CMLS, Cell. Mol. Life Sci. 54 (1998) 965–978
1420-682X/98/090965-14 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Review

Endothelial cells as antigen-presenting cells: role in human transplant rejection

M. L. Rose

National Heart and Lung Institute, Imperial College School of Medicine, Heart Science Centre, Harefield Hospital, Harefield, Middlesex UB9 6JH (UK), Fax +44 1895 828 900

Received 30 March 1998; received after revision 4 May 1998; accepted 4 May 1998

Abstract. The immunological properties of human endothelial cells suggest they perform a pivotal role in acute and chronic rejection following solid organ transplantation. In this review the basic features of acute and chronic rejection are described as are the cellular and molecular requirements for antigen presentation. Traditionally, antigen-presenting cells are considered to be bone marrow-derived cells. However, these conclusions have been derived from rodent models of allograft rejection where bone marrow-derived passenger leukocytes are the only source of donor major histocompatibility complex (MHC) class II in the grafted organ. In contrast, in humans, virtually all the microvascular and small vessel endothelial cells are 'constitutively' positive for MHC class II antigens. The phenotypic properties of human endothelial cells, their response to cytokines and their ability to stimulate resting T cells are described. Unlike bone marrow-derived antigen presenting cells (APCs), which utilise B7/CD28 interactions, human endothelial cells utilise lymphocyte function antigen 3 (LFA3)/CD2 pathways to stimulate T cells. They activate a CD45RO + B7-independent subpopulation of T cells. Their effect on allogeneic T cells is compared with other non-bone marrow-derived cells such as fibroblasts, epithelial cells and smooth muscle cells, which are unable to stimulate resting T cells. Evidence is presented suggesting that release of MHC and non-human leukocyte antigens (HLA) from endothelial cells stimulates an alloantibody and autoimmune response leading to chronic rejection.

Key words. Endothelial cells; transplantation; rejection; antigen presentation.

Introduction

Approximately 36,000 solid organ transplants are performed throughout the world each year, of which the majority are kidney transplants. About 5000 hearts, 6500 livers and 1200 lung transplants are performed. Rejection remains the most common complication following transplantation and is the major source of morbidity and mortality.

It has been recognized for many years that cells of the immune system which bear class II antigens of the major histocompatibilty complex (MHC) are potent stimulators of the rejection process. In particular, bone marrow-derived monocytes or dendritic cells (also found in donor organs as passenger leukocytes) are potent stimulators of an antiallograft response. However, it is now appreciated that many human parenchymal cells either constitutively express MHC class II molecules (such as endothelial and epithelial cells) or can be induced to express MHC class II (smooth muscle cells and fibroblasts) after exposure to appropriate cy-

tokines. In view of these observations there is considerable interest in the role of donor-derived MHC class II-positive parenchymal cells in stimulating alloreactive T cells of the host. Endothelial cells (ECs) forming the interface between donor and recipient are the first donor cells to be recognized by the host's immune system. This fact plus the observation that they are highly responsive to cytokines and express numerous molecules which interact with ligands on lymphocytes has stimulated much research into their precise role in transplant rejection. It is our view that ECs are pivotal both in controlling the egress of inflammatory cells into the allografted organ and as specific antigen-presenting cells (APCs), by presenting foreign molecules to the immune system (fig. 1). The purpose of this review is to compare the characteristics of ECs and other parenchymally derived cells as APCs. First it is necessary to briefly describe the features of acute and chronic rejection before explaining the role of ECs in this process.

Basic mechanism of rejection

The major stimulus for rejection of allografted organs is recognition of foreign antigens coded by the major histocompatibilty complex (MHC). Class I (HLA-ABC) and Class II (HLA-DR, DP, DQ) antigens are highly polymorphic glycoproteins encoded by the MHC locus found on chromosome 6 in humans. The frequency of circulating T cells which recognize foreign MHC molecules is very large (estimated at an astounding 0.1–1% of circulating T cells) – a fact which almost certainly accounts for the vigour of the antiallograft response.

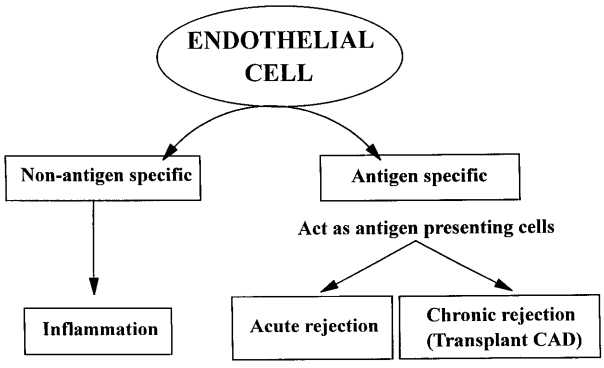

Figure 1. Diagram to illustrate role of ECs in transplant rejection. Reprinted with permission from: Rose M. L. (1998), Role of endothelial cells in allograft rejection. In: An Introduction to Vascular Biology, pp. 248–261, Hunt B., Poston L., Halliday A., Schachter M. (eds), © 1998 Cambridge University Press, Cambridge.

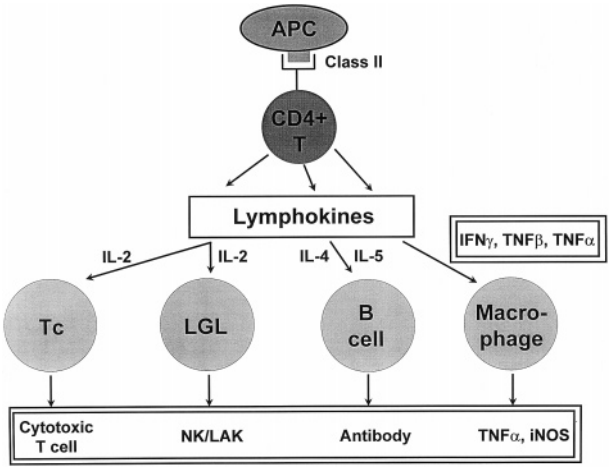

Figure 2. Diagrammatic representation of T-cell activation, illustrating the pivotal role of MHC class II antigens (presented by APCs within the graft) in initiating rejection. Activation of CD4+ T cells results in a cascade of lymphokines, causing the maturation of a number of possible effector mechanisms (in double-lined boxes). Note that the cytokines IFNγ, TNFβ and TNFα may be directly damaging to tissues. Reprinted with permission from Rose M. L. (1998). Role of endothelial cells in allograft rejection. In: An Introduction to Vascular Biology, pp. 248–261, Hunt B., Poston L., Halliday A., Schachter M. (eds), © 1998 Cambridge University Press, Cambridge.

Rejection is initiated by the CD4+ T cell subset recognizing MHC class II antigens on APCs within the graft (fig. 2). Recognition of foreign MHC molecules results in CD4+ T cell activation and release of cytokines (interleukin (IL)-2, IL-4, IL-5, IL-6, interferon (IFN)γ, tumour necrosis factor (TNF)α, TNFβ) which allow maturation of the effector mechanisms of rejection, namely maturation of CD8+ cytotoxic T cells, infiltration of macrophages, maturation of NK cells and lymphokine-activated killer cells (LAK), and antibody formation (fig. 2). These effector mechanisms have been listed for the sake of completeness; there is little evidence that natural killer (NK) or LAK cells are important in allograft rejection. Indeed, the precise effector mechanisms which cause graft dysfunction are unknown; although CD8+ cytotoxic T cells are invariably found in allografts, experimental studies have shown that CD8+ T cells are not essential for rejection [1]. It is quite possible that a direct effect of cytokines, in particular TNFα and IFNγ, may be toxic to allografted cells. For example, TNFa has a negative ionotropic effect on cardiac myocytes [2], and elevated levels of TNFa have been reported in the serum of patients in heart failure [3]. Similarly, induction of inducible nitric oxide synthase by activated macrophages and ECs may be an important effector mechanism, and

has been associated with contractile dysfunction after cardiac transplantation [4].

Activation of CD4+ T cells is thus pivotal in initiating acute rejection (fig. 2). In view of the fact that CD4+ T cells are activated by foreign MHC class II molecules, understanding the quantitative and qualitative distribution of these molecules on the allografted organ is of considerable importance. The advent of monoclonal antibodies, use of frozen sections and advances in immunocytochemical techniques have revolutionized knowledge about the normal distribution of MHC molecules in different tissues. Class II (HLA-DR and DP) antigens, originally thought to be restricted to macrophages, dendritic cells, monocytes and activated T cells, have now been described on human endothelial and epithelial cells [5, 6]. The expression of class II on human ECs has been described in every organ [5, 7], and it is particular striking on the microvessels, that is capillaries, arterioles and venules. The large vessel endothelium (such as aorta, pulmonary artery, saphenous vein) are negative for MHC class II expression [7].

Pathways of antigen presentation and APCs

The term antigen-presenting cell has a specific meaning to immunologists: it means the cell is able to present antigen to resting T cells, that is, it is able to cause activation of resting T cells. Only specialized cells (traditionally recognized as B cells, dendritic cells and monocytes) can perform this task. T cells recognize nominal antigen as processed peptides presented by self-MHC molecules. An important step in the understanding of alloreactivity came with the discovery that T cells can engage and respond to allogeneic MHC molecules directly (fig. 3). This form of antigen recognition, termed direct presentation or the direct pathway, is responsible for the strong proliferative response to alloantigens seen in vitro and quite possibly the early acute rejection seen in nonimmunosuppressed animals after transplantation of MHC mismatched organs. However, T cells can also recognize allogeneic peptides that have been processed and presented within self-MHC molecules by recipient APCs, in the same manner that T cells recognize nominal antigen (fig. 3). This pathway is termed the indirect route or indirect pathway of T-cell activation. Alloantigens shed from the graft are likely to be treated as exogenous antigen by recipient APCs and will therefore be presented within MHC class II molecules to activate recipient CD4+ T cells. A number of experimental studies have exemplified this phenomenon [8–10].

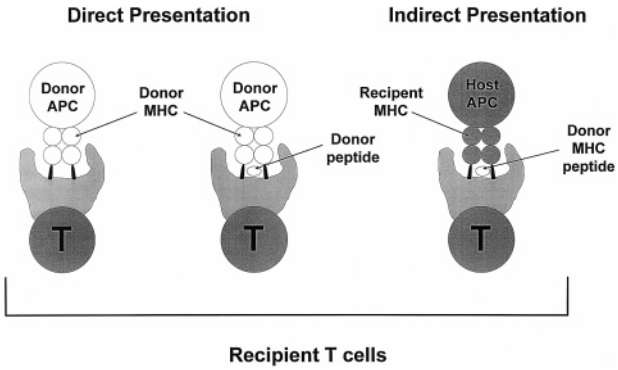

Figure 3. Diagrammatic representation of mechanisms whereby recipient T cells recognize allo-class II determinants. Recipient T cells recognize donor MHC determinants on donor APC (direct presentation) or they recognize donor MHC peptides which have been released from donor cells and processed and presented by host APCs within self-MHC molecules (indirect presentation). Reprinted with permission from: Shoskes D. A. and Wood K. J. (1994). Indirect presentation of MHC antigens in transplantation, Immunol. Today **15:** 32–38, © 1998 Elsevier Science, Oxford, UK.

Any graft cell expressing class II antigens will be able to activate the indirect pathway – it is likely that damaged ECs are an important source of graft-derived MHC class II antigens – since these are the only parenchymal cells expressing class II in the heart. The contribution indirect recognition of endothelial MHC class II makes to cellular rejection is currently not known. However, the question which has absorbed a number of groups in recent years is whether ECs can cause direct allostimulation of resting T lymphocytes (see below and ref. 11 for review). There are two reasons for this: First is that direct recognition of allo-MHC molecules results in a 'strong' response, the number of T cells recognizing MHC molecules directly is 10–100 times higher than those recognizing nominal antigen, resulting in a strong in vitro proliferative response. Second is that it is known that expression of MHC class II is not sufficient to cause T-cell activation.

One of the important concepts to emerge in recent years is the knowledge that T cells require two signals to become activated [12]: one is occupancy of the T-cell receptor; the second is activation of one of the many 'accessory molecules' present on T cells (fig. 4). Much attention has focused on the B7 family of receptors [13; CD80, CD86], known to be essential as second signals on APCs of bone marrow origin (e.g. monocytes, B cells and dendritic cells); B7 receptors interact with CD28 molecules on the surface of resting T cells, and blockade of this pathway inhibits dendritic cell-stimulated mixed lymphocyte responses in vitro [14]. It is thought that interaction of resting T-cell receptor with antigen in the absence of costimulation results in T cells being rendered anergic [15] or depleted by apoptosis [16]. Another T-cell surface antigen, CTLA4, binds CD80 and CD86 with 10–20

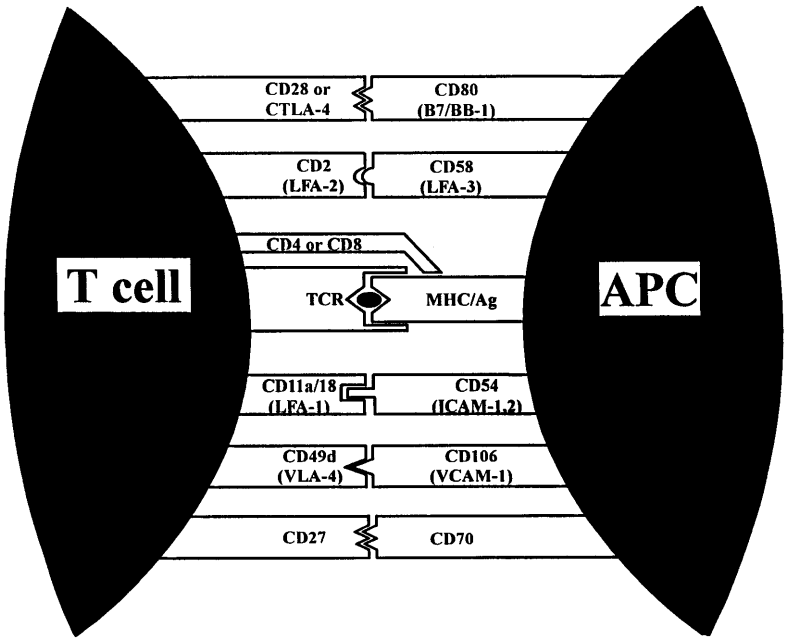

Figure 4. Diagrammatic representation of possible interactions between receptors on T cells and their appropriate ligands on APCs. Reprinted with permission from: Rose M. L. (1998), Role of endothelial cells in allograft rejection. In: An Introduction to Vascular Biology, pp. 248–261, Hunt B., Poston L., Halliday A., Schachter M. (eds), © 1998 Cambridge University Press, Cambridge.

times greater affinity than CD28 [17]. Thus, CTLA4-Ig acts as a strong competitive inhibitor of CD28-mediated T-cell activation. Injection of this fusion protein into rodents results in long-term survival of MHC-mismatched cardiac allografts [18–20] and human islet xenografts [21].

Acute rejection

The consequences of T-cell activation described above lead to infiltration of the graft with inflammatory cells (T cells and monocytes) – a process termed acute rejection. The majority of patients have one or two acute rejection episodes in the first 6 months following transplantation. Acute rejection may be suspected clinically, but it is always confirmed by histological assessment of biopsies. In particular, endomyocardial biopsy is an essential part of the management of patients following cardiac transplantation. Acute rejection invariably responds to anti-T-cell depletion therapy, steroids and cyclosporine.

A characteristic feature of biopsies showing histological signs of rejection is the upregulation of MHC and adhesion molecules (see ref. 22 for review). After cardiac transplantation, there is massive upregulation of MHC class I antigens (normally found only on the interstitial cells), so that cardiac myocytes become MHC class I positive [6]. Upregulation of MHC class I antigens has also been described on hepatocytes after liver transplantation [23]. In kidneys, the majority of structures constitutively express MHC class I molecules [22]; therefore, studies have focused on changes in MHC class II expression [24, 25], where increased expression is found on renal tubules and ECs. There is also upregulation of adhesion molecules during acute rejection (see below). The upregulation of these molecules is almost certainly mediated by local production of cytokines by infiltrating cells; thus, some cytokines (such as TNFα and IFNγ) have been directly visualized in graft biopsies using immunocytochemical methods [26]. Others (IL-1, IL-2, IL-4, IL-6, IL-10) have been detected using polymerase chain reaction to amplify cytokine messenger RNA (mRNA) [27].

Chronic rejection

Chronic rejection, presenting as a rapidly progressing obliterative vascular disease occurring in the transplanted heart, is the major cause of late death and repeat transplantation after cardiac transplantation. This disease is variously termed cardiac allograft vasculopathy or transplant associated coronary artery disease (TxCAD).

Table 1. Risk factors associated with transplant associated coronary artery disease.

Immunological	Nonimmunological
Numbers of acute rejection episodes	hypercholesterolaemia
Numbers of mismatches at the HLA locus	hypertriglyceridaemia
Anti-HLA antibodies	hypertension
Antiendothelial antibodies	CMV infection
	increased donor age
	smoking
	diabetes

This same phenomenon is also present in renal, lung and liver allografts and has been designated chronic rejection, obliterative bronchiolitis and vanishing bile duct syndrome, respectively. Chronic rejection does not respond to currently used immunosuppressive drugs (cytolytic anti-T cell agents, steroids and cyclosporine) – which primarily inhibit T-cell-mediated immune responses. The reported incidence of TxCAD, detected by routine angiography, varies greatly between cardiac transplant centres. Incidences of 18% at 1 year progressing to 44% at 3 years have been reported [28]. Use of more accurate and sensitive methods of detecting intimal hyperplasia, such as intracoronary ultrasound [29], is likely to increase the reported incidence of the disease.

Histology of TxCAD

There are a number of reviews which describe the histological differences between TxCAD and spontaneous CAD, and the various risk factors, both immunological and nonimmunological, have been described [30]. Importantly, the lesions are limited to the allograft and involve both arterial and venous structures – demonstrating the role of the alloimmune response in the aetiology. It is interesting that TxCAD is a much more diffuse disease than spontaneous CAD, affecting the entire length of the epicardial vessels; the intimal proliferation is concentric as opposed to the eccentric plaque found in spontaneous CAD. These differences suggest that the whole endothelium is the target of damage in TxCAD. Because the epicardial branches, including the intramyocardial branches, are affected by TxCAD, coronary artery bypass surgery for revascularization is usually precluded.

Immunobiology of TxCAD

The occurrence of a vasculopathy, affecting the allografted organs, is almost certainly of multifactorial aetiology. It is highly likely that the obstructive vascular lesions progress through repetitive endothelial injury followed by repair, smooth muscle cell proliferation and hypertrophy, all of which gradually produce luminal obliteration. It is useful to think of the disease in term of the Ross hypothesis [31], namely an initial damage to the endothelium resulting in release of growth factors and intimal proliferation. The latter process will be assisted by risk factors (table 1) some of which are common to both TxCAD and spontaneous CAD (the nonimmunological risk factors) and some of which are only found in transplant recipients (immunological risk factors). Of the nonimmunological risk factors, the role of hypercholesterolaemia is firmly established [32], with high levels of lipoprotein (a) being an important predictive factor [33, 34]. The role of cytomegalovirus (CMV), a common opportunistic infection in transplant recipients, is less certain. Although a number of clinical studies show an association between CMV infection and TxCAD [35–38], the association is not always clear [39]. It is possible to infect cultured ECs with CMV in vitro, where it downregulates MHC class I expression [40] and fails to induce MHC class II antigens [41]. These findings support the hypothesis that ECs might act as a reservoir for the virus, rather than enhancing immunogenicity of the foreign endothelium. Interestingly, it has been shown that CMV-infected endothelial cells cause proliferation of memory T cells from CMV-seropositive individuals in the absence of MHC class II antigens on the stimulator cells [42], suggesting that molecules other than class II are presenting viral antigens in this situation.

Most investigators would acknowledge that the initial damage to the endothelium is mediated by the alloimmune response, although it can also be argued that nonimmunological damage such as ischaemia, surgical manipulation and perfusion/reperfusion injury could also initially damage the ECs [43].

ECs as antigen-presenting cells

Endothelial expression of MHC class II molecules in vivo

Since MHC class II antigens initiate allograft rejection, it is of interest to describe the distribution of these molecules on ECs of different origins (table 2). All ECs constitutively express MHC class I molecules, and many ECs constitutively express MHC class II molecules. However, there is an interesting heterogeneity with regard to constitutive expression of class II antigens; the large vessels (aorta, pulmonary artery, endocardium, umbilical vein, umbilical artery) are negative, but the capillaries within all organs examined are strongly positive (table 2). Thus, foreign MHC class II antigens are introduced into a host every time a vascularized organ is transplanted. Arterioles and venules within the heart show weak or patchy basal expression of MHC class II antigens.

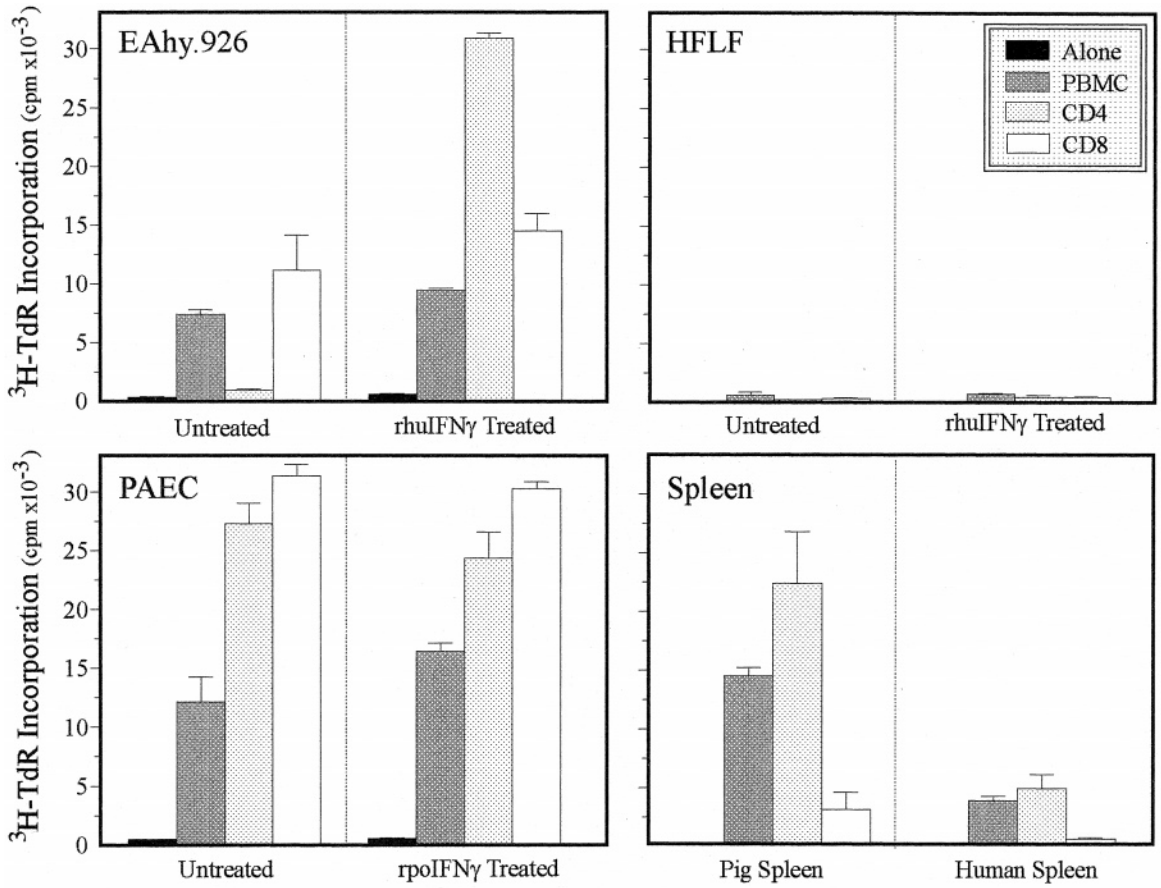

Figure 5. Human PBMC and $CD4^+$ and $CD8^+$ T cell proliferative responses to human and pig EC (EAhy.926, and PAEC, respectively), human fibroblasts (HFLF), and human and pig splenocytes. Results are shown for day 6 proliferation only; representative of three experiments. Bars show mean ± s.d. of triplicated wells. Thanks to Dr. C. A. Bravery for his permission to reproduce this figure.

MHC class II expression on cultured ECs

The most common ECs used in cell culture are those derived from human umbilical vein (HUVECs); these do not express MHC class II antigens in situ [7], and it is therefore not surprising that they are also negative in vitro. Treatment of HUVECs with IFNα, IFNβ, TNF,

Table 2. Distribution of adhesion molecules, MHC molecules and vWf in ECs derived from microvessels and large vessels of the human cardiovascular system. Summarized from refs 6, 7 and 71. ++ Strong, even expression. + Strong but patchy expression. +/− Weak and patchy expression.

	Myocardial biopsies			Large vessels		
	Capillaries	Arterioles	Venules	Coronary	PA	Aorta
CD31	++	++	++	++	++	++
ICAM-1	++	+	+	++	+	+
VCAM-1	neg	+/−	+/−	+	neg	neg
E-selectin	neg	neg	+/−	+	+/−	+/−
vWf	+/−	++	++	++	++	++
Class I	++	+	+	++	+	+
Class II	++	+/−	+/−	++	neg	neg

lymphotoxin or CD40 ligand increases the level of MHC class I antigen without inducing MHC class II molecules [44, 45]. An exception to this is that TNFα enhances MHC class II expression in porcine ECs [46]. IFNγ is uniquely able to induce MHC class II antigens on HUVECs [47]; HLA-DR is the most strongly expressed class II locus, followed by DP antigens. DQ is induced by IFNγ, but at very low levels. When used in combinations, the IFNs can act interchangeably with each other and synergistically with TNF and LT to increase class I. IFNα and IFNβ strongly inhibit the ability of IFNγ to induce class II antigens, as does TGFβ [48, 49]. The experiments to date using transforming growth factor (TGF)β have used monocytes or astrocytes as target cells [48, 49], recent studies confirm that TGFβ inhibits IFNγ-mediated upregulation of class II on human ECs (D. Moyes and M. Rose, unpublished results). The vast majority of studies on human ECs have been performed using HUVECs because of their plentiful supply. However, ECs can be cultured from any human vascular bed, and where experiments have been performed on aorta or pulmonary artery cells, the results are the same as with HUVECs [50]. Interestingly, the exception to this rule appears to be microvascular ECs derived from the adult human heart. These cells are MHC class II-positive in situ, and they lose their class II after 2 weeks in culture [51]. This observation raises the interesting possibility that factors in normal serum act to maintain microvascular MHC class II expression in vivo. This hypothesis has been confirmed by our observation that microvascular ECs are 10–100 times more sensitive to IFNγ than HUVECs (or other large-vessel ECs) in terms of MHC class II upregulation [50]. This suggests that the apparent 'constitutive' expression of microvascular endothelial MHC class II in vivo is a response to a tiny amount of circulating IFNγ. This idea is supported by the observation that in vivo treatment of dogs with high doses of cyclosporine (which would inhibit production of IFNγ) led to decreased expression of class II on the dog's ECs [52]. Interestingly, observations from IFNγ knockout mice suggest that class I expression is dependent on IFNγ in the mouse, not MHC class II molecules [53]. NK cells are also able to induce MHC class II molecules on HUVECs [50, 54] and microvascular ECs in a contact-dependent manner. In our hands this effect was inhibited by neutralizing antibodies to IFNγ [50], but others have reported the effect is not dependent on IFNγ [54].

Stimulation of alloreactive T cells

The fact that human microvascular ECs constitutively express MHC class II molecules has stimulated a number of workers to ask whether ECs directly cause allo-stimulation of resting T cells. We and others [55, 56] have cultured stringently purified CD4+ T cells with pure passaged HUVECs and looked for T-cell proliferation (measured by uptake of 3H-thymidine) at day 6. The ECs are treated with mitomycin C to stop them proliferating; any proliferation detected is thus due to responding T cells. The results in figure 5 show the response of CD4+ T cells to human ECs (Eahy.926), porcine aortic ECs (PAECs) and fetal lung fibroblasts. It can be seen that provided IFNγ is used to upregulate MHC class II, there is a strong proliferative response to human ECs, but not to fibroblasts. There is also a strong response to PAECs which is independent of IFNγ treatment. The reason for this is that PAEC class II expression persists in culture. That the response was direct and not indirect was proven by the findings that responder T cells were free of contaminating APCs [55, 56] and could not be inhibited by CTAL-4-Ig [57]. The response of CD4+ T cells depends on pretreatment of the ECs with IFNγ and is inhibited by antibodies to MHC class II determinants [57]. It is not affected by antibodies to MHC class I. In contrast, isolated CD8+ T cells proliferate to untreated ECs, and the response is inhibited by antibodies to MHC class I determinants [56, 58].

The majority of studies use ECs of foetal origin (from the umbilical vein); we have confirmed these findings using ECs from adult aorta, coronary artery and microvascular beds and in addition have shown that smooth muscle cells derived from the same vessel fail to stimulate allogeneic T cells [59]. When restimulated, T cells respond to ECs giving kinetics similar to that seen in the primary response [60]. There is no evidence for anergy induction.

It must be concluded, therefore, that donor ECs can present alloantigen to recipient T cells. It is interesting to note that there is a species difference between rodents and humans. Rodents do not constitutively express MHC class II antigens on their ECs. This is an important species difference and may explain why it is easier to suppress transplant rejection in rodents than it is in humans. Thus, in rodents the only MHC class II-positive cells of donor origin in the graft are cells (dendritic cells and macrophages) of bone marrow origin. These cells have a finite life span and in addition they migrate from the graft to lodge in other organs. Thus, after about 2 weeks the rodent allografted organ is bereft of donor MHC class II-presenting cells. In contrast, in humans, endothelial expression of donor MHC class II antigens ensures persistence of donor class II for long periods (if not indefinitely) after transplantation [61]. It follows that understanding the signals that allow human ECs to stimulate T cells may lead to new strategies of preventing rejection.

Second-signal requirements of ECs

We have questioned whether ECs utilize the B7 pathway to stimulate T cells, and our results [57] and those of others [62] demonstrate that endothelial cells do not

express B7 receptors. Thus, umbilical vein, aorta, coronary artery and heart microvascular ECs do not bind CTLA-4-immunoglobulin (Ig), as assessed by flow cytometry, and do not bind monoclonal antibodies against CD80/CD86 [57, 59, 62]. EC/T-cell interactions are not inhibited by doses of CTLA-4-Ig which inhibit splenocyte/T-cell interactions [57, 62]. There has been a single report that HUVECs and dermal microvascular ECs express CD86 antigens [63]; this study used commercially derived ECs and has not, as yet, been corroborated. However, EC/T cell interactions are inhibited by antibodies to LFA/3 (CD58, ref. 64), the ligand for which, found on all T cells, is CD2. It is interesting that the affinity of CD2 for the human ligand CD58 is much greater than its affinity for the mouse ligand CD48 [65]. The possibility that second signals other than B7 could be important in allograft rejection is supported by the evidence of skin allograft rejection in CD28-deficient mice [66]; blocking the receptor for LFA3 prolongs cardiac allograft survival in primates [67], and use of antibodies against intercellular adhesion molecule (ICAM)-1 induces tolerance in a murine model of allograft rejection [68]. Indeed, it has been shown that 'adhesion molecules' such as vascular cell adhesion molecule (VCAM)-1, ICAM-1 and ICAM-2 can act as costimulatory signals inducing T-cell proliferation in vitro [69, 70]. Human ECs constitutively express ICAM-1 and ICAM-2, each of which can bind to T-cell LFA-1(CD11aCD18). ICAM-1 is constitutively expressed at quite low levels, but unlike ICAM-2 it can be rapidly and markedly upregulated in response to IL-1, TNFα, LT, CD40 ligand or IFNγ [71]. VCAM-1 is not normally expressed on ECs but is upregulated by IL-1, TNFα, LT, CD40 ligand and IL-4. VCAM-1 binds to T-cell VLA4 (CD49/CD29). Several groups have demonstrated upregulation on VCAM-1 on microvascular ECs within rejecting cardiac allografts [72, 73]. Cytokines also induce ECs to express selectins (both E and P, refs 74 and 75) as well as ligands for L-selectin [76]; but there is no evidence that these molecules can act as costimulators for T-cell activation. However, the possibility that extracellular matrix molecules (fibronectin, collagen, lamenin) can act as second signals via interaction with $\beta 1$ integrins on lymphocytes should also be considered [77]. There remains much to learn about the nature of the second signals presented by ECs.

Other molecules necessary for antigen presentation

The actual structure recognized by T cells is a complex of the MHC molecule and a bound peptide. Class I molecules acquire their peptides predominantly from proteins degraded in the cytoplasm by the proteosome; cytoplasmic peptides are translocated into the endoplasmic reticulum and loaded on to nascent class I molecules by the action of transporter in antigen processing (TAP) proteins [78]. Although peptide loading has not been examined directly in ECs, it has been shown that ECs express TAP proteins [79] and that alloreactive CD8 + cytotoxic T cell (CTL) lines can specifically lyse ECs, implying that the TAP system is functional [80, 81]. One of the effects of IFNγ on ECs is to upregulate TAP functional activity [82]. Class II MHC molecules associate with a non-MHC-coded protein, the invariant chain, until they reach an intracellular compartment that fuses with late endosomes/lysosomes. Here, the invariant chain is degraded, and peptides derived from endocytosed proteins are loaded. The loading of peptides into class II molecules depends on an MHC-encoded molecule called DM [83, 84]. ECs have been shown to express invariant chain after IFNγ treatment [47], and we have recently shown that DM is expressed coordinately with DR and DP molecules in human ECs (D. Moyes and M. Rose, unpublished results). These results strongly suggest that ECs must be able to process antigen and present it within MHC class I and class II molecules. It has been shown that human ECs are able to process exogenous proteins to produce a peptide-class II MHC molecule complex recognized by antigen-specific CD4 + T cells [85, 86]. These results imply that ECs must be able to form specific peptide complexes with IFNγ-induced class II MHC molecules.

ECs compared with other APCs

It is well established that bone marrow-derived APCs such as dendritic cells, B cells and monocytes (professional APCs) are very potent stimulators of alloreactive T cells. In rodents, where the only class II-bearing cells in donor organs are derived from the bone marrow, depletion of these cells prior to transplantation results in prolonged allograft survival [87] in certain rat strain combinations. Mature dendritic cells and monocytes constitutively express MHC class II antigens. The B7 molecules (CD80 and CD86) are constitutively expressed by dendritic cells and upregulated on activated monocytes and B cells. It is clear that, at least in vitro, much of the activation of alloreactive T cells by dendritic cells and monocytes is through CD28, since these responses are abrogated by CTLA-4-Ig [14]. Costimulation through CD28 induces tyrosine phosphorylation of a number of specific substrates [88] and greatly enhances T-cell activation. This effect involves stabilization of mRNA for several cytokines, including IL-2 [89]. In contrast, activation of T cells by ECs involves interaction between endothelial LFA-3 and CD2 on the T cell. $\beta 1$ integrins on the T-cell surface may also be involved in signalling via ECs. Interaction between CD2 and its ligand increases the affinity of the T cell for the APC and also regulates a number of cellular responses [90, 91].

Because of the greater affinity of CD2 for CD58 than CD48 [65], it is likely that costimulation via CD2 is a more important pathway in humans than rodents. In view of the lack of involvement of CD28 stimulation, it is not surprising that EC stimulation of T cells produces less IL-2 than stimulation by professional APCs. Studies [92, 93] have shown some IL-2 is produced from HUVEC/T-cell cultures, but far less than is produced from culturing porcine aortic ECs with human T cells [91]. Porcine aortic ECs express B7 receptors and therefore stimulate via CD28 [92, 93]. The response of human T cells to allogeneic ECs is, however, highly sensitive to cyclosporine (Batten and M. Rose, unpublished results) and the sensitivity is reversed by addition of B7 transfectants to culture wells, a result which accords entirely with the reported cyclosporine resistance of CD28-mediated stimulation of T cells [94]. In terms of numbers of T cells which are activated by ECs, far fewer are activated than by professional APCs. Thus use of limiting dilution analysis to investigate numbers of CD4+ T cells stimulated to produce IL-2 showed monocytes stimulate 1/232 T cells, whereas HUVECs treated with IFNγ to express MHC class II stimulated 1/1801 T cells [95]. The relatively small number of CD4+ T cells which respond to allogeneic HUVECs is explained by the fact that only T cells with a memory phenotype (CD45RO+) respond to ECs [96]. Thus, numbers of circulating CD45RO+ B7-independent T cells will be relatively small.

ECs compared with other parenchymal cells

Fibroblasts [56], epithelial cells from lung [97] or kidney [98] and smooth muscle cells from blood vessels [51, 99] can all be induced to express MHC class II antigens by in vitro treatment with IFNγ, achieving levels of expression similar to that found on IFNγ-treated HUVECs. Nevertheless, these cells do not cause proliferation of highly purified CD4+ T cells [51, 56, 97–99]. A direct comparison between microvascular ECs and alveolar and small airway epithelial cells derived from the same source (lungs) showed proliferation of T cells to endothelial but not epithelial cells [97]. Similarly, a direct comparison between ECs or smooth muscle cells from saphenous vein [99] or coronary artery [59] showed only the ECs caused cell proliferation. The smooth muscle cells failed to stimulate T-cell proliferation; they did not anergize the T cells. Some have reported exposure of CD4+ T cells to thyroid and renal epi-thelial cells anergizes both the CD45RO and CD45RA population of T cells [98]. The same authors have reported that HUVECs anergize the CD45RA+ population [100], but such a phenomenon has never been reported by any other group [60]. The reason non-EC parenchymal cells fail to stimulate T cells is not known, but it might relate to density of expression of accessory molecules. The accessory molecules of ECs (LFA-3 and possibly ICAM-1) are not as strongly expressed on lung epithelial cells compared with lung microvascular ECs [97].

Consequences of endothelial activation of T cells

It is well established that in vitro activation of T cells by allogeneic professional APCs results in a vigorous proliferative response accompanied by production of substantial amounts of IL-2 and IFNγ and small amounts of IL-4. As a result of a primary proliferative response, T cells are generated with a memory phenotype that gives a different cytokine profile on restimulation (more IL-2 and IFNγ). In addition, cytotoxic T cells are generated with specificity for class I MHC antigens present on the original stimulator cells. Pober [96] has reported that EC stimulation of allogeneic T cells results in IL-2, IL4 and IFNγ production, but upon secondary stimulation there is no evidence for a maturation of the T-cell response: the second proliferative response is no different from the first one in terms of cytokines produced. It has also been suggested that endothelial stimulation of T cells results in poor production of cytotoxic T cells [95]. The model used by Pober consisted of comparing ECs and monocytes as accessory cells in the presence of the polyclonal activator phytohaemagglutinin (PHA). The results obtained using this model may not be the same as directly investigating endothelial stimulation of T cells, and more work needs to be done in this area. That a virally induced murine EC line activates cloned Th2 cells and not cloned Th1 cells [101] also suggests ECs may activate T cells to produce a different cytokine profile than that produced by professional APCs. Pober has suggested that the deficient antigen-presenting ability of ECs leads to an aberrant immune response when T cells are present in vessels walls; he suggests that cytokines are produced which result in intimal thickening as opposed to an immune response resulting in tissue destruction (as happens in acute rejection of the heart).

Role of ECs in chronic rejection

It is likely that ECs are involved in chronic rejection via a number of different mechanisms. This review has focused on class II/CD4+ T-cell interactions, but it is clear from rodent models that MHC class II is not essential for chronic rejection. Thus, severe vasculopathy occurs in the coronary arteries of mice transplanted with hearts from MHC class I or non-MHC mismatched donors [102], and vasculopathy can occur (albeit to a lesser degree) in CD4+ T-cell-deficient recipients [103]. In these cases it is likely that immune responses mediated by CD8+ T cells and/or alloantiobody against MHC class I antigens are the important factors. Rodent and human ECs constitutively express MHC class I antigens.

Interestingly, it appears that very little MHC class II is induced on mouse arterial and capillary ECs even during chronic rejection [102]; thus rodents may be providing us with a model of rejection in the absence of endothelial class II. The expression of CD40 on normal EC and induced expression of CD40L on ECs in coronary artery disease (CAD) [104] suggests that ECs may be able to stimulate B cells and monocytes by a T-independent mechanism. Functional CD40 has been demonstrated on ECs by their ability to induce B7.2 on a B-cell line [104]. Indeed it has been shown that anti-CD40L antibody inhibits allograft rejection [105], and not only by inhibiting CD80 expression on recipient monocytes.

In patients the paradox is that, whereas T-cell damage to the myocardium is limited and controlled by immunosuppressive drugs, initial damage to the coronary endothelium, in some patients, progresses to TxCAD. It must be remembered that the mainstay immunosuppressive drug, cyclosporine, acts by inhibiting early events in T-cell activation, namely transcription of IL-2 [106]. T-cell activation leads to maturation of a number of different effector pathways (fig. 2) depending on the release of different cytokines. Thus, production of cytokines IL-4 and IL-5 will lead to antibody production. Some studies report that production of these cytokines is less sensitive to cyclosporine than to IL-2 production [107], and proliferating B cells are known to be less sensitive to cyclosporine (CsA) [108]. It is clear, therefore, that chronic rejection is driven by mechanisms which are relatively resistant to CsA; such mechanisms may include the T-independent mechanisms described above. One of the effector mechanisms which is little affected by immunosuppression is antibody production. Despite the heavy immunosuppression received by patients after solid organ transplantation, the majority make a vigorous antibody response against the allografted organ (reviewed in ref. 109).

It is our hypothesis that a sustained antibody response against HLA and non-HLA antigens on donor ECs is one of the factors which leads to TxCAD. The most common way of detecting antibodies formed after transplantation is a complement-dependent cytotoxicity test against a panel of HLA-typed leukocytes (termed panel reactive antibodies, or PRA test) or donor cells (termed a donor-specific response). Many clinical studies have reported an association between antibody producers and development of chronic rejection [110]. Thus Suciu-Foca et al. reported a 90% 4-year actuarial survival in patients who had not made antibody following cardiac transplantation versus 38% 4-year survival in the antibody producers [111]. These authors looked for anti-HLA antibodies, but our own studies have shown a correlation between antiendothelial antibodies and chronic rejection [112]. Using gel electrophoresis to separate endothelial peptides according to molecular weight, followed by probing blots with patients' sera, we found that the majority of patients who had TxCAD had antibodies against endothelial peptides of 56 and 58 kDa. We have subsequently confirmed this association in a separate study of new patients using both western blotting and flow cytometry (unpublished results). A similar association between antiendothelial antibodies, detected by flow cytometry, and chronic rejection has been reported after renal transplantation [113, 114]. Since this test [112] detected antibodies against unrelated HUVECs, it is clear that donor-specific HLA antigens could not be involved. Use of SDS-polyacrylamide gel electrophoresis and amino acid sequencing revealed that the most immunogeneic endothelial peptide (at 56/58 kDa) was the intermediate filament vimentin, and other immunoreactive peptides were identified as triose phosphate isomerase and glucose-regulating protein – in all 40 different proteins were identified which reacted with patients, IgM [115]. Vimentin is the intermediate filament characteristic of but not restricted to ECs and fibroblasts. Whereas smooth muscle cells predominantly express desmin as their intermediate filament, they coexpress desmin and vimentin when migrating or proliferating. Vimentin is diffusely expressed in the intima and media of normal and diseased coronary arteries. Our working hypothesis is that antibodies to vimentin reflect disease activity in the coronary arteries. But the outstanding questions are how vimentin, a cytosolic protein, is exposed to the immune system and whether and how the antibodies are damaging.

It is highly likely that ECs are damaged early after transplantation (possibly by nonimmunological factors such as ischaemia/reperfusion injury), and some vimentin is released into the circulation to be bound by host B cells. Use of the MHCPEP database of MHC-binding peptides has revealed a sequence homology between epitopes of vimentin and class II-presented peptides, these being an HLA-DRα peptide and a heat shock protein peptide (HSP65). Our hypothesis is that host T cells will recognize vimentin fragments, presented indirectly by host B cells and other APCs as a consequence of cross-reactivity with MHC class II-presented peptides (such as DRα). The hypothesis asssumes that vimentin is not normally exposed to the immune system, and it will therefore be recognized as an autoantigen. Such cross-reactions between DRα and infectious agents/normal components of tissues has been suggested as a mechanism for a number of auto-immune diseases [116]. It is likely that damaged ECs are a source of many peptides which will be presented indirectly to recipient T cells. It is also possible that CD40 ligand-expressing ECs directly stimulate B cells to make antibody.

A number of experimental models support an important role for antibody in pathogeneisi of TxCAD (see ref. 117 for review). Thus, Russel and colleagues reported that transplantation of B10A hearts into B10.BR recipients

(across an MHC class I mismatch) resulted in severe coronary lesions, and the recipients made complement-dependent cytotoxic antibody against donor MHC class I antigens. That antibody could induce arterial lesions was shown by use of severe combined immunodeficient mice as recipients of B10.BR hearts; arterial lesions were induced in these hearts by injections of anti-B10.BR antiserum [118]. Recently, elegant experiments have used transplantation of carotid arteries from murine B10.A(2R) donors into mutant MHC mismatched recipients with various genetic defects of their immune system [103]. The neointimal thickening characteristic of Tx-CAD was dependent on the presence of CD4 + T cells, antibody formation and macropha-ges. Cytotoxic CD8 + T cells and NK cells were not involved in the process.

One of the major drawbacks to ascribing a role for antibodies in pathogenesis is lack of understanding about the way antibodies interact with their cellular targets. The serum derived from our patients does not exhibit complement-dependent cytotoxicity against ECs derived from HUVECs or aorta, nor does it exhibit antibody-dependent cellular cytotoxicity. This is not surprising with the exception of serum from patients with Kawasaki disease, where IgM antibodies are directly cytotoxic to ECs in the presence of complement [119]. Anti-endothelial antibodies have not been found to mediate complement-mediated damage to ECs. However, complement-mediated lysis is a severe and acute form of damage, usually associated with hyperacute rejection. It is more important to investigate whether antiendothelial antibodies can mediate more subtle forms of damage. Recently a number of reports have demonstrated that antibodies from patients with autoimmune disease [120] or transplant patients [121] can upregulate adhesion molecules on ECs. Ligation of monomorphic regions of MHC class I molecules on ECs results in tyrosine phosphorylation (J. Smith and M. Rose, unpublished results), EC proliferation and upregulation of fibroblast growth factor receptor [122]. We believe the information that antibodies can activate ECs is very promising and should be explored as a mechanism whereby antibodies could damage ECs in both autoimmune disease and chronic rejection after solid organ transplantation.

In conclusion, the immunological properties of ECs suggest they perform a pivotal role in acute and chronic rejection following solid organ transplantation. Expression of MHC class I and class II molecules allows them to activate recipient T cells by both direct and indirect routes. Release of non-HLA antigens as a result of immunological or nonimmunological damage provides a stimulus for antibody formation which may further damage or activate donor ECs. The costimulatory molecules used by ECs appear to differ from those used by traditional APCs such as B cells and dendritic cells. Further understanding of the molecules involved is warranted, as development of specific strategies to block EC recognition may provide better ways of preventing rejection than methods currently used.

1 Steinmuller D. (1985) Which T cells mediate allograft rejection? Transplantation **40:** 229–233
2 Ungureanu-Longrois D., Balligand J. L., Simmons W. W., Okada I., Kobzik L., Lowenstein C. J. et al. (1995) Induction of nitric oxide synthase activity by cytokines in ventricular myocytes is necessary but not sufficient to decrease contractile responses to beta-adrenergic agonists. Circ. Res. **77:** 494–502
3 Matsumori A., Yamada T., Suzuki H., Matoba Y. and Sasayama S. (1994) Increased circulating cytokines in patients with myocarditis and cardiomyopathy. Br. Heart J. **72:** 561–566
4 Lewis N. P., Tsao P. S., Rickenbacher P. R., Xue C., Johns R. A., Haywood G. A. et al. (1996) Induction of nitric oxide synthase in the human cardiac allograft is associated with contractile dysfunction of the left ventricle. Circulation **93:** 720–729
5 Daar A. S., Fuggle S. V., Fabre J. W. and Morris P. J. (1984) The detailed distribution of MHC class II antigens in normal human organs. Transplantation **38:** 292–297
6 Rose M. L., Coles M. I., Griffin R. J., Pomerance A. and Yacoub M. H. (1986) Expression of class I and class II major histocompatibilty antigens in normal and transplanted human heart. Transplantation **41:** 776–780
7 Page C. S., Rose M. L., Piggott R. and Yacoub M. H. (1992) Heterogeneity of vascular ECs. Am. J. Pathol. **141:** 673–683
8 Parker K. E., Dalchau R., Fowler V. J., Priestly C. A., Carter C. A. and Fabre J. W. (1992) Stimulation of CD4 + T lymphocytes by allogeneic MHC peptides presented on autologous antigen presenting cells. Transplantation **53:** 918–924
9 Terness P., Dufter C., Otto G. and Opelz G. (1996) Allograft survival following immunisation with membrane bound or soluble peptide MHC class I donor antigens: factors relevant for the induction of rejection by indirect recognition. Transplant. Int. **9:** 2–8
10 Liu Z., Colovai A. I., Tugulea S., Reed E. F., Fisher P. E., Mancini D. et al. (1996) Indirect recognition of donor HLA-DR peptides in organ allograft rejection. J. Clin. Invest. **98:** 1150–1157
11 Pober J. S., Orosz C. G., Rose M. L. and Savage C. O. S. (1996) Can graft endothelial cells initiate a host anti-graft immune response? Transplantation **61:** 343–349
12 Janeway C. A. J. and Bottomly K. (1994) Signals and signs for lymphocyte responses. Cell **57:** 275–285
13 June C. H., Blustone J. A., Nadler L. M. and Thompson C. B. (1994) The B7 and CD28 receptor families. Immunol. Today **15:** 321–331
14 Tan P., Anasetti C., Hansen J. A., Melrose J., Brunvand M., Bradshaw J. et al. (1993) Induction of alloantigen specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. J. Exp. Med. **177:** 165–173
15 Schwartz R. H. (1990) A cell culture model for T lymphocyte clonal anergy. Science **248:** 1349–1356
16 Liu Y. and Janeway C. A. J. (1990) Interferon g plays a critical role in induced cell death of effector T cells: a possible third mechanism of self-tolerance. J. Exp. Med. **172:** 1735–1739
17 Linsley P. S., Brady W., Urnes M., Grosmire L. S., Damle N. K. and Ledbetter J. A. (1991) CTLA-4 is a second receptor for the B cell activation antigen B7. J. Exp. Med. **147:** 561–569
18 Turka L. A., Linsley P. S., Lin H., Brady W., Leiden J. M., Wei R. Q. et al. (1992) T cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. Proc. Natl. Acad. Sci. USA **89:** 11102–11105
19 Lin H., Bolling S. F., Linsley P. S., Wei R. Q., Gordon D., Thompson C. B. et al. (1993) Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4-Ig plus donor-specific transfusions. J. Exp. Med. **178:** 1801–1806

20 Pearson T. C., Alexandre D. Z., Winn K. J., Linsley P. S., Lowry R. P. and Larsen C. P. (1994) Transplantation tolerance induced by CTLA4-Ig. Transplantation **57:** 1701–1706
21 Lenschow D. J., Zeng Y., Thistlethwaite J., Montag A., Brady W., Gibson M. G. et al. (1992) Long term survival of xenogeneic pancreatic islet grafts induced by CTLA4-Ig. Science **257:** 789–792
22 Fuggle S. V. (1989) MHC antigen expression in vascularised organ allografts: clinical correlations and significance. Transplant. Rev. **3:** 81–102
23 So S. K. S., Platt J. L., Ascher N. L. and Snover D. C. (1987) Increased expression of class I major histocompatibilty complex antigens on hepatocytes in rejecting human liver allografts. Transplantation **43:** 79–84
24 Fuggle S. V., Errasti P., Daar A. S., Fabre J. W., Ting A., Morris P. J. et al. (1983) Localisation of major histocompatibilty complex (HLA-ABC and DR) antigens in 46 kidneys: differences in HLA-DR staining of tubules among kidneys. Transplantation **35:** 385–390
25 Milton A. D., Spencer S. C. and Fabre J. W. (1986) Detailed analysis and demonstration of differences in the kinetics of induction of class I and class II major histocompatibilty complex antigens in rejecting cardiac and kidney allografts in the rat. Transplantation **41:** 499–508
26 Arbustini E., Grasso M., Diegoli M., Bramerio M., Foglieni A. S., Albertario M. et al. (1991) Expression of tumour necrosis factor in human acute cardiac rejection. An immunohistochemical and immunoblotting study. Am. J. Pathol. **139:** 709–715
27 Cunningham D. A., Dunn M. J., Yacoub M. J. and Rose M. L. (1994) Local production of cytokines in the human cardiac allograft. Transplantation **57:** 1333–1337
28 Gao S. J., Schroeder J. S., Alderman E. L., Hunt S. A., Valantine H. A. and Weiederhold V. (1989) Prevalence of accelerated coronary artery disease in heart transplant survivors. Comparison of cyclosporine and azathioprine regimens. Circulation **8:** III100–105
29 Johnson J. A. and Kobashigawa J. A. (1995) Quantitative analysis of transplant coronary artery disease with use of intracoronary ultrasound. J. Heart Lung Transplant. **14:** S198–202
30 Hosenpud J. D., Shipley G. D. and Wagner C. R. (1992) Cardiac allograft vasculopathy: current concepts, recent developments and future directions. J. Heart Lung Transplant. **11:** 9–23
31 Ross R. (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature **362:** 801–809
32 Barbir M., Banner N., Thompson G. R., Khaghani A., Mitchell A. and Yacoub M. H. (1991). Relationship of immunosuppression and serum lipids to the development of coronary arterial disease in the transplanted heart. Int. J. Cardiol. **32:** 51–56
33 Barbir M., Kushwaha S., Hunt B., Mackin A., Thompson G. R., Mitchell A. et al. (1992) Lipoprotein (a) and accelerated coronary artery disease in cardiac transplant recipients. Lancet **340:** 1500–1502
34 Kobashigawa J. A., Katznelson S., Laks H., Johnson J. A., Yeatman L., Wang X. M. et al. (1995) Effect of pravastatin on outcome after cardiac transplantation. N. Engl. J. Med. **333:** 621–627
35 Gratten M. T., Moreno-Cabral C. E., Starnes V. A., Oyer P. E., Stinson E. B., Shumway N. E. et al. (1989) Cytomegalovirus is associated with cardiac allograft rejection and atherosclerosis. JAMA **261:** 3561–3566
36 McDonald K., Rector T. S., Braunlin E. A., Kubo S. H. and Olivari M. T. (1989) Association of coronary artery disease in transplant recipients with cytomegalovirus infection. Am. J. Cardiol. **64:** 359–362
37 Loebe M., Schiller S., Zaus O., Warnecke H., Flecke E. and Hetzer R. (1990) Role of cytomegalovirus in the development of coronary artery disease in the transplanted heart. J. Heart Lung Transplant. **9:** 707–711
38 Everett J. P., Hershberger R. E., Norman D. J., Chou S., Ratkovec R. M., Cobanoglu A. et al. (1992) Prolonged cytomegalovirus infection with viremia is associated with development of cardiac allograft vasculopathy. J. Heart Lung Transplant. **11:** S133–S137
39 Kendall T. J., Wilson R. E., Radio S. J., Kandolph R., Gulizia J. M., Winters G. L. et al. (1992) Cytomegalovirus and other herpesviruses: do they have a role in the development of accelerated coronary artery disease in human heart allografts? J. Heart Lung Transplant. **11:** S14–20
40 Beersma F. C., Bijlmakers M. J. E. and Ploegh H. L. (1993) Human cytomegalovirus down regulates HLA class I expression by reducing the stability of class I chains. J. Immunol. **151:** 4455–4464
41 Sedmak D. D., Roberts W. H., Stephens R. E., Buesching W. J., Morgan L. A. and Davis D. H. (1990) Inability of cytomegalovirus infection of cultured ECs to induce HLA class II antigen expression. Transplantation **49:** 458–462
42 Waldman W. J., Adams P. W., Orosz C. G. and Sedmak D. D. (1992) T lymphocyte activation by cytomegalovirus infected allogeneic cultured human ECs. Transplantation **54:** 887–896
43 Tullius S. G. and Tilney N. J. (1995) Both alloantigen-dependent and independent factors influence chronic allograft rejection. Transplantation **59:** 313–318
44 Collins T., Lapierre L. A., Fiers W., Strominger J. L. and Pober J. S. (1986) Recombinant human tumour necrosis factor increases mRNA levels and surface expression of HLA-A,B antigens in vascular ECs and dermal fibroblasts in vitro. Proc. Natl. Acad. Sci. USA **83:** 446–450
45 Lapierre L. A., Fiers W. and Pober J. S. (1988) Three distinct classes of regulatory cytokines control endothelial cell MHC antigen expression: interactions with immune (γ) interferon differentiate the effects of tumor necrosis factor and lymphotoxin form those of leukocyte (α) and fibroblast (β) interferons. J. Exp. Med. **167:** 794–804
46 Batten P., Yacoub M. H. and Rose M. L. (1996) Effect of human cytokines (IFN-γ, TNF-α, IL-1β, IL-4) on porcine ECs: induction of MHC and adhesion molecules and functional significance of these changes. Immunology **87:** 127–133
47 Collins T., Korman A. J., Wake C. T., Boss J. M., Kappes D. J., Fiers W. et al. (1984) Immune interferon activates multiple class II major histocompatibilty complex genes and the associated invariant chain gene in human ECs and dermal fibroblasts. Proc. Natl. Acad. Sci. USA **81:** 4917–4921
48 Panek R. B., Lee Y.-J. and Benveniste E. N. (1995). TGF-β suppression of IFN-γ induced class II MHC gene expression does not involve inhibition of phosphorylation of JAK1, JAK2 or signal transducers and activators of transcription, or modification of IFN-γ enhanced factor X expression. J. Immunol. **154:** 610–619
49 Nanden D. and Reiner N. E. (1997). TGF-β attenuates the class II transactivator and reveals an accessory pathway of IFN-γ action. J. Immunol. **158:** 1095–1101
50 McDouall R. M., Batten P., McCormack A., Yacoub M. H. and Rose M. L. (1997) MHC class II expression on human microvascular endothelial cells: exquisite sensitivity to IFNγ and NK cells. Transplantation **64:** 1175–1180
51 McDouall R. M., Yacoub M. H. and Rose M. L. (1996) Isolation, culture and characterisation of MHC class II positive microvascular ECs from the human heart. Microvasc. Res. **51:** 137–152
52 Groenewegen C., Buurman W. A. and van der Linden C. J. (1985) Lymphokine dependence of in vivo expression of MHC class II antigens by endothelium. Nature **316:** 361–363
53 Goes N., Sims T., Urmson J., Vincent D., Ramassar V. and Halloran P. F. (1995) Disturbed MHC regulation in the IFNγ knockout mouse. Evidence for three states of MHC expression with distinct roles for IFNγ. J. Immunol. **155:** 4559–4566
54 Watson C. A., Petzelbauer P., Zhou J., Pardi R. and Bender J. (1995) Contact dependent endothelial class II gene activation induced by NK cells is mediated by IFN gamma dependent and independent mechanisms. J. Immunol. **154:** 3222–3233

55 Savage C. O. S., Hughes C. C. W., McIntyre B. W., Picard J. K. and Pober J. S. (1993) Human CD4 + T cells proliferate to HLA-DR + allogeneic vascular endothelium: identification of accessory interactions. Transplantation **56:** 128–134

56 Page C. S., Holloway N., Smith H., Yacoub M. H. and Rose M. L. (1994) Alloproliferative responses of purified CD4 + and CD8 + T cell subsets to human vascular ECs in the absence of contaminating accessory cells. Transplantation **57:** 1628–1637

57 Page C. S., Thompson C., Yacoub M. H. and Rose M. L. (1994) Human endothelial cell stimulation of allogeneic T cells via a CTLA-4 independent pathway. Transplant. Immunol. **1994: 2:** 342–347

58 Epperson D. E. and Pober J. S. (1994) Antigen-presenting function of human endothelial cells. Direct activation of resting CD8 + T cells. J. Immunol. **153:** 5402–5412

59 McDouall R. M., Page C. S., Hafizi H., Yacoub M. H. and Rose M. L. (1996) Alloproliferation of purified CD4 + T cells to adult human heart ECs and study of second signal requirements. Immunology **89:** 220–226

60 Savage C. O. S. and Brooks C. J. (1995). Human vascular ECs do not induce anergy in allogeneic CD4 + T cells unless costimulation is prevented. Transplantation **60:** 734–740

61 Rose M. L., Navarette C., Yacoub M. H. and Festenstein H. (1988) Persistence of donor specific class II antigens in allografted human heart two years after transplantation. Human Immunol. **23:** 179–182

62 Murray A. G., Khodadoust M. M., Pober J. S. and Bothwell A. L. M. (1994) Porcine aortic ECs activate human T cells: direct presentation of MHC antigens and costimulation by ligands for human CD2 and CD28. Immunity **1:** 57–63

63 Seino K., Azuma M., Bashuda H., Fukao K., Yagita H. and Okumura K. (1995) CD86 (B70/B7–2) on ECs co-stimulates allogeneic CD4 + T cells. Int. Immunol. **7:** 1331–1337

64 Savage C. O. S., Hughes C. C. W., Pepinsky R. S., Wallner B. P., Freedman A. S. and Pober J. S. (1991) Endothelial cell lymphocyte function associated antigen-3 and an unidentified ligand act in concert to provide costimulation to human peripheral blood CD4 + T cells. Cell. Immunol. **137:** 150–163

65 Arulanandam A. R., Moingeon P., Concino M. F., Recny M. A., Kato K., Yagita H. et al. (1993) A soluble multimeric recombinant CD2 protein identifies CD48 as a low affinity ligand for human CD2: divergence of CD2 ligands during evolution of humans and mice. J. Exp. Med. **177:** 1439–1450

66 Kawai K., Shahinian A., Mak T. W. and Ohashi P. S. (1996) Skin allograft rejection in CD28-deficient mice. Transplantation **61:** 352–355

67 Kaplon A. G., Hochman P. S., Michler R. E., Kwiatkowski P. A., Edwards N. M., Berger C. L. et al. (1996) Short course single agent therapy with an LFA-3-IgG1 fusion protein prolongs primate cardiac allograft survival. Transplantation **61:** 356–363

68 Isobe I. M., Suzuki J., Yamazaki S., Horie S., Okubo Y. and Sekiguchi M. (1997) Assessment of tolerance induction to cardiac allograft by anti-ICAM-1 and anti-LFA-1 monoclonal antibodies. J. Heart Lung Transplant. **16:** 1149–1156

69 VanSeventer G. A., Shimizy Y., Horgan K. J. and Shaw S. (1990) The LFA-1 ligand ICAM-1 provides a costimulatory signal for T cell receptor mediated activation of resting T cells. J. Immunol. **144:** 4579–4586

70 Damle N. and Aruffo A. (1991) Vascular cell adhesion molecule induces T cell antigen receptor dependent activation by CD4 + T lymphocytes. Proc. Natl. Acad. Sci. **88:** 6403–6407

71 Karmann K., Hughes C. C. W., Schechner J., Fanslow W. C. and Pober J. S. (1995) CD40 on human ECs: inducibility by cytokines and functional regulation of adhesion molecule expression. Proc. Natl. Acad. Sci. **92:** 4342–4346

72 Taylor P. M., Rose M. L., Yacoub M. H. and Piggott R. (1992) Induction of vascular adhesion molecules during rejection of human cardiac allografts. Transplantation **54:** 451–457

73 Briscoe D. M., Schoen F. J., Rice G. E., Bevilacqua M. P., Ganz P. and Pober J. S. (1991). Induced expression of endothelial-leukocyte adhesion molecules in human cardiac allografts. Transplantation **51:** 537–539

74 Pober J. S., Bevilacqua M. P., Mendrick D. L., Lapierre L. A., Fiers W. and Gimbrone M. A. (1986) Two distinct monokines, interleukin 1 and tumour necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured ECs. J. Immunol. **136:** 1680–1687

75 Luscinskas F. W., Ding H. and Lichtman A. H. (1995) P-selectin and vascular adhesion molecule 1 mediate rolling and arrest, respectively, of CD4 + T lymphocytes on tumour necrosis factor a activated vascular endothelium under flow. J. Exp. Med. **181:** 1179–1186

76 Spertini O., Luscinskas F. W., Kansas G. S., Munro J. M., Griffin J. D. et al. (1991) Leukocyte adhesion molecule-1 (LAM-1, L-selectin) interacts with an inducible endothelial cell ligand to support leukocyte adhesion. J. Immunol. **147:** 2565–2573

77 Yamada A., Nikaido T., Nojima Y., Schlossman S. and Morimoto C. (1991) Activation of human CD4 + T lymphocytes. Interaction of fibronectin with VLA-5 receptor on CD4 cells induces the AP-1 transcription factor. J. Immunol. **146:** 53–56

78 Germain R. N. and Margulies D. H. (1993) The biochemistry and cell biology of antigen processing and presentation. Ann. Rev. Immunol. **11:** 403–450

79 Epperson D. E., Arnold D., Spies T., Cresswell P., Pober J. S. and Johnson D. R. (1992) Cytokines increase transporter in antigen processing-1 (TAP-1) expression more rapidly than HLA class I expression in ECs. J. Immunol. **149:** 3297–3301

80 Clayberger C., Uyehara T., Hardy B., Eaton J., Karasele M. and Krensky A. M. (1985) Target specificity and cell surface structures involved in the human cytolytic T cell response to endothelial cells. J. Immunol. **135:** 12–18

81 Pardi R. and Bender J. R. (1991) Signal requirements for the generation of CD4 + and CD8 + T cell responses to human allogeneic microvascular endothelium. Circ. Res. **69:** 1269–1279

82 Ayalon O., Hughes E. A., Creswell P., Lee J., O'Donnel L., Pardi R. et al. (1998) Induction of transporter associated with antigen processing by interferon g confers endothelial cell cytoprotection against natural killer-mediated lysis. Proc. Natl. Acad. Sci. USA **95:** 2435–2440

83 Fling S. P., Arp B. and Pious D. (1994) HLA-DMA and DMB genes are both required for the MHC class II/peptide complex formation in antigen presenting cells. Nature **368:** 554–558

84 Ceman S., Rudersdorf R. A., Peteran J. M. and DeMars A. (1995) DMA and DMB are the only genes in the class II region of the human MHC needed for class II associated antigen processing. J. Immunol. **154:** 2545–2556

85 Vora M., Yssel H., de Vries J. E. and Karasek M. A. (1994) Antigen presentation by human dermal microvascular ECs. Immunoregulatory effects of IFNγ and IL-10. J. Immunol. **152:** 5734–5741

86 Savage C. O. S., Brooks C. J., Harcourt G. C., Picard J. K., King W., Sansom D. M. et al. (1995) Human vascular endothelial cells process and present autoantigen to human T cell lines. Int. Immunol. **7:** 471–479

87 Lechler R. I. and Batchelor J. R. (1982) Restoration of immungenicity of passenger cell depleted kidney allografts by the addition of donor strain dendritic cells. J. Exp. Med. **155:** 31–41

88 Vandenberghe P., Freeman G. J., Nadler L. M., Fletcher M. C., Kamoun M., Turka L. A. et al. (1992) Antibody and B7/BB1-mediated ligation of the CD28 receptor induces tyrosine phosphorylation in human T cells. J. Exp. Med. **175:** 951–960

89 Lindstein T., June C. H., Ledbetter J. A., Stella G. and Thompson C. B. (1989) Regulation of lymphokine messenger RNA stability by a surface mediated T cell activation pathway. Science **244:** 339–343

90 King P. D., Sadra A., Han A., Liu X. R., Sunder-Plassmann R., Reinherz E. L. et al. (1996) CD2 signalling in T cells involves tyrosine phosphorylation and activation of the Tec family kinase, EMT/ITK/TSK. Int. Immunol. **8:** 1707–1714

91 Gollob J. A., Li J., Kawasaki H., Daley J. F., Groves C., Reinherz E. L. et al. (1996) Molecular interactions between

CD58 and CD2 counter receptors mediates the ability of monocytes to augment T cell activation by IL-12. J. Immunol. **157:** 1886–1893
92 Bravery C. A., Batten P., Yacoub M. H. and Rose M. L. (1995) Direct recognition of SLA and HLA-like class II antigens on porcine endothelium by human T cells results in T cell activation and release of Interleukin-2. Transplantation **60:** 1024–1033
93 Hughes C. C. W. and Pober J. S. (1993) Costimulation of peripheral blood T cell activation by human ECs. Enhanced IL-2 transcription correlates with increased c-fos synthesis and increased fos content of AP-1. J. Immunol. **150:** 3148–3160
94 June C. H., Ledbetter J. A., Gillespie M. M., Lindsten T. and Thompson C. B. (1987) T cell proliferation involving the CD28 pathway is associated with cyclosporine resistant interleukin-2 gene expression. Mol. Cell. Biol. **7:** 4472–4481
95 Adams P. W., Lee S. H., Waldman W. J., Sedmak D. D., Morgan C. J., Ward J. S. et al. (1992) Alloantigenicity of human endothelial cells. 1. Frequency and phenotype of human T helper lymphocytes that can react to allogeneic endothelial cells. J. Immunol. **148:** 3753–3760
96 Pober J. S., Ma W., Biedermann B. and Libby P. (1997) Vascular cells have limited capacities to activate and differentiate T cells: implications for transplant vascular sclerosis. Transplant. Immunol. **5:** 251–255
97 Cunningham A. C., Zhang J.-G., Moy J. V., Ali S. and Kirby J. A. (1997) A comparison of the antigen-presenting capabilities of class II MHC expressing human lung epithelial and ECs. Immunology **91:** 458–463
98 Marelli-Berg F. M., Weetman A., Frasca L., Deacock S., Imami N., Lombardi G. et al. (1997) Antigen presentation by epithelial cells induces anergic immunoregulatory CD45RO + T cells and deletion of CD45RA + T cells. J. Immunol. **159:** 5853–5861
99 Murray A. G., Libby P. and Pober J. S. (1995) Human vascular smooth muscle cells poorly stimulate and actively inhibit allogeneic CD4 + T cell proliferation in vitro. J. Immunol. **154:** 151–161
100 Marelli-Berg F. M., Hargreaves R. E. G., Carmichael P., Dorling A., Lombardi G. and Lechler R. I. (1996) Major histocompatibility complex class II expressing endothelial cells induce allospecific non-responsiveness in naive T cells. J. Exp. Med. **183:** 1603–1612
101 St. Louis J. D., Lederer J. A. and Lichtman A. H. (1993). Costimulator deficient antigen presentation by an endothelial cell line induces a non-proliferative T cell activation response without anergy. J. Exp. Med. **178:** 1597–1605
102 Russel P. S., Chase C. M., Winn H. J. and Colvin R. B. (1994). Coronary atherosclerosis in transplanted mouse hearts. 1. Time course and immunogenetics and immunopathological considerations. Am J. Pathol. **144:** 260–274
103 Shi C., Lee W. S., He Q., Zhang D., Fletcher D. L., Newell J. B. et al. (1996) Immunologic basis of transplant-associated arteriosclerosis. Proc. Natl. Acad. Sci. USA **93:** 4051–4056
104 Mach F., Schonbeck U., Sukhova G. K., Bourcier T., Bonnefoy J. Y., Pober J. S. et al. (1997) Functional CD40 ligand is expressed on human vascular ECs, smooth muscle cells and macrophages: implications for CD40-CD40 ligand signalling in atherosclerosis. Proc. Natl. Acad. Sci. USA **94:** 1931–1936
105 Hancock W. W., Sayegh M. H., Zheng X. G., Peach R., Linsley P. S. and Turka L. A. (1996) Costimulatory function and expression of CD40 ligand, CD80 and CD86 in vascularised murine cardiac allograft rejection. Proc. Natl. Acad. Sci. USA **93:** 13967–13972
106 Clipstone N. A. and Crabtree G. R. (1992) Identification of calcineurin as a key signalling enzyme in T lymphocyte activation. Nature **357:** 695–697
107 Han C. W., Imamura M., Hashino S., Zhu X., Tanaka J., Imai K. et al. (1995) Differential effects of the immunosuppressants cyclosporin A, FK506 and KM2210 on cytokine expression. Bone Marrow Transplant. **15:** 733–739
108 Morris R. E. (1991) Rapamycin: FK506's fraternal twin or distant cousin? Immunol. Today **12:** 137–140
109 Rose M. L. (1993) Antibody mediated rejection following cardiac transplantation. Transplant. Rev. **7:** 140–152
110 Rose E. A., Smith C. R., Petrossian G. A., Barr M. L. and Reemtsma K. (1989) Humoral immune responses after cardiac transplantation: correlation with fatal rejection and graft atherosclerosis. Surgery **106:** 203–208
111 Suciu-Foca N., Reed E., Marboe C., Harris P., Yu P. X., Sun Y. K. et al. (1991) The role of anti-HLA antibodies in heart transplantation. Transplantation **51:** 716–724
112 Dunn M. J., Crisp S. J., Rose M. L., Taylor P. M. and Yacoub M. H. (1992) Antiendothelial antibodies and coronary artery disease after cardiac transplantation. Lancet **339:** 1566–1570
113 Al Hussein K. A., Talbot D., Proud D., Taylor R. M. R. and Shenton B. K. (1995) The clinical significance of post transplantation non-HLA antibodies in renal transplantation. Transplant. Int. **8:** 214–220
114 Ferry B. L., Welsh K. I., Dunn M. J., Law D. L., Proctor J., Chapel H. et al. (1997) Anti-cell surface endothelial antibodies in sera from cardiac and kidney transplant recipients: association with chronic rejection. Transplant. Immunol. **5:** 17–24
115 Wheeler C. H., Collins A., Dunn M. J., Crisp S. J., Yacoub M. H. and Rose M. L. (1995) Characterisation of endothelial antigens associated with transplant associated coronary artery disease. J. Heart Lung Transplant. **14:** S188–197
116 Baum H., Davies H. and Peakman M. (1996) Molecular mimicry in the MHC: hidden clues to autoimmunity? Immunol. Today **17:** 64–70
117 Rose M. L. (1996) Role of antibody and indirect antigen presentation in transplant associated coronary artery disease. J. Heart Lung Transplant. **15:** 342–349
118 Russel P. S., Chase C. M., Winn H. J. and Colvin R. B. (1994) Coronary atherosclerosis in transplanted mouse hearts. II. Importance of humoral immunity. J. Immunol. **152:** 5134–5141
119 Leung D. Y., Collins M. T., Lapierre L. A., Geha R. S. and Pober J. S. (1986) Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. J. Clin. Invest. **77:** 1428–1435
120 Carvalho D., Savage C. O. S., Black C. M. and Pearson J. D. (1996) IgG antiendothelial cell autoantibodies from scleroderma patients induce leukocyte adhesion to human vascular endothelial cells in vitro. J. Clin. Invest. **97:** 1–9
121 Pidwell D. W., Heller M. J., Gabler D. and Orosz C. (1995) In vitro stimulation of human ECs by sera from a subpopulation of high percentage panel reactive antibody patients. Transplantation **60:** 563–569
122 Harris P. E., Bian H. and Reed E. F. (1997). Induction of high affinity growth factor receptor and proliferation in human endothelial cells by anti-HLA antibodies: a possible mechanism for transplant atherosclerosis. J. Immunol. **159:** 5697–5704