Establishment of T-Helper-2 immune response based gerbil model of enteric infection


* Intestinal Disease Research Programme, McMaster University, Hamilton, Ontario, Canada

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Establishment of T-Helper-2 Immune Response Based Gerbil Model of Enteric Infection

M. Sagar, I. Padol, W. I. Khan, R. P. Bonin, P. A. Blennerhassett & R. H. Hunt

Intestinal Disease Research Programme, McMaster University, Hamilton, Ontario, Canada


Background: The reciprocal antagonism of T-helper-1 (Th-1) and Th-2 type immune responses suggests that helminth parasitic infection may ameliorate disease where a Th-1 type response dominates. The Mongolian gerbil has been useful in the investigation of the pathogenesis of gastric cancer, since long-term infection of gerbils with Helicobacter pylori induces adenocarcinoma. In this study the kinetics of worm expulsion and associated immune responses in gerbils infected with Trichinella spiralis were investigated in an attempt to establish an animal model of parasitic infection that could be helpful when investigating the effect of a Th-2 type response on Th-1-based intestinal disorders. Methods: Gerbils were infected with various doses of infective T. spiralis larvae and were euthanized on different days after infection to investigate the intestinal worm recovery, goblet cell population, eosinophil response and serum IgG1 responses. Results: The number of worms recovered from the intestine was dependent on the number of larvae used for the infection. Almost all worms were expelled spontaneously by day 26 post-infection, when the gerbils had been infected with 375 or 750 larvae. The number of intestinal goblet cells, eosinophils and the serum IgG1 level significantly increased following infection compared with the control. Conclusion: This is the first comprehensive report on the time-course of T. spiralis infection in gerbils. The data indicate that the T. spiralis-infected gerbil could be used as a model of the Th-2-based response to investigate the effect of a parasite-induced Th-2 response on various Th-1-mediated intestinal disorders such as H. pylori-induced gastritis and gastric carcinoma.

Key words: Eosinophils; gerbils; goblet cells; IgG1; Th-2; Trichinella spiralis

Richard H. Hunt, Dept. of Medicine, Division of Gastroenterology, McMaster University, Health Science Centre, Room 4W8, 1200 Main Street West, Hamilton, Ontario L8N 3Z5 Canada (fax. +1 905 521 5072, e-mail. huntr@mcmaster.ca)

CD4+ T cells constitute an important component of many immune responses, including those associated with Helicobacter pylori and intestinal nematode infections. T-helper (Th) cell-dependent immune responses are generally divided into two major subsets, Th-1 and Th-2 (1). Th-1 cells predominantly secrete interferon gamma (IFN-\(\gamma\)) and interleukin-2 (IL-2), while Th-2 cells secrete IL-4, IL-5, IL-9, and IL-13. Th-1 and Th-2 cells cross-regulate one another. IFN-\(\gamma\), secreted by Th-1 cells, directly suppresses IL-4 production, and thus inhibits the differentiation of naïve Th cells into Th-2 cells (2, 3). In contrast, IL-4 inhibits the secretion of IFN-\(\gamma\), blocking the polarization into Th-1 cells (4). The dichotomous split of Th lymphocytes into Th-1 and Th-2 cells has provided a convenient conceptual framework to characterize T-cell responses in different intestinal diseases.

Infection with H. pylori is associated with the development of gastritis and peptic ulcer disease as well as gastric carcinoma (5). H. pylori infection is now considered to be one of the most common enteric infectious diseases in humans, with about 50% of the human population reported to be infected with this bacterial agent (6). H. pylori infection in both humans and the mouse model is associated with a Th-1 type immune response (7–9). It has been reported that in C57Bl/6 mice, chronic Helicobacter infection produces higher amounts of IFN-\(\gamma\) as compared to IL-4 and induces considerable gastric inflammation and corpus atrophy, which is considered a premalignant gastric lesion. In contrast to infection with H. pylori, helminth parasite infections generate a strong Th-2 type immune response in the mouse host with characteristic features of intestinal mastocytosis, eosinophilia, goblet cell hyperplasia, and muscle hypercontractility (10–13). The reciprocal antagonism of a Th-1 and Th-2 type immune response suggests that helminth parasitic infections may ameliorate disease where a Th-1 type response dominates. Infection with the helminth, Heligmosomoides polygyrus in mice attenuates the Th-1 response as well as the severity of the inflammatory response to subsequent infection with Helicobacter felis (14). It has also been shown recently that previous infection with a Th-2-based intestinal nematode infection ameliorated Th-1-mediated experimental colitis in mice (15).
The Mongolian gerbil is a fascinating animal model with which to explore the relationship between H. pylori infection and gastric carcinoma, which is induced by long-term infection with this pathogen (16, 17). The aim of the present study was to establish a gerbil model of helminth parasite infection that could be helpful to investigate the effect of a Th-2 type response on subsequent Th-1-based gastrointestinal disorders. For the first time, we demonstrate the time-course of Trichinella spiralis infection in gerbils. Our data reveal that gerbils infected with T. spiralis generate a predominantly Th-2 type immune response, which indicates potential suitability of this model for future studies. For example, the T. spiralis gerbil model can be used to investigate the effect of a parasite-induced Th-2 response on various Th-1-mediated disorders such as H. pylori-induced gastritis and gastric carcinoma.

Materials and Methods

Animals

Specific pathogen-free 5–7-week-old male Mongolian gerbils (Charles River, USA) were housed in a biohazard room, with a 12-h light and 12-h dark cycle. The animals were provided with an autoclaved rodent diet and water ad libitum. The protocols employed were in direct accordance with guidelines drafted by the McMaster University Animal Care Committee and the Canadian Council on the Use of Laboratory Animals.

T. spiralis infection

The T. spiralis parasite used in the study originated in the Dept. of Zoology at the University of Toronto, and the colony was maintained through serial infections alternating between male Sprague-Dawley rats and male CD1 mice. The larvae were obtained from infected rodents 60–90 days post-infection (PI), using a modification of the technique described by Castro and Fairbairn (18). Gerbils were infected orally with either 750, 375, or 50 T. spiralis larvae per gerbil and were killed at various time-points after infection. Adult worms were recovered from gerbils after the intestine had been opened longitudinally, rinsed, and placed in Hanks’ buffer (100 mL washing buffer and 2 g bovine serum albumin (BSA)). The plate was incubated for 1 h at 37 °C. Following washing of the wells, serum diluted with blocking buffer was added to each well. After incubation at 37 °C for 1 h, the wells were washed and rabbit anti-mouse IgG1 or IgG2a diluted 1:1000 with the blocking buffer was added to each well. The plate was incubated at 37 °C for 1 h. After washing five times, goat anti-rabbit IgG horseradish peroxidase conjugated diluted 1:1000 with blocking buffer was added to each well. Following incubation for 1 h at 37 °C, the wells were washed and enzyme reaction was performed at room temperature for 15–30 min using 2,2-azino-di-(3-methylbenzthiazoline sulfonic acid) (ABTS, Sigma, St. Louis, Mo., USA) as a substrate. An ELISA plate reader was used to record development of absorbance at a wavelength of 405 nm. Serum IgG1 and IgG2a results are reported as absorbance value at a sample dilution of 1:1000. Standard curves could not be generated because gerbil immunoglobulins were not available.

Statistical analysis

Data were analyzed using Student’s t test, with a P value of <0.05 considered to be significant. All results are expressed as the mean ± s.e. (standard error of the mean).

Results

We observed larvae dose-dependent worm expulsion in the Mongolian gerbils. Following infection with 375 or 750 infective larvae, the number of worms recovered from the intestine of gerbils reached a peak on day 12 PI, and then gradually decreased. Almost all worms were expelled from the intestine by day 26 PI (Fig. 1). The number of worms recovered from the intestine of gerbils infected with 750 larvae remained higher compared with those infected with 375 larvae at all the time-points investigated. Moreover, we experienced a gerbil mortality rate of 15% with 750 larvae/gerbil and 10% with 375 larvae/gerbil and no mortality using 50 larvae/gerbil.

As goblet cell hyperplasia is also a hallmark of intestinal nematode infection, we investigated the intestinal goblet cell response during T. spiralis infection in gerbils. There was a significant increase in the number of goblet cells in T. spiralis-infected gerbils on days 20, 22, 26, and 34 PI as compared with non-infected control gerbils (Fig. 2). There was no significant difference in the number of goblet cells on day 42 PI.

We also investigated intestinal tissue eosinophilia, which is
considered to be a Th-2-mediated characteristic of intestinal nematode infection. As shown in Fig. 3, we observed a significantly higher number of eosinophils in the intestines of gerbils on day 20 after infection with 375 or with 750 infective larvae as compared to the count in non-infected control gerbils.

We then investigated serum levels of IgG1 and IgG2a during *T. spiralis* infection in gerbils. At all time-points between day 12 and day 41 PI with *T. spiralis*, there was significant up-regulation in Th-2 dependent IgG1 serum antibody compared to the Th-1 dependent IgG2a response in infected gerbils (Fig. 4). Interestingly, we observed a significant increase in serum IgG1 level on day 30 PI even when the gerbils were infected with 50 larvae (Fig. 5).

**Discussion**

Mongolian gerbils are not as popular as mice or rats in animal research, and are mainly used as a model for studying epilepsy (19) or cerebral infarction (20). Although their hematological and immunological characteristics have not been studied in detail, Mongolian gerbils are highly susceptible to various infectious agents. The results reported here illustrate for the first time the time-course of *T. spiralis* infection in gerbils, which suggests that this model can be used for investigating various parasite immunological parameters and to study infection-induced inflammatory changes in the intestine.

To our knowledge, there is only one study in which the use...
of *T. spiralis* in gerbils is investigated, using 1000 and 500 larvae for infection (18). However, that study focused on the recovery of larvae from the cardiac tissue of infected gerbils, and did not go beyond day 21 PI. In the current study, we used two doses of *T. spiralis*, 750 and 375 larvae/gerbil, and found that most of the worms were cleared by day 26 in both dose groups. However, there was a 15% mortality rate when we used 750 larvae/gerbil. Autopsy of these gerbils revealed eosinophil-rich granulomas and heavy infestation with *T. spiralis* larvae of the following organs; heart, lung, brain, skeletal muscle, liver, pancreas, adrenal glands, trachea, esophagus and diaphragm, duodenum, stomach, and reproductive glands. Our study shows that the safety threshold for *T. spiralis* infection is lower in gerbils than in the mouse, where doses of up to 500 larvae per mouse are well tolerated. Therefore, we suggest that gerbils should be infected with a significantly lower dose of no more than 300 *T. spiralis* larvae/gerbil to prevent unwanted side effects and loss of animals. The time-course for the worm expulsion in gerbils is similar to that observed in mice and rats, peaking on day 12, and mostly cleared by day 26. Another commonly used nematode larva, *Nippostrongylus brasiliensis*, has also been studied in Mongolian gerbils, and again the time-course of infection was essentially the same as that in rats (21, 22). In our study using gerbils, we stress that the dose of *T. spiralis* larvae should be significantly lower than that in mice and rats in order to preclude severe side effects and subsequent loss of animals.

Although immunological studies in gerbils have been limited due to the lack of availability of reagents to investigate gerbil-specific cytokine profiles, this study confirms that various Th-2 cell-mediated cellular changes such as the up-regulation of eosinophils and goblet cells are present in Mongolian gerbils. Recently, it has been reported that...

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**Fig. 3.** Intestinal eosinophils cell response in *T. spiralis*-infected gerbils. Gerbils were infected with either 375 or 750 larvae orally and killed on days indicated, to investigate intestinal eosinophils cell numbers. Bars represent mean ± s.e. (standard error of the mean) from 4 animals. VCU = villus crypt units.

**Fig. 4.** Serum IgG1 and IgG2a levels expressed as Abs at 405 nm in Mongolian gerbils infected with either 375 *T. spiralis* larvae/gerbil at different time-points post-infection. Mice sera infected with 375 infective larvae were used as a positive control for IgG1. Bars represent mean ± s.e. (standard error of the mean) from 4 animals.
infection with another helminth, *Taenia solium*, also promotes eosinophilia and goblet cell hyperplasia in gerbils (23). Studies in mice and rats suggest that both intestinal eosinophilia and goblet cell hyperplasia, which occur during intestinal nematode infection, are Th-2 dependent (10–13). We also observed an elevation of the Th-2-mediated IgG1 response in the gerbils following infection. Everything considered, it seems likely that infection with *T. spiralis* in Mongolian gerbils is associated with a predominant Th-2 type immune response. As the gerbil model is becoming increasingly popular in *Helicobacter* research for studying the relationship between the occurrence of gastritis and subsequent gastric adenocarcinoma, and the fact that the manipulation of a type T-helper cell response can have a significant bearing on the severity of observed gastritis (14), our study of the use of *T. spiralis* in Mongolian gerbils provides an essential reference point for further research in this area.

### References


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