Author’s response to reviews

Title: First case report of M1 macrophage polarization in an untreated symptomatic patient with toxoplasmosis

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Version: 4 Date: 13 Jan 2018

Author’s response to reviews:

BMC Infectious Diseases Editorial Office
Dr Jason Mooney
Editor,
Biomed Central
Chieti, 12 / 1 / 2018

Dear Editor,

We are submitting a revision of our manuscript No. INFD-17-01445R3 entitled “First report of M1 macrophage polarization in a symptomatic untreated case human Toxoplasmosis” by Graziano De Luca, Chiara Di Lisio, Giuseppe Lattanzio, Tommaso D’ Antuono, Marcella Liberatore and Francesca Bianca Aiello We have revised the manuscript on a point by point basis along the lines suggested by our Reviewers.
Specifically:

Comments from Reviewer #1

This is a case report highlighting the presence of M1 type macrophages in the lymph node in a case of human toxoplasmosis. Because most studies on macrophage polarization during Toxoplasma gondii infection have been carried out in the mouse model, a brief note highlighting M1 macrophage polarization is a good addition to the field. Nevertheless, the interpretation that the cells identified are M1 macrophages needs to be more cautious, because only a single (phosphorylation of STAT1) marker was used. Additional markers (iNOS or interferon-induced GBPs) should be investigated to reinforce the conclusion.

In the revised version of the manuscript iNOS immunoreactivity is shown in a new figure (Figure 2B). In the text (Case presentation, page 5, lines 114-115) it is now reported that iNOS positive mononuclear cells (36.4 ±14.9 per HPF) were observed between the microgranulomas and in germinal centers.

1) Abstract: Remove the sentence-"M2 polarization in rat toxoplasma infection is associated with chronic persistence:. This is out of place in the Conclusion section and not very meaningful, because the study did not investigate whether the macrophages were negative or positive for M2 markers.

The sentence "M2 polarization in rat toxoplasma infection is associated with chronic persistence”

has been removed from the abstract.
2) Reference citation need to be consistent and accurate. For example, on page 2, sentence starting with "M2 macrophages are activated by various cytokines...." ends with a reference citation (#2), which is formatted differently from the rest of the manuscript. It is also not a relevant paper with respect to M2 cells. Similarly on page 5, reference 1 is cited for cytokine-activated murine endothelium contributes to clearance of T. gondii through recruitment of CD8 lymphocytes, which is also not a matching article for this statement.

We have corrected the inappropriate citation (Background, page 2, line 44) and added another reference concerning the role of M2 macrophages (page 2, line 44, reference 3) and an appropriate reference concerning activated murine endothelial cells (Discussion, page 6, line 129, reference 11)

3. Figures and legends: Instead of stating the magnification of the lens, scale bars should be depicted in the photomicrographs.

In the revised version of the manuscripts scale bars are depicted in the microphotographs, and the magnification of the lens has been removed.

Also, they should indicate what color is used to designate CD68 vs. pSTAT1 reactivity.

The color used to designate CD68 vs. pSTAT1, CD8 vs.pStat1, and CD31 vs pSTAT1 reactivities is now indicated in the legends to Figures 1 and 3.

Finally, in Figure 1 C, not all the arrows are pointing to double positive cells.

We have changed the orientation of the triangles to point more specifically to double positive cells. As now described in the legend to Figure 1, in Figure 1 C triangles point to double positive cells, arrows to phospo-Stat1 positive cells, and guillemets to CD68 positive cells.
It would have been informative to know whether the positive sites also contained parasites/parasite antigens.

We did not mention in the text that tachyzoites were not present because in acute toxoplasma lymphadenitis of immunocompetent patients parasites are usually not found, whereas in immunosuppressed patients they can be occasionally observed in macrophage cytoplasm (Frenkel JK, Toxoplasmosis. In: Binford CH, Connor DH eds Pathology of Tropical and Extraordinary Diseases, Washington DC, Armed Forced Institute of Pathology, 1976; p.284-307; Pritt BS, Protozoal infections, Toxoplasmosis. In: Procop GW, Pritt BS, eds. Pathology of infectious diseases, 1st ed. Philadelphia, PA: Elsevier Saunders. 2015; p. 641-642. In our patient, lymph node sections stained with Pas and Giemsa techniques were examined at high magnification and tachyzoites were not observed.

Comments from Reviewer #2

1) Please clarify rationale for CD68 staining - as 'all macrophages express this' isn't a clear scientific fact that doesn't merit a reference. Yes, CD68 is a well-used histochemical marker but low levels have been found on other cell types (albeit low levels) which could impact the interpretation of this data set. The link of CD68/pSTAT1 co-staining needs to be solid to the reader. Essentially, the conclusion hinges on Figure 1C. Do you have control stained tissues to show alongside Figure 1C using your same protocol?

Although the CD68 antigen was found to be expressed at low levels by cells other than macrophages (fibroblasts and carcinoma cells) the antibody we used (anti-CD68, Dako, clone PG-M1) is widely considered the most specific marker for macrophages. These concepts are now reported in the text with the related references (Background, page 3, line 61, references 6 and 7). We have sent 3 microphotographs as supplementary files: a microphotograph showing that the anti-CD68 monoclonal antibody we used recognized mononuclear cells with macrophage morphology, and two microphotographs showing a positive control for the anti-phospho-Stat1
monoclonal antibody alone and a positive control for the combination of anti-CD68 and anti-phospho-Stat1 monoclonal antibodies in ulcerative colitis, as reported in reference 5.

We have not added these microphotographs to Figure 1 because in the revised manuscript a new figure (new Figure 2) showing iNOS expression and IFN-gamma expression support our findings but we are willing to add these microphotographs to Figure 1 if requested.

2) Can the authors better describe/reference methods (Line 85)? How many sections were screened? Can you insert the antibody clone in ()?

We have briefly described the staining methods, specified the number of sections screened and inserted the antibody clones in parenthesis (Case presentation, page 4, lines 86-95, page 5, lines 96-100).

3. Was it possible to look at circulating IFN-gamma in the patient, better describing TH1 polarization? Is this microenvironment specific?

We did not look at circulating IFN-gamma levels because this analysis is not routinely performed in our Center and the diagnosis of toxoplasmosis was performed in the late phase of the disease. In the revised manuscript we show that mononuclear cells scattered between microgranulomas and in germinal centers displayed IFN-gamma immunoreactivity in a new figure (Figure 2A), and report these data in the text (Case presentation, page 5, lines 113-114).

4. The method of detection in the abstract is missing and should be added for clarity.

We have added the method of detection to the abstract (Abstract, page 1, lines 18-19)

5) Reference text in line 44 is different than others.

We have corrected the reference format (Background, page 2, line 44)
We hope that in its present form the paper will be acceptable for publication in “BMC Infectious Diseases”, and thank you for your time and attention to our work.

Francesca Bianca Aiello