Chapter 10

Summary
Chronic transplant dysfunction, histologically characterized by obliterator vascular occlusive vasculopathy referred to as transplant arteriosclerosis (TA), is today’s most important cause of long-term graft loss after solid organ transplantation. Although several risk factors have been identified, the etiology of TA is largely unknown and precise pathogenetic mechanisms still remain obscure. Aim of this thesis was to analyze the contribution of several risk factors supposedly involved in the development of TA, namely alloreactivity directed against the graft (Chapter 2 & Chapter 3), cytomegalovirus (CMV) infection (Chapter 4) and host genetics (Chapter 5). Moreover, we aimed at gaining more insight in the precise pathogenetic mechanisms leading to vascular narrowing by focusing on the origin (donor vs. recipient) of neointimal vascular smooth muscle (VSM) cells and endothelial cells (EC’s) (Chapter 6, Chapter 7 & Chapter 8).

Chapter 1 gives an overview of the clinical and histopathological hallmarks of CTD after solid organ transplantation. Moreover, risk factors for the development of TA and subsequent CTD are described. The current paradigm of the development of TA is discussed and the supposed cascade of pathological events leading to TA is presented. Finally, the aim of this thesis is formulated.

Alloreactivity of the host immune system against the graft seems to be one of the key factors in the pathogenesis of TA. Eliminating this alloreactive response after transplantation by induction of transplant tolerance would therefore be a possible solution to prevent development of TA. In the course of a series of studies on tolerance induction against cardiac allografts, several years ago a model was developed using intrathymic (IT) immune modulation and a short course of immunosuppression. IT immune modulation is effective in preventing acute rejection of MHC-incompatible vascularized cardiac allografts transplanted in rats, suggesting that transplant tolerance was achieved. However, experiments described in Chapter 2 clearly demonstrated that the development of TA was not prevented: TA started to develop several weeks after transplantation and an increase in the severity of TA and the number of affected graft coronary arteries was observed with time. In vitro mixed lymphocyte reactions and immunohistochemical analysis revealed that IT immune modulation did not completely abolish alloreactivity, but rather altered the type of immune response against the graft. Intrgraft cytokine mRNA expression, as analyzed with semi-quantitative RT-PCR, showed decreased IL-2 and IFN-γ expression indicative of a down-regulated Th1-type cytokine response two weeks after transplantation. In long-term surviving allografts, high intrgraft mRNA expression levels of IL-10, IFN-γ and TGF-β were measured. In conclusion, despite induction of long-lasting graft-survival, IT immune modulation did not prevent the development of TA.

Using the same IT immune modulation protocol as in Chapter 2, in Chapter 3 it was studied whether IT immune modulation was effective in prolonging graft survival after non-vascularized allogeneic tissue transplantation. In contrast to vascularized cardiac allografts, IT immune modulation did not prolong survival of MHC-incompatible skin grafts which may be caused by tissue-specific antigens.
which are presumably not expressed on splenocytes. Survival of allogeneic neonatal cardiac tissue transplanted subcutaneously in the ear-pinnae of recipient rats was significantly prolonged after IT immune modulation. Long-term surviving neonatal cardiac tissue was characterized by the appearance of large-cavernous like blood vessel structures which appeared to originate from the graft (neo-angiogenesis). In contrast to the coronary arteries in vascularized cardiac allografts, newly formed blood vessels in long-term surviving neonatal cardiac tissue did not develop TA.

One of the factors supposed to be involved in the process of TA development is CMV infection. In Chapter 4 the effect was analyzed of recipient genotype and timing of infection on the severity of TA development in aortic allografts transplanted in rats. Development of TA differed among different rat strain combinations, in which the Lew to BN combination appeared to be a high TA responder combination. The effect of timing of CMV infection was analyzed in the BN to Lew combination, since this appeared to be a low TA responder combination. Infection with Rat CMV (RCMV) only enhanced the development of TA when the developing acute rejection episode and acute infection occurred simultaneously, i.e. when the allograft recipients were infected with RCMV 1-5 days after transplantation. The enhancing effect of RCMV infection on TA development could not be generalized since, in other weak TA responder combinations, RCMV infection did not enhance the development of TA. Obviously there are more factors than timing of infection and weakness of TA development that determine the CMV-enhanced development of TA.

In Chapter 4 it was already shown that differences exist in the severity of TA development between different rat strain combination after aortic transplantation. This observation was further analyzed in Chapter 5, in which the contribution of recipient encoded MHC and non-MHC determinants on the rate of TA development was studied. The high TA responsiveness of BN rats appeared to be determined by non-MHC encoded determinants, since also MHC congenic BN.1IL rats still showed the high TA responder phenotype. Which non-MHC encoded determinants precisely determine the high TA responsiveness remained unclear from this study, however, data indicated that it is at least unlikely that the immunologic responder status of the host (Th1 vs. Th2 status) plays an important role.

A central element in the process of TA development is uncontrolled proliferation of neointimal VSM cells, resulting in obliteration of intragraft arteries. Current thinking on the process of TA holds that, in response to cytokines, growth factors and other inflammatory mediators, medial VSM cells start to migrate and proliferate into the subendothelial space. According to this generally applied working hypothesis, neointimal VSM cells and EC’s are graft (donor)-derived. In Chapter 6 and Chapter 7 it was analyzed whether the neointimal VSM cells and EC’s are truly graft-derived. MHC class I haplotype specific immunohistochemistry revealed that neointimal EC’s in aortic allografts transplanted in non-immunosuppressed recipients were of host- and not of donor-origin, indicating that EC-replacement had taken place (Chapter 6). However, in cardiac allografts transplan-
ted following IT immune modulation (see also Chapter 2), neointimal EC’s were still of graft origin indicating that in cardiac allografts no EC-replacement occurred (Chapter 6). In both aortic and cardiac allografts severe TA developed, indicating that EC replacement is not a prerequisite for eventual TA development. Immunosuppressive treatment with high dosages of cyclosporin A for a period of 4 weeks completely prevented EC destruction and TA development in aortic allografts indicating that adequate immunosuppression in the short run is effective in preventing development of TA.

To further analyze the origin (donor vs. recipient) of neointimal VSM cells a male-specific nested PCR procedure was developed, based on the genomic sequence of the Y-chromosome, which was sufficiently sensitive to detect male-derived cells at the single cell level. Since all transplantations were performed using female donor and male recipient rats, a positive PCR signal indicated recipient-origin of the regarding cell. PCR analysis of single nuclei of α-actin positive neointimal VSM cells microdissected from both aortic allografts (Chapter 6) and cardiac allografts (Chapter 7) revealed that these VSM cells were of recipient and not of donor origin. These results were confirmed by another PCR which was based on polymorphisms present in the genomic sequence encoding the Tap2-gene. So, in contrast to current thinking we feel these data to indicate that neointimal VSM cells are of recipient- and not of donor-origin.

As in Chapter 6 it was shown that in aortic allografts with TA neointimal EC’s and VSM cells were recipient-derived, in Chapter 8 the anatomical origin (bone marrow [BM] vs. non-BM derived) of host-derived neointimal cells was analyzed. Confocal laserscanning microscopy on DA aortic allografts transplanted in Lew<sub>BM</sub>−→BN and BN<sub>BM</sub>→Lew chimeric rats revealed that in advanced TA EC’s were primarily non-BM derived, although BM-derived EC’s can contribute to the development of TA in developing lesions. Also neointimal VSM cells were most-likely non-BM derived. These results indicate that a non-marrow source provides the neointimal EC’s and VSM cells in TA.

In Chapter 9 the results described in this thesis are discussed at large and, based on the findings, a sequence of events leading to the development of TA and subsequent CTD is proposed. Instead of looking at TA as an undesirable element in CTD, we propose that the process leading to TA is basically part of a normal healing response which proceeds, however, beyond the needs of functional repair. This view on the development of TA has led to the identification of possible new targets of therapeutic intervention to prevent or treat TA.