

## **The role of TNF- $\alpha$ in rheumatoid arthritis: a focus on regulatory T cells**

Mark Farrugia, Byron Baron

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Handling editor:

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Date: 8-Aug-2016

Ref.: Ms. No. JCTRes-D-16-00015

The role of TNF- $\alpha$  in Rheumatoid Arthritis: a focus on Regulatory T-Cells  
Journal of Clinical and Translational Research

Dear Dr Baron,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Sep 07, 2016.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

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Yours sincerely

Rowan van Golen  
Associate Editor  
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1:

In this review the authors discuss the role of Treg cells in RA, and the effects of anti-TNF therapy on Treg cells in RA which is an important and controversial topic. The following points should be addressed:

1. The figures would benefit from a descriptive legend
2. Reference number 29/32 is repeated twice
3. Treg cells include various types including FoxP3+ (so called nTreg/tTreg as well as peripherally induced FoxP3+ Treg cells) and FoxP3- Tregs such as IL-10 producing Tr1 cells. It is not always clear in the review what type of Treg cells are being referred to by 'Treg', and the review would benefit from more clarity on whether FoxP3+ or FoxP3- Tregs are being discussed.
4. Similarly, information on whether the studies discussed used Treg cells from PB or SF would add clarity to the review.
3. The recent study by Nguyen and Ehrenstein (JEM 2016) is relevant and should be discussed in the review.
3. Page 3, line 42/43: The authors state that 'in all scenarios, however, the lack of function of the Tregs themselves seem to be consistently observed'. I believe that this viewpoint does not accurately summarise the relevant literature. For example a recent very detailed and robust study has showed that there is no discernible defect in RA peripheral blood Treg cell function (Walter et al. Arthritis Rheumatol. 2016). These conflicting studies should be discussed together with possible reasons for the discrepancies between studies.

Reviewer #2:

“The role of TNF- $\alpha$  in Rheumatoid Arthritis: a focus on Regulatory T-Cells” – Review paper

General comments:

1. This is a review paper that concerns the effect of TNF in Treg cells in an RA setting. It is a focused literature review, however it is a little difficult to read.
  2. The authors should avoid using reviews as references in a review paper.
  3. To facilitate the reading ‘TNF- $\alpha$ ’ should be changed to ‘TNF’ and “Anti-TNF- $\alpha$  therapies” should be substituted by ‘TNF-blocker’ or ‘TNF blocking therapies’
  4. We understand that in the same study human and animal samples might be used. The authors should clarify in the text if a study/experiment they refer to is conducted: in
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humans/animals/cell lines; in vivo/in vitro; healthy/control/RA patients/RA model

5. The review organization and written English should be thoroughly revised. The use of expressions as 'bring about' should be avoided.

Title:

6. The use of capital letters should be consistent – I suggest removal of all capital letters (Should read: The role of TNF- $\alpha$  in rheumatoid arthritis: a focus on regulatory T-cells

Abstract:

7. English should be revised. Removal of 'bring about' and change 'medicinals' to therapies.

8. TNF-blockers are used systemically and are not specifically used 'to block the inflammation of the joints' – re-write.

Introduction

9. The sub-headings should include one about the TNF molecule itself with details of the structure of TNF, its processing and membrane bound expression/secretion and one about TNF blockers explaining the different used molecules and their modes of action - I suggest adding the first sub-heading (about the TNF molecule) after "Synovial Immunological processes" and the 'TNF blocking therapies' subheading before "anti-TNF therapy effects and prospects."

10. Pg 3, line 6 'rheumatoid factor' – add the acronym RF for further use (pg 6)

11. Pg3, line 10 should include a reference to the risk factors for RA development.

12. Although epidemiology is important it is more important to describe the signs and symptoms of the disease since further in the review there are sentences about bone and cartilage destruction and no previous reference to the mechanisms by which it occurs.

13. Last paragraph of pg 3:

a. Therapies should be by order of clinical use – I understand that different countries have different treatment regimens but it is unusual to see the corticosteroids in the end of a therapy list.

b. DMARDs include immunosuppressants, steroids and biological therapies – this should be clarified

c. IL-1 are not mentioned in the text but are mentioned in the figure.

14. Figure 1: Inhibitor arrows should be thicker to be more prominent

15. Figure 1 legend: typo in glucocorticoids (line 39, pg 4), English should be revised – line 40 'The rest of the possible therapies' is not proper scientific English

Synovial Immunological Processes

16. The title should have only the first letter as a capital letter

17. Pg 5, ln 7 'brought about' should be revised

18. Figure 2: Neutrophil action should be included in the figure

19. Figure 2 legend: review the use of 'bring about' (ln 51); ln 53 change 'regional' to 'local'; ln 54 the use of 'destruction of cartilage and bones' is too broad – The effect of RA in bone can be local or systemic, an earlier elucidation (in the introduction to RA paragraphs) of this process will make it clearer.

20. Pg 6, ln 22-24 – If you talk here about IL-1 cytokines, you need to introduce here IL-6 and IL-17 too

Role of T-cells in RA

21. The authors should explain briefly the difference between effector T cells and regulatory T cells here.

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22. Pg 6 ln42 – rheumatoid factor to RF
23. Pg 6 ln 45-47: Re write this paragraph in light of the comments above with the introductory paragraph about bone damage in RA. ‘OPG ligands’ is not the correct and current used name for RANKL and none was written before to introduce this concept. The implication and effects of inflammation in bone destruction should be clarified both local and systemically.
24. Pg6, ln 45 – joint damage does not occur only by T-cell activation and does not occur only in animal models of RA.
25. Pg7, ln 22 – reference is not formatted like others  
Effect of TNF on Treg function in RA
26. Pg 7 ln 40 – Clarify where the high levels of TNF were found, in the serum? Synovial fluid? In patients with therapy?
27. Reference to the study with infliximab might be reconsidered to be used here since the TNF blocking effects are used in the next chapter (I understand the importance of referring to the FoxP3+CD62L- cells here)
28. Pg7 ln 59 – refrain from saying ‘in the other study’ – write ‘in the previous study’ and add the reference (41 I believe)
29. Figure 3 should only be referenced after the authors talk about PkC and Dlg1 in the text (either in pg9, after line 42 or in the end of that subsection)
30. Pg 8, ln 59 – clarify in what setting this study was conducted (in humans/animals/cell lines; in vivo/in vitro; healthy/control/RA patients/RA model)
31. Pg 9 1st paragraph: This paragraph is confusing and it should be clarified from the beginning that this was study in human cells and clarify if they are normal or from RA patients - the last sentence (ln 9) should be backed up with the references to the ‘other studies’.
32. Pg 9 ln16 – ‘Addition of TNF increased...’ addition of TNF to what? Where? Animal model? Cell culture? Humans?
33. Pg 9 ln 38 - clarify in what setting this study was conducted (in humans/animals/cell lines; in vivo/in vitro; healthy/control/RA patients/RA model)
34. Pg9 ln 47 - clarify in what setting this study was conducted (in vivo/in vitro; healthy/control/ RA model) – ‘Activated mouse peripheral FoxP3+ Tregs’ is not enough. In ln 48 clarify if the co-cultures are from the same study.
35. Pg 9 ln 55 – refrain from repeating ‘this study’ change to ‘work’ so it is not repetitive.
36. Pg 9, ln 57 - clarify in what setting this study was conducted (cells from human/mouse; normal or RA?)

#### Anti-TNF therapy effects and prospects for the future

37. Title should only have the first capital letter reading “Anti-TNF therapy effects and prospects...” and should be changed to ‘future works’ or ‘further studies’
  38. If the author does not create a sub-heading with the different types of TNF blockers and their mode of action these should be described here, in the beginning of this sub-heading.
  39. Pg10 ln19 – remove ‘do’ – ‘studies show’.
  40. Pg10 ln24 – should read ‘monocyte membrane bound TNF’
  41. Pg 10, ln33 – do not use ‘quell’, use ‘inhibit’ or a more common synonym.
  42. Pg 10 paragraph ln32-37 – There are other side effects to the use of TNF blockers that are more common than the ones linked to autoimmunity – The author should comment on both kinds
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of side effects the ones related to immunosuppression and the ones related to auto-immunity.

43. Pg 10 paragraph ln 39-46 – This should be in a separate sub-heading (see comment 9) or in the end of the ‘synovial immunological processes’ sub-heading

44. Pg 10 ln 54-59:

a. These lines should be re-written – the adverse effects patients experience are not a result of the poorly understood system – the sentence is far-fetched and not appropriate for a review.

b. ‘It would be wise to look for alternate routes’ – should also be re-written in a more scientific way.

c. Ln 59 remove ‘is obtained’ – does not make sense with ‘whilst working to obtain a broader...’

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

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Authors’ rebuttal

Dear Editor,

Hereunder please find the list of changes made to the manuscript entitled “The role of TNF- $\alpha$  in rheumatoid arthritis: a focus on regulatory T-cells” following the suggestions of Reviewer 2.

General comments:

1. This is a review paper that concerns the effect of TNF in Treg cells in an RA setting. It is a focused literature review, however it is a little difficult to read.  
**We have made small amendments to the flow of the text and included the suggested corrections in order to make the review easier to read.**
2. The authors should avoid using reviews as references in a review paper.  
**Where reviews had been referenced, these were replaced by the original studies in question.**
3. To facilitate the reading ‘TNF- $\alpha$ ’ should be changed to ‘TNF’ and “Anti-TNF- $\alpha$  therapies” should be substituted by ‘TNF-blocker’ or ‘TNF blocking therapies’  
**We do not agree the removal of the alpha would improve the readability of the text since there are other TNFs apart from TNF- $\alpha$  and readers might get confused. Moreover “anti-TNF- $\alpha$  therapies” is a frequently used phrase in the literature, and thus we believe readers can follow better with the inclusion of the alpha.**
4. We understand that in the same study human and animal samples might be used. The authors should clarify in the text if a study/experiment they refer to is conducted: in humans/animals/cell lines; *in vivo/in vitro*; healthy/control/RA patients/RA model  
**We understand the importance of this point and have made adequate clarifications for the different studies quoted where these might have been missing.**
5. The review organization and written English should be thoroughly revised. The use of expressions as ‘bring about’ should be avoided.  
**The English syntax and phrasal verbs used in the text were re-evaluated and amended where necessary.**

Title:

6. The use of capital letters should be consistent – I suggest removal of all capital letters (Should read: The role of TNF- $\alpha$  in rheumatoid arthritis: a focus on regulatory T-cells  
**We have amended the title accordingly**

Abstract:

7. English should be revised. Removal of ‘bring about’ and change ‘medicinals’ to therapies.  
**Changed as suggested**
8. TNF-blockers are used systemically and are not specifically used ‘to block the inflammation of the joints’ – re-write.  
**Re-worded to make it clearer**

Introduction:

9. The sub-headings should include one about the TNF molecule itself with details of the structure of TNF, it’s processing and membrane bound expression/secretion and one about TNF blockers explaining the different used molecules and their modes of action - I suggest adding the first sub-heading (about the TNF molecule) after “Synovial Immunological processes” and the ‘TNF blocking therapies’ subheading before “anti-TNF therapy effects and prospects.”  
**In accordance with this request, a new subheading “TNF-  $\alpha$ ” was added in which the TNF- $\alpha$  structure and receptors were discussed. However, we do not believe a separate section for anti-TNF- $\alpha$  therapies is needed, but instead revised the section already present which dealt with these therapies.**
10. Pg 3, line 6 ‘rheumatoid factor’ – add the acronym RF for further use (pg 6)  
**Amended**
11. Pg3, line 10 should include a reference to the risk factors for RA development.  
**Amended**
12. Although epidemiology is important it is more important to describe the signs and symptoms of the disease since further in the review there are sentences about bone and cartilage destruction and no previous reference to the mechanisms by which it occurs.  
**To clarify this point and improve the link with later material, we added a paragraph prior to the epidemiology studies in which cartilage and bone damage by FLS, MCSF and RANKL is discussed**

Last paragraph of pg 3:

13. Therapies should be by order of clinical use – I understand that different countries have different treatment regimens but it is unusual to see the corticosteroids in the end of a therapy list.  
**Corticosteroids were originally included at the end because they were not a topic of relevance to the diagram but we thought they could be mentioned as a sideline. We have now removed them from this paragraph since they are not mentioned in the diagram.**
14. DMARDs include immunosuppressants, steroids and biological therapies – this should be clarified  
**This sentence has been re-written to make it clearer**
15. IL-1 are not mentioned in the text but are mentioned in the figure.  
**This has been changed to include IL-1 in the text**

16. Figure 1: Inhibitor arrows should be thicker to be more prominent  
**The inhibitor arrows have been doubled in thickness to make them more prominent**
17. Figure 1 legend: typo in glucocorticoids (line 39, pg 4), English should be revised – line 40 ‘The rest of the possible therapies’ is not proper scientific English  
**The sentence has been rewritten**

Synovial Immunological Processes:

18. The title should have only the first letter as a capital letter  
**Amended as requested**
19. Pg 5, ln 7 ‘brought about’ should be revised  
**Changed accordingly**
20. Figure 2: Neutrophil action should be included in the figure  
**We did not include this change since throughout this review our focus was on T-cells and neutrophils were only occasionally mentioned, thus we do not believe the addition of neutrophils is necessary or helpful to the focus of this review.**
21. Figure 2 legend: review the use of ‘bring about’ (ln 51); ln 53 change ‘regional’ to ‘local’; ln 54 the use of ‘destruction of cartilage and bones’ is too broad – The effect of RA in bone can be local or systemic, an earlier elucidation (in the introduction to RA paragraphs) of this process will make it clearer.  
**Replaced ‘bring about’ and made the necessary changes to the legend to improve clarity, together with the inclusion of the paragraph mentioned in pt 12.**
22. Pg 6, ln 22-24 – If you talk here about IL-1 cytokines, you need to introduce here IL-6 and IL-17 too  
**To avoid confusion, the focus on Il-1 was removed from this section.**

Role of T-cells in RA:

23. The authors should explain briefly the difference between effector T cells and regulatory T cells here.  
**We believe that, even without extensive prior knowledge, through reading our review the reader can easily distinguish the main differences between T effector cells and T regulatory cells and thus no further detailed explanation was included as we feel this would be beyond the scope of this review.**
24. Pg 6 ln42 – rheumatoid factor to RF  
**Amended accordingly**
25. Pg 6 ln 45-47: Re write this paragraph in light of the comments above with the introductory paragraph about bone damage in RA. ‘OPG ligands’ is not the correct and current used name for RANKL and none was written before to introduce this concept. The implication and effects of inflammation in bone destruction should be clarified both local and systemically.  
**Included the paragraph mentioned in pt 12 and substituted “OPG ligands” with RANKL to make this clearer.**
26. Pg6, ln 45 – joint damage does not occur only by T-cell activation and does not occur only in animal models of RA.

We never stated the above in our review. However this section has been re-written altogether anyway so it has now been made clear.

27. Pg7, ln 22 – reference is not formatted like others  
Amended to match

Effect of TNF on Treg function in RA:

28. Pg 7 ln 40 – Clarify where the high levels of TNF were found, in the serum? Synovial fluid? In patients with therapy?  
We have reworded this sentence to clarify
29. Reference to the study with infliximab might be reconsidered to be used here since the TNF blocking effects are used in the next chapter (I understand the importance of referring to the FoxP3+CD62L-cells here)  
Modified accordingly
30. Pg7 ln 59 – refrain from saying ‘in the other study’ – write ‘in the previous study’ and add the reference (41 I believe)  
Changed appropriately and included the reference
31. Figure 3 should only be referenced after the authors talk about PkC and Dlg1 in the text (either in pg9, after line 42 or in the end of that subsection)  
Changed such that Figure 3 is referenced at the end of the section
32. Pg 8, ln 59 – clarify in what setting this study was conducted (in humans/animals/cell lines; *in vivo/in vitro*; healthy/control/RA patients/RA model)  
Amended to include the setting
33. Pg 9 1<sup>st</sup> paragraph: This paragraph is confusing and it should be clarified from the beginning that this was study in human cells and clarify if they are normal or from RA patients - the last sentence (ln 9) should be backed up with the references to the ‘other studies’.  
Paragraph rewritten and clarified by additional references
34. Pg 9 ln16 – ‘Addition of TNF increased...’ addition of TNF to what? Where? Animal model? Cell culture? Humans?  
Clarified the sentence and added the setting
35. Pg 9 ln 38 - clarify in what setting this study was conducted (in humans/animals/cell lines; *in vivo/in vitro*; healthy/control/RA patients/RA model)  
Amended to include the setting
36. Pg9 ln 47 - clarify in what setting this study was conducted (*in vivo/in vitro*; healthy/control/ RA model) – ‘Activated mouse peripheral FoxP3+ Tregs’ is not enough. In ln 48 clarify if the co-cultures are from the same study.  
Amended to include the setting
37. Pg 9 ln 55 – refrain from repeating ‘this study’ change to ‘work’ so it is not repetitive.  
Reworded the sentence

38. Pg 9, Ln 57 - clarify in what setting this study was conducted (cells from human/mouse; normal or RA?)  
**Amended to include the setting**

Anti-TNF therapy effects and prospects for the future:

39. Title should only have the first capital letter reading “Anti-TNF therapy effects and prospects...” and should be changed to ‘future works’ or ‘further studies’  
**Changed accordingly**
40. If the author does not create a sub-heading with the different types of TNF blockers and their mode of action these should be described here, in the beginning of this sub-heading.  
**We believe that there is already enough literature focusing in detail on the different anti-TNF- $\alpha$  therapies available, and further focus in our review would be beyond our scope**
41. Pg10 ln19 – remove ‘do’ – ‘studies show’.  
**Amended accordingly**
42. Pg10 ln24 – should read ‘monocyte membrane bound TNF’  
**Changed as indicated**
43. Pg 10, ln33 – do not use ‘quell’, use ‘inhibit’ or a more common synonym.  
**Replaced as indicated**
44. Pg 10 paragraph ln32-37 – There are other side effects to the use of TNF blockers that are more common than the ones linked to autoimmunity – The author should comment on both kinds of side effects the ones related to immunosuppression and the ones related to auto-immunity.  
**We focused only on the side effects inducing auto-immunity since this emphasises the point that not understanding fully the entire mechanisms/pathways involved, might produce results which can seem to contradict what one would expect. Reviewing all major side effects of all therapies mentioned is beyond the scope of this review.**
45. Pg 10 paragraph ln 39-46 – This should be in a separate sub-heading (see comment 9) or in the end of the ‘synovial immunological processes’ sub-heading  
**Moved this paragraph to the end of the section “Effect of TNF- $\alpha$  on Treg function in RA”**
46. Pg 10 ln 54-59:  
These lines should be re-written – the adverse effects patients experience are not a result of the poorly understood system – the sentence is far-fetched and not appropriate for a review.  
**This sentence was written to make it clearer**
47. ‘It would be wise to look for alternate routes’ – should also be re-written in a more scientific way.  
**Reworded to fit the style**
48. Ln 59 remove ‘is obtained’ – does not make sense with ‘whilst working to obtain a broader...’  
**Removed as requested**

Editor's note:

To avoid confusion, an earlier version of manuscript with all modifications based on Reviewer 1' comments highlighted and/or marked with track changes was also sent to the reviewer for re-review.

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2<sup>nd</sup> editorial decision:

Date: 12-Sep-2016

Ref.: Ms. No. JCTRes-D-16-00015R1  
The role of TNF- $\alpha$  in rheumatoid arthritis: a focus on regulatory T-cells  
Journal of Clinical and Translational Research

Dear Dr Baron,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

The final comments from reviewers can be found below.

Thank you for submitting your work to JCTR.

Kindest regards,

Rowan van Golen  
Associate Editor  
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #1: The authors need to reference the revised figure legends to indicate the sources of information.

Reviewer #3: Thank you for addressing the majority of my comments