Shared Expression of Mucin12 ~~contribute~~in both ~~common antigenicity of host-parasite relationship between~~ *Ascaris Lumbricoides* and Human Small Intestine

Authors

Affiliations

*Corresponding author:

Conflict of interest:

Comment [SC ED1]: The scientific names of species should be italicised.

Comment [SC ED2]: The title of the paper should be concise and yet present the main research focus of the paper. We have therefore, revised it to make it impressive and informative for the readers.

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Comment [SE3]: Please mention the names of the authors who were involved in conducting this research.
Please follow the below format for the names:
First Author[1], Second Author[2]*, Third Author[3]

If available, please provide the e-mail address of each author.

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Comment [SC ED4]: Please provide full postal address of all the affiliations of the authors you have mentioned above.
An example of the format is given below:
[1]First Department, First University, Address, City, Country Name

Comment [SE5]: Please provide the details of corresponding author from among the authors that you have listed above. The corresponding author will be the one who will communicate with the journal after submission, on behalf of remaining authors in the paper.

Comment [SE6]: Please provide a declaration of any conflict of interest. If there are no conflict of interest to declare, the authors can mention "There are no conflict of interests to declare."

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~~Summary~~Abstract

~~*Ascaris lumbricoides* is one of the most common parasites in the world.~~ ~~The purpose of t~~this ~~research~~ study is to focuses on the host specificity of human Ascaris~~.~~ lumbricoides, which is a ~~parasitic~~ parasite of~~in~~ the small intestine and is also one of the commonest parasites worldwide. As part of this investigation, ~~we examined,~~ at ~~a~~ the genetic level, we examined the common antigenicity existing in *A. lumbricoides* and human small intestinal mucosa to unravel ~~the~~ this host–parasite relationship. We obtained three DNA clones ~~after~~ by screening ~~analysis~~ for common antigenicity ~~of~~ using a human colon cDNA library ~~on common antigenicity using~~and anti-*A. lumbricoides* polyclonal antibodies~~y~~. After sequencing analysis, ~~we identified one of them is~~ the transmembrane mucin12 gene was identified as a gene of interest. S~~The s~~pecific signals of immuno~~e~~-staining with polyclonal anti-mucin12 antibodies~~y~~ were observed in the mucous secretory organs, epidermis, and intestinal canal of *A. lumbricoides*. These signals ~~were~~ disappeared when immunohistochemistry was performed using pre-absorbed polyclonal antibodies~~y~~ with a specific peptide. These results suggest~~ed~~ that ~~mucin12~~mucin12 ~~was~~ is localized in the mucous secretory organs ~~to~~ in the epidermis of *A. lumbricoides*. Furthermore, we examined the site of ~~mucin12~~mucin12 localization ~~on~~ in the host ~~side,~~; ~~the~~ specific mucin12 signals ~~of mucin12~~ were observed on the muco~~us~~al epithelia~~l~~ present around intestinal crypts and villi of the small intestine. Therefore, ~~it is~~we suggest~~ed~~ that ~~mucin12~~mucin12 is ~~one of the~~a protein~~s~~ that show ~~the~~s common antigenicity in both ~~parasites, *A. lumbricoides*~~ and its host. It is presumed that adult *A. lumbricoides* live in ~~its~~ their ~~ideal~~ preferred environment, which is the small intestine, by secreting ~~mucin12~~mucin12 ~~, which is the common antigenicity in the small intestine,~~ to avoid being attacked by the host immune system.

Keyw~~W~~ords:
Human *Ascaris Lumbricoides*, ~~Human~~ human ~~Small~~ small ~~Intestinal~~ intestinal ~~Mucosa~~mucosa, ~~Mucin12~~mucin12, ~~Host~~host ~~-~~parasite relationship, sequencing.

~~Abbreviations~~

1. Introduction

Infection with the parasite *A. lumbricoides* ~~infection is a disease caused by parasitizing of *A. lumbricoides* and is~~is widespread throughout the world. ~~Many~~ Several ~~cases~~ infections develop in tropical, subtropical, and temperate regions, although ~~a few also develop in~~few may also occur in cool regions. In Japan, the ~~there were many~~prevalence of *A. lumbricoides* ~~patients~~ infection increased to such an extent after World War II ~~and~~ that it was

Comment [SC ED7]: The abstract reflects the aim of the study and the methods implemented to achieve the study's objectives. However, you may consider to provide a brief introductory statement about already known host-pathogen interaction mechanism that would help in conveying the focus of the study clearly.

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Comment [SC ED8]: This is a lengthy sentence and is filled with jargons. Hence, we have edited it to make a concise yet clearer and meaningful context to your research.

Comment [SC ED9]: It would be ideal to mention the molecular weight of this glycoprotein and present a meaningful sentence.

Comment [SC ED10]: Although you have concluded the Abstract by stating the defensive mechanism of the parasite in human small intestine, please note that this is quite a known fact reported in several papers. Hence, the final statements here should reflect the potential of your findings from a diagnostic and prognostic view to appeal the reader's attention.

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Comment [SC ED11]: We have added a relevant keyword to enhance the paper's chances of visibility and searchability by readers online after it gets published.

Comment [SE12]: Note that as per journal guidelines, you are required to place a list of abbreviations in a footnote on the first page of the article.

Comment [SE13]: When referring to your data and describing your data do not use third person. This is very confusing. Please note the changes we have done by switching to saying "we" or "our" etc.

Comment [SE14]: Tip: Serial comma
In American English, a comma (called serial or oxford comma) is inserted before "and" in a series.

called deemed a national affliction;, but however, the number of patients prominently has decreased considerably, thanks to group disinfestation, usage of chemical fertilizers, and the improved conditions of livingenvironment. However, iIncreased international travel ing has been causing new problems in recent years because infection sources have increased due toof infected travelers from overseas countries entering Japan.

Infection begins when mature Looking at the life history of *A. lumbricoides* , at first mature eggs were are ingested orally ingested. Once they reach the , and reached to the small intestine, they and hatches., and Hatching the larvae invade the intestinal walls. of the small intestine, andThey are then able to enter the systemic circulationthe circulatory system via the portal vein and reach the lungs from the heart. They break rupture the alveoli and areis swallowed once more by through the pharynx via the, bronchus, and the trachea, and enabling them to returns to the small intestine again. Finally, they become adultsreach maturity in the small intestine, where they mature and remain and stay. The human small intestine is considered to be theI ideal habitat for *A. lumbricoides* can be the human small intestine, but although its immunological escape mechanism has not yet been sufficiently clearly elucidated yet. Few gene level studies of the genetics of *A. lumbricoides* exist, and no research has been conducted regarding concerning the common antigenicity between *A. lumbricoides* and the human small intestine has been conducted.

However, some studies have investigated the biochemistry of the intestinal mucosa and its relevance in *A. lumbricoides* infection. As for research concerning *A. lumbricoides* and human intestinal mucosa, Scientists have, proved reported the existence of an antibody against nematodes in the blood serum of ulcerative colitis patients through using the Ouchterlony method. The researcher reported the existence of common antigen substances in the cortical laminae and basal laminae of the cuticles on of *A. lumbricoides* side. Antigens common to *A. lumbricoides*, local existenceare exist of common antigens with *A. lumbricoides* around the basement membrane in normal human intestinal mucosa, and that a protein with a molecular weight of 41.38 kDa in a crude antigen of normal intestinal mucosa antigen is the substance that owns shares a common antigen with *A. lumbricoides*.

2. Materials and mMethods

2.1 Preparation of *A. lumbricoides* crude antigen and polyclonal antibody

AThe adult warm of *A. lumbricoides* were homogenized and the crude antigen was extracted using a homogenizer with PBS at 4°C℃. RThe rabbit polyclonal antibody specific for to *A. lumbricoides* were was kindly donated from by Dr. Ishida [5].

2.2 Screening using against a human colon cDNA library

In order to obtainWe screened for the a cDNA clone that show determines the

Comment [SC ED15]: This sentence is too lengthy and the flow of research findings reported in unclear. We have edited the sentence and added punctuations wherever applicable to enhance the overall readability.

Comment [QA16]: I have edited this sentence to bring the focus of the reader to the 'infection' rather than the 'life history' of the parasite.

Comment [SC ED17]: This is a non-specific inclusion of texts and hence we have edited it to present a concise context.

Comment [SC ED18]: This is a well-known fact and hence, please provide a suitable reference to it.

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Comment [SE19]: Please add the appropriate citation.

Comment [QA20]: Do you want to depict any specific significance of this molecular weight of protein in the parasite as well as human small intestine mucosa?

Comment [SC ED21]: This sentence should be referenced for the author to refer to the existing findings reported in similar context.

Comment [SE22]: Please provide details regarding the affiliation. E.g., Department/university/state/country.

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~~common~~common antigenicity between ~~host and~~ *A. lumbricoides* and its host ~~, we used the screening method of~~using a λ-phage method ~~using~~ and polyclonal anti-~~-~~*A. lumbricoides* antibod~~y~~ies. ~~–~~Briefly, ~~the~~ plaques ~~that were~~ produced ~~by~~ using λ-phage human colon cDNA librari~~es~~y were transferred to ~~the~~ a nitrate cellulose membrane, which was saturated with 10 mM IPTG. The membranes were screened with polyclonal anti-*A. lumbricoides* ~~antibody~~ antibodies and ~~some of~~ positive phage clones were ~~obtained~~ identified by ~~developing color using~~ development using 3.3-diaminobenzidine~~s~~-4-hydrochloride~~s~~ (DAB).

~~3. ~~2.3 Sequence a~~Analysis ~~of the sequence~~

~~—~~The positive plaques ~~for~~ reactive against *A. lumbricoides* ~~antibody~~ antibodies were ~~picked up and~~ transformed into an~~the~~ *E.coli* host. These clones were constructed as a plasmid, pExcell. The sequences of positive clones were analyzed using the BigDye Terminator v3.1 cycle sequencing kit (Becton Dickinson Biosciences). The plasmid sequences were then compared with ~~the~~ sequences in the GenBank database (National Institute for Biotechnology Information) to determine the identity of genes.

~~4.~~2.4 Identifying ~~the site of mucin12~~mucin12 localization in *A. lumbricoides*

~~The~~ A 14 amino acid synthetic peptide ~~of 14 amino acid~~ (HREQYDVPQEWRKE) from amino acid 396 to 409 of ~~mucin12~~mucin12 ~~were~~ was synthesized and administered ~~it~~ to rabbits to prepare anti-~~mucin12~~mucin12 polyclonal ~~antibody~~ antibodies by Sigma-Ggenosys (Hokkaido, Japan). On day 0, ~~The first administration to~~ the rabbit ~~on day 0~~ was given a 200 μg dose of the peptide, ~~then boosted by~~ followed by an additional 100 μg in 5 subsequent administrations on days 7, 14, 21, 27, and 42 with incomplete Freud~~'s~~'s adjuvant. On day 49, exsanguination was conducted. ~~Furthermore, t~~The serum was purified by ammonium sulfate precipitation.

To identify the localization of mucin12 in *A. lumbricoides*, we conducted immuno-staining with sections of *A. lumbricoides*. Three frozen sections were used: ~~one~~ from the head, the quarter point ~~of~~from the head, and the quarter point ~~from of~~ the tail ~~of A. lumbricoides~~. The sections of *A. lumbricoides* were cut into ~~sliced with μ of thickness~~slices and stained with HE. ~~In order to identify the localization of mucin12 in A. lumbricoides, we conducted immuno-staining with sections of A. lumbricoides.~~ After blocking, the sections were stained with the polyclonal anti-~~mucin12~~mucin12 antibodies~~y~~ (~~×~~x150 dilution) and mucin12 ~~antibody~~ antibodies after the absorption treatment with the synthetic peptide described ~~above~~ earlier in this section to identify the specificity of the primary antibody. After washing with PBS with Tween 20, FITC anti-rabbit IgG (Sigma-Aldrich, ~~×~~x150 dilution) ~~were~~ was used ~~for~~ as the secondary antibody. The~~se~~ sections were observed ~~by~~ using a laser confocal microscope LSM510 (Carl Zeiss).

Comment [SE23]: What is the company/clone of the secondary antibody used for final detection?

Comment [SC ED24]: The abbreviation should be elaborated to help the reader understand the chemical composition of this reagent.

Comment [QA25]: At what phase (beginning of stationary phase or in the stationary phase, etc.) was IPTG added to the membrane. This is an important aspect of induction protocol that needs to be clarified.

Comment [QA26]: Is the plasmid sequence for pExcell published? If so please cite it in this section.

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Comment [SE27]: Please provide precise details on the care and use of animals and of experimental procedures.

Comment [SE28]: The "Ethical approval" aspect of this paper is blank. Approval for the use of rabbits for generation of polyclonal serum and human tissue for staining of mucin isoforms is not described in the materials and methods. No major journal will accept these studies without statement of ethical approval.

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Comment [SE29]: "The quarter point" does not make it clear where the sections were taken from. Please clarify.

Comment [QA30]: How thick were these sections cut for immunostaining? Was the polyclonal antibody to Muc12 validated in any other way independent of peptide absorption?

To confirm the mucin12 existing in *A. lumbricoides* crude antigen, SDS-PAGE was performed using a crude extract of *A. lumbricoides*. For western ~~-~~blotting, crude extract was transferred onto nitrocellulose membrane by a semidry transfer system (ATTO, Japan) and incubated with anti-mucin12 polyclonal antibodies~~y~~ (~~×~~1,000 dilution) for primary antigen after blocking. ~~Furthermore, the~~The membrane was incubated with HRP a~~A~~nti-rabbit IgG for the secondary antibodies~~y~~ and the reacted bands were visualized ~~by~~ using the ECL method (GE healthcare) ~~using~~ and X-ray films.

~~In order t~~To confirm that the results of immuno~~e~~-staining were not affected by ~~mucin12~~mucin12 ~~that were derived from~~of human origin, w~~W~~estern ~~-~~blotting was performed with protein extracts of *A. lumbricoides* crude antigen and mouse Embryonic Stem (ES) cell lines using ~~R~~a~~A~~nti-GAPDH polyclonal antibodies~~y~~ that could cross-react with ~~some~~ mammalian GAPDH (human, mouse, and rat, etc.) (Cat: ab9485-25, Abcam, ~~×~~1,000) for the primary antibodies~~y~~ and HRP anti-rabbit IgG (Sigma-Aldrich, as ~~described~~ above) for the secondary antibodies~~y~~.

3. Results

~~1.~~3.1 Screening ~~with~~ against a human cDNA library

~~As the results of homology search, t~~A~~he~~ fragments of 389 bp ~~was identified~~matched with ~~as from~~base 756 ~~bases~~ to 1144 ~~base~~ of human transmembrane ~~mucin12~~mucin12 (AF147790) with ~~and its consistent was~~ 99.5% consistency (Fig. 1).

[Other text deleted]

~~2.~~3.2 Identifying the site of mucin12 localization in *A. lumbricoides*

In order to identify the localization of ~~mucin12~~mucin12 in~~on~~ *A. lumbricoides*, immunohistochemistry was performed with the anti-~~mucin12~~mucin12 polyclonal antibodies~~y~~. ~~As the results, the~~ FITC-~~-~~labeled signals were detected in the mucous secretory organs, epidermis, and intestinal canal (Fig. 2b and 2c). ~~In order t~~To confirm the specificity of the antibody, the ~~mucin12~~mucin12 antibody~~ies~~ ~~was~~ were pre-absorbed with a synthetic peptide that was used for the preparation of polyclonal antibodies~~y~~ and ~~done the staining as same~~stained as described ~~above~~in section 2. As shown~~ed~~ in Fig. 2d and 2e, the signals ~~were clearly~~ disappeared. These results ~~were~~ suggested that the protein ~~which~~ that reacts with anti-human ~~mucin12~~mucin12 is localized in the mucous secretory organs, epidermis, and intestinal canal ~~in~~ of *A. lumbricoides*.

[Other text deleted]

Comment [SC ED31]: Please specify the quantity of crude extract used.

Comment [SC ED32]: Please mention the name of the company from where you have purchased this anti-immunoglobulin. Also, provide the dilution of the antibody used in this experiment.

Comment [SC ED33]: Please mention the source to which this polyclonal antibody belongs.

Comment [SE34]: Please describe how the screening of "clones" or "plaques" was done at the beginning of the results section to help clarify the data in this section with the reader.

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Comment [SE35]: Should this perhaps be in section 2? Please check.

Comment [QA36]: Although you have indicated the locations of the protein reactive mechanism in the parasite, it would be ideal to emphasize on which of these locations showed the most promising outcome.

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4. Discussion

It ~~was discussed~~has been suggested that ~~one of~~ an ~~the parasite's~~ immunological escape mechanism utilized by~~s~~ *A. lumbricoides* is the expression of ~~the matter~~an antigen very similar to ~~to its~~a host antigen [6]. However, the parasite's immunological escape mechanisms ~~have~~ have not yet been ~~sufficiently~~ clearly ~~unraveled yet~~elucidated, so this hypothesis has not been definitively confirmed. ~~–~~Therefore, the ~~researchers~~ authors of the present report decided to ~~reveal~~ examine ~~the~~ this potential immunological escape mechanism using a molecular ~~biological~~ biology tool. ~~–~~After ~~conducting a~~ screening against a human colon cDNA library ~~of human colon~~ using anti-*A. lumbricoides* polyclonal ~~antibody~~ antibodies and checking for a common antigen, ~~3~~ three positive clones were obtained.~~–~~ ~~One of the r~~Results of ~~the~~ analysis of each sequence showed that one clone possessed high ~~homologous~~ homology with transmembrane ~~mucin 12~~mucin12.

Mucins are ~~mucous~~ glycosylated proteins that are important components of mucous that cover~~ing~~ inner cavities such as the trachea, the digestive tract including the stomach and intestines, and the gonads [11].

[Other text deleted]

~~–~~At the time of writing~~Currently~~, 18 different mucin ~~genes~~ proteins have been reported to exist in humans. ~~There are secreted mucins, which are secreted from epithelial cells, and membrane-associated mucins, which have transmembrane sections and exist under cellular membrane-bound conditions.~~

We examined transmembrane mucin12 ~~In order to observe the~~in the context ~~association of transmembrane mucin12 in~~of the rest of the mucin family.~~,~~ ~~G~~genomic mucin12 DNA can be found on chromosome 7, along with that of ~~of mucin12 including in~~ mucin 3 and mucin 17 ~~are existed on same chromosome 7~~ [20], and ~~mucin12~~mucin12 ~~protein is comprised of~~ comprises a total of 588 amino acid sequences.

~~Mucin 12~~Mucin12 was localized in the mucous secretory organs and the epidermis of hypodermis of *A. lumbricoides* by immunostaining with m~~Mucin12~~ucin12 polyclonal antibody (Fig. ~~)~~). ~~The mucous secretory organs are connected to the epidermis of hypodermis and the lateral cord, and are involved in excretion and form the cluster-like [21]. There is an excretory canal in the lateral cord, and it is connected to the surface of the worm body.~~

Then, w~~W~~estern blotting was conducted to examine the existence of common antigenicity of ~~mucin12~~mucin12 in *A. lumbricoides* crude antigen.~~–~~ A band was detected around 37 kDa. Additionally, a specific band was detected in the protein extracted from cultured mouse cell but not in human *A. lumbricoides* crude antigen.

~~Researchers~~ The authors would like to further examine the protein~~,~~ which is cross-reactive with ~~mucin12~~mucin12 in the *A. lumbricoides* crude antigen, and the ~~matter~~

Comment [QA37]: Although your meaning is sufficiently evident through this paragraph, I have inserted further edits in this section to depict the core intended research approach of this study in comparison to existing literature. Also, it not very well correlates to the finding of 3 positive clones after screening of the cDNA library.

Comment [SE38]: This sentence has been deleted to avoid redundancy.

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Comment [QA39]: The intended importance of the term 'transmembrane' should be explained further because scientists have explored the membrane properties in context of the parasite's ability to defy the host's immune mechanism. This aspect should be clearly depicted by you through these lines.

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Comment [SE40]: Please provide the figure number.

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Comment [QA41]: This explanation about lateral cord does not add significance to the explanation you have provided about mucous secretory organs in relation to mucin12. We have thus deleted it to make the paragraph more concise and focussed.

Comment [SE42]: Is it known what the sequence of the 37KDa protein from the helminth is and how it is modified (glycosylated or a receptor) during infection?

Comment [QA43]: This is a non-specific expression and should be elaborated with proper context.

substance similar to actin and beta-casein-like-protein ~~detected as~~noted to have common antigenicity, both detected in this study. We would also like~~And~~ to ~~research which~~ investigate whether any *A. lumbricoides* hosts are~~is~~ immune to avoidance by ~~mucin 12~~mucin12, ~~of the mechanism~~ and ~~the~~ other mechanisms of immune avoidance utilized by *A. lumbricoides*.

5. Conclusion

In this study, analysis of common antigenicity between *A. lumbricoides* and intestinal mucosa obtained three DNA clones. ~~After analyzing~~Analysis of each clone sequence indicated ~~, it was clarified~~ that one of them has high homology with transmembrane ~~mucin12~~mucin12.

Localization of ~~mucin12~~mucin12 was confirmed in the ~~mucous~~mucosal epithelial~~l~~ present around the intestinal crypts and villi of the human small intestine. These data suggest that expression of mucin proteins by helminths may be one mechanism ~~by~~ through which the ~~helminth~~ parasite evades immunological detection within the mammalian host.

6. Acknowledgments

~~**Ethical approval**~~

[Other text deleted]

Figure ~~l~~Legends:

Figure 1. Sequence analysis of a newly ~~found~~ identified clone after screening ~~of~~ of the *A. lumbricoides* cDNA library. TM12: human transmembrane ~~mucin 12~~mucin12.

Figure 2. ~~Identification of the l~~Localization ~~for~~ of m~~Mucin12~~ucin12 in ~~human~~ the *A. ~~Lumbricoides~~ lumbricoides* adult worm. ~~The s~~Sections of *A. ~~Lumbricoides~~ lumbricoides* were stained with HE (a) and anti-m~~Mucin12~~ucin12 polyclonal antibodiesy ~~. The sections of A. lumbricoides were stained with anti-Mucin12 polyclonal antibody that was pre-treated~~pretreated with synthetic m~~Mucin12~~ucin12 peptide (d and e). The scale bar ~~indicates~~ represents 200 μm (b and d)~~,~~ and 100 μm (a, c and e)~~, respectively~~.

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Comment [SE44]: Please check if the edit retains your intended meaning.

Comment [QA45]: There are papers that have reported about expression of mucin proteins being one of the pathogenic mode of overcoming the antigenic defence mechanism of the host. Hence, this concluding remark should be more focussed on how your findings can contribute to the next level of analysis by other researchers in this field. You might probably refer to the continuous evolution of parasitic immune regulatory pathways that can be explored towards obtaining a novel therapeutic outcome.

Comment [SE46]: Please provide information about those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Comment [SE47]: Remark: Please ensure the figures included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.