Chapter 1

General Introduction

SOLID ORGAN TRANSPLANTATION

Since the late 50’s, transplantation of solid organs has become an increasingly successful therapy for patients suffering from end-stage organ failure. In the year 2000, more than 13.000 kidneys, almost 5.000 livers, more than 2.000 hearts, and almost 1.000 lungs were transplanted in the USA. In the Netherlands, 346 cadaveric kidneys, 95 livers, 43 hearts and 17 lungs were transplanted in the year 1999. The short-term results of organ transplantation have significantly increased over the last decades. This improvement is primarily due to the introduction of new, more effective, immunosuppressive agents. Especially the introduction of cyclosporine A (CsA) in the late 70’s caused an enormous decrease in morbidity and mortality after solid organ transplantation. Moreover, improved HLA tissue-typing assays and surgical techniques as well as advances made in donor-organ preservation contributed to the decreased morbidity and mortality after solid organ transplantation.

ALLOGRAFT REJECTION

After transplantation of allogeneic solid organs, grafts can be rejected resulting in graft loss in three different ways: hyperacutely, acutely, and chronically. Grafts will be hyperacutely rejected if pre-existing circulating anti-donor-HLA antibodies are present in the recipient at the time of transplantation. Binding of these antibodies to HLA-antigens expressed on donor-endothelium results in severe damage of the graft endothelial cells (EC’s), and platelet aggregation and complement activation occurs. Today, however, potential transplant recipients placed on waiting-lists are frequently panel-tested for the presence of circulating anti-HLA antibodies before transplantation, thereby minimizing the risk of hyperacute rejection after transplantation.

Unlike hyperacute rejection, which is antibody-mediated, acute rejection is a cell-mediated pathological inflammatory response, which occurs most frequently in the first months after transplantation. Acute rejection occurs when HLA-mismatches between donor and recipient are present. Because of limitations in the time of donor-organ preservation as well as the shortage of donor-organs, prospective HLA-typing of the donor is not possible except for kidney grafts. Therefore, the presence of HLA-mismatches between donor and recipient can not be prevented, thereby increasing the risk of acute rejection. To date, however, acute rejection episodes can be adequately treated since new immunosuppressive agents have become available (e.g., CsA and FK506). Despite the use of these new drugs, however, it has become clear that clinical transplantation has not achieved its goals as a long-term treatment. Long-term success has remained at the same level as in the pre-cyclosporine era, and so far no drugs are available that can further extend graft survival time. Chronic graft dysfunction (CTD) is now recognized as the primary cause of allograft loss after the first year after transplantation.

CHRONIC TRANSPLANT DYSFUNCTION

As mentioned above, CTD has become today’s most important cause of late (>1 year post transplantation) graft fail-
In its clinical context, CTD can be defined as the progressive irreversible loss of graft function that occurs late in the post transplant period (months to years after transplantation). Formerly, CTD was also known as ‘chronic rejection’. The term ‘rejection’, however, implies the alloimmune response of the recipient against the graft to be the basis for the deterioration in graft function. However, data have been reported indicating that also non-alloimmune associated factors involved in organ transplantation can cause similar functional and histopathological changes. Referring the whole process to as chronic rejection is therefore not satisfactory, and as long as the progressive deterioration in graft function can not exclusively be attributed to an alloimmune mediated pathway it is recommended to name the process CTD, leaving any causative factor as yet out of consideration.

The incidence of CTD after transplantation depends on the type of organ grafted. Five years after kidney transplantation, 30%-50% of the grafts are lost due to CTD. After cardiac and lung transplantation, the incidence of CTD is more than 50% and more than 70%, respectively, five years after transplantation. In liver transplantation, CTD seems to be less of a problem compared to kidney, cardiac, and lung allografts. Five years after transplantation, the incidence of CTD after liver transplantation varies from 3% to 26% depending on the transplant center.

The clinical presentation of CTD depends on the transplanted organ. CTD in kidney grafts presents as a progressive decline in the glomerular filtration rate in conjunction with increasing plasma creatinine concentrations, proteinuria and arterial hypertension. In cardiac allografts, CTD presents itself with myocardial infarction, arrhythmia’s and sudden death, and in liver transplants CTD is clinically characterized by elevation of liver enzymes and bilirubin in blood. Finally, CTD in the transplanted lung is characterized by a decline in pulmonary function, referred to as bronchiolitis obliterans syndrome (BOS).

CHRONIC TRANSPLANT DYSFUNCTION ASSOCIATED HISTOPATHOLOGY

The histopathological characteristics associated with CTD depend on the organ that has been transplanted. CTD in kidney allografts is characterized by inflammation, excessive interstitial fibrosis, tubular atrophy and glomerular sclerosis, whereas in cardiac transplants CTD is characterized by inflammation, fibrosis and an aggressive form of coronary artery disease (coronary allograft vasculopathy), that limits survival after transplantation. In liver allografts, CTD is histologically characterized by inflammation and disappearance of bile ducts and arthritis (vanishing bile duct syndrome), whereas in lung allografts the presence of fibroproliferative obliteration of bronchioles (obliterative bronchiolitis) is indicative of CTD.

The common histomorphological feature of CTD in solid organ transplants (kidney, heart, and lung) is the development of transplant vascular sclerosis or transplant arteriosclerosis (TA), which is, however, most prominent in cardiac al-
TA is characterized by vascular lesions in the graft that consist of concentric myointimal proliferation resulting in the development of an occlusive neointima in the arterial structures of the graft\textsuperscript{13}. The neointima primarily consists of $\alpha$-actin positive vascular smooth muscle (VSM) cells which are believed to be derived from the vessel media\textsuperscript{15}. This progressive blood vessel occlusion could lead to downstream ischemic tissue damage and disruptive fibrosis, and has therefore generally been accepted as the main cause of progressive deterioration in graft function (CTD).

Other findings coinciding with TA include a persistent focal perivascular inflammation (perivasculitis), endothelial swelling, disruption of the internal elastic lamina, focal myocyte necrosis, foam cell accumulation in the neointima, and presence of macrophages and T lymphocytes in the neointimal lesion\textsuperscript{33,34}. In contrast to ordinary atherosclerosis, which is usually focal and eccentric, the common form of TA is concentric and generalized. Figure 1 shows a typical example of the histopathological characteristics of CTD in a cardiac allograft i.e., luminal occlusion and perivascular inflammation.

**Figure 1.** Microphotographs of a normal coronary artery (A) and a coronary artery with severe transplant arteriosclerosis (B) in normal rat cardiac tissue and a rat cardiac allograft, respectively. Note the presence of severe neointima formation containing $\alpha$-actin positive vascular smooth muscle (VSM) cells, and perivascular inflammation. Double staining for elastin (internal elastic lamina) and VSM cell $\alpha$-actin. Abbreviations: IC, inflammatory cells; IEL, internal elastic lamina; M, media; MC, myocardium; NI, neointima. Magnification x200.
ETIOLOGY OF CHRONIC TRANSPLANT DYSFUNCTION

The presence of persistent perivascular inflammation in TA suggests that an allogeneic injury to the graft vessels is the prime cause for development of TA. In 1963, Porter et al. already showed presence of TA in kidney allografts from patients who had experienced early episodes of acute rejection. These results indeed suggest that the process of TA development can evolve from early acute cellular infiltration of the graft (acute rejection) to a more chronic inflammatory process, eventually resulting in the development of TA. However, the etiology of TA is still poorly defined, and the precise mechanism(s) of the development of this vasculopathy still remain obscure. The pathogenesis of TA seems to be multifactorial and both an ongoing alloimmune response of the host and non-alloantigen driven tissue-damage of the graft seem to contribute to the development of TA. Several risk factors for the development of TA have been identified which can roughly be divided in alloantigen dependent factors (i.e., donor-recipient combination), donor-related factors, recipient-related factors, and viral infections.

COMPONENTS INVOLVED IN TRANSPLANT ARTERIOSCLEROSIS

Alloantigen dependent risk factors (donor-recipient combination)

Transplant arteriosclerosis is characterized by interstitial and persistent perivascular inflammation, suggesting that alloreactivity of host immune cells against the MHC-incompatible graft plays an important role in the development of TA. Several factors supporting the contribution of an alloimmunologic mechanism in the development of TA have been identified.

Histoincompatibility. The presence of HLA-disparities between donor and recipient is associated with rejection of the graft, and long-term graft survival appeared to be strongly correlated with the presence of HLA-incompatibility between the donor and the recipient. However, a clear association between the number of HLA mismatches and the development of TA remains a matter of controversy. Using experimental transplant models it has been shown that in the absence of mismatches (isografts) these grafts develop no or minor TA after transplantation, suggesting that HLA-incompatibility indeed plays a role in the development of TA. It is, however, unclear whether matching of donor and recipient HLA directly affects the development of TA or whether this results from a decreased incidence of acute rejection episodes.

Acute rejection. Although no direct association between the number of HLA-mismatches and the development of TA is observed, one of the most important risk factors for the development of TA is the onset, frequency, and severity of acute rejection episodes after transplantation. This effect has been demonstrated in many retrospective studies analyzing all types of organ transplants. These clinical data have been confirmed by experimental studies using animal transplant...
models in rats and rabbits\textsuperscript{54-56}. However, the occurrence of acute rejection episodes early after transplantation is not an absolute prerequisite for the development of TA since development of TA without prior acute rejection episodes has also been described in some studies\textsuperscript{57-59}.

\textit{Inadequate immunosuppression.} Since acute rejection is associated with the development of TA, one might expect that inadequate immunosuppression to treat acute rejection episodes is also related to the development of TA. In several clinical studies it indeed has been shown that a low dose of maintenance CsA was associated with CTD\textsuperscript{38,60,61}, whereas in others it was not\textsuperscript{62}. Studying human renal allografts, Isoniemi et al. showed that patients receiving triple-drug immunosuppression developed less pronounced CTD related pathology compared to patients that received double-drug immunosuppression\textsuperscript{63}. These results support the role of inadequate immunosuppression in the development of TA. Moreover, in rat studies it has been shown that high dose CsA treatment prevents or inhibits development of TA after allogeneic aorta transplantation\textsuperscript{64-67}. Although very effective in rats, in man it would be impossible to maintain high doses of CsA for prolonged periods of time because of the nephrotoxic side-effects\textsuperscript{68}. (See also paragraph \textit{Prevention of chronic transplant dysfunction}). Non-compliance of patients may result in inadequate immunosuppression, and indeed it has been shown that also non-compliance is associated with late deterioration in graft-function\textsuperscript{69,70}.

\textit{Anti-donor antigen antibodies.} Although many patients develop antibodies reactive with donor-HLA antigens and other donor-tissue antigens after transplantation, a clear correlation between antibodies and the development of CTD is, however, not consistently found\textsuperscript{71-74}. After experimental kidney transplantation in rats, antibodies reactive with glomerular and tubular basement membranes, mesangial cells and endothelial cells (EC’s) were found in sera of allograft recipients\textsuperscript{75,76}. Also after allogeneic aorta transplantation, donor reactive antibodies have been found\textsuperscript{77,78}. The exact role of donor reactive antibodies in the pathogenesis of TA has to be further explored.

\textbf{Donor- and recipient-related risk factors}

Although alloreactivity of the host against the graft appears to be the most important factor contributing to the development of TA, also alloantigen independent factors seem to be associated with the pathogenesis of TA. In the late 60’s it has been shown that kidney isografts transplanted between identical twins developed glomerular and vascular lesions\textsuperscript{79}, and today several alloantigen independent risk factors for the development of TA have been identified\textsuperscript{80,81}.

\textbf{Donor-related risk factors}

\textit{Ischemia.} Whether or not ischemia plays an important role in the development of CTD and TA is still unclear, since both studies favoring\textsuperscript{6,82} and discounting\textsuperscript{83,84} the effect of ischemia have been described. In experimental kidney and aorta transplant models in rats, it has been shown that prolonged ischemic time induces the development of TA in isografts\textsuperscript{81,85,86}. 
Moreover, the severity of the vascular lesions seems to correlate with the duration of the ischemic period\textsuperscript{87,88}. Although a clear effect of ischemia on the development of TA in isografts has been described, it is less clear whether the length of the ischemic period plays a role in the development of TA in allografts.

\textit{Brain death.} Over the last years it has been shown that recipients of kidney allografts from brain dead donors show decreased long-term graft survival compared to grafts obtained from living-related and unrelated donors\textsuperscript{89}. It has been hypothesized that brain death increases the cytokine induced expression of surface molecules on peripheral organs, and in experimental models it has been shown that brain death indeed activates EC’s and increases the influx of inflammatory cells several hours after the induction of brain death\textsuperscript{90,91}. Moreover, accelerated acute rejection of kidney and cardiac allografts in rats has been observed after transplantation of grafts from brain dead donors\textsuperscript{91,92}. Whether brain death also accelerates the development of TA has still to be proven.

\textit{Donor age.} Kidney and cardiac allografts obtained from older donors have been found to be associated with poorer survival rates and show an earlier onset of CTD\textsuperscript{18,93}.

\textit{Recipient-related risk factors}

\textit{Cytokine gene polymorphisms.} Among organ recipients, transplanted with similar HLA-incompatible grafts and receiving similar immunosuppression, variation exists in both the rejection rate and long-term outcome. This variation has not been fully explained, but data indicate that the immune response to an allograft varies from one individual to another. In general, from an immunological point of view, different individuals might display different responses upon stimulation (different responder status). This individual variation is, at least in part, due to genetic variation in the regulation of cytokine gene expression\textsuperscript{94}. High and low cytokine responses \textit{in vitro} for TNF-\(\alpha\), TGF-\(\beta\), IFN-\(\gamma\), and IL-10 can be predicted from an individual’s cytokine genotype\textsuperscript{95}.

Acute rejection of kidney and liver allografts has been shown to correlate with the presence of the high TNF-\(\alpha\) production genotype\textsuperscript{96,97}. Also the high IFN-\(\gamma\) and IL-10 production genotypes have been correlated with increased acute rejection after kidney transplantation, however, also controversial results have been reported\textsuperscript{96-99}.

Also CTD and TA have been shown to correlate with cytokine genotypes. In several studies, presence of the high TGF-\(\beta\) production genotype has been correlated with accelerated onset of TA in cardiac allografts\textsuperscript{100,101}. Also in lung transplantation, the high TGF-\(\beta\) production genotype has been shown to be a risk factor for the development of obliterative bronchiolitis\textsuperscript{102}. Taken together, these studies indicate that it might be possible to identify high and low responders to allografts, and to predict who will suffer from acute rejection and CTD.

\textit{Hypertension and hyperlipidemia.} In clinical kidney and heart transplantation, systemic hypertension has been shown to be associated with CTD\textsuperscript{103,104}. Moreover, in experimental kidney transplantation systemic hypertension acceler-
General Introduction

ates CTD, whereas antihypertensive drugs inhibit or reduce TA and CTD after kidney and aorta transplantation\textsuperscript{105-107}. Also hyperlipidemia has been identified as a risk factor for CTD\textsuperscript{108}, however its role remains controversial.

**Gender and race.** Male recipients are more vulnerable to CTD compared to female recipients\textsuperscript{18}. This gender effect might reflect a role for oestrogen, as it has been shown that oestradiol effectively inhibits TA after allogeneic aorta transplantation in rats\textsuperscript{109}. Moreover, long-term graft survival of cardiac and kidney allografts seem to be related to race\textsuperscript{18,110}.

**Viral infections**

*Cytomegalovirus (CMV).* Studying human cardiac allografts, Grattan et al. in 1989 for the first time showed a positive correlation between the presence of cytomegalovirus (positive serology, CMV inclusion bodies, and positive CMV culture) and graft atherosclerosis\textsuperscript{111}. Subsequently, similar results have been reported by others\textsuperscript{112}. Also in human kidney, liver, and lung transplants CMV has been shown to be related to CTD\textsuperscript{53,113-115}. On the other hand, also data have been described indicating that CMV does not contribute to enhanced CTD after solid organ transplantation\textsuperscript{116-119}. It would be interesting to investigate whether these different clinical outcomes might be related to differences in antiviral treatment regimens (e.g., prophylactic treatment with antiviral drugs).

Also in several experimental transplant models in rats (heart, kidney, liver, and trachea), using Rat Cytomegalovirus (RCMV), data have been collected that indicate that viral infection accelerates the development of CTD related pathology\textsuperscript{120-123}. In the aortic transplant model in rats, it has been shown that RCMV infection results in enhancement of both the perivascular inflammatory response\textsuperscript{124-126} and neointima formation\textsuperscript{44,45,126-128}. Inhibition of viral replication and inflammation using antiviral\textsuperscript{45,127} and immunosuppressive drugs\textsuperscript{129}, respectively, prevent RCMV enhanced development of TA, indicating that CMV indeed may affect the process of TA development. The exact mechanism by which CMV is involved in the development of TA and CTD is still unclear.

**PREVENTION OF CHRONIC TRANSPLANT DYSFUNCTION**

The standard therapy to prevent acute rejection in cardiac transplantation is triple therapy, consisting of cyclosporineA (CsA), azathioprine, and prednisone\textsuperscript{130}. CsA is a small fungal peptide that interferes with the synthesis of a variety of cytokines, particularly IL-2, which is critical for T cell maturation and clonal expansion (proliferation), and IFN-\(\gamma\), which is critical for macrophage activation\textsuperscript{131}. Azathioprine is a purine antagonist and inhibits cell proliferation\textsuperscript{132}. Prednisone is a corticosteroid and among others influences the transcription of several genes thereby inhibiting T cell proliferation\textsuperscript{133}. This triple therapy is very effective in treatment of acute rejection episodes, however CsA therapy has some serious side-effects. CsA induces severe nephrotoxicity, and even at minimal effective concentrations of CsA evidence of nephrotoxicity may be present\textsuperscript{131}. Moreover, CsA therapy does not prevent development of CTD which might be attributed
to the fact that CsA directly upregulates fibrogenic cytokines such as TGF-β. Over the last decade, several new immunosuppressive drugs have been developed of which some are now being used in clinical practice. FK506 (Tacrolimus) is more potent than CsA, but has the same mode of action. Compared to CsA, unfortunately FK506 has not been found to prolong long-term graft survival after kidney transplantation. Mycophenolate mofetil (MMF) and Rapamycin (Sirolimus) are newly approved immunosuppressive drugs both inhibiting T cell proliferation. These new immunosuppressive drugs also have side-effects and appear not to prevent or control CTD. Several studies in rats have been reported, however, indicating that treatment of allograft recipients with CsA, rapamycin, or other immunosuppressive agents can block the development of CTD related vasculopathy.

In addition to immunosuppressive drugs, polyclonal antibodies specific to T cells, such as anti-thymocyte globin (ATG) and anti-CD3 (OKT3), are mainly used as rescue therapy resulting in functional T cell blockade.

Since alloreactive T cells seem to play an important role in the development of CTD-related histopathology, it has been suggested that the key to preventing CTD involves inducing immunologic tolerance to the graft. The creation of transplantation tolerance would obviate the need for long-term immunosuppression and theoretically could prevent CTD. Blockade of T cell costimulation pathways not only results in suppression of the immune response, but has also been shown to be effective in inducing antigen-specific transplantation tolerance in rodents. Optimal T cell activation requires both antigen-specific signals (signal 1, interaction TCR-MHC/peptide complex) and non-antigen-specific, costimulatory, signals (signal 2). The best understood costimulatory pathway is provided through the T cell surface molecule CD28 and its ligands CD80 (B7-1) and CD86 (B7-2) on antigen-presenting-cells (APC’s). Blockade of this costimulatory CD28-B7 pathway early after transplantation by using CTLA4-Ig fusion protein has been shown to ameliorate development of TA and other, graft specific, histopathological findings of CTD in cardiac and kidney allografts in rodents. Even blocking CD28-B7 mediated T cell costimulatory activation late after transplantation, after initial graft injury, has been shown to prevent progression of CTD related histopathology. When CTLA4-Ig treatment was suboptimal, and antidonor reactivity (partly) remained, the development of TA could not be prevented. Another T cell costimulatory pathway is mediated through CD40, expressed on a variety of cell types including APC’s, B cells and EC’s), and its ligand CD154 which is expressed on activated T cells. Although blockade of the CD40-CD154 pathway using monoclonal antibodies prolonged graft survival, its effect on the development of CTD related histopathology remains controversial. Moreover, cardiac allografting in CD154-/- knock-out mice did not reduce the development of TA.

Although both treatment with new immunosuppressive drugs and blockade of costimulatory pathways might be (partly) effective in the amelioration of TA,
one should remember that in most animal studies the development of TA is analyzed several weeks to months after transplantation. Since TA is primarily caused by alloreactive T cells, it is predictable that suppressing T cell alloreactivity inhibits the development of TA. However, in clinical practice, transplant recipients need long-term treatment with immunosuppressive drugs. So far, it is not known whether the new immunosuppressive drugs and costimulation blockers only prevent development of TA on the short-term, and therefore TA deceleration should not be confused with TA elimination.

Probably all protocols, which do not eliminate alloreactivity completely but rather suppress the immune response against the graft, will fail to prevent the eventual development of TA. According to this view, only those interventions in which antidonor reactivity is completely blocked or deleted (i.e., ‘true tolerance’), e.g., the creation of donor bone marrow chimeras, will prevent the development TA.

**PATHOPHYSIOLOGY OF TRANSPLANT ARTERIOSCLEROSIS**

Although the exact pathogenesis of TA is unknown, data from clinical and experimental studies suggest that its development is an immune-mediated process modified by nonimmunologic features. Despite discrepancies in histopathology between ordinary atherosclerosis and TA, the ‘response-to-injury’ paradigm applicable to atherosclerosis and originally proposed by Ross et al. has been accepted widely for the development of TA. This paradigm proposes that transplant-related trauma (alloantigen-dependent and independent) causes activation and damage of EC’s along the graft arterial system. Important insults leading to EC damage may include preservation/ischemia injury, reperfusion injury, acute rejection episodes (i.e., activation alloreactive T cells), antibody deposition, and complement fixation. The thus damaged and activated endothelium subsequently initiates a generalized vascular repair process that is coordinated by proinflammatory and histogenic factors produced by the activated EC’s itself as well as vessel wall parenchymal cells and inflammatory cells. Moreover, the immune response characterized by perivascular inflammation induces further low-grade damage to the vascular endothelium. This cascade of events eventually results in replication of VSM cells in the vascular wall, influx of VSM cells from the media into the subendothelial space (intima), and generation of the neointimal lesion. So, according to this working hypothesis graft endothelium seems to be one of the key players in the development of TA.

**Activation of graft endothelium**

Endothelial cells play an important role in recruiting lymphocytes into sites of inflammation by molecular adhesion events. Upon stimulation, graft endothelium upregulates the expression of adhesion molecules, thereby increasing interactions between polymorphonuclear neutrophils (PMN’s) and lymphocytes, and EC’s. Important adhesion molecules expressed by activated EC’s are E-selectin, P-selectin, VCAM-1 and ICAM-1. Moreover, several chemokines produced by both endothelium and interstitial cells...
(e.g., interleukin (IL)-8, MCP-1, MIP-1α, MIP-1β, and RANTES) can promote the binding of lymphocytes and macrophages to endothelium\textsuperscript{162,163}.

The initial damage to the graft endothelium, caused by alloantigen independent factors (such as ischemia/reperfusion injury and brain death, resulting in infiltration of free-oxygen radical producing PMN’s), may be followed by influx of macrophages into the subendothelial space which may subsequently be activated by IFN-γ. Activated macrophages produce a number of inflammatory mediators including IL-1, IL-6, IFN-γ, TNF-α and the chemokines IL-8, MCP-1, MIP-1α, MIP-1β. These cytokines and chemokines in turn further activate the graft endothelium and increase the expression of adhesion molecules, thereby attracting more macrophages and lymphocytes in a positive feedback loop\textsuperscript{164}. Thus, as a result of the transplant procedure, a complete network of cytokines is already activated, even before the allogeneic reactions develop.

In addition to its capability to attract and activate macrophages, graft endothelium can activate alloreactive T cells by the direct allorecognition pathway of antigen presentation. Nominal antigens are recognized as peptides, presented to (self-restricted) T cells by self-MHC molecules (indirect pathway)\textsuperscript{165}. However, direct allorecognition implies that a T cell recognizes foreign MHC molecules per se independent of the peptide present in the groove of this MHC molecule\textsuperscript{166}. In vitro studies have demonstrated that EC’s express both MHC class I and II antigens upon stimulation with IFN-γ and are able to directly stimulate allogeneic T cells\textsuperscript{167-169}. In addition to MHC molecules, EC’s also express costimulatory molecules which are necessary to induce full T cell activation. The best defined of these molecules is LFA-3, which interacts with T cell CD2\textsuperscript{170,171}. Once activated, T cells produce leukotrienes and several cytokines including IL-1, IL-4, IFN-γ, TNF-α, and TGF-β, which increases the expression of E-selectin, ICAM-1 and VCAM-1 on graft endothelium\textsuperscript{172,173}. Moreover, TNF-α and IFN-γ further increase the expression of MHC class I and II on graft EC’s. This cross-talk between lymphocytes and EC’s suggests that a positive feedback loop may be established.

One central element of the TA paradigm is that smooth muscle cells from the vessel media are the progenitors of the neointima, i.e. medial VSM cells are supposed to migrate from the media to the intima in response to activation by cytokines and growth factors\textsuperscript{15}. The ongoing perivascular response induces persistent low-grade damage to the graft endothelium, which in turn begins to secrete growth factors to repair the damage. Upon activation, EC’s have been shown to produce multiple growth factors such as PDGF, IGF-1, bFGF, and TGF-β as well as pro-thrombotic molecules (tissue factor and plasminogen activator inhibitor) and metabolic products such as prostaglandins, nitric oxide, and low-density lipoproteins\textsuperscript{15,159}. Also activated T cells and macrophages produce a range of these factors. The ‘response-to-injury’ paradigm now implies that these factors together induce migration of VSM cells to the intima, during which they transform their phenotype from ‘contractile’ to ‘secretory’ and the cells become capable of replication\textsuperscript{159}. 
DONOR VS. RECIPIENT ORIGIN OF NEOINTIMAL CELLS IN TRANSPLANT ARTERIOSCLEROSIS

Transplant arteriosclerosis consists of progressive concentric intimal thickening coinciding with ongoing perivascular inflammation. Current thinking on the process of TA holds that in response to cytokines, growth factors and other inflammatory mediators produced by inflammatory cells and damaged/activated graft endothelium, donor-derived medial vascular smooth muscle (VSM) cells of affected arteries start to proliferate and migrate from the media into the subendothelial space just beneath the endothelial cell layer. According to this concept, neointimal EC’s and VSM cells in TA originate from graft tissue and therefore should be donor-derived. Figure 2 shows a schematic representation of the hypothetical migration of donor medial VSM cells into the subendothelial space in response to the ongoing perivascular inflammation, resulting in obliteration of the vascular lumen eventually.

However, if the neointimal EC’s and VSM cells do indeed originate from the donor, they should demonstrably be graft derived. Already in the early 60’s, Woodruff and Medawar proposed that replacement of graft endothelium with host-derived EC’s might be the reason why long-term allograft survivors experience relatively few rejection episodes (i.e., graft adaptation). Since then, several groups studied whether EC’s covering the luminal surface of the graft vessels after transplantation are indeed of donor-origin or had been replaced by host-derived EC’s. Donor-origin of the graft vessels endothelium is supported by findings reported by several groups. However, also repopulation of graft vessels by host-derived EC’s has been reported, suggesting...

Figure 2. Current paradigm on the development of intimal hyperplasia in transplant arteriosclerosis.
gesting heterogeneity of the underlying processes.

Only few studies have addressed the question whether the neointimal VSM cells in TA are indeed graft-derived. Donor-origin of neointimal VSM cells has been described in human solid organ transplants, favoring the current paradigm that neointimal VSM cells are derived from the graft’s media\textsuperscript{182,188}. Alternatively, data exist indicating that also the host might give rise to $\alpha$-actin positive VSM cells which are derived from the bloodstream. Studying Dacron-hubs implanted in the aorta of young pigs, Stump et al. showed that these hubs were covered with EC’s and VSM-like cells which originated from cells in the blood\textsuperscript{189}. Moreover, implantation of biodegradable compliant vascular grafts in rats show development of new vascular wall structures, including a neomedia (with $\alpha$-actin positive VSM cells) and a neointima (with EC’s)\textsuperscript{190-192}. In the Dacron-hubs as well as the synthetic biodegradable vascular grafts in rats show development of new vascular wall structures, including a neomedia (with $\alpha$-actin positive VSM cells) and a neointima (with EC’s)\textsuperscript{190-192}. In the Dacron-hubs as well as the synthetic biodegradable vascular grafts, the EC’s and VSM cells should by definition be host-derived, indicating that EC’s and VSM cells can originate from the host. Some data have been reported suggesting that host-derived VSM cells also contribute in the process of neointima formation after organ transplantation. Plissonnier et al. showed that neointimal VSM cell proliferation and medial VSM cell disappearance in rat aortic allografts start at the same time. According to the TA paradigm, the medial VSM cells now migrate to the intima, resulting in an acellular media and the formation of a neointima. Légaré et al. demonstrated that there is a striking loss of medial VSM cells by apoptotic cell death even before the development of a neointima\textsuperscript{193}. It seems therefore at least a bit contradictory that the VSM cells proliferate at one side (neointima) and die by apoptosis at another, nearby, side. Moreover, if migration should be the only mechanism by which medial VSM cells disappear from the media, one could wonder why all medial VSM cells migrate to the intima since then a dramatic loss of vascular wall function is warranted.

So, a major role for the media-derived VSM cells in the process of neointima formation seems unlikely. In their study, Plissonnier et al. indeed showed by FACS analysis using allo-antisera the neointimal VSM cells to be of host-origin and not of donor-origin\textsuperscript{77}. However, neointimal VSM cells and neointima infiltrating host-derived inflammatory cells could not be distinguished due to the procedure they used.

Based on the observations that new vascular wall structures develop after implantation of biodegradable vascular grafts, as well as the notion that medial VSM cells disappear before neointimal VSM cells appear, we hypothesized that, analogous to the development vascular wall structures in biodegradable grafts, the development of TA in allogeneic grafts is in principal the same implying that neointimal EC’s and VSM cells are host-derived\textsuperscript{194}.

Although some data on the origin of neointimal cells have become available in the past years, no conclusive results have been described on the origin of EC’s and VSM cells after solid organ transplantation so far.
AIM OF THE THESIS

Chronic transplant dysfunction (CTD) has become today’s most important cause of late graft failure and cannot be prevented by current immunosuppressive protocols. CTD can be defined as the progressive irreversible loss of graft function that occurs months to years after transplantation. Irrespective of the organ grafted (kidney, heart, liver, lung), graft vessels eventually develop so-called transplant arteriosclerosis (TA) consisting of concentric intimal thickening (neointima formation) coinciding with ongoing perivascular inflammation. Pathogenesis of TA seems to be multifactorial, but precise mechanisms involved in the development of this remodeling process still remain obscure. Both alloantigen-dependent factors (e.g. histoincompatibility, acute rejection, and inadequate immunosuppression) as well as alloantigen-independent factors (e.g. ischemia, brain death and cytomegalovirus infection) have been identified as probable risk factors for the development of TA.

Current thinking on the process of TA holds that in response to cytokines, growth factors and other inflammatory mediators produced by activated endothelium and inflammatory cells, donor-derived medial VSM cells start to proliferate and migrate into the subendothelial space. This response-to-injury with time results in occlusion of the vascular lumen, eventually leading to progressive deterioration of graft function.

The introduction of this thesis (Chapter 1) presents the clinical and histopathological hallmarks of CTD after solid organ transplantation. Moreover, risk factors for the development of CTD are described. The current paradigm of the development of TA is discussed and the cascade of pathological events leading to TA is presented.

In the subsequent chapters of this thesis a series of experiments is described in which several aspects of the pathophysiology of TA after allogeneic organ transplantation are addressed. First we introduced and validated several experimental transplant models in rats, and analyzed their applicability to study development of TA (Chapters 2, 3, and 4). Alloreactivity of the host immune system against the graft seems to be one of the key factors in the pathogenesis of TA. Theoretically, inducing transplant tolerance, and thereby abolishing alloreactivity against the graft, might be a possible strategy to prevent the development of TA. Intrathymic (IT) immune modulation has been shown to significantly prolong graft survival of MHC-incompatible cardiac allografts in rats. In Chapter 2 we studied whether induction of hyporesponsiveness by IT immune modulation prevents the development of TA after allogeneic heart transplantation in rats.

Since transplantation of a vascular cardiac allograft is a rather complicated and time-consuming operational technique, we analyzed in Chapter 3 whether a more simplified model could be used to study IT immune modulation in rats after allogeneic organ transplantation. Therefore, neonatal cardiac tissue was transplanted subcutaneously in the ear-pinnae of adult recipient rats. Moreover, development of vascular lesions in neonatal cardiac allografts transplanted in the ear-pinnae of IT immune modulated rats was analyzed. In Chapter 4 we describe a series of expe-
riments in which we used the aortic transplant model in rats which is a well-described and reproducible model to study development of TA. We studied the effect of several risk factors for the development of TA. In clinical organ transplantation variation exists in both the rejection rate and long-term outcome among organ recipients transplanted with similar HLA-incompatible grafts and receiving similar immunosuppression. Data indicate that the immune response to an allograft varies from one individual to another, and these variations might be involved in the different long-term outcome. We therefore analyzed the severity and kinetics of TA development in several rat strain combinations, representing recipients with different immune responses to an allograft.

Cytomegalovirus (CMV) appears to be a risk factor for the development of accelerated and enhanced TA. Using Rat CMV (RCMV), we analyzed whether additional RCMV infection after allogeneic aorta transplantation in different strain combinations enhanced the development of TA. Moreover, the effect of timing of RCMV infection of the donor or recipient on the development of TA was analyzed.

Since considerable differences exist in the kinetics of the development of TA in different rat strains (Chapter 4), we further analyzed which factors might be responsible for this observation. Using MHC-congeneric rat strains we therefore studied in Chapter 5 the kinetics of the development of TA after aortic transplantation. In addition, we used bone marrow chimeric rats to study whether allogeneic bone marrow transplantation between high and low TA responders altered the severity of TA development in aortic allografts.

According to the current paradigm of the development of TA, the neointimal EC’s and VSM cells originate from graft tissue and therefore should be donor-derived. Sufficient experimental evidence supporting this paradigm is, however, lacking, and we hypothesized that the neointimal EC’s and VSM cells are not donor but recipient-derived. Knowing the exact origin of these neointimal cells might have implications for the development of new strategies to prevent or ameliorate TA. To test this hypothesis, a series of experiments in which we studied the origin of neointimal EC’s and VSM cells was performed and results are described the second part of this thesis (Chapters 6, 7 and 8).

We analyzed the origin of neointimal EC’s and VSM cells in aortic and cardiac allografts (Chapters 6 and 7). The origin of EC’s was determined using MHC class I haplotype specific immunohistochemistry, whereas the origin of neointimal VSM cells was determined using a PCR based analysis which was sufficiently sensitive to detect host-derived cells at the single cell level.

Since we showed that the neointimal EC’s and VSM cells are of host-origin and not of donor-origin, the question remained what the anatomical origin of the host-derived EC’s and VSM cells is: ingrowth from the recipient side of anastomosis or derived from recirculating, possibly bone marrow derived, progenitor cells. To address this question, we analyzed in Chapter 8 the anatomical origin (bone marrow vs. non-bone marrow) of the host-derived EC’s in TA in aortic allografts transplanted in bone marrow chimeric rats using confocal laserscanning microscopy (CLSM).

In Chapter 9, all data are discussed in
their context and a concluding view on the development of TA is given.

REFERENCES

Chapter 1


Chapter 1


General Introduction


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