclerosis, Endothelium, Growth factor, Inflammation, Transplantation.

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