Tissue and Cell 46 (2014) 21–26

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

# The immediate response of jejunal mucosa to small bowel heterotopic allotransplatation in rats

Z. Jonecová [a], Š. Tóth [a,*], J. Varga [b], P. Staško [c], B. Kovalčinová [a], M. Maretta [a], J. Veselá [a]

[a] Department of Histology and Embryology, Faculty of Medicine, Pavol Jozef Šafárik University, Šrobárova 2, 041 80 Košice, Slovakia
[b] 2nd Department of Gynaecology and Obstetrics, Faculty of Medicine, Pavol Jozef Šafárik University, Rastislavova 73, 041 90 Košice, Slovakia
[c] Department of Vascular Surgery, Faculty of Medicine, Pavol Jozef Šafárik University, Trieda SNP 1, 040 11 Košice, Slovakia

ARTICLE INFO

*Article history:*
Received 14 June 2012
Received in revised form 30 August 2013
Accepted 30 August 2013
Available online 29 September 2013

*Keywords:*
Transplantation
Graft
Jejunum
Histopathology
Morphometry
Apoptosis

ABSTRACT

The course of histopathological alterations within jejunal graft architecture during the initial adaptation phase in the host body was investigated. Graft tissues were compared to the intestinal tissues of the recipients. This study demonstrates: (1) renewal of intestinal epithelial lining in the graft biopsies during initial hours after transplantation is more likely caused by migration and extension of remaining epithelial cells than by their increased mitotic division. (2) Distinct decrease in histopathological injury was observed in transplanted grafts after 6 h, but the morphometrical parameters, particularly villus height and wall thickness, remained altered. (3) Significant decrease in apoptotic cell death in the epithelial lining within 6 h of graft recirculation was accompanied by no effect on apoptosis levels of the cells in lamina propria connective tissue. (4) Although the apoptosis level in the connective tissue cells was not modulated in the grafts within the first hour after transplantation, caspase-3 dependent apoptosis was decreased significantly.



## 1. Introduction

Control of graft rejection and maintenance of the mucosal barrier function represent pivotal issues for the outcome of small bowel transplantation (SBTx), and their mutual balance is crucial in postoperative management. The integrity of the intestinal graft mucosal barrier plays a key role in prevention of bacterial translocation and maintenance of the absorptive capacity, even if many factors could reduce its function after transplantation (Mueller et al., 2002). Ischaemia-reperfusion (IR) or preservation injury represents the most critical event during SBTx that may affect the integrity of the mucosal barrier and promote postoperative complications (Zonta et al., 2007). Ischaemia-induced endothelial cell injury has been described as the pivotal causative event leading to an array of pathophysiological issues such as microvascular vasoconstriction, adhesion and aggregation of platelets and neutrophils, and decreased blood flow inclusively described as the "no-reflow phenomenon" soon after reperfusion (Koo et al., 1992). Paradoxically it is the restoration of blood flow that causes the subsequent cell and tissue damage which is referred to as reperfusion/recirculation injury (Sasaki and Joh, 2007). This is characterized by increased microcirculatory cellular damage and mucosa barrier dysfunction. Rupture of the mucosal barrier promotes bacterial translocation, causing severe infectious episodes within a transplanted organism already weakened by its hyper-catabolic state.

The aim of the present experimental study was to analyze the histopathological changes in jejunal graft tissue in the initial hours after heterotopic allotransplantation in rats. IR injury is one of the main factors affecting the function and structure of transplanted intestinal grafts. The intestinal mucosa may play a more sophisticated role in allograft responses through its ability to present alloantigens to immunocompetent cells and to promote intercellular communication within the epithelium as well as with underlying connective tissue proper. Recent evidence suggests that the intestinal epithelium has machinery to present antigens under inflammatory conditions (Adams and Afford, 2005). Histopathological changes in the grafts have usually been studied in connection with peracute or acute graft rejection. There is no evidence about what happens immediately after the start of recirculation in the graft and how the tissue responses participate in the process of graft adaptation. Our objectives were to evaluate (1) the time course of graft histopathological injury and morphometric parameters, (2) tissue repair ability and (3) the participation of apoptotic cell death in this process.

## 2. Materials and methods

This study was approved by the Committee for Ethics on Animal Experiments at the Faculty of Medicine, Pavol Jozef Šafárik

* Corresponding author. Tel.: +421 552343381.
*E-mail address:* stoth2000@gmail.com (Š. Tóth).

0040-8166/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tice.2013.08.004

University, Košice, Slovakia, and the experimental protocol was approved by the State Veterinary and Food Administration of the Slovak Republic No. 854/10-221/3.

### 2.1. Experimental design

Twenty-four adult male Wistar rats ($n = 24$, 12 donors, 12 recipients) weighing between 250 and 350 g were used in the experiment. The animals were maintained under standard conditions of controlled temperature and humidity. All animals were fed standard rat chow and water *ad libitum* and were given only water for 12 h before surgery. After the graft harvest, donor animals were killed by exsanguination. The recipients were randomly assigned to one of the experimental groups – group T1 survived 1 h and group T6 survived 6 h after transplantation.

### 2.2. Surgical procedures and sampling

The animals were anesthetized with intraperitoneal injection of ketamine 60–80 mg/kg (Narketan 10 inj. ad us. vet., Vétoquinol S.A., Lure Cedex, France), and xylazine 8–10 mg/kg (Xylariem inj. ad us. vet., Riemser Arzneimittel, Greifswald-Insel Riems, Germany). After 1 h, 1/3 of the total dose of anaesthetic mixture was administered intramuscularly.

The harvest of jejunal graft and intestinal heterotopic allotransplantation was performed using the technique adapted from Baláž et al. (2003). After dissection of the aorta, the jejunal graft was placed in preservation solution (saline solution, 20 $cm^3$) at 4 °C for 1 h. Aorto-aortal and porto-caval anastomoses were performed using the end-to-side microsurgery technique. The oral end of the jejunal graft was occluded and the aboral end of the graft was exteriorized as jejunostomy.

### 2.3. Tissue specimen preparation

After the one or 6-h reperfusion, the recipient animals were sacrificed. Small intestine samples of recipient animals 1–2 cm long (R) were taken 10 cm from the Trietz ligament, washed with cold saline and fixed in 4% *p*-formaldehyde. Similar samples of the graft from the same animal (G) were taken and processed in the same way. The tissues were then embedded in paraffin, cut into 4–5 μm sections and mounted. After deparaffinization, the tissues were stained with haematoxylin-eosin (HE) for histological examination.

### 2.4. Histopathological evaluation

The histopathological injury index (HII) of the intestine was evaluated on a graded scale from 0 to 8 according to the Park/Chiu scoring system adapted by Quaedackers et al. (2000): 0 = normal mucosa; 1 = subepithelial space at villus tip, often with capillary congestion; 2 = more extended subepithelial space with moderate epithelial lifting; 3 = epithelial lifting along villus sides; 4 = denuded villi; 5 = loss of villous tissue; 6 = crypt layer destruction; 7 = transmucosal infarction; 8 = transmural infarction. This index is based on the extent and severity of histopathological abnormality or lesion in the section.

### 2.5. Morphometrical analysis

Morphometry was performed using an Olympus BX50 light microscope with Olympus Camera SP350 (Olympus, Tokyo, Japan) and QuickPHOTO Industrial 2.3 image analyser software (Promicra, Prague, Czech Republic).

Villus height (VH) from the villus base to the tip and crypt depth (CD) from the villus base to the muscular layer was measured in 12 axially-oriented villi in at least three different quadrants of each intestinal sample. The thicknesses of the muscular layer (MT) and the intestinal wall (WT) were measured in the same manner. All measurements were done using magnification 200×.

Density of cells in the *lamina propria mucosae* was calculated as the number of cells per unit of mucosal tissue area ($mm^2$). The mucosal tissue area (*lamina propria mucosae*) was measured from villi tip to the *lamina muscularis mucosae*.

### 2.6. Immunohistochemical analysis of regeneration/reparation capacity

#### 2.6.1. PCNA method (proliferating cell nuclear antigen)

Histological sections (4–5 μm) were deparaffinised and rehydrated. Endogenous peroxidase activity was blocked with 3% $H_2O_2$ with methanol. Pre-treatment was performed in a microwave oven at 600 W for 15 min in 0.01 M citrate buffer at pH 6.0. This yielded the best results in terms of antigen retrieval. Primary Anti-PCNA Rabbit Polyclonal Antibody was labelled using a two-stage indirect immunoperoxidase technique. Primary antibody was applied at the appropriate titre (Table 1). Biotinylated secondary anti-goat anti-mouse antibody IgG (H + L) (Millipore Bioscience Research Reagents, Billerica, MA, USA) was used in labelling with IHC Select® Immunoperoxidase Secondary Detection System (Millipore Bioscience Research Reagents, Billerica, MA, USA) for detection of proliferative activity. Positive cell populations were visualized with diaminobenzidine, DAB (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and counterstained with Mayer's haematoxylin. Omitting the primary antibodies was considered as the negative control.

### 2.7. Immunofluorescence analysis of apoptotic cell populations

#### 2.7.1. In situ TUNEL method

Detection of apoptosis was performed using terminal deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate *in situ* nick end labelling (TUNEL) in paraffin-embedded 4-μm-thick sections, applying the method specified the manufacturer's instructions (*In situ* Cell Death Detection Kit, Fluorescein, Roche, Germany). The fluorescein-labelled dUTP (Table 1) was detected by alkaline phosphatase and antifluorescein conjugation and visualized with NBT/BCIP. After fluorescence staining as described above, sections were incubated with 10 mg/ml Hoechst 33342 (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 5 min, washed three times in PBS, cover-slipped in fluorescent mounting medium

**Table 1**
Antibodies employed in the experimental study.

| Primary & secondary antibodies | Antibody working dilution | Incubation parameters[a] | Source/code |
|---|---|---|---|
| Anti-PCNA rabbit polyclonal antibody | 1:100 | 1 h, RT | Thermo Scientific (RB-9055-P0) |
| Biotinylated goat anti-mouse IgG/goat anti-rabbit IgG | 1[b] | 40 min, RT | Millipore IHC Select (DAB150) |
| Rabbit polyclonal to Caspase 3 | 1:200 | ON, 4 °C | Abcam (AB44976) |
| FITC-conjugated affiniPure goat anti-rabbit IgG (H + L) | 1:500 | 2 h, RT | Jackson ImmunoResearch (111-095-003) |

[a] RT: room temperature; ON: overnight.
[b] The antibody is supplied ready-to-use by the manufacturer.