In **chapter 1** a short overview of the history of transplantation tolerance is given followed by a discussion of the various mechanisms currently thought to mediate tolerance and the ways to induce transplantation tolerance. **Section 1.1** ends with a review of the literature on intrathymic tolerance induction. **Section 1.2** introduces our practical work. Current immunosuppressive protocols are very successful in preventing or abrogating acute rejection. However, these regimens have a number of serious side effects (e.g. increased susceptibility to infection, increased risk of neoplastic disease and renal and myocardial toxicity). These problems could be prevented by the induction of donor-specific tolerance which would eliminate the need for long-term immunosuppression. One of the protocols with potential clinical application is tolerance induction via intrathymic inoculation with donor-type antigens and short term immunosuppression with antilymphocyte serum (ALS). However, the obligatory interval between tolerance induction and the actual transplantation precludes clinical use. We have tried to create an intrathymic tolerance induction protocol with clinical by adding a short course of cyclosporin A (CsA) treatment to allow us to perform tolerance induction and transplantation simultaneously.

In **section 2.1** the results with our simultaneous transplantation and intrathymic tolerance induction (STITTI) protocol are described. This protocol is highly efficient (> 90%) and can be used to induce tolerance for cardiac allografts in several different fully MHC disparate ('high' responder) rat strains. As the STITTI protocol allows us to perform tolerance induction simultaneously with the actual transplantation it may have clinical potential.

Section 2.2 describes our skin transplant experiments. The unmodified STITTI protocol does not induce tolerance to skin allografts in the PVG to AO rat strain combination. However, substitution of keratinocytes for splenocytes in the intrathymic inoculum does induce long-term acceptance of skin allografts in approximately 50% of the recipients. The lower efficiency is probably due to the higher immunogenicity of skin as compared to heart allografts. To investigate wether tolerance could 'spread' to new antigens we transplanted donor-type skin grafts onto long-term cardiac allograft acceptors. As approximately 50% of these allografts are accepted long-term and the unmodified STITTI protocol does not induce tolerance to skin allografts we concluded that spreading had indeed occurred.

As the cardiac allograft in our model is transplanted heterotopically the beating of the graft does not indicate if it is fully functional and could support life. To evaluate the status of the cardiac allograft we performed histological studies on grafts explanted at different times after tolerance induction and transplantation. Results from this study are reported in **chapter 3**. Early after transplantation and tolerance induction a parenchymatous type infiltrate is observed which is indistinguishable from the

infiltrate seen in rejecting grafts. Later the infiltrate is more peripheral, blood vessel associated, and there has been a shift from $CD8^+$ T cells and macrophages to $CD4^+$ T cells and B cells. We also noted a gradual deterioration of the heart tissue but are unsure wether this is due to the inflammation or the blood vessel associated pathology (chronic rejection) resulting in an insufficient oxygen supply.

Two of the most important questions remaining at this time were: (i) how is tolerance induced, and (ii) what mechanism is responsible for the maintenance of tolerance. To investigate the first of these two questions we evaluated the survival of allogeneic splenocytes in the recipient's thymus with and without immunosuppression. Results presented in **chapter 4** indicate that allogeneic splenocytes injected into the thymus of a recipient treated with our standard ALS + CsA regimen are present only in a restricted area for a limited time (< 28 days). The allogeneic splenocytes are eliminated by a reaction in which large numbers of recipient B cells and macrophages are observed. However, no polymorphonuclear neutrophils were seen. Recipient B cells and macrophages collocate with the allogenic cells. There is some morphological damage to the thymus due to either the (very limited) graft versus host reaction or the 'inflammatory' response. In contrast, allogeneic cells are eliminated much faster and with much more damage to the thymus in the ALS treated and non immunosuppressed animals while congenic cells survive at least 35 days with no sign of any reaction.

The reaction to the injection of allogenic cells may in turn induce a regulatory response to prevent damage to the thymus. We think this regulatory response is peripheralized, becomes thymus independent and subsequently is responsible for the maintenance of tolerance.

Chapter 5 deals with the investigation of the mechanism responsible for the maintenance of tolerance. Results presented in **section 5.1** show that in animals treated according to the STITTI protocol the proliferative response early (up to 90 days) after tolerance induction is non-specifically depressed but not absent after stimulation with donor antigens. This indicates that the induced tolerance is not mediated solely by an central (i.e. intrathymic) mechanism.

Results from the experiments reported in **section 5.2** show that long-term acceptance of cardiac allografts induced with the STITTI protocol can be transferred to sublethally irradiated naive syngeneic recipients using splenocytes, thoracic duct lymphocytes or purified T cells. Therefore the mechanism mediating tolerance is probably a mechanism of active suppression. Furthermore, the cell mediating 'tolerance' is most likely a recirculating CD4 positive T cell.

Transferred cells comprise only a small percentage of the peripheral population in the (secondary) recipients. As it is unlikely that donor derived cells are solely responsible for the tolerance seen in these animals we postulated that the recipient's peripheral population has become functionally tolerant, thereby fulfilling the criteria for

'infectious tolerance'. We tested wether the peripheral population of the (secondary) recipient had become tolerant by again attempting to transfer tolerance to new naive (tertiary) recipient. This is indeed possible. We therefore postulate that functional tolerance induced by the STITTI protocol is mediated by a mechanism of active suppression with the hallmarks of 'infectious tolerance'.
These results are discussed in the light of current literature in **chapter 6**.

Conclusions

- Simultaneous transplantation and intrathymic tolerance induction (STITTI) protocol induces stable long-lasting tolerance.
- While the STITTI protocol completely prevents or abrogates acute rejection there is still chronic rejection which might be prevented by providing the 'right' antigens in the thymus.
- Tolerance induction is possible in recipients predisposed to a cellular (Th1) response but becomes difficult when recipients are predisposed to a humoral (Th2) response.
- Tolerance is not mediated by a central (i.e. intrathymic) mechanism but instead by an mechanism of active suppression with 'infectious' properties.
- Tolerance is probably mediated by a $CD8^-$ recirculating T cell.

Furthermore, the literature has provided evidence for the following:

- Tolerance is probably mediated by a $CD45RC^-RT6^+CD4^+CD8^-$ recirculating T cell.
- Intrathymic tolerance induction is not dependent upon either MHC class I or MHC class II antigens.
- Intrathymic tolerance induction can prevent and reverse cellular autoimmunity and may do the same for humoral autoimmunity.
- Intrathymic tolerance induction may also tolerize the humoral response and can prevent hyperacute rejection.

Despite its current drawbacks (i.e. an extra operation to deliver the intrathymic inoculum and chronic rejection of the graft) we feel that the simultaneous transplantation and intrathymic tolerance induction protocol has clinical potential.