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Trichuris muris system

Analisis of IL-13 producing cells in intestine during nematodes infection

28th September 28, 2012

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1. Introduction

The human natural gastrointestinal parasite, *Trichuris trichiura*, has been estimated to infect over 1 billion people all around the world (Crompton, 1999) and the worm infection has been a large burden of health and economy in developing countries. For the further understanding and control of *T. trichiura* infection, *Trichuris muris*, the mouse whipworm, has been used as a laboratory model of *T. trichiura* over the last 10 years because they have a similar life cycle and *T. muris* is easy to maintain (Cliffe and Grencis, 2004).

Interestingly, it was found that *T. muris* infection is capable of inducing two kinds of immune responses in mice, T_H1 response and T_H2 response (Bancroft et al., 2001). TH2 response is characterised by the production of IL-4, IL-5, IL-9 and IL-13 (Arthur and Manson, 1986, Mosmann and Coffman, 1989, Mosmann and Sad, 1996, Paliard et al., 1988, Paul and Seder, 1994, Romagnani, 1991) resulting in mastocytosis, eosinophilia and IgE, IgG1 production (Abbas et al., 1996, Mosmann and Sad, 1996) that can lead to worm expulsion, whereas TH1 response is characterised by the production of IFN-r, IL-2, tumour necrosis factor (TNF) and lymphotoxin (LT) (Abbas et al., 1996, Mosmann and Sad, 1996, Ware, 2002) that can lead to the activation of macrophage and the production of IgG2a (Romagnani, 1991, Abbas et al., 1996, Mosmann and Sad, 1996) and result in chronic T. muris infection. Consequently, these findings led to the interest in TH2 type cytokines because they are thought to play important roles in the host protection against gastrointestinal helminth infection.

Bancroft et al. (1998) have reported that IL-13 may play central role in TH2 response by finding that IL-13 knock-out mice could not expel their worm and harboured chronic infection. Thus, it is essential to explore the main sources of IL-13. Several studies have shown CD4+ T cells are the main source of IL-13 and in addition, nuocytes have shown to be a new source of IL-13 during *Nippostrongylus brasiliensis* infection (Neill et al. 2010).

However, what kind of cells are the main source of IL-13 and in what part of caecum do they exist during T. muris infection is still unkown.

This study will focus on IL-13 producing cells in caecum during T. muris infecton based on immunofluorescenthistology, and FACS analisis methods to reveal its location and quantity.

2. Materials and Methods

Animals

BALB/c mice were purchased from Charles River Laboratories as required. In individual experiments, all mice were matched as the same age, gender and background strain. In addition, mice were maintained in animal unit which is kept in specific pathogen-free conditions and all animal experiments undertaken in this study were done so with the approval of the UK Home Office.

Il13-eGFP mice

Il13-eGFP mice were provided by Daniel R. Neill, MRC laboratory of Molecular Biology, University of Cambridge. The Il13-dGFP mice were generated by recomnineering (Liu, 2003, Warming, 2005).
Neomycin-negative, Cre-recombinase-negative mice were backcrossed into the BALB/c backgrounds. Genotyping of IL13-eGFP mice used PCR primers
(forward, 5′-TCAACAGGCTAAGGCCACAAGCC-3′),
(forward, 5′-CATGGTCCTGCTGGAGTTCGTG-3′)
and (reverse, 5′-GCTTCGTCTGTCACTCACACAGG-3′), giving a wild-type product of 300 bp and a targeted product of 522 bp.

Helminth infection

Mice were injected into mouse *T. muris* eggs or *T. spiralis* nurse cells that has passed hatching test, which has more than 50 % of hatching.

Immunofluorescence on cryosections and fluorescent microscopy

Mice were killed and caecum was taken for the histology for *T. muris* infection and small intestine for *T. spiralis* infection. After the dissection, samples were placed into 4 % PFA for 3 hours to fixing. After fixing, samples were moved to

10 %, 20 %, 30 % sucrose for 3 hours each. Envelopping by OCT is followed and 6 µm cryosection is performed. Slides were treated with Avidin-Biotin blocking kit for 15 minits each and then treated with 5 % bovine serum for 1 hour. Next, samples were incubated for 3 hours with conjugated antibody: anti mouse CD4-biotin. Samples are then rinsed with PBS and then incubated with AlexaFluor 488 biotin and then, sections were mounted with mounting medium.

3. Life cycle of *Trichuris muris*

The life cycle of *T. muris* is a cycle of a direct faeco-oral route, starting from the release of infective eggs into host faeces and which in turn, released into outer world to mature and then hatch in host ceacum once ingested when it is infective (Figure 1).

Once ingested, embryonated eggs accumulate in the ceacum and hatch. Panesar and Croll have suggested that hatching occurs in the caecum and that the pH in the caecum and colon plays an important role in this hatching process (Panesar and Croll, 1980). According to the study of Wakelin, *T. muris* eggs hatch within a very short time, as L1 larvar can be observed 90 min post infection (Wakelin, 1967).

Recently, Hayes has found that the hatching of T. muris eggs depends on microflora in the host gut and concident with remodulating of the host immune response (Hayes et al, 2010).

Once hatched in the host ceacum, larvae start penetrating the mucosal layer of the large intestine and move into the crypt gland, where they apply the anterior end to the epithelial cells and keep penetrating for a stable environment, otherwise they will get expelled.

The larvae mature through 4 moulds. The first occurring on days 9~11 post infection, the second at day 17 pi, the third on day 22 pi and the final one on day 32 pi. Once matured, adult worms (Figure 2) start mating with their anterior end applying into the mucosa and their posterior end released into the lumen of the large intestine, which enables male worms and female worms to mate with each

other. Once mated, female worms release eggs into the lumen, which will move along by peristalsis and released outside with the faeces.

The eggs take about 2 months to embryonate and once they are ingested, it follows the same life cycle.

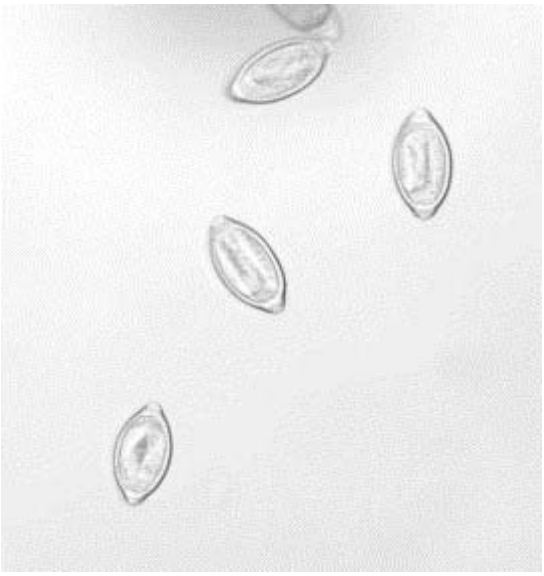

Fig. 1 Embryonated *T. muris* eggs

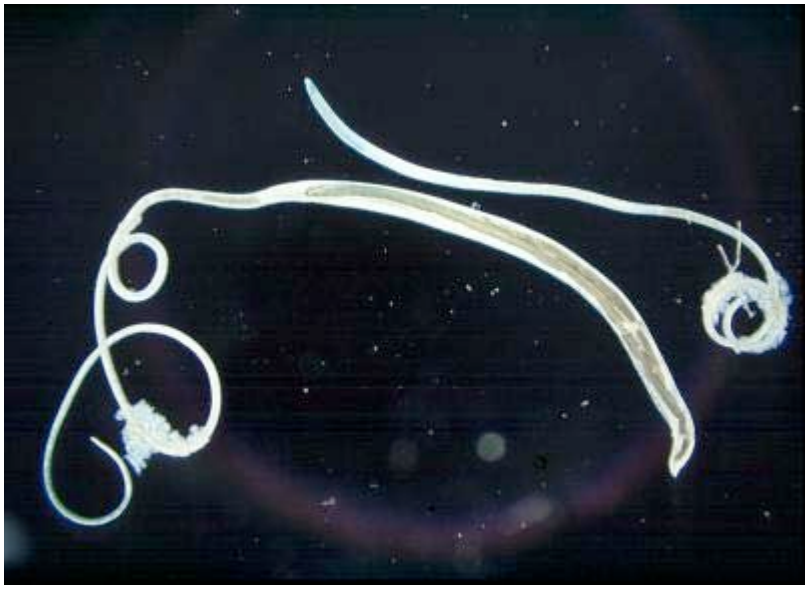

Fig. 2 Adult male and female worm

4. Life cycle of *Trichi spiralis*

Trichinella spiralis is a type of helminths that can infect a wide range of mammalian. It's lifecycle is very unique, First, it completes within a single host, and for second, it establish chronic infection in skeletal muscles which will cause severe damage on host.

The infection occurs when a skeletal muscle contaminated nurse cells, first stage larvae which form a cell like structure and reside in skeletal muscles, are consumed. After consumption, larvae in nurse cells will be released in stomach. After the release, young larvar will move into small intestine where they mature into adult worms and mate. After mating, female worms will release new born larvae into bloodstream ot lymphatics which enables them to enter most organs, but preferably in skeletal muscle tissue (Wang and Bell, 1986, Despommier et al., 1975). Once in muscle tissue, larvae generate a structure called nurse cell in which the larvae will be encapsulated and remains infectious for months to years (Froscher et al., 1988). Once contaminated skeletal muscle tissue is consumed, the life cycle follows.

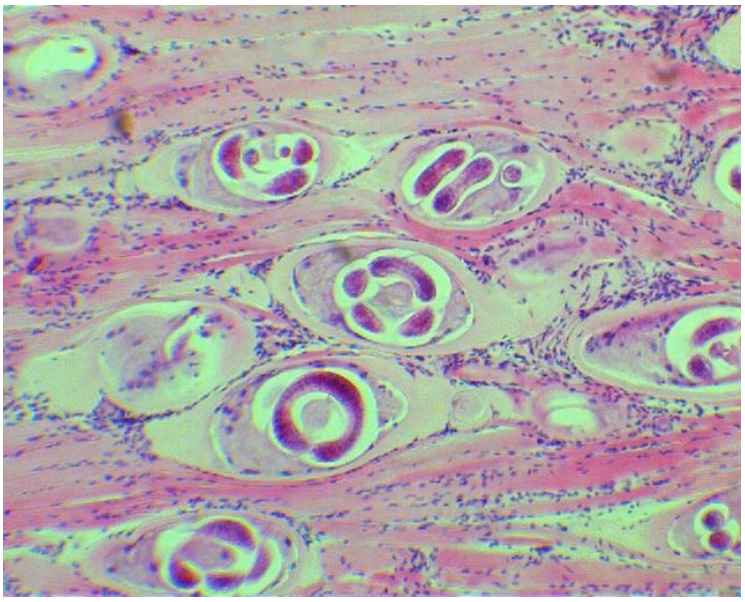

Fig 3.*T. Spiralis* nurse cells

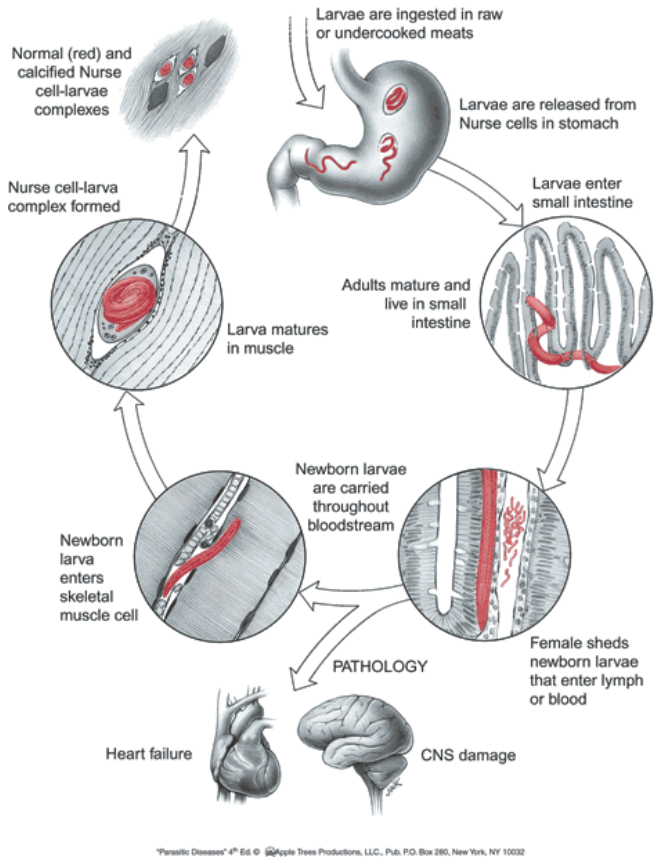

Fig 4.Life Cycle of *T. spiralis*

(Dr. Dickson Despommier, Environmental Health Science of Columbia University)

5. Immune response against Helminth infection

T helper 2 (Th2) immune response, as characterized by the expression of the type-2 cytokines IL-4, IL-5, IL-9 and IL-13 is essential for the control of parasitic helminth such as *T .muris*, *T. spiralis* and *Nippostrongylus brasiliensis* (Neill, 2011).The process of type-2 immune response against helminth infection is composed of mainly fourse parts. First is the recognition of helminth antigens and the activation of innate immmunity. Second, initiation and maintain of the type-2 immune response by innate effector cells. At last, the activation and regulation of worm expulsion by effector mechanism.

Recognition of helminths by innate innate effector cells

The initiation of adaptive type-2 immune response needs antigen presentation from innate effector cells to CD4+ T helper cells visa MHC class □. Unlike Th1 and Th17, in which pathogens are processed by phagocytosis, pathogens that are associated with Th2 are too large to be phagocytosed. Therefore, it is likely that antigens that are produced by pathogens such as T. muris and T. spiralis are phagocytosed by innate effector cells and presented to MHC class Ⅱ (Oliphant et al., 2011).

At first, helminthes antigens are recognized by pathogen recognition receptors (PRRs) such as TLRs, C-type lectin-like receptors (CLRs) and NOD like receptors (NLRs) which are expressed witin many types of innate cells such as dendritic cells (DCs) and epithelial cells (Moussion, 2008). Once activated by PPRs, DCs present antigens to T cells via MHC class Ⅱ which will cause T cells to differentiate into Th2 cells.

Initiation of the type-2 immune response by innate effector cells

Not only DCs but also other innate immune cells play important role in the initiation of type-2 immune response. Mast cells (Bischoff, 2009), basophiols (Ohnmacht, 2010), eosinophils (Spencer, 2010), macrophages (Gordon, 2010), NKT cells (Umetsu, 2010) and epithelial cells (Saenz, 2008) also play important role in the regulation of Th2 differentiation, eosinophilia, mastocytosis, hyperplasia of intestinal goblet cells and smooth muscle contraction by the production of type-2 cytokines such as IL-4 and IL-13(Finkelman et al., 2004, Klion, 2004, Moro, 2010).

Effector mechanisms

Muscle Hypercontractility

Khan et al. has found that there is an increase in muscle hypercontractility during T. muris infection which is caused by IL-9 function. It is also suggested that the function of IL-9 is triggered by the production of IL-4 and IL-13 (Khan et al., 2003).Furthermore, the blocking of IL-9 during T. muris infection caused both

susceptibility and decreased muscle hypercontractility. In addition, it has been found that not only IL-9 but also both IL-4 and IL-13 are able to cause an increase in muscle hypercontractility (Akiho et al., 2002).
Based on these evidences, it is reasonable so conclude that muscle hypercontractility is an effective mechanism for helminth expulsion by creating a more rapid transit through the intestine, which will make an unstable environment for helminth to reside.

Goblet cells

Goblet cells are one of the the most important effector mechanisms for worm expulsion. Goblet cells secret sticky mucus into the intestine which not only protect epithelium from infectious attack but also weaken worm vitality and make it difficult for worms to penetrate epithelium for its desired niche (Artis et al., 2002, Hasnain et al., 2011). In addition to mucus secretion, goblet cell hyperplasia can be also observed during *T. muris* infection (Artis et al., 2002). Again, it has reported that IL-13 and IL-9 in particular are the main players that control goblet cell differentiation and mucus production (Kondo et al., 2002, Whittaker et al., 2002, Taube et al., 2002).

Epithelial cells

Epithelial cells are considered to be the most important effector cells in worm expulsion because of its close interaction with parasites. It has been shown that the percentage of cells undergoing proliferation increases after infection and that crypt cell hyperplasia in controlled cytokines such as IFN-γ (Artis et al., 1999). One unique characteristic of the intestinal epithelium is its dynamic continual renewal. Stem cells continuously produce daughter cells, which will proliferate and differentiate and then move up to the crypt axis until they fall off from intestine wall and released into faeces. This process suggests that gut dwelling parasites such as *T. muris* need to continually penetrate the epithelium to keep its position in desired niche (Cliffe and Grencis, 2004). Therefore, it is reasonable to suggest that this escalator like epithelial cell turnover is a strong and effective

mechanism for worm expulsion with the help of mascle hypercontractility and mucus secreted from goblet cells.

6. Immunofluorescent staining on IL13eGFP mouse large and small intestine after T. muris and T. spiralis infection

In both small intestine and large intestine sections, there were hardly any CD4+ /Il13+ cells except for mesenteric lymph nodes (mLN) and some lymphoid tissue in large intestine. Through which, it can be suggested that CD4+ T helper cells are not the main source of IL-13.

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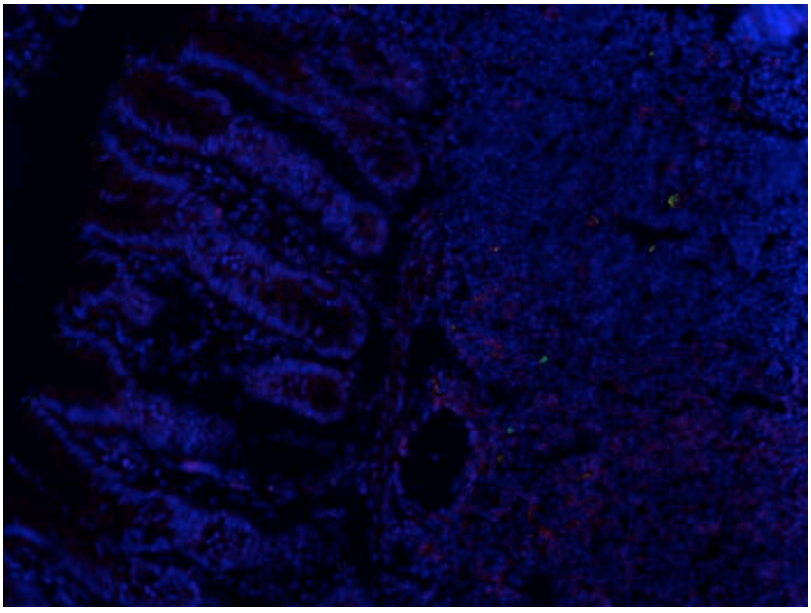

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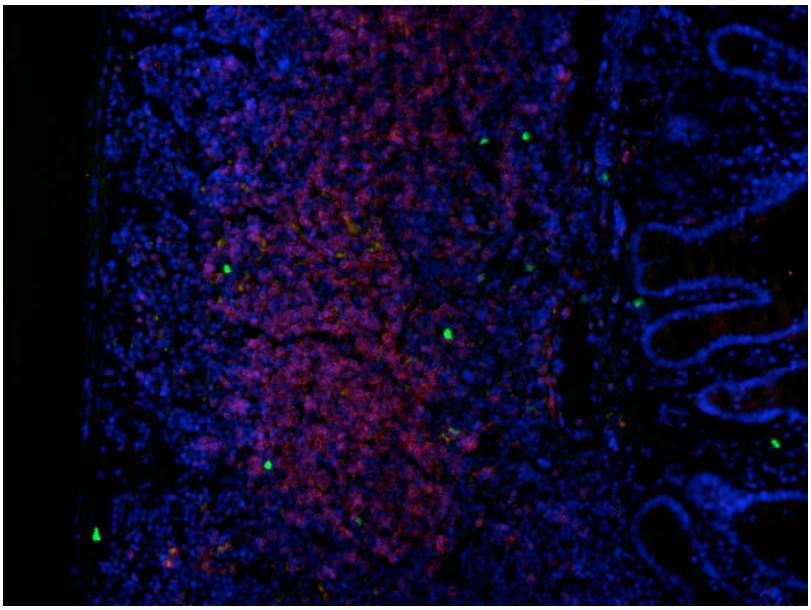

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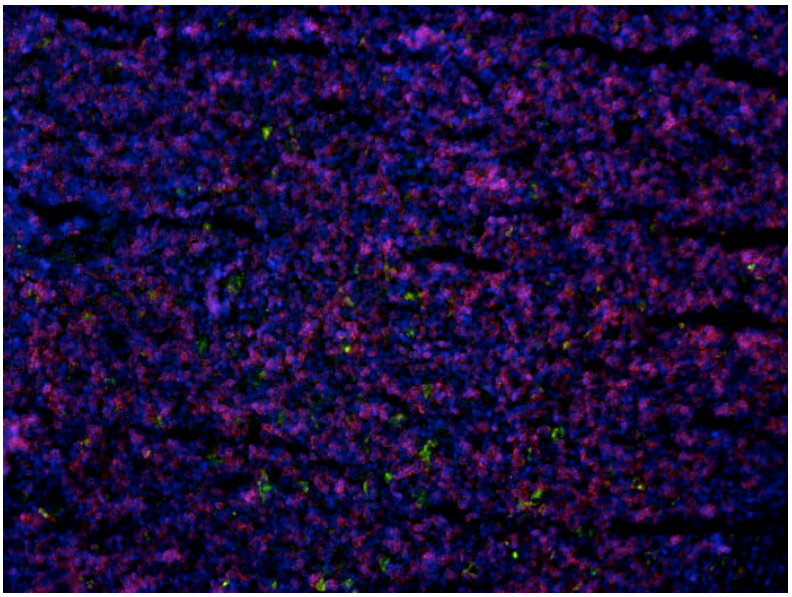
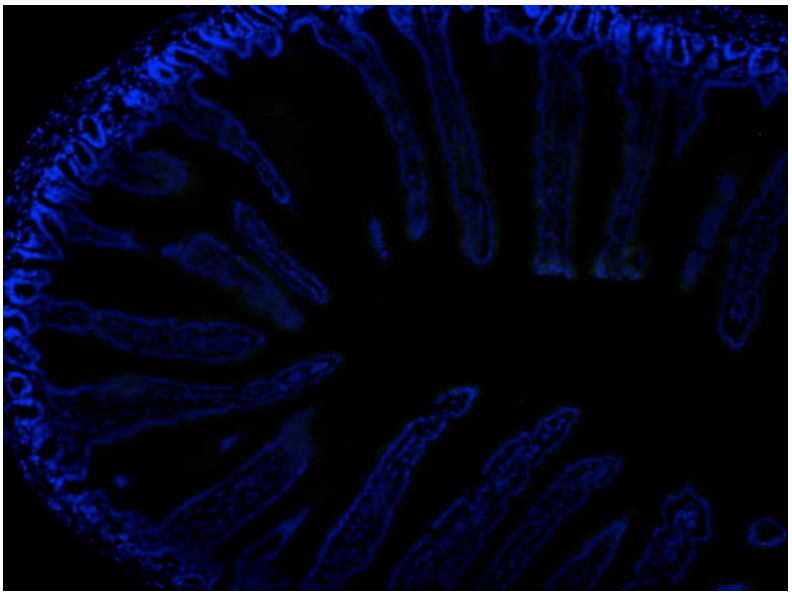


Fig 5.*T. muris* infection day 14 pi (A: naïve mouse, B: infected mouse, C: mLN of infected mouse)

A.



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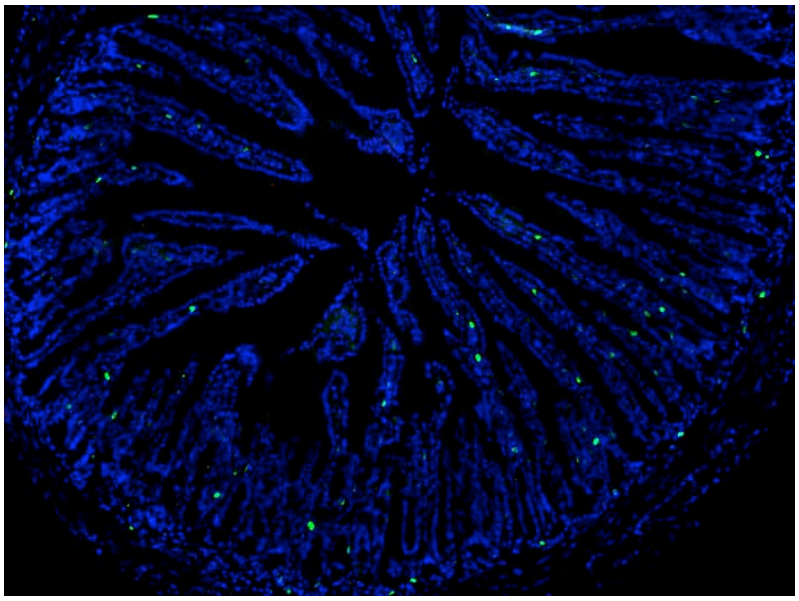

Fig 6.*T. spiralis* infection day 7 pi (A: naïve mouse, B: infected mouse)

7. Conclusion

It has been shown that there are many players that initiate and maintain Th2 immune response and there are multiple ways of expelling gut dwelling nematodes such as skeletal muscle hypercontractility, secretion of mucus by goblet cells and epithelial cells turnover. Since it has been studied that CD4+ T helper cells are not the main source of IL-13 in caecum during *T. muris* and *T. spiralis* infection except for mLN and lymphoid tissue in large intestine, however the main source has yet to be identified.

There are some possible cell types that are expected to be associated with IL-13 production such as nuocytes and other innate immune cells which have been recently found (Neill, 2010).

For the further study in this project, samples from different infection stages are crucial. In addition, in order to analyze multiple cell types in large and small intestine during nematodes infection, there is a technical need for flow cytometry and a statistical method for analyzing the number of Il13eGFP cells during nematodes infection since it is of a strong interest.

It is hoped that this study could contribute to the identification of those cells in humans and accordingly, to a new possible way of controlling nematodes infection.

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