• pISSN: 2085-1545
• eISSN: 2339-093X

Sains Medika Journal of Medicine and Health

http://jurnal.unissula.ac.id/index.php/sainsmedika

RESEARCH ARTICLE

No Structural Change in Mice Intestinal Gut-Associated Lymphoid Tissue After *Trichuris muris* Egg Infestation

Ahmad Husairi[1], Istiana[2], Oski Illiandri*[1]

[1]Department of Biomedicine, School of Medicine, University of Lambung Mangkurat Banjarmasin, South Kalimantan, Indonesia

[2]Department of Parasitology School of Medicine, University of Lambung Mangkurat Banjarmasin, South Kalimantan, Indonesia

*corresponding author, email: oilliandri@ulm.ac.ac.id

ABSTRACT

Introduction: It has been known that the infestation of *Trichuris muris* egg in the intestinal mice may induce a host immune response to eliminate the worms. Worms antigen will stimulate host immune response in the intestinal secondary lymphoid organs. One of the secondary lymphoid organs in the intestinum is GALT (gut-associated lymphoid tissue). Worm antigen activate naive T cells and naive B cells in GALT and trigger differentiation of T cells into Th2 and stimulate B cell to produce gut IgE. However a structural change of GALT structure is still poorly understood. **Objective:** This study aims to find influence of embrionic *Trichuris muris* egg infestation to the structural changes of mice intestinum GALT.
Methods: This research uses true experimental method with post-test only with control group design. Mice are grouped into 3 groups. The first group was given 40 *Trichuris muris* embryonic egg peroral, second group was given 200 eggs, and third was control group. On the 30th day after treatment, the mice were sacrificed and intestinal GALT structural change has been analyzed ($p<0.05$).
Results: There was no effect of *Trichuris muris* embryonic egg infestation on the structure of mice intestinum GALT. However, there is a unique finding of GALT in the form of a Peyer's patch in the basal plica transversalis colon proximalis of 1 mice given egg T. peroral murmus, at low doses or high doses.
Conclusion: There was no effect of *Trichuris muris* embryonic egg infestation on the structure of mice intestinum GALT

Keywords: *GALT, Peyer's patch, Trichuris muris, sel Th2, sel B*

• pISSN: 2085-1545
• eISSN: 2339-093X

Sains Medika Journal of Medicine and Health

http://jurnal.unissula.ac.id/index.php/sainsmedika

ABSTRAK

Latar Belakang: Telah diketahui bahwa infestasi telur *Trichuris muris* pada usus mencit dapat menginduksi respon imun inang untuk mengeliminasi cacing. Antigen cacing akan merangsang respon imun pejamu pada organ limfoid sekunder usus. Salah satu organ limfoid sekunder di usus adalah GALT (jaringan limfoid terkait usus). Antigen cacing mengaktifkan sel T naif dan sel B naif di GALT dan memicu diferensiasi sel T menjadi Th2 dan merangsang sel B untuk menghasilkan IgE usus. Namun mekanisme yang menyebabkan perubahan struktur GALT masih kurang dipahami. **Tujuan:** Penelitian ini bertujuan untuk menganalisis pengaruh infestasi telur berembrio *Trichuris muris* terhadap perubahan struktur GALT usus mencit.
Metode: Penelitian ini menggunakan metode eksperimen sejati dengan rancangan *post-test only with control group design*. Tikus dikelompokkan menjadi 3 kelompok. Kelompok pertama diberi 40 butir telur berembrio *Trichuris muris* per oral, kelompok kedua diberi 200 butir telur, dan kelompok ketiga adalah kelompok kontrol. Pada hari ke-30 setelah perlakuan, mencit dikorbankan dan dianalisis perubahan struktur GALT usus ($p<0,05$).
Hasil: Tidak terdapat pengaruh infestasi telur berembrio *Trichuris muris* terhadap struktur GALT usus mencit. Namun terdapat temuan unik GALT berupa peyer's patch pada basal plica transversalis colon proximalis 1 mencit yang diberi telur *Trichuris muris* peroral baik pada dosis rendah maupun dosis tinggi.
Kesimpulan: Tidak terdapat pengaruh akibat infestasi telur Trichuris muris terhadap struktur GALT usus mencit.

INTRODUCTION

Gastrointestinal tract has two tremendous properties; first, the combination of the small intestine and colon mucosa has a total surface area of more than 200 $m^{2.}$ Second, the lumen intestinum is filled with microbes. It is estimated there are more than 500-1000 species of bacteria living in mammalian intestinum (Gilbert et al., 2018). In addition to commensal organisms, there are pathogenic organisms that live in the intestinum, namely bacteria, viruses, protozoa, and worm parasites. The presence of organisms in the lumen intestinum can produce an immune response, both innate immune responses and adaptive immune responses (Zheng *et al.*, 2020)

An adaptive immune response in the intestinum begins in the secondary lymphoid tissue in a collection of organized lymphocytes and in lymphatici mesenterica nodi. Lymphoid tissue located near the epithelium of the intestinal mucosa is referred to as GALT (gut-associated lymphoid tissue). It has been known that GALT is part of immune system that is immunoreactive and distributed throughout the intestinal tract (Kong et al., 2018). The most prominent GALT structure is the Peyer's patch (Peyer's patches). The Peyer's patch structure is similar to the lymphatici nodule structure, but overall, the ratio of B cells to T cells in GALT is five times greater than that in lymphatici nodi (Senda et al., 2019).

• pISSN: 2085-1545
• eISSN: 2339-093X
Sains Medika Journal of Medicine and Health
http://jurnal.unissula.ac.id/index.php/sainsmedika

The microbial antigen in the intestinal lumen can be sampled by lamina propria dendritic cells that extend the cytoplasmic processus between epithelial intestinal cells. This cell extends dendrites through the junction between adjacent epithelial cells without damaging the tight junction. Dendritic cells that accompany these antigens can enhance protective adaptive immune responses to pathogens within the lumen. These dendritic cells are capable of processing and presenting antigens to T cells in GALT (Stagg, 2018).

Since GALT is involved in the initiation of an adaptive immune response, these secondary lymphoid tissues are not static but varying in structure, depending on the presence or absence of infection. Diffuse lymphoid mucosa tissue can appear in response to infection and then disappears, while the organized network architecture changes in a firmer way during infection (Linden et al., 2012)

One of the organisms that often infects the intestinum is a worm. Trichuris sp is one of the most widely spread metazoa parasites in the human population, notably Trichuris trichiura. This worm is estimated to infect 1.049 million people, especially in the tropics and subtropics. This worm can cause anemia especially in children because it is inherent in the intestinal mucosa and causes bleeding (Viswanath et al., 2021) .

One of the most frequently observed species of Trichuris worm in experimental animals is Trichuris muris (*Trichuris muris*). The nature of this worm infection varies among strains (strains) of mice. Most strains of mice are resistant to infection by removing parasites before the adult worm develops. However, a small number of mice strains are unable to improve protective immune responses and have chronic infections. A worm-resistant mouse strain shows predominant Th2 cells, whereas a mice strain that is unable to excrete parasites shows predominant Th1 cells (Houlden *et al.*, 2015). Interleukin-4 (IL-4) and interleukin-13 (IL-13) secreted by Th2 cells protect mice against live worms in the intestinal lumen. Interleukin-4 can induce class transfer in B cells into plasma cells that produce IgE (Bao and Reinhardt, 2015).

Recent research has shown that worms can provide adjuvant function that controls Th2 differentiation in vivo. The adjuvant structure of the worms that controls Th2 differentiation is still unclear. However, the data confirm that glycan, lipids, lipoproteins, and proteases expressed by certain worms may function as pathogen-associated molecular pattern (PAMP) to control Th2 cell differentiation. In a recent important study, chitin has been identified as a potential Th2-cell-stimulating PAMP (Elieh Ali Komi et al., 2018)

• pISSN: 2085-1545
• eISSN: 2339-093X
Sains Medika Journal of Medicine and Health
http://jurnal.unissula.ac.id/index.php/sainsmedika

Naïve lymphocytes (T naïve lymphocytes and B naïve lymphocytes) present in secondary lymphoid tissue such as GALT may be exposed to antigen. The exposed naïve lymphocytes will proliferate and differentiate into effector cells and memory cells (Kato et al., 2014).

Based on the previous background, the infestation of *Trichuris muris* in the intestinum may potentially alter GALT structure via immune response initiation and proliferation differentiation of T cells and B cells. However, in our previous knowledge no research has been done to dig the effect of *Trichuris muris* egg infestations on the GALT structure. It is important to analyze the influence of *Trichuris muris* egg infestation on GALT intestinum mouse's structure.

METHODS

This research uses true experimental method with post-test design with control group (post-test only control group design). The subjects were BALB/c males aged 2 months with weight between 20-30 gram. The research materials used in this study were embryonated egg *Trichuris muris*, pellet food, 70% ethanol solution, standard solution of eosin hematoxylin staining for histology preparations, ether solution, and 10% neutral buffered formalin solution. The structure of GALT taken in this study is the Peyer's patch structure and solitary lymphoid intestinal follicles. Plaque Peyeri is a lymphoid tissue aggregate in the lamina propria intestinum with a total number of follicles of at least 2 fruits. Solitary lymphoid follicles are a single lymphoid tissue in the lamina propria intestinum. The dosage of embryonated eggs *T. muris* peroral is the number of embryonated egg of *T. muris* given through the mouth of mice. This dose is divided into two, i.e low dose at 40 eggs and high dose at 200 eggs.

The research procedure performed is as follows. Ethical clearance issued by research ethics committee at Ulin Banjarmasin Hospital letter no. 080/KEPK-FK UNLAM/EC/IX/2015. Mice adapted at Veterinary Care Center Banjarbaru for 1 week. Furthermore, 30 mice were grouped randomly into 3 groups, i.e control group (X0), low dose treatment group (X1), and high dose treatment group(X2). Low dose group was given 40 *T. muris* embrionic eggs orally. Group X2 was given 200 Trichuris muris embryonic eggs perorally. No treatment has been given to control group X0. Base on Holm methods, on the 30 days after treatment, the mice were anesthetized with ether inhalation solution. Furthermore, mice were sacrificed by cervical dislocation. The ventral surface of the abdominal mouse is antisepted with a 70% ethanol solution. Abdominal skin is held with tweezers and made a small incision with scissors. The contents of the abdomen then opened and intestinum tenue, cecum, and colon were identified.

• pISSN: 2085-1545
• eISSN: 2339-093X
http://jurnal.unissula.ac.id/index.php/sainsmedika

Cecum is held with tweezers and pulled out of the abdominis cavity. Ileum is cut with scissors at 5 cm orally from the ileocecal junction. Colon is cut with scissors at 5 cm of an ileocecal junction. These small intestine and colon pieces are dissected and released from their peritoneal attachment. Ileum is cut at 2 cm (for transversal cut preparation) and 3 cm (for the preparation of longitudinal pieces) orally (proximal) from the ileocecal junction. Colon is cut at 2 cm (for transversal cut preparation) and 3 cm (for anal bititudinal preparation) distal from ileocecal junction(Holm et al., 2015, Antignano et al., 2011)

The ileum and colon pieces are included in a 10% neutral buffer buffer solution, as modified from Antignano (Antignano et al., 2011). This intestinal piece is then sent to the Histopathology Laboratory of Veterinary Hall of Banjarbaru for making transverse and longitudinal histologic preparations. After the preparation of histologic preparations using Haematoxillin Eosin staining, the structure of GALT (Peyer's patchand solitary lymphoid follicles) of transverse and longitudinal stripe histological preparations was identified in each sample by light microscopy. Each GALT was define as composite of 3 follicle in each intestine segment and count mean in each group then compared between groups and analyzed using ANOVA statistic test and difference will be test using Tukey ad hoc test. Mean difference significancy was $p<0.01$

RESULTS

From what has been obtained from the results of this study, it shows that the amount of GALT obtained in mice that get infestation is not significantly different between groups. In group X0, both the ileal side and the colon side, the mean obtained was not significantly different from the X1 and X2 groups (p=0.02).

Table 1. GALT count in mice intestinum after 30 days *Trichuris muris egg infestation* orally, X0: control group, X1: treatment group 40 egg *Trichuris muris* orally, X2: treatment group 200 egg *Trichuris muris* orally

Group	GALT counting			
	Ileal		Colon	
	longitudinal section	transversal section	longitudinal section	transversal section
X0	1	1	1	2
X1	2	1	2	2
X2	1	1	1	2

• pISSN: 2085-1545
• eISSN: 2339-093X
Sains Medika Journal of Medicine and Health
http://jurnal.unissula.ac.id/index.php/sainsmedika

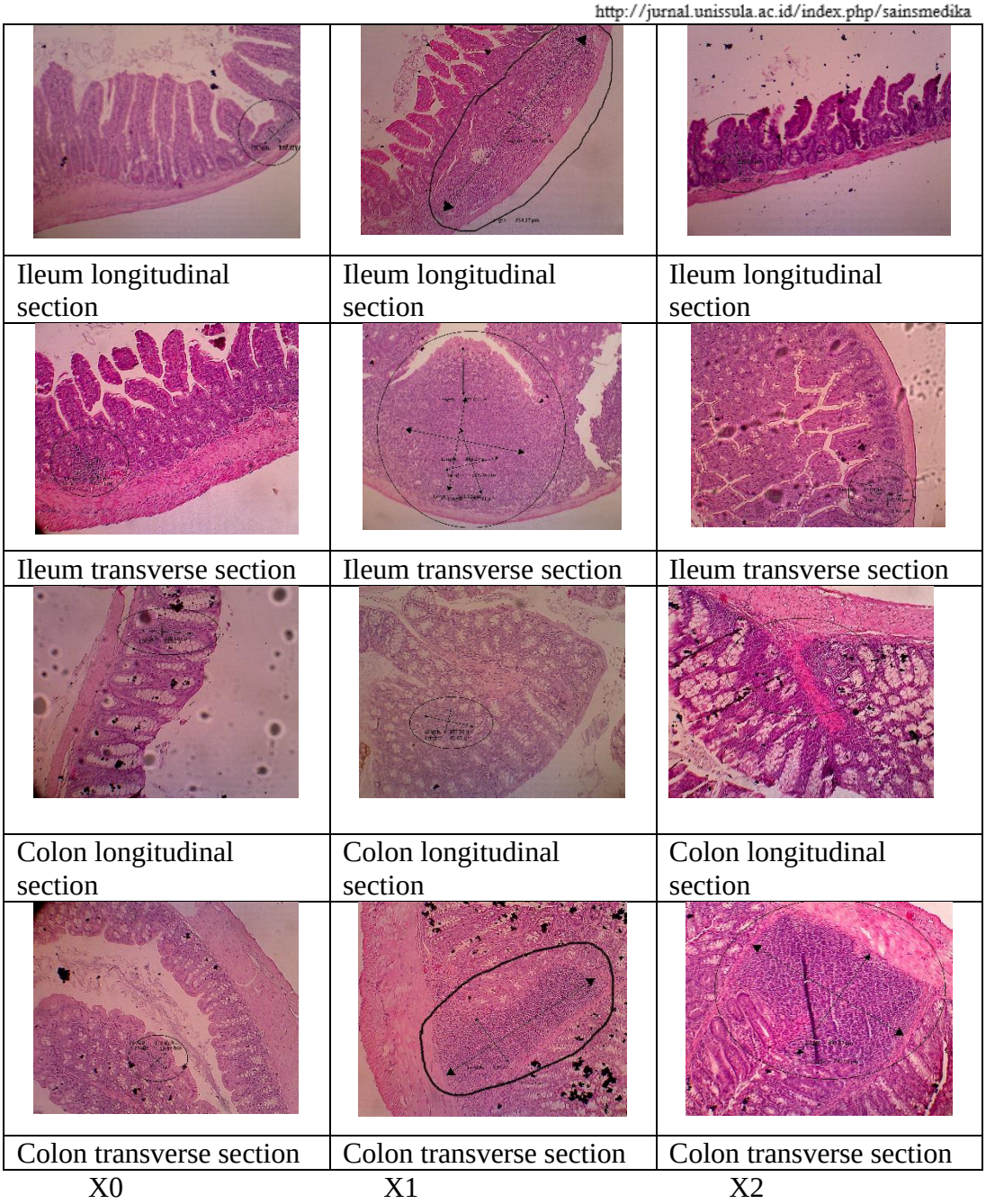

Figure 1. Mice intestinal GALT structureI 30 days after *Trichuris muris egg infestation.* X0: control group, X1: treatment group 40 egg *Trichuris muris* orally, X2: treatment group 200 egg *Trichuris muris* orally

DISCUSSIONS

This study showed that there was no significant effect of infestation of *Trichuris muris* egg in the structure of GALT intestinum mice on day 30 because the adult worm of *Trichuris muris* in the lumen intestinum can't pass through transitosis of M-cell. The anterior part of adult *Trichuris muris* are several mm in diameter, whereas M-cells are only several microns

• pISSN: 2085-1545
• eISSN: 2339-093X
http://jurnal.unissula.ac.id/index.php/sainsmedika

(Mestecky et al., 2015, Klementowicz et al., 2012) The size of the anterior part of the adult T. murine is much greater than that of M-cells. Thus, there were no proliferation and differentiation of T cells and B cells in GALT so its structure does not change. However, an interesting finding was found in this study. We found a unique GALT in proximal colon of mice infected with infective eggs of the oral murine, at low and high doses. GALT appears to be organized in the basal section of the transcatis colon proximalis plica. According to the literature, GALT in mice is usually located in the submucosa antimesenterica. Colon contains only isolated (solitary) lymphoid follicles called cryptopatches (Mörbe et al., 2021). In this study, GALT was found in control mice in the form of cryptopatches and located in the middle of transversalis plica, whereas GALT in mice fed with low-dose, high-dose T. embryoned eggs was lower location in the form of Peyer's patch (PP) and located in the basal plica transversalis. However, these new findings can't be generalized because they are only found in 1 of 8-10 mice. Further research still needs to be done to confirm this. Limitations of this study were intestinal samples taken only from a small portion of ileum and a small fraction of colon. This small intestinal sample may be a cause of GALT not found in most of the study subjects. In addition, sampling and cutting of histologic preparations is not guided first with macroscopic examination of the entire intestinum. Plaque Peyeri is usually located in the submucosa antimesenterica and can actually be seen macroscopically from the serous surface (Mörbe et al., 2021) .

Gut Assosiated LT is a MALT (Mucosa-associated lymphoid tissue) located near the epithelium of the intestinal mucosa. It is abundant in mice and is usually located in the submucosa antimesenterica. In contrast to other secondary lymphoid tissue such as lymphatici nodi in the groin and neck fold, GALT has a common characteristic in the absence of a fibrous tissue capsule firmly around the lymphoid tissues or afferent lymphatic vessels. Thus, the peripheral aspect of the follicle of GALT is dispersed into the surrounding lamina propria. GALT in humans consists of Peyeri's patches (Peyer's patches / PP), small intestinal isolated lymphoid follicles, appendix, and lymphoid colon follicles (Mörbe et al., 2021). PP can be found in the jejunum, ileum, and cecum. Smaller lymphocyte aggregates called cryptopatches or isolated lymphoid follicles can be found along the length of the intestinum (Scudamore, 2014).

Structurally, PP is an area of lymphoid tissue that is organized in the mucosa intestinum tenue. Peyer patch coated by a special lymphoepithelium (follicle-associated epithelium, FAE) without crypta or villi. In this FAE there is a special cell called M-cell . Under the epithelium,

• pISSN: 2085-1545
• eISSN: 2339-093X
Sains Medika Journal of Medicine and Health
http://jurnal.unissula.ac.id/index.php/sainsmedika

there is a dome region containing dendritic cells, T cells, B cells, and macrophages. PP has a lymphoid follicle structure, with centrum germinale containing B lymphocytes, follicular Th (T helper) cells, follicular dendritic cells, and macrophages. B cells become denser in the mantle zone. There is a large germinale centrum on PP. Centrum germinale is surrounded by a follicular naïve B cell that expresses Ig M and Ig D. The parafolicular area is present between the follicles. T cells are present among the follicles that surround the high endothelial venule.

Peyer's patch tends to be more prominent in young mice (Scudamore, 2014). Mice have 8-10 PP, while humans have hundreds of PPs. According to Cornes 1965 there are about 100-300 PP throughout the adult intestinum tenue (late adolescene), and decreases with age (Cornes, 1965). However, Cornes only counts PP with more than 5 follicles. The research of Van Kruiningen (2002) in Meier et al (2014) calculates the PP in the 2 m end of the human intestinum tenue. In this study, PP was defined as having at least 3 follicles. The result is only about 30 PPs identified

Colon also contains thousands of isolated (solitary) lymphoid follicles with the highest density in the rectum. Normal human jejunum also contains a structure called lymphocyte-filled villi. In histology, this structure appears as a short squat villi with T cells and a number of B cells. Mice have thousands of cryptopatches, while humans do not have cryptopatches (Nochi et al., 2013)

The structure of GALT is largely determined by the role of M-cells. M cells are found scattered over the dome of GALT. M-cells are surrounded by absorptive cells. The main way of transporting antigens from the intestinal lumen to GALT is the M-cells. M cells play an important role in antigen sampling and transport it to lymphocytes and macrophages. Macromolecular transitosis through M cells carries the antigen from the mucosa to the lymphoid follicles beneath it. Glycoprotein 2 (GP2), which is specifically expressed on the apical plasma membrane of M cells, acts as a transitotic receptor for mucosa antigen (Kimura, 2018). M-cells can capture various groups of infectious agents. Viruses that can be transported via M-cells include retrovirus, poliovirus, HIV type 1, and mouse mammary tumor virus (MMTV). M-cells can also capture a variety of bacterial species, including Vibrio cholera, Salmonella typhimurium, Mycobacterium paratuberculosis, Brucella abortus, Campylobacter jejuni, Yersininia enterocolitica, Shigella flexneri, Escherichia coli strains RDEC-1, and Lysteria monocytogenes. Some parasites can also enter M cells, such as Cryptosporidium parvum, Eimeria coecicola, and Giardia muris (Mestecky et al., 2015).

Microbial antigens that have passed through M-cells are processed and presented by dendritic cells to lymphocytes. T-lymphocytes and B lymphocytes proliferate and differentiate into effector cells and memory cells. These effector cells and memory cells potentially alter the structure of GALT (Stahl and Belkind-Gerson, 2021).

CONCLUSIONS

There was no significant effect of embryonic egg infestation on the GALT structure of intestinal mice. However, there is a unique finding of GALT in the form of a Peyer's patch in the basal plica transversalis proximal colon of a mice given embrionic egg Trichuris muris perorally, either at low doses or high doses.

ACKNOWLEDGMENT

We would like to thanks to Mr.Frans Antignano from The Biomedical Research Center, University of British Columbia who has provided embryonic eggs of *Trichuris muris.*

REFERENCES

Antignano, F., Mullaly, S. C., Burrows, K. & Zaph, C. 2011. *Trichuris muris* infection: a model of type 2 immunity and inflammation in the gut. *J Vis Exp*.

Bao, K. & Reinhardt, R. L. 2015. The differential expression of IL-4 and IL-13 and its impact on type-2 immunity. *Cytokine,* 75**,** 25-37.

Cornes, J. S. 1965. Number, size, and distribution of Peyer's patches in the human small intestine: Part I The development of Peyer's patches. *Gut,* 6**,** 225-9.

Elieh Ali Komi, D., Sharma, L. & Dela Cruz, C. S. 2018. Chitin and Its Effects on Inflammatory and Immune Responses. *Clin Rev Allergy Immunol,* 54**,** 213-223.

Gilbert, J. A., Blaser, M. J., Caporaso, J. G., Jansson, J. K., Lynch, S. V. & Knight, R. 2018. Current understanding of the human microbiome. *Nature Medicine,* 24**,** 392-400.

Granger, D. N., Grisham, M. B. & Kevil, C. G. 2006. CHAPTER 46 - Recruitment of Inflammatory and Immune Cells in the Gut: Physiology and Pathophysiology. *In:* JOHNSON, L. R. (ed.) *Physiology of the Gastrointestinal Tract (Fourth Edition).* Burlington: Academic Press.

Holm, J. B., Sorobetea, D., Kiilerich, P., Ramayo-Caldas, Y., Estellé, J., Ma, T., Madsen, L., Kristiansen, K. & Svensson-Frej, M. 2015. Chronic Trichuris muris Infection Decreases Diversity of the Intestinal Microbiota and Concomitantly Increases the Abundance of Lactobacilli. *PLOS ONE,* 10**,** e0125495.

Houlden, A., Hayes, K. S., Bancroft, A. J., Worthington, J. J., Wang, P., Grencis, R. K. & Roberts, I. S. 2015. Chronic *Trichuris muris* Infection in C57BL/6 Mice Causes Significant Changes in Host Microbiota and Metabolome: Effects Reversed by Pathogen Clearance. *PLOS ONE,* 10**,** e0125945.

Kato, LM., Kawamoto, S., Maruya, M., Fagarasan S. 2014. The role of the adaptive immune system in regulation of gut microbiota. *Immunol Rev*. 260(1):67-75. doi: 10.1111/imr.12185. PMID: 24942682.

Kimura, S. 2018. Molecular insights into the mechanisms of M-cell differentiation and transcytosis in the mucosa-associated lymphoid tissues. *Anat Sci Int,* 93**,** 23-34.

Klementowicz, J. E., Travis, M. A. & Grencis, R. K. 2012. Trichuris muris: a model of gastrointestinal parasite infection. *Semin Immunopathol,* 34**,** 815-28.

Kong, S., Zhang, Y. H. & Zhang, W. 2018. Regulation of Intestinal Epithelial Cells Properties and Functions by Amino Acids. *BioMed research international,* 2018**,** 2819154-2819154.

Linden, M., Ward, J. M. & Cherian, S. 2012. 19 - Hematopoietic and Lymphoid Tissues. *In:* TREUTING, P. M. & DINTZIS, S. M. (eds.) *Comparative Anatomy and Histology.* San Diego: Academic Press.

Meier, DG., Docena, H., Ramisch, D., Toscanini, U., Berardi, G., Gondolesi, GE., M. Rumbo, M. 2014. Immunological Status of Isolated Lymphoid Follicles After Intestinal Transplantation, *American Journal of Transplantation*, 10.1111/ajt.12797, **14**, 9, (2148-2158), (2014).

Mestecky, J., Strober, W., Russel, M., BL, B. K., Cheroutre, H. & Volume, B. L. 2015. *Mucosal Immunology* USA, Elsevier

Mörbe, U. M., Jørgensen, P. B., Fenton, T. M., von Burg, N., Riis, L. B., Spencer, J. & Agace, W. W. 2021. Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal Immunology*.

Nochi, T., Denton, P. W., Wahl, A. & Garcia, J. V. 2013. Cryptopatches are essential for the development of human GALT. *Cell Rep,* 3**,** 1874-84.

Senda, T., Dogra, P., Granot, T., Furuhashi, K., Snyder, M. E., Carpenter, D. J., Szabo, P. A., Thapa, P., Miron, M. & Farber, D. L. 2019. Microanatomical dissection of human intestinal T-cell immunity reveals site-specific changes in gut-associated lymphoid tissues over life. *Mucosal Immunology,* 12**,** 378-389.

Stagg, A. J. 2018. Intestinal Dendritic Cells in Health and Gut Inflammation. *Frontiers in Immunology,* 9.

Stahl, M. G. & Belkind-Gerson, J. 2021. 1 - Development of the Gastrointestinal Tract. *In:* WYLLIE, R., HYAMS, J. S. & KAY, M. (eds.) *Pediatric Gastrointestinal and Liver Disease (Sixth Edition).* Philadelphia: Elsevier.

Viswanath, A., Yarrarapu, S. N. S. & Williams, M. 2021. Trichuris Trichiura. *StatPearls.* Treasure Island (FL): StatPearls Publishing.

Zheng, D., Liwinski, T. & Elinav, E. 2020. Interaction between microbiota and immunity in health and disease. *Cell Research,* 30**,** 492-506.