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Induction of indoleamine 2,3-dioxygenase in small intestine of mouse infected with parasitic helminth, *Hymenolepis nana*

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Abstract. One hundred and fifty crushed eggs of *Hymenolepis nana* were administrated to 5–6 week old ddY mice, and the animals were killed at 0, 7, 14, 21 or 28 days post-infection (dpi). The small intestines were recovered, and both halves, proximal and distal, were examined, observed for immunohistochemistry, and assayed on enzyme activity of IDO. Enzyme activity of IDO was increased by *H. nana* infection, and was parallel to a decrease of recovered worms. The expression pattern of IDO-positive cells in the proximal intestine was also the same as those of IDO activity with a little change earlier. Furthermore, IDO-positive cells coincided with goblet cells, but not all goblet cells were IDO-positive. Present findings apparently suggest that IDO might be involved in the mucous excretion process in the expulsion of worms.

Keywords: IDO; Indoleamine 2,3-dioxygenase; Mouse; Intestine; *Hymenolepis nana*; *Goblet* cell

1. Introduction

Indoleamine 2,3-dioxygenase (IDO; EC 1.13.11.42) is an initial and rate-limiting enzyme in the metabolism of tryptophan along the kynurenine pathway. IDO is widely induced in many tissues under various pathological conditions associated with immune activation, and it has been suggested that the induced IDO may inhibit proliferation of some pathogens,

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including tumors and intracellular parasites, such as Toxoplasma gondii, by deprivation of tryptophan [1,2].

However, while IDO is emerging as an important immunoregulatory enzyme as shown in the mouse and human placenta [3–6], there have been few works on the multicellular parasitic helminthes. Recently the DNA array data have shown that the up-regulation of IDO was found in the colon of the susceptible mouse infected with a kind of helminth, *Trichuris muris* [7], but the localization of the IDO-positive cells was not clear. We aimed to localize the cells in which IDO was up-regulated under the infection of intestinal parasitic helminth, *Hymenolepis nana*.

2. Materials and methods

One hundred and fifty crushed eggs of *H. nana* were administrated to 5–6 week old ddY mice, and at 0, 7, 14, 21 or 28 days post-infection (dpi) animals were killed, and the small intestines were recovered, and both halves, proximal and distal, were examined. The small parts of each tissue were fixed in Carnoy's fluid and stained with anti-IDO antibody with immPress reagent (Vector Laboratories, INC) according to manufacturing procedure. The sections were counterstained with haematoxylin and/or alcian blue, and then dehydrated and mounted. Negative controls were prepared using non-immunized rabbit IgG as the primary antibody. The rest of each tissue was used for IDO activity (assay of kynurenine by HPLC). Worm burdens were assessed at 7, 14, 21, 28 dpi.

3. Results

The recovery rates of worms from mice were 33% at 14 dpi and 26 % at 21 dpi, and decreased to 4% at 28 dpi (Fig. 1).

IDO activities of both proximal and distal parts of intestine from infected groups were increased at 7, 14 and 21 dpi, but decreased at 28 dpi. Immunohistochemically, IDO-positive cells in the infected mice were detected in the epithelial layer in the luminal side of the intestinal villi, but not detected at the one third of basal side of the villi (Fig. 2A). IDO-positive cells were alcian blue-positive ones, which indicated goblet cells in the luminal side of villi (Fig. 2B). On the contrary, goblet cells at the one third of basal side were IDO-negative. In the uninfected mouse, few IDO-positive cells were

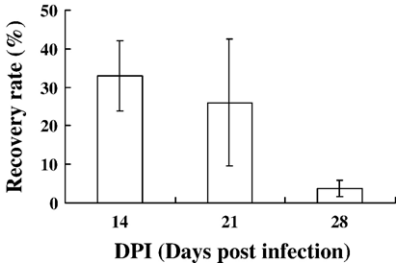

Fig. 1. Recovery rate of adult worms.