Mesenchymal stromal cells to induce tolerance to solid organ transplantation

CHAPTER 9

SUMMARY

Successful solid organ transplantation would be a realistic therapeutic option only whether donor-specific immunologic tolerance could be reliably and safely induced.

Encouraging results have emerged from many tolerance induction strategies in experimental models, but translating these protocols across species from rodents to the clinic is providing a formidable task and so far we are still unable to induce tolerance in a routine intention-to-treat protocol. Mesenchymal Stromal Cells (MSC) have recently emerged as one of the most promising candidates for cell-based immunotherapy because they modulate the immune response via an array of direct and indirect interaction with a broad range of cell types in various ways. In particular, MSC drive T cells toward a regulatory phenotype and have the unique capability to control proliferation and activation of memory T cells which represent a major barrier to tolerance induction in transplant patients.

A number of studies either in experimental models of solid organ transplantation or in kidney transplant recipients were designed and performed in this thesis with the aim to establish the tolerogenic potential of MSC, their mechanisms of action as well as to find out the best infusion protocol to be applied in clinical transplantation.

The tolerogenic potential of MSC has been first evaluated in a murine model of semi-allogeneic heart (B6C3 heart in B6 recipient mice) transplantation (**Chapter 2**). Results showed that donor-derived MSC were effective in prolonging heart graft survival when infused into the portal vein 7 days before surgery. The same tolerogenic potential was shared by recipient-derived MSCs when given pretransplant via tail vein. Both donor-derived and recipient-derived MSCs mediated in vivo expansion of regulatory T cells (Tregs). These results provided the basis for the design of a safety and clinical feasibility pilot clinical studies of autologous bone marrow derived MSC in kidney transplant recipients with a living donor. Results of the first two patients, reported in **Chapter 3**, indicated that post-transplant MSC infusion induced a transient renal insufficiency characterized at histological analysis by an inflammatory infiltrate of neutrophils and C3 deposition but no evidence of graft rejection. It was hypothesized that the subclinical inflammatory environment of the graft in the few days post-surgery could have favoured the prevalent intragraft recruitment and activation of the infused MSC promoting a proinflammatory environment with eventual acute renal dysfunction. This hypothesis has been confirmed back into a murine kidney transplant model (**Chapter 4**) showing that a single administration of syngeneic MSC before (one day before surgery) but not after renal transplantation avoided the acute deterioration of graft function, while maintaining the immunomodulatory effects associated with MSC treatment, including a marked Treg expansion.

These experimental findings did represent a gain of knowledge to further implement our clinical protocol, aimed at creating favourable conditions for MSC-promoting immunomodulation avoiding

any possible side-effects associated with cell infusion. Thus two subsequent patients living-related kidney transplant recipients received pre-transplant (day-1) intravenous infusion of bone-marrow derived autologous MSC before T cell-depleting induction therapy. In the fist patient studied, MSC treatment was uneventful and graft function remained normal during 1 year follow-up. In the second patient, acute cellular rejection occurred 2 weeks post-transplant. Both patients had excellent graft function at the last observation. Circulating memory $CD8^+$ T cells and donor-specific $CD8^+$ T-cell cytolytic response were reduced in MSC-treated patients. Thus, pre-transplant MSCs no longer negatively affect kidney graft and maintained MSC-immunomodulatory properties (**Chapter 5**).

Finally, **chapters 6 and 7** are review chapters aimed at making the focus on the more recent acquaintance on MSC immunomodulatory effects in vivo in experimental transplant models as well as in early clinical experiences in kidney transplantation, and discuss topics of crucial importance for the future clinical use of MSC as immunotherapy in solid organ transplantation.