APPEARANCE OF PNAD+ HEVS IN THE ABSENCE OF MADCAM-1 IN NKX2-3-/- MICE IS DEPENDENT ON LTβR ACTIVATION

Zoltán Kellermayer and Péter Balogh
University of Pécs, Department of Immunology and Biotechnology

INTRODUCTION: In the absence of Nkx2-3 transcription factor mice lack endothelial mucosal addressin cell adhesion molecule (MAdCAM-1) expression, needed for lymphocyte homing to the mucosa. Previously we have shown that this leads to the compensatory upregulation of PNAd only in the mesenteric lymph nodes (mLN) and Peyer's patches (PP) of the intestine, but not the lamina propria vessels. We hypothesized that the appearance of PNAd+ high endothelial venules (HEVs) requires activation of the lymphotoxin beta receptor (LTβR) by various ligands expressed on mature lymphocytes. We also examined how the altered addressin profile affects PP cellular composition and homing velocity.
MATERIALS AND METHODS Double mutant *Nkx2-3-/-xRag1-/-* mice were created by crossing single mutant mice and were identified by PCR with primers for Nkx2-3, Rag1 and Neomycin phosphotransferase genes. Nkx2-3 deficient neonatal mice were injected intraperitoneally at P1, P3, and P5 with LTβR-Ig fusion protein. Multicolor immunofluorescence was performed with anti-CD45, anti-MadCAM-1 and anti-PNAd mAbs. Adult PPs were analyzed with flow cytometry with anti-CD3, anti-B220, anti-FoxP3, anti-CD25, and anti-CD4 mAbs to compare lymphocyte subsets in Nkx2-3 deficient and wild type mice. Fluorescently labeled donor lymphocytes were injected intravenously. Distribution of homed cells was examined with immunofluorescence by costaining tissue sections with anti-endothelial mAbs.
RESULTS Primordial PPs in young adult *Nkx2-3-/-xRag1-/-* mice contained few PNAd+ HEVs. Treatment of neonatal Nkx2-3 mice with LTβR-Ig fusion protein led to the complete absence of PNAd and the disappearance of residual MAdCAM-1. Cell extravasation through PNAd+ vascular segments in mutant PPs was significantly slower compared to wild type samples. Adult PPs in Nkx2-3 mutant mice contained higher number of CD3+ T cells and regulatory T cells and significantly less B cells.
CONCLUSIONS Our results indicate that appearance of PNAd+ HEVs requires activation of LTβR. However, this signal can be delivered by non-T/non-B cells as *Nkx2-3-/-xRag1-/-* mice lacking mature B and T cells expressed PNAd+ vessels. The switch in addressin profile influences the lymphocyte composition and also the rate of cell homing to mutant PPs.
Supported by OTKA K108429

ORAL PRESENTATION
THEORETICAL IMMUNOLOGY