Peer Community In Microbiology

Interaction of bovine macrophages with Mycoplasma mycoides subsp. mycoides

Pablo Zunino *based on peer reviews by 2 anonymous reviewers*

Philippe Totté, Tiffany Bonnefois, Lucia Manso-Silvan (2023) Interactions between *Mycoplasma mycoides* subsp. *mycoides* and bovine macrophages under physiological conditions. bioRxiv, ver. 2, peer-reviewed and recommended by Peer Community in Microbiology. https://doi.org/10.1101/2022.12.06.519279

Submitted: 15 December 2022, Recommended: 09 May 2023

Cite this recommendation as:

Zunino, P. (2023) Interaction of bovine macrophages with Mycoplasma mycoides subsp. mycoides. *Peer Community in Microbiology*, 100005. 10.24072/pci.microbiol.100005

Published: 09 May 2023

Mycoplasma *mycoides* subsp. *mycoides* (*Mmm*), a pathogenic wall-less bacterium, is the etiological agent of contagious bovine pleuropneumonia (CBPP). This highly contagious respiratory disease may develop in severe pneumonia, with associated high mortality rates in cattle. Mmm can display different immune evasion mechanisms; in addition, a host uncontrolled inflammatory response stands for lung lesions and chronic carrier animals.

Macrophages are among the most important lines of defense against *Mmm* of the lower respiratory tract. Although their importance in defense and immune response modulation is known, results about their role and mechanisms of action are scarce and sometimes conflicting.

In the present study, Totté et al. (1) aimed to investigate the interaction of bovine macrophages (isolated from cattle peripheral blood mononuclear cells) with *Mmm*, under in vitro conditions. The authors highlight that the study was performed under physiological conditions (in the presence of complement prepared from the same cell donor).

In their study, using different approaches, the authors provide interesting and original results, proposing a pivotal role of complement in controlling the inflammatory response, which is crucial in the CBPP pathogenesis.

The authors reported that macrophages did not kill Mmm in the presence of a non-bactericidal concentration of bovine serum. However, Mmm inactivation was observed when antiserum from CBPP convalescent animals was used. They also observed that Mmm induced the production of TNF by macrophages (when a high MOI was assessed). However, complement could even abolish Mmm-induced TNF response when used at bactericidal activity concentrations. This role of complement could be combined with the development of potentially protective antibodies against particular Mmm antigens involved in the interaction with identified macrophage receptors to propose control strategies against CBPP.

Overall, the study by Totté et al. provides new fundamental insight for the research on preventive or therapeutic strategies for a poorly understood disease that still represents a serious concern for livestock production.

References:

1. Totté, P., Bonnefois, T., Manso-Silván, L. Interactions between *Mycoplasma mycoides* subsp. *mycoides* and bovine macrophages under physiological conditions. bioRxiv 2022.12.06.519279, ver. 2 peer-reviewed and recommended by Peer Community In Microbiology. https://doi.org/10.1101/2022.12.06.519279

Reviews

Evaluation round #2

Reviewed by anonymous reviewer 2, 04 May 2023

The authors have addressed all the reviewer's comments. I accept the manuscript in this form.

Reviewed by anonymous reviewer 1, 28 April 2023

All the comments have been adressed in the revised version of the manuscrit.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2022.12.06.519279
Version of the preprint: 1

Authors' reply, 11 April 2023

Download author's reply

Decision by Pablo Zunino, posted 23 February 2023, validated 23 February 2023

This preprint merits a revision

Dear Authors:

Two Reviewers have evaluated your article. As you can see, both of them have recognized the scientific merit of your work (which I agree), although they have some concerns, comments, and suggestions to be addressed before recommendation (please see comments below).

Also, please take into account these few comments of my own:

I noticed that there is a dead link at the end of Totté et al. ms. on BioRxiv, in the "Data, scripts, code, and supplementary information availability" section: "Supplementary File1 is available online: XXXXDOI of the webpage hosting the data https://doi.org/10.5802/fake.doi"

It must be clear whether this "Supplementary File1" refers to the supplementary figure available on the BioRxiv page (i.e. https://www.biorxiv.org/content/10.1101/2022.12.06.519279v1.supplementary-material) or if it refers to the data used for the study and available on Zenodo (i.e. https://doi.org/10.5281/zenodo.7442581)

In any case, you must clarify this and give the correct web address in the manuscript. The link to the data must be available in the manuscript.

The surname of the first author must be Totté (not totté).

In the section “Data, scripts, code, and supplementary information availability,” the authors refer to a “Supplementary File1,” but it is not cited in the text; please clarify.

Please consider the inclusion of additional references that could enhance the scientific support of the study.

Reviewed by anonymous reviewer 2, 17 February 2023

The manuscript presents interesting data concerning interactions between *Mmm*, an aetiological agent of CBPP, and macrophages isolated from cattle *in vitro* conditions. However before publishing, the following comments should be addressed:

Methods:

Line 74: the method for CFU counting should be presented in detail

Line 78: the detailed information about the cattle form which the sera were collected should be added (the origin, breeding conditions, epizootic status especially for mycoplasma infections)

Line 80: the range for room temperature should be added

Line 82: the authors should explain such concentration range

Line 85: the authors should add more information about the animals from which the sera were collected (the origin, medical history, the methods used for the status confirmation)

Line 91: the information about the Ethics Committee agreement should be transferred to sub-section of ’bovine complement and antiserum’)

Line 108: there should be added the detailed conditions for culturing

Line 140: the detailed information about the ELISA reader used here should be added

Results

Lines 157, 175: a statements ’results not shown’ or ’data not shown’ - the authors should give the reason for such explanation, it is not possible to reliably evaluate such presented statement

Line 184: ’Mmm’ in ’Anti-Mmm’ should be in italics

Discussion

Lines 310, 335: ’not shown’ should be avoided; the authors should describe it in another way or based on the published data

Reviewed by anonymous reviewer 1, 14 February 2023

Mycoplasma mycoides subsp. mycoides (Mmm) is the causal agent of contagious bovine pleuropneumonia. During bovine lung infection, Mmm encounters macrophages, which play an important role in the first lines of lung defenses. As the interaction between Mmm and macrophages is far from being characterized, the authors proposed to study this interaction and the role of the opsonization by proteins of the complement system or by antibodies from CBPP convalescent animals.

The article is well written and interesting results were obtained and clearly illustrated. The authors demonstrated that macrophages do not killed Mmm or Mmm in the presence of non bactericidal concentration of bovine serum. However Mmm killing was observed with antiserum from CBPP convalescent animals. Mmm induced the production of TNF by macrophages (at high MOI) and decreasing the MOI or the viability of Mmm (using non-decomplemented bovine serum) abrogated the TNF secretion by macrophages.

Comments:

The authors used macrophages purified from the blood instead of alveolar macrophages obtained directly from the bovine lungs. Will the results be the same if the authors used alveolar macrophages instead of circulating macrophages? May be this point should be discuss in the discussion section.

Did the authors quantify the opsinization of Mmm when using non bactericidal concentration of non decomplemented bovine serum? May be the difference between non decomplemented sera and antiserum come from a low binding of the complement proteins onto the Mmm cells?

Regarding TNF production experiments: Is the effect of bovine complement on TNF production specific or not? The production of TNF by macrophages seems to be dependent of the titer of viable Mmm, reducing the MOI or killing them by adding bovine complement lead to similar results. Do you think that similar result will be obtain with heat-killed Mmm?

Finally, did the authors observe that Mmm is able to reduce the viability of macrophages?

Minor comments

Line 1: ”subsp.” between mycoides mycoides.

Line 78: maybe it would be worth to mention that the animals are CBPP-free.

Line 84: remove the dot after CO2.

Line 157: The effect of bovine serum decomplementation (30 mn at 56°C) has to be shown, it is an important result as innate defenses are present in serum other than complement (Bacterial self-defence: how Escherichia coli evades serum killing by Helen Miajlovic & Stephen G. Smith).