ANTIGEN-INDUCED MESENTERIC NERVE ACTIVATION IN RATS DOES NOT OCCUR UNTIL THREE WEEKS OR MORE AFTER INFECTION WITH *NIPPOSTRONGYLUS BRASILIENSIS* (Nb)

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Introduction

Nippostrongylus brasiliensis (Nb) is an intestinal nematode that produces a mild transient inflammation. Chronic changes in gut morphology have been demonstrated in the weeks after inflammation (Stead, RH: Ann. N.Y. Ac. Sci. 1992; 664; 443-55). Other models using demonstrate that challenge with antigen in sensitized animals induces mesenteric nerve activation (Jiang, W, *et al*, Gastroenterology 119:1267-75). We have previously shown in the rat Nb model that secretory antigen induces marked and prolonged mesenteric nerve firing in rats five and ten weeks post infection. However it is not known how early after infection such a response can be obtained. The aim of this study was to characterize the timecourse of afferent responses to Nb antigen challenge.

Methods

Male Lewis rats were infected with 3000 L3 Nb larvae and data collected at various time points post infection (PI). Rats were anesthetized with pentobarbital (60-80 mg/kg i.v.) and extracellular mesenteric afferent recordings were obtained using conventional techniques. Animals were challenged with secretory antigen (200 & 600 µg/kg, i.v.) and 2-methyl-5HT (2me5HT; 100µg/kg, i.v.). For histological analysis, sections of jejunum were removed, fixed in Acetic Acid:Alcohol (1:9), embedded in paraffin and stained with Alcian blue for visualization of mast cells. Qwin software was used to count the number of mast cells per 5mm.

Data are expressed in mean ± SEM (n). A p-value of <0.05 was considered significant. Statistical analysis were performed using one-way ANOVA with Tukey's post-hoc test.

Results

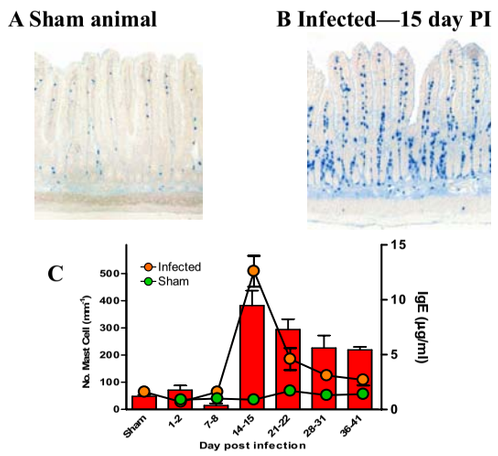

Figure 1 - Changes in mast cell and IgE levels. Jejunal sections showing mast cell distribution in sham (A) and infected (B) animals. C) Group data showing the typical pattern of mast cell changes observed, i.e. a loss of stainable intestinal mucosal mast cells (IMMC) occurred Days 7-8 PI followed by subsequent IMMC hyperplasia. The maximum IMMC numbers were determined at Days 14-15 PI, which coincides with the time of maximum IgE levels.

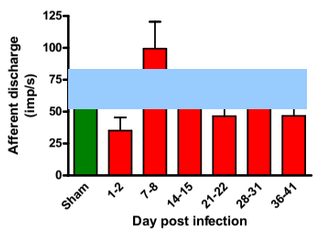

Figure 2 - Responses to 2methyl 5HT were seen throughout the entire time course of the study, demonstrating a functional vagal mucosal afferent innervation.

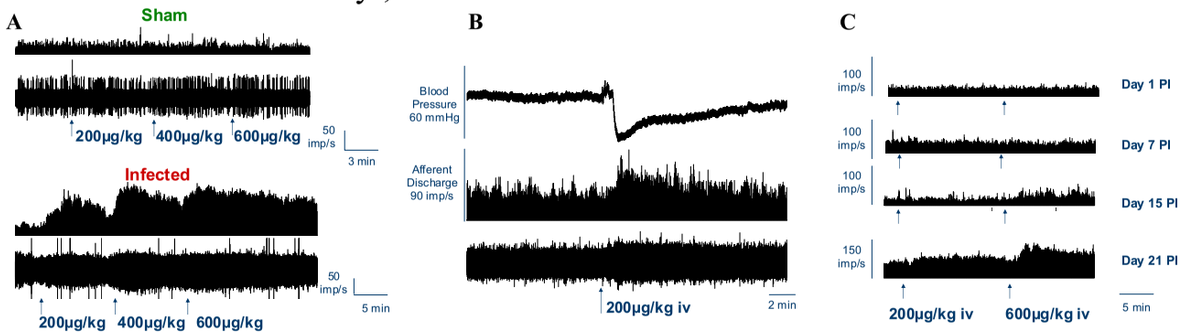

Figure 3 - Secretory antigen in sham and infected rats. A) Nb antigen (200-600 µg/kg iv) increased mesenteric afferent activity in Nb infected (bottom panel), but not sham (top panel) animals. B) Nb antigen (200 µg/kg iv) increased afferent discharge and had a depressor response on blood pressure. C) Rats were administered Nb antigen (200 and 600 µg/kg iv) 1, 7, 15 and 21 days post infection (PI). No afferent responses were observed 1 and 7 days PI. A slight response was detected 15 days PI, with a significant response to the highest dose (600 µg/kg) being observed 21 days PI.

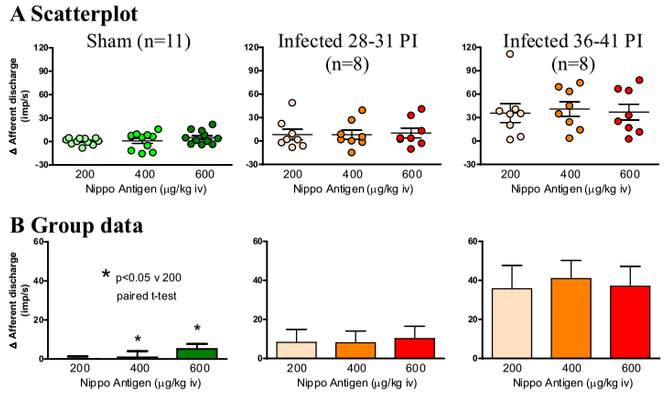

Figure 4 - Response to antigen in Sham vs Infected rats. Scatterplot (A) and group data (B) demonstrate a small response to Nb antigen at days 28-31 PI, but with a much larger response at days 36-41 PI in Nb infected rats.

Conclusions

- The increase in intestinal mucosal mast cells (IMMC) peaks 2 weeks post-infection, concurrent with maximum IgE levels.
- However, there is no appreciable afferent response to Nb antigen 2 weeks post-infection, even though nerves are still viable and active.
- Small response to secretory Nb antigen in infected rats are present 3 weeks post-infection. Sham treated animals do not show any response to Nb antigen at any timepoint.
- Large afferent responses to Nb antigen are only consistently observed 5-6 weeks post-infection.
- This delay in afferent sensitivity to Nb antigen challenge may reflect ongoing nerve and/or mast cell remodeling.