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Generation of tolerogenic dendritic cells for suppression of allograft rejection in organ transplantation.

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Development of immunosuppressive drugs have revolutionized organ transplantation, giving to patients suffering from terminal organ failure the chance of a new life. In the long run however the prognosis of transplant patients is shadowed by the occurrence of chronic infections, malignant diseases or chronic rejection. Some of these dreadful events represent “adverse effects” of the actual pharmacological therapies which are “shutting down” the entire immune system of the organ recipient, achieving on the one hand enough immunosuppression to prevent rejection but on the other hand disabling the immune response of the patient to adequately respond to ubiquitary antigens.
During the last decade, along with a better understanding of the alloimmune response after organ transplantation, novel therapies have been designed which induce targeted, long-time immunosuppression to transplanted organs while keeping the physiological immune response of the organ recipient “untouched”.
Among others, based on the results of experimental studies, generation of “designer” dendritic cells (DCs) with tolerogenic capacities have been proposed as a realistic strategy for induction of tolerance in clinical transplantation. It is thought that DCs play essential roles in central and peripheral T-cell tolerance under physiological conditions. Following organ or cell transplantation DCs present antigens to host T cells by direct or indirect pathways of

allorecognition. As a consequence, they activate the immune response and thus are responsible for rejection of the transplanted tissue.

Mitomycin C is a commonly used alkylating agent which induces genotoxic stress and elicits various effects upon cell function, including modulation of the immunogenicity of transplanted cells. The purpose of our study was to investigate the effect of Mitomycin C treatment upon DCs and to delineate the mechanism of their conversion into “suppressive” cells.

Initially rat DCs were generated in-vitro from peripheral blood monocytes in the presence of GM-CSF, IL-4 and additional TNF-alpha and PGE2. After 8 to 9 days of culture the isolated cells expressed a typical mature DC morphology and phenotype (MHC – II $^{+++}$, CD86 $^{+++}$, CD80 $^{+++}$, ICAM – 1 $^{++}$, OX62^{++}) showing a strong T-cell allostimulatory capacity in cell cultures.

We show that upon treatment with Mitomycin C, rat DCs lose their allostimulatory capacity in-vitro and this effect is neither attributed to MMC-induced cytotoxicity upon DCs, nor to the presence of MMC in the culture supernatants. T-cell unresponsiveness was found to be induced by MMC-mediated downregulation of costimulatory (CD86, CD80) and adhesion (ICAM-1) molecules on the DC cell surface. Functional blocking of these molecules with specific monoclonal antibodies confers to DCs the same T-cell suppressive properties in-vitro as MMC treatment. Most importantly, suppressed T-cells cannot be restimulated by re-challenge with untreated donor DCs whereas by addition of third party DCs, a significant allostimmune response is induced. After co-incubation with allogeneic MMC-DCs, purified CD4^{+} T cells presented a percentage of 17% apoptotic cells suggesting clonal deletion rather than anergy as a mechanism for MMC-mediated suppression. Thus, MMC-treated DCs suppress allogeneic T-cell proliferation in-vitro in an antigen-specific fashion by eliminating T cells carrying donor-specific TCRs. These results support previous findings which state that absent or insufficient costimulation in the presence of TCR:MHC engagement can inactivate the allospecific T-cell response and induce immunological tolerance.

In-vivo administration of DCs, pre-treated either with MMC or mAbs to ICAM-1, CD86 and CD80 strongly inhibited T-cell proliferation in an allogeneic lymph-node assay. When prospective heart transplant recipients were treated by a single intraportal injection of donor-derived MMC-DCs, animals tolerated their allografts for 30 days. Significant prolongation of graft survival (52 days) was also achieved after pre-treatment of DCs with mAbs to ICAM-1, CD86 and CD80 arguing for the involvement of the same molecules in MMC-DCs induced

suppression. Moreover, this process was donor-specific since no significant effect could be noted on third party heart allograft survival.

This study shows that treatment with Mitomycin C generates suppressive DCs which are able to induce donor-specific prolongation of rat heart allograft survival. We consider MMC-treated donor DCs as pertinent candidates for inducing immunological tolerance in clinical organ transplantation.