Galectin-1 modulates mucosal immune response against *Yersinia enterocolitica* infection

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Introduction

The mucosa of the gastrointestinal tract is continuously exposed to a myriad of antigens and microorganisms, however, only a limited number of which enter the body and cause disease. The gut-associated lymphoid tissue (GALT) comprise organized tissues such as the Peyer's patches (PP) and mesenteric lymph nodes (MLN) that are generally considered to be inductive sites of the intestinal mucosa immune system.

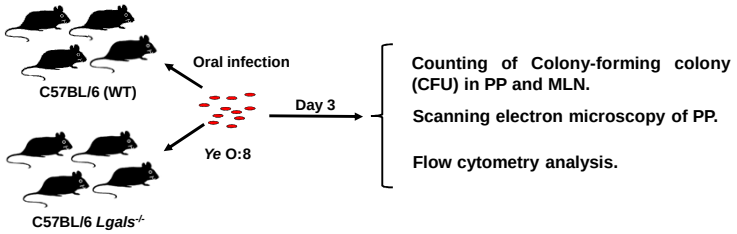
Galectin-1 (Gal-1), an evolutionarily conserved *β*-galactoside-binding protein, has key roles in a variety of physiologic and pathologic processes. These functions include suppression of T-cell responses through selective induction of T_H1 and T_H17 cell apoptosis and activation of tolerogenic circuits on dendritic cells. These glycosylation-dependent functions account for the capacity of this lectin to dampen inflammation in autoimmune, chronic and acute inflammatory disorders. Gal-1 is expressed in different portions of the gastrointestinal tract, and has been implicated in different intestinal disorders.

Yersinia enterocolitica (*Ye*) is a Gram-negative, predominantly extracellularly located pathogen that causes food-borne acute or chronic gastrointestinal diseases. During the course of an infection with *Yersinia*, the bacteria colonize the intestinal tract, enter through the M cells of PP, colonize the PP and may eventually disseminate to the MLN, and subsequently, to spleen, liver, and lung. The role of Gal-1 in *Ye* infection has not been fully explored yet .

Objetive

The purpose of the present work was to investigate the role of Gal-1 in the mucosal immune response against oral infection with *Ye*.

Materials and Methods



Results

Recovery of *Ye* from PP and MLN

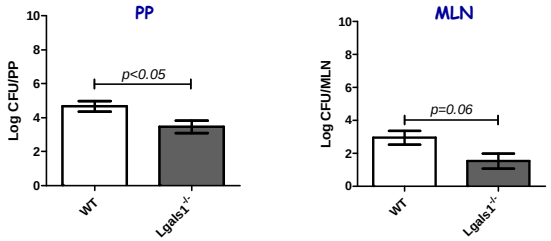


Fig 1. Recovery of *Ye* from PP and MLN. Three days after infection, the mice were killed and PP and MLN were aseptically obtained. Dilutions of the PP and MLN homogenates were plated onto MacConkey with Irgazan or Mueller Hinton agar, respectively. The number of colony forming units (CFU) in the plates was determined after incubation for 48 h at 25 ºC. The results indicated that *Ye* invades PP and MLN of both groups of mice. The CFU number was significantly lower in PP showing a tendency toward reduction in MLN in infected *Lgals1*$^{-/-}$ compared to WT mice.

Ultrastructure of PP from WT and *Lgals1*$^{-/-}$ mice

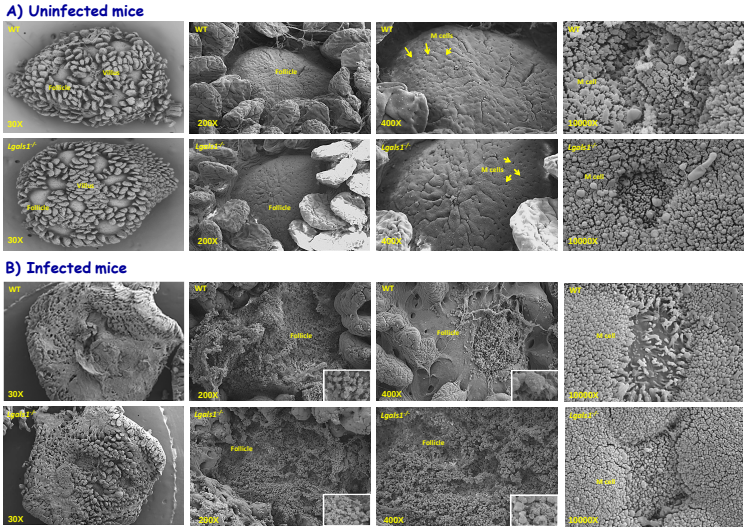


Fig 2. **Ultrastructure of PP from WT and *Lgals1*$^{-/-}$.** The PP were removed and fixed in ice-cold glutaraldehyde in Sorensen's buffer. The tissues were dehydrated and coated with a gold layer. The samples were examined with scanning electron microscope (SEM). Between 5 and 9 PP were found macroscopically in the normal small intestine of uninfected WT and *Lgals1*$^{-/-}$ mice. By SEM, 6-9 domes could be observed on the luminal surface of each PP of WT and *Lgals1*$^{-/-}$ mice. The M cells could be identified easily in follicle-associated epithelium (FAE) by their short microvilli. Interestingly we observed a suggestive reduction in the number and size of M cells in *Lgals1*$^{-/-}$ mice (A). To characterize the early events during invasion of the PP by *Ye*, WT and *Lgals*$^{-/-}$ mice were infected orogastrically with 1 x 10^9 bacteria. At 3 days after infection, cytoarchitecture of the infected PP was almost completely destroyed and *Ye*-induced abscesses were detected in the FAE and follicle of both WT and *Lgals1*$^{-/-}$ mice. Many leukocytes were observed on the surface of the FAE (insert). By higher magnifications, bacteria attached to the FAE could be recognized. These results suggest that there are differences in the cytoarchitecture of the PP *Lgals*$^{-/-}$mice as compared to WT, and these differences do not affect the invasive capacity of *Ye*.

Cell recruitment in PP and MLN

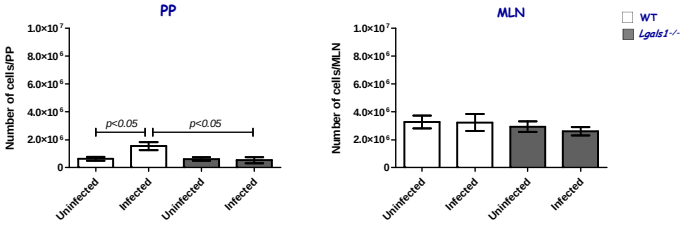


Fig 3: **Number of total cells in PP and MLN.** The PP and MLN were removed at 3 days after infection and the total cell number was counted and compared with uninfected mice. We observed that *Ye* infection induced cell influx in PP of WT. In contrast, the increase in the total cell number was not detected in *Lgals1*$^{-/-}$ mice after *Ye* infection.

Analysis of PP and MLN cellular populations by flow cytometry

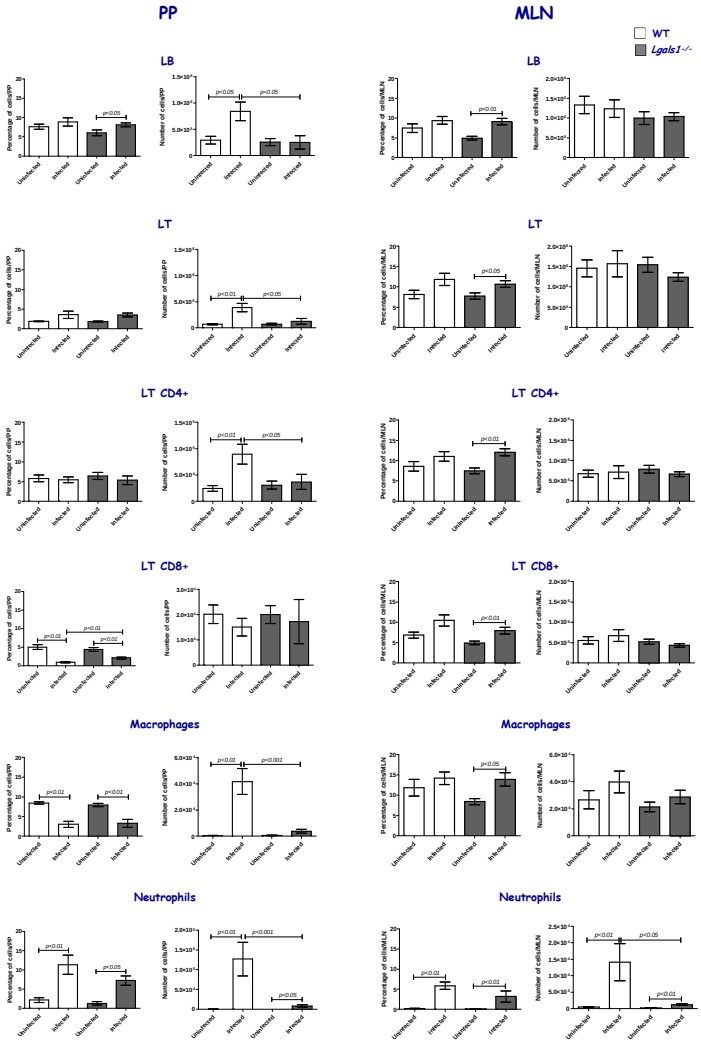


Fig 4: Flow cytometry analysis of cell population in PP and MLN. WT and *Lgals1*$^{-/-}$ mice were orogastrically infected with *Ye*. On day 3 after infection, MLN and PP were removed and cell infiltration (LT, LB, macrophages and neutrophils) were analyzed by flow cytometry. Lower numbers of LT CD4$^+$, LB, macrophages and neutrophils were detected in PP of infected *Lgals1*$^{-/-}$ mice. Furthermore, we observed after infection reduction in the LT CD8$^+$ frequency in PP of both groups of mice; however, this reduction was lower in *Lgals1*$^{-/-}$ mice. On the other hand, an increase in the number of neutrophils was observed in MLN from WT mice compared to *Lgals1*$^{-/-}$.

Conclusions

We conclude that Gal-1 may be involved in M cell development and in the control of mucosal immune response after *Ye* infection. The absence of Gal-1 may favor CD8 T cell response which could act to maintain protective immune response contributing to *Ye* eradication.