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Immunomodulation with MHC Class II Peptides after Small Bowel Transplantation: Influence on Rejection and Outcome

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T-cell recognition of allogeneic major histocompatibility complex (MHC) peptides presented by self-MHC molecules initiates allograft rejection. Under certain conditions, synthetic MHC peptides are able to inhibit the allogeneic T-cell response. The present study was designed to evaluate the effect of MHC class II peptides RT1.D2 and RT1.B2, corresponding to the hypervariable domain of Wistar Furth (WF) rat, combined with low-dose cyclosporine A (CsA) in experimental small bowel transplantation. In the rat strain combination WF-to-LEW, preoperatively administered RT1.B2 peptide (50 µg per rat) (but not RT1.D2 peptide) showed immunoinhibitory effects were seen and allograft survival was prolonged to a mean of 106 ± 24.3 days in 43% of the Lewis graft (LEW) recipients. The graft survival after CsA application alone was limited to 49.9 ± 8.3 days. In combination with peptide RT1.D2, the survival time was 47.1 ± 3.8 days. The authors have evidence that the combination of CsA with peptide RT1.B2 induces specific unresponsiveness in alloreactive T-cells. These promising results indicate that it is possible to suppress the T-cell-dependent alloresponse by the indirectly presented MHC class II peptide RT1.B2 in combination with low-dose CsA, to prolong small bowel allograft survival.

Introduction

Allograft rejection is mediated by T-cell recognition of allo-MHC (major histocompatibility complex) molecules through 2 distinct pathways of allorecognition. In the so-called indirect pathway, alloreactive CD4⁺ T cells are stimulated by self-antigen-presenting cells that present MHC peptides of the graft. Synthetic MHC peptides are able to inhibit the allogeneic response under certain conditions, for example, if they are administered via thymic or oral route.¹⁻³ The present study was designed to evaluate the effects of the MHC class II peptides RT1.D2 and RT1.B2 in combination with low-dose cyclosporine A (CsA) in indirect allorecognition after experimental small bowel transplantation (SBT). More specifically, our strategy to

control the T-cell-dependent allogeneic response is based on the activation of recipient alloreactive T cells before transplantation by immunization with 1 of the 2 MHC class II peptides. The short-term administration of low-dose CsA after small bowel transplantation is expected to eliminate or inhibit these activated T cells.

Materials and Methods

Inbred male rats of the strains Wistar Furth (WF, RT1^u) and Lewis (LEW, RT1^l) were purchased from Charles River Laboratories (Sulzfeld, Germany). All animals were cared for according to national guidelines for animal care. One-step orthotopic small bowel transplantation was performed in the allogeneic rat strain combination WF-to-LEW

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as previously described.⁴ Seven days before, and on the day of transplantation, the immune system of LEW recipients was stimulated by subcutaneous injection of 50 µg of MHC class II peptides RT1.B2 and RT1.D2. Both synthetic peptides correspond to residues 20 to 44 of the hypervariable domains of the RT1.B^a and RT1.D^a class II molecules of WF¹. Low doses of CsA (Novartis Pharma GmbH, Nürnberg, Germany) were administered from days 0 to 7 (5 mg/kg/day) and from days 8 to 30 (1 mg/kg/day). LEW rats undergoing SBT were divided into 3 groups.

- Group 1 ($n = 7$): Animals received only CsA after SBT.
- Group 2 ($n = 7$): Animals were immunized pre- and intraoperatively with peptide RT1.B2 and given immunosuppression as indicated.
- Group 3 ($n = 7$): Animals were immunized pre- and intraoperatively with peptide RT1.D2 and given immunosuppression as indicated.

The postoperative monitoring of transplanted animals was performed as described.⁴ Peptide-induced *in vitro* restimulation of effector T cells was measured with an indirect T-lymphocyte proliferation assay as described previously.⁵ These cells were activated *in vivo* either after peptide immunization or by the allograft.

Results

Both synthetic peptides, RT1.D2 and RT1.B2, led to accelerated graft rejection. The mean survival time (MST) of allografts in animals immunized preoperatively with either peptide RT1.D2 or RT1.B2 was limited to 3.3 ± 0.5 days, as compared with 5.3 ± 0.5 days in nonimmunized animals. With the immunosuppressive protocol consisting of low-dose CsA alone, the allograft function was maintained for an MST of 49.9 ± 8.3 days. Administration of peptide RT1.D2, in combination with postoperative immunosuppression, did not influence allograft function. With MST of 47.1 ± 3.8 days, the animals in this group did not differ significantly from the CsA control group. In contrast, a combination of peptide RT1.B2 and low-dose CsA resulted in a significant prolongation of allograft survival to an MST of 106 ± 24.2 days in

43% of the transplanted LEW rats ($P = 0.004$). These animals showed no signs of chronic rejection.

We then analyzed if and for how long RT1.B2- and RT1.D2-specific effector T cells persist *in vivo*. We checked for their presence on days 20 and 40 postoperatively. Both RT1.D2- and RT1.B2-specific T cells were detected on day 20 in all 3 groups. On day 40, RT1.D2-specific T cells were detected in RT1.D2-immunized, as well as in nonimmunized, allograft recipients, whereas in RT1.B2-immunized animals, RT1.B2-specific T cells could not be detected at this time point.

Discussion

We describe here a strategy to specifically control T-cell-mediated alloresponse. This strategy is based on the activation of recipient alloreactive T cells before transplantation by immunization with allo-MHC class II peptide RT1.B2, followed by subsequent elimination or inhibition of these T cells by temporarily administered low-dose immunosuppression with CsA. CsA has major effects on the calcineurin-mediated signal transduction pathway in T cells. Recent evidence suggests that CsA plays a direct role in mediating programmed cell death in mature lymphocytes.⁶

Several investigators have identified immunomodulatory properties of MHC-derived peptides *in vivo*. Nisco et al. showed that allograft survival could be induced in rats by peptides corresponding to residues 75 to 84 of the α -helix of a highly conserved region of human MHC class I molecules.⁷ Willetts and others showed an efficacy of these so-called Allotrap peptides in allogeneic small bowel transplantation.⁸ We could not detect any RT1.B2-reactive T cells on day 40 after transplantation in recipients treated with the MHC class-II-derived peptide RT1.B2 and CsA. It appears that selective elimination or inhibition of RT1.B2-specific T lymphocytes may be associated with prolongation of small bowel allograft survival. Further investigation is necessary to clarify this important effect of the synthetic MHC peptide RT1.B2.

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