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Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat

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ABSTRACT

Objective(s): : Research on uterine transplantation (UTx) is conducted in preparation for its introduction in the human as a treatment for absolute uterine factor infertility. A major area of research in experimental animals is to ascertain that immunosuppressants that will be used at UTx do not negatively affect the potential of the uterus to implant an embryo and to carry a pregnancy to term. This study investigates the effects on a uterine transplant in the rat of the calcineurin inhibitor, cyclosporine A (CsA), on uterine morphology and expression patterns of some mediators involved in implantation/inflammation.

Study design: : Donor rats were of Brown Norway strain and recipients were of Lewis strain. The uterus was transplanted to an orthotopic site by vascular anastomosis. The recipients were given CsA (10 mg/kg) sc once daily or no CsA until they were sacrificed at postoperative day 7. Syngenic transplanted Lewis rats were used as controls. Uteri were analyzed regarding histology, immunohistochemistry against T-cells and mRNA levels of the implantation/inflammation-related markers leukaemia inhibitory factor (LIF), galectin-1, CD200, interleukin (IL)-1α, and IL-15.

Result(s): : There was pronounced inflammation with abundance of CD8-lymphocytes in uterine grafts of non-CsA-treated animals and only mild inflammation in treated animals. The uterine mRNA levels of IL-1α were decreased after CsA in comparison to uteri of non-treated transplanted animals. The mRNA levels of galectin-1 were decreased in the rejected uteri and were higher in the CsA-treated. The levels of mRNA of IL-15 were lower in the syngenic transplanted group compared to the CsA-treated transplanted. There was no difference between the groups concerning mRNA levels of CD200, or LIF, with wide variation of the levels of the two latter mediators in all groups.

Conclusion(s): : Cyclosporine A suppresses rejection of an allogenic rat uterine transplant, with normalization of mRNA levels of the proinflammatory cytokine IL-1α and the glycan-binding protein galectin-1.

1. Introduction

Research on uterine transplantation (UTx) has been ongoing since the 1960s when experiments in the dog [1–5] explored utero-tubal-ovarian transplantation. Due to lack of effective immunosuppressants at that time, however, allograft rejection [6,23] was seen. There has been renewed interest in UTx research during the last decade [7–9], initiated by the first and so far only human UTx attempt [10], which ended when a necrotic uterus was removed three months after transplantation. Recently, several UTx animal models have been presented, exemplified by the first offspring from a transplanted uterus in the syngenic mouse model [7] and survival of uterine allograft in the pig [11] under immunosuppression by tacrolimus/cyclosporine A (CsA). Initial studies on CsA in the mouse UTx model showed that rejection was retarded by CsA, but very high doses were needed [12].

Recently, rat UTx models were developed by us [13] and others [14]. These models use similar (by bodyweight) doses of immunosuppressants to suppress rejection [15], as compared to what is applicable in the human [16]. Moreover, rat uterine and pregnancy physiology is extremely well described [17].

Although several experiments on UTx have been presented recently [8,18], the key results that are lacking are live offspring after allogenic UTx under immunosuppression. In this first study on effects of immunosuppression after rat UTx, we have examined the effects of CsA on uterine morphology and expression of mediators linked to implantation/inflammation. The cytokine IL-15 is produced by activated macrophages, endothelial cells, keratinocytes, renal cells [19] and trophoblastic cells [20]. During early pregnancy, IL-15 induces maturation of uterine NK-cells that

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may be vital in the local immune tolerance of the semiallogenic fetus [21,22]. Interleukin (IL)-1 has multiple proinflammatory actions. Leukaemia inhibitor factor (LIF) is an anti-inflammatory cytokine [23] which is crucial for implantation in the mouse [24]. CD200, expressed by dendritic cells [25], is linked to development of local graft tolerance [26] at the maternal–fetal interface [27]. Galectin-1 (Gal-1), a member of the glycan-binding family [28], is generally immunosuppressive and may be involved in the cross-talk between the immune and endocrine systems, as exemplified by progesterone regulation of decidual Gal-1 [29].

2. Material and methods

2.1. Animals

Female Brown Norway rats and Lewis rats (Charles River, Sulzfelt, Germany) were used. The animals (weight 150–170 g) were exposed to a light/dark cycle of 12/12 h. The study was approved by the Animal Ethics Committee in Gothenburg, Sweden.

2.2. Surgery

The surgery is essentially the same as described by us previously [13] and is outlined with the modifications described more in detail. All surgical procedures were performed with the aid of a stereomicroscope (Leica M651; Leica Microsystems, Wetzlar, Germany), bipolar diathermy (CoaComp Biocoagulator, Instrumenta AB, Billdal, Sweden) and microsurgical instruments.

The animals were anaesthetized with isoflurane in air and O_2. The objective of donor-surgery was to isolate a graft containing the right uterine horn, the common uterine cavity, the cervix, and the upper vagina with a vascular pedicle including the vessels from the right uterine up to and including the common iliacs. After dissection of the uterus and the vessels, with continued blood flow to the organ, a catheter was inserted into the arterial side and the uterus was flushed with 1 mL of Perfadex (+4 °C) solution (Vitrolife Sweden AB, Kungsbacka, Sweden) supplemented with xylocaine (0.4 mg/mL; Astra Zeneca, Mölndal, Sweden) and heparin (50 IU/mL; Leo Pharma AB, Malmö, Sweden). When the uterus was blanched at flushing, the graft was removed from the animal and placed in Perfadex (+4 °C).

The recipient-surgery was initiated when the graft was under cold ischemia in the preservation solution. A hysterectomy was performed with placement of titanium clips between the uterus and the ovaries. The vaginal vault was opened just below the cervix and the right common iliac vessels were mobilised and separated from each other up to their branching from the aorta/vena cava. The graft was positioned intra-abdominally and clamps were placed on each side of the intended anastomosis sites on the right common iliac vessels. The vessels of the graft were anastomosed end-to-side to the right common iliac vessels of the recipient. The vaginal cuff was sutured to the vaginal vault. The tip of the uterine horn of the graft was fixed to the tissue surrounding the clip, which had previously been placed at the right utero-tubal junction. Three mL of 4% icodextrin solution (Adept®; Baxter, Deerfield, IL, USA) was placed intra-abdominally before closure to prevent postoperative adhesions. The recipients were treated post-operatively for 2 days with analgesia, anti-thrombotic agent and antibiotics (1 mL of bupivacain (5 mg/mL; Marcain®, Astra Zeneca, Mölndal, Sweden) sc; buprenorphine (0.005 mg/kg; Temgesic®, Schering-Plough, Kenilworth, NY, USA) sc; carprofen (5.0 mg/kg; Rimadyl®, Pfizer, New York, NY, USA) sc; low molecular weight heparin (100 IU/kg; Fragmin®; Pfizer. Täby, Sweden) sc; trim-sulfa (100 + 20 mg/kg; Bimotrim®, CEVA Vetpharma, Lund, Sweden) sc.

2.3. Experimental protocol

Three experimental groups were used. Two groups were Lewis rats that had undergone hysterectomy and UTx with uteri from animals of the Brown Norway strain, with one group of animals receiving CsA ($n = 5$) and one group with no immunosuppression ($n = 5$). The control group consisted of syngenic transplanted Lewis to Lewis ($n = 6$), undergoing simultaneous hysterectomy. The CsA-treated animals received sc10 mg/kg of CsA (Sandimmun®, Novartis Pharma AG, Basel, Switzerland) diluted in 90% propylene glycol from the day of surgery until postoperative day 7 when they were sacrificed. The UTx animals were relaparotomized on day 2 for inspection to ascertain graft viability.

At day 7 post-UTx, the rats were anaesthetized and the macroscopic appearance of the uterus was noted in regard to texture (firm or normal), colour (pale/grey, normal, dark), size (normal, small enlargement (>150% of size of syngenic controls) or marked enlargement (>200% of size of syngenic controls)), and bleeding on incision (bleeding or no bleeding). The uterus was then removed and the uterine horn was divided into three equally large parts with the upper part (nearest the clip) being discarded to avoid use of tissue that may have been influenced by the titanium clips; the midsection was used for histology and the lower part used for analysis of mRNA levels.

2.4. Histology

The tissue was fixed in 4% formaldehyde and then embedded in paraffin. Sections (≈4 (mm) were stained with haematoxylin for histological evaluation and for immunohistochemistry (IHC) with antibodies against CD4 (1:100; Serotec, Dusseldorf, Germany), CD8 (1:100; Serotec), CD3 (1:100; Abcam, Cambridge, UK) and macrophage antigen – ED2 (1:100; Serotec), according to the manufacturers' protocols. A magnification of ×400 was used for the histological examination and 10× × 40× quantification of cells was done using a grid (10 × 10 squares) in five random fields of the myometrium and the endometrium.

2.5. Expression levels of mRNA

Uterine tissue was immediately immersed in RNAlater® (Ambion, Huntingdon, UK) and frozen (−20 °C) until analysis. Total RNA was later extracted from the tissue using Trizol (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using 2 μg total RNA with 0.5 μg random primer (Promega Corp., Madison, WI, USA) in a total volume of 17 μL. This mixture was denatured at 70 °C for 5 min. Then, 0.5 mM deoxy-NTP, 20 U RNAsine, RT-buffer and 200U Moloney murine leukaemia virus reverse transcriptase (Promega Corp., Madison, WI, USA) were added to a final volume of 25 μL. The cDNA synthesis was performed for 60 min at 37 °C.

Real-time quantitative PCR was done with the ABI Prism 7000 Sequence Detector (Applied Biosystems, Foster City, CA, USA). Taqman MGB probes targeting IL-15 (Rn00689964_m1), IL-1((Rn00566700_m1), CD200 (Rn01646320_m1), LIF (Rn00573491_g1) and Gal-1 (Rn00571505_m1) and control (β-actin) were purchased as TaqMan Gene expression assays (Applied Biosystems). Each amplification reaction consisted of 20 ng cDNA, 1× probe-mix and 1× TaqMan Universal PCR mastermix (Applied Biosystems) to a final volume of 25 μL. After control of amplification efficiency of the targets genes and control, the relative expression was presented with the comparative Ct method (ΔΔCt) [30]. Expression of the target gene mRNA was normalised for the expression of the control (β-actin).