

TOP IMPACT EDITING SERVICE REPORT

Journal name and URL: Molecular and Biochemical Parasitology / <https://www.journals.elsevier.com/molecular-and-biochemical-parasitology>

Manuscript Title (Edited): Shared Expression of Mucin12 in both *Ascaris Lumbricoides* and Human Small Intestine

Summary

This article is focused towards identification of the antigen secreted by human *Ascaris lumbricoides* in small intestine. This commonest parasite in the world infects the human small intestine after the humans ingest mature eggs of the parasite. It is presumed that adult *A. lumbricoides* live in their preferred environment, which is the small intestine, by secreting mucin12 to avoid being attacked by the host immune system. The authors obtained three DNA clones by screening for common antigenicity using a human colon cDNA library and anti-*A. lumbricoides* polyclonal antibodies. After sequencing analysis, the transmembrane mucin12 gene was identified as a gene of interest.

Research Strength

Originality and Significance of Research [Rating: Excellent, Good, Fair, or Poor]

Good

The study appears to be novel. The reason behind this study is incredibly sound and the research into this area is very interesting. Determining how helminths evade the immune system is an important mechanism in understanding type 2 immunity. Additionally, generation of antibodies to mucins seem to be implicated in autoimmune diseases. Thus, this study is important to the field. However, the authors need to improve the manuscript based on the above suggestions in order to improve the weightage of his findings.

The research content is acceptable but the study would be highly beneficial and would have better prospectus, if all revisions as mentioned are done and the missing data provided.

- The article has all the required sections for an article, but needs some revision.
- The study is very relevant to the field of drug industry, pharmaceuticals as well as healthcare industry.

Content Review

Title, Abstract, and Keywords

The title adequately reflects the theme of the paper, is concise, and fits well within the guidelines of the selected journal in this research area. However, title page according to journal standards is not fulfilled. In addition, the title "Mucin12 contribute common antigenicity of host-parasite relationship between *Ascaris Lumbricoides* and Human" contains some grammatical issues and doesn't best represent what the core research focus of the manuscript is about. Muc12 doesn't technically contribute to the common antigenicity but instead is the antigen expressed by both *Ascaris* and human. Instead, we suggest a more succinct and detailed title such as "Shared Expression of Mucin12 in both *Ascaris lumbricoides* and human small intestine"

- Add the following details:

1. Author names and affiliations
2. Corresponding author details
3. Complete postal address of corresponding author

The abstract does present the purpose and significance of the study. However, the structure of the abstract is very poorly defined. It does not properly summarize the content of the article. Start the abstract on its own page

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instead of continuing on the title page. The heading “summary” should be changed to “Abstract” as per journal’s requirement. We have edited the abstract to make the content easily understandable for the readers. Also, we have reduced the word count from 239 to 225 to adhere to the journal guidelines.

Overall, the abstract contains long and confusing sentences and is filled with jargon. For example, the sentence “The purpose of this research is to focus on the host specificity of human *A. lumbricoides* which is parasitic in the small intestine and to examine, at a genetic level, the common antigenicity existing in *A. lumbricoides* and human small intestinal mucosa to unravel the host-parasite relationship” is very long. This sentence would be cleaner and easier read as “The purpose of this research is to focus on the host specificity of human *A. lumbricoides*, a parasitic in the small intestine. As part of this investigation, we examined, at a genetic level, the common antigenicity existing in *A. lumbricoides* and human small intestinal mucosa to unravel the host-parasite relationship.” Additionally, use of the word “clones” is jargon and confusing. Were these clones of the genome, cells, or tissues? When describing mucin12 expression or antibody binding to mucin12 it would be most ideal if you clarify which organism (helminth or mammalian host) you are referring it in the sentence.

○ Introduction and Literature review

The structure of this section should be revised. The author should revise the introduction and make it more concise in the light of those findings. The whole purpose of this study is to understand the mechanism of molecular mimicry that *A. lumbricoides* uses to go undetected within the mammalian host. Understanding of this concept would be greatly heightened if the authors described how *A. lumbricoides* is contracted and its life cycle within the mammalian host within the introduction. The subject of the second paragraph is a little confusing at first. This is mostly due to the very confusing first sentence: “From the host’s biological defence reaction, it is described that the host’s immunological escape mechanism evades immunity by mutating glycoprotein on the body surface, the parasite expressing matter quite similar to it on the host’s body surface, thus avoiding immunity by inhibiting the host’s immune system1.” How can the host have an immunological escape mechanism if it is the host? Are you describing the parasites? We are not exactly sure the point the authors are trying to make here but we think it is about how the parasite mutates glycoproteins (unsure of whether this is on its surface or the host’s surface) to evade immunological detection. To help clarify, in the beginning of the second paragraph, please state that *A. lumbricoides* uses multiple mechanisms to go undetected within the host and then proceed to state examples. Some of these examples are given in the next paragraph such as how helminth can extract sialic acid from the host, express hyaluronidase receptor, and induce protective and remodelling cytokines. Thus, you might be able to combine the paragraphs and make this section more concise.

The language of the article needed revisions, which we have now addressed in the edited manuscript. We have also made the article more concise to avoid unnecessary lengthy discussions. Many vital details have not been addressed. Other information that would be helpful in the introduction is description of the expression of mucins in *A. lumbricoides* and other helminths and expression of Muc12, and other isoforms within the mammalian host. Descriptions of these data are included in the discussion. A few sentences about these concepts would be a great way to start to frame the data. The author should also refer to the detailed comments in the manuscript for sections applicable and revise.

○ Methods and Statistical analysis

The experiments are adequate to answer the research question. However, the author has not mentioned very important details about methodologies. Approval for the use of rabbits for generation of polyclonal serum and health human tissue for staining of mucin isoforms is not described in the materials and methods. How was healthy intestinal tissue obtained and what are the characteristics of this human cohort (age, sex, and definition of “health”).

Parts of the materials and methods section are very unclear. Under “Preparation of *A. lumbricoides* crude antigen and polyclonal antibody” it was stated that “rabbits were immunized using the crude antigen of *A. lumbricoides*

for 4 times a week interval and the sera were collected.” It would be ideal to know the amount of antigen that was injected during these times and whether an adjuvant was used. The plaque assay described in “Screening using human colon cDNA library” is complicated and confusing. Is there a previous study that has published on this method? Can you cite it in the methods and state others have used it before?

While identifying the site of mucin12 localization in *A. lumbricoides*, it would be good to know where the *A. lumbricoides* was obtained from, what form of the parasite (infective or intestinal?) was frozen, and how the helminth was frozen. These unclear contents were missed by the author to mention in this section. All such details have been elaborately discussed in the comments section. The author has not mentioned anything about whether a commercial Muc12 antibody used to confirm the polyclonal antibody. For details, kindly refer to the Comments section in the manuscript.

○ Results and Discussion

The results appear complete and are represented adequately. The results have been explained with discussion but the findings are not properly interpreted. 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. Were false positive plaques obtained by accident or on purpose? If on accident just state that overall 3 positive plaques were obtained. Was the fragment of homology (756 bases to 1144 base) used to immunize rabbits? If not, how homologous are these fragments to each other?

All interpretations seem to be based on the previous literature. Overall, the findings of the present study in the light of previous findings are clearly defined. The supporting literature is sufficient enough and the conclusions hence drawn are understandable. However,

Proper detailed suggestions are provided in the comments. Please refer to them.

No statistical analyses are performed and significance values are all missing for the biological assays.

The discussion part is detailed and clearly presents the results. Be sure that when describing an organism for the first time to state both the genus and species. In the discussion, *C. parvum* is described for the first time as such, instead of as *Cryptosporidium parvum*. Please check and make the changes accordingly.

Additionally, sometimes new paragraphs are started after a sentence or two. This results in a longer section about the same topic. Please try to cluster 5-6 related sentences in paragraphs. Overall, the information in the discussion is very interesting and very relevant however it is disorganized. Antibodies to different mucin isoforms and their association with disease and expression of these mucins in parasites are described and then a few paragraphs later are a description of what mucins, their composition, and different isoforms. This would obviously be the most helpful and descriptive earlier on.

The limitations of the study have not been highlighted. The significance of the findings in light of the past studies and present novelty has to be emphasized. Do helminth infected individuals have increased antibodies to helminth-derived mucins? Detailed similar comments have been added in the manuscript file. The author should address all mentioned issues.

○ Figures and Tables

The figures provided are represented well, however, one of the figures was cited without a figure number.

In figure 1, the homologous sequence is highlighted in the ORF but should also be highlighted in the amino acid sequence. Was a portion of this sequence used to immunize rabbits? In the figure legend of figure 1, more detailed information should be given about the methods of how clones were screened and how this homologous sequence was identified. In the legend of figure 2, *A. Lumbricoides* should be stated as *A. lumbricoides* (with a lower case L). This figure legend should include more detail about how the worm was frozen, how thick sections were cut, and for what concentration and time the sections were stained with antibody. In figure 2, the figure legend should include information about for how healthy tissue was obtained, treated, and fixed for staining. Additionally, labelling the pathology or cellular morphology of the Muc12 stained *A. lumbricoides* and human intestine sections on the actual images would be incredibly helpful to the interpretation of this data.

No tables were included in this manuscript.

○ Conclusion

The conclusion is concise. However, the authors have not been able to highlight the significance and use of this method in clinical uses. Even though we understand now how the “clones” were obtained, use of this term is still confusing. It might be easier to say “peptide library” or “antigen screen” to describe the assay. Since you came to the same conclusion about antibodies to Muc12 binding to *A. lumbricoides* and human small intestines, and these binding were subsided with pre-absorption using peptide, it may be easier to combine these concepts and sentences for the conclusion. A sentence about the broad implications of this study such as “these data suggest that expression of mucin proteins by helminths may be one mechanism by which the helminth evades immunological detection within the mammalian host.

- No future prospectus of the study has been detailed out.

○ References

Most of the recent findings have been cited in the manuscript.

Citations in the following section are lacking:

“Mucins are mucous glycosylated proteins covering inner cavities such as the trachea, the digestive tract including the stomach and intestines, and the gonads. There is secretory mucin which is the main component of mucus and transmembrane mucin which is associated with the cell membrane. Mucin has high molecular weight, higher than 1 million holding numerous sugar chains associated with polypeptide through O-linked glycoside. The protein core of mucin is coded by various mucin genes. Most mucins are rich in serine and threonine, formed by a variable number of tandem repetition.”

“Regarding the homology with the mucin family, mucin3 showed homologous with amino acid sequence from 238 to 585 (30%) in mucin12, and mucin17 showed homologous with amino acid sequence from 238 to 581 (38%) in mucin12.”

Structure, Style, and Format Review

○ Clarity of Presentation

The quality of presentation in the article is average. Although the aim of the study is highlighted adequately, there are many sections including introduction that were not appropriately structured. The writing throughout the article is not clear and concise. The flow of language throughout the article appears ambiguous at many places. Additionally the article had many grammatical errors, which we have rectified in the edited manuscript. Many sections of this paper were very confusing. This was the result of long sentences, short paragraphs, confusion between description of the helminth or human, use of jargon, incomplete methods, and disorganization of sections. We have reworked the sections as described and have provided suggestions wherever applicable. Data described in the discussion are ideal for the broad understanding of these data. Emphasis on the life cycle of the parasite in the introduction would be beneficial. Many sections as pointed out in the comments needed major revision/rephrasing which we have addressed. Please check and confirm if the research presentation is in line with your experimental makeup of the study.

○ Organization and Structure

The article is structured into logical sections. The appropriate data were included in the ideal section of this manuscript. The data included in the discussion section frame the data obtained from this study well, however this section is disorganized. With a reorganization of paragraphs, and consolidation of this information, this discussion section will be great. Please check the revisions done by us in the manuscript.

○ Format and Style

The length of the article is as per standard guidelines of a journal that publishes similar research findings, although this journal has not specified any word count limit. Include page and line numbers.

Try to start sections on a new page

Change “summary” to “abstract”

In the “abbreviations” section, two different fonts are used. Please clean this up in preparation for submission. Some parts of the text are in different colors, but it is unclear about why. The abstract had been edited by us to bring the total word count within the limit specified in the journal guidelines.

There is no limit of word count in introduction section. The sections in main manuscript should be numbered.

Divide the article into numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc.

We have formatted the manuscript according to the journal’s instructions.

When describing data generated by your lab or by the author, please state “we” or “our” instead of talking about the data in 3rd person.

○ Authorship

It complies with the journal’s guidelines; however, the author should provide a declaration of interest. It is mandatory for the journal.

SUITABILITY TO JOURNAL INSTRUCTIONS

○ Journal Scope

‘Molecular and Biochemical Parasitology’ is a journal within the field of molecular biology and biochemistry that is interested in papers that report about host-parasite relationships focussed on the parasite, particularly as related to specific parasite molecules. It also welcomes papers that deal with analysis of genes and genome structure, function and expression, analysis of variation in parasite populations relevant to genetic exchange, pathogenesis, drug and vaccine target characterization, and drug resistance, and parasite protein trafficking, organelle biogenesis, and cellular structure especially with reference to the roles of specific molecules.

Since the authors investigate the mechanism of molecular mimicry between *A. lumbricoides* and mammalian host and demonstrate that a homologous protein (muc12) is responsible for this, it fits the scope of the journal perfectly.

○ Journal Quality and Coverage

- Impact factor – 2.158
- Indexed databases – BIOSIS Citation Index, PubMed/Medline, Elsevier BIOBASE, and Scopus.
- Quartile ranking – Q2 ranking

○ Manuscript Compatibility (based on journal’s scope, quality and coverage)

The scope of this study is in line with the aims/scope of the recommended journal. However, the author should make all the revisions as suggested. The manuscript is a pioneer in the field of molecular biology, host pathogenicity and parasitology, and many similar studies have been published recently.

The journal’s indexing in well-reputed databases will ensure wide readership.

This journal specifically publishes data on molecular and biochemical studies on the mode of action of antiparasitic drugs, molecular and biochemical aspects of membrane structure and function, molecular and biochemical aspects of host parasite relationships including analysis of parasitic escape mechanisms. This fits well with the scope of the current study.
