Format for ANSWERING REVIEWERS

July 28, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4489-review.doc).

Title: Decoy receptor 3 (DcR3): its role as a biomarker for chronic inflammatory diseases

Author: Spyros I. Siakavellas and Giorgos Bamias

Name of Journal: *World Journal of Immunology*

ESPS Manuscript NO: 4489

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 1:

The manuscript provides a comprehensive review and update on the role of DcR3, its role in chronic inflammation and whether levels in the serum could act as a biomarker for disease. There are few recent reviews on this topic therefore submission is timely. I have a few suggestions to strengthen the manuscript.

1. It is often unclear as to whether Dcr3 is protective or pathogenic in particular diseases e.g. prevents T cell apoptosis in activated cells but ameliorates T cell responses to alloantigens and decreases T cell activation – what is overall effect? DcR3 has a dual role in pathogenesis and this should be highlighted throughout.

Reply: we have added specific comments on the dual role of DcR3 in immunological responses

2. A diagram or summary table of whether DcR3 is pathogenic or protective and the references to this should be included. Also a table of the diseases in which serum levels of DcR3 is increased would strengthen the manuscript.

Reply: in the revised manuscript we have followed the reviewer's recommendations and added a Table and a Figure with relevant information.

3. Mouse and rat do not express DcR3 and the relevance of rodent studies should be discussed.

Reply: we have added specific comments

4. ancylosing spondylitis should read ankylosing spondylitis

Reply: this was corrected

Reviewer 2:

The manuscript by Siakavellas and Bamias reviews the current knowledge on DcR3, a decoy receptor for FasL, LIGHT and TL1A. They describe the immune modulatory functions of DcR3 and its possible relevance as biomarker in inflammatory diseases. The manuscript is well written and the paper is the first to give a good summary of current available literature with respect to DcR3 in inflammatory diseases. The review could be improved by the

a. addition of the following tables and figure: a. Table with cells expressing DcR3;

b. Table with normal serum DcR3 concentration in serum/plasma versus DcR3 concentrations in different diseases;

c. Figure depicting the diverse biological activities of DcR3 (ligand binding, target cells, immune modulatory effects, etc).

Reply: in the revised manuscript we have followed the reviewer's recommendations and added a Table and a Figure with relevant information.

Reviewer 3:

Spyros I. Siakavellas et.al. summarized DCR3 well in this review paper. However, it might be more helpful to readers if they can put some figures/tables on the paper.

For example, using tables, they can summarize expression levels of DCR3 in tissues or cells and functions based on cell types.

Furthermore, serum levels of DCR3 in various disease could be summarized in a figure.

The sensitivity and specificity of diagnostic tool detecting DCR3 in the sera for prediction of prognosis & diagnosis could be summarized in a table. Finally, mode of action of DCR3 could be schematically depicted in a figure. That is, inhibition of ligand-receptor interaction, effects on downstream signalling, effects on expression of effector molecules.

Reply: in the revised manuscript we have followed the reviewer's recommendations and added a Table and a Figure with relevant information.

Reviewer 4:

The manuscript by Spyros I. Siakavellas and Giorgos Bamias reviews the role of Decoy receptor 3 (DcR3, alternative names TR6 or M68) in several inflammatory conditions. There is good description of the levels of expression of DcR3 in several pathologies and the implications of this are discussed. The potential use of this molecule as a biomarker during infection and inflammation is also discussed. The manuscript is organized clearly and is also well written.

However, the lack of tables to summarize some relevant data and figures to make important points clearer makes the reading of this review tiresome. Tables indicating the different levels of expression in different tissues and/or diseases would be helpful.

Also some figures on the normal role/function of DcR3 and on the altered function in disease will also be very helpful to the readers. DcR3 can be beneficial or detrimental in different conditions. This dual role in pathogenesis should be discussed further and the relevant references clearly indicated.

Reply: in the revised manuscript we have followed the reviewer's recommendations and added a Table and a Figure with relevant information.

Reviewer 5:

To Authors The authors provide a review of the literature on DcR3 relevant to chronic inflammatory diseases as it is believed that there has been less emphasis on this than its role in cancer. The two aspects elaborated on are its role in pathogenesis and its immunomodulatory role. There appears to be inconsistencies in the literature regarding the pathophysiological vs beneficial roles of DcR3, making it difficult to determine its precise role. The field is further complicated because of the question as to whether the increased level in serum/fluids is the cause or consequence of the inflammatory conditions? Its potential use as a diagnostic/prognostic marker is highlighted by the review.

The review may need reconstruction to prevent repletion of the varying characteristics of DcR3 with each disease and might be better dealt with as the different role and bringing the various inflammatory conditions under such headings. While some aspects appeared to be well written other showed clarity and English expression. I have made specific statement below.

Reply: we thank the reviewer. We believe, however, that an effort to present the available data on DcR3 under general, function-based subheadings would lead to a confusing presentation as many areas are still unclear. Therefore, we kept the disease-centered approach. In our revised manuscript we have made several modifications as suggested by the reviewer (see below our point-to-point respone).

Title; The most convincing data relates to its use as biomarker for inflammatory conditions. This is not evident in the title.

Reply: we thank the reviewer. We have modified the title of our manuscript to emphasize the potential role of DcR3 as a biomarker.

Abstract Page 2 – Line 6: it cannot be said to induce anti-apoptotic effects. I think it stops the signals for apoptosis from being initiated?

Reply: we thank the reviewer. We have corrected accordingly.

Page 2, Line 16 / What does 'usually' mean

Reply: the word usually has been deleted

Line 17; what about normal people?

Reply: a sentence has been added

Page 3 Line 1: 'factor' or 'marker?

Reply: we replaced "factor" with "marker"

Background Page 3, line 6; rewrite t ' with osteoprotegerin, in that they lack a transmembrane region…"

Reply: the sentence has been modified

Page 3 Line 14: "In contrast" is not to be needed. Has the anti-inflammatory/Biomarkers effects only recently been appreciated? Should mention some of the major diseases that are relevant to DcR3 at the end of the Introduction.

Reply: the paragraph has been modified

Line 15: Can you refer to DcR3 is an "immunological-mediator"? The review has no section to a detailed discussion on its structure. I think that this would be useful.

Reply: We thank the reviewer. The possible role of DcR3 as a mediator of pathogenic immunological phenomena is presented in the individual diseases sections.

Page 4: Weak expression in tissues is this because it is a secreted protein?

Reply: We thank the reviewer. Although this is a possible explanation there cannot be a

definitive statement about it

Page 4, Line 15: Change "was" to were"

Reply: we replaced "was" with "were"

Page 4, Line 14-16: rewrite this sentence. Do you mean that the findings vary according experimental system and from cell type to cell type?

Reply: we have changed the text accordingly

Page 4 Line 18: "α" to "x"

Reply: we do not understand the comment

Page 4Line 19: Which TLRs?

Reply: we have added the information

Page 4 Lines 18 – 21. This sentence is not clear – rewrite And is the section of DcR3 induced by engagement of TLRs dependent on ERK1/2, JNK, Src-like protein kinases, PI3K, NFkB? Otherwise what is the point of adding this aspect?

Page 4, Last sentence: Do you discuss what this actually means?

Reply: we apologize for the confusion that arose from this paragraph. We have re-written it and presented it with more clarity.

Page 5, First paragraph: "Is this an in introduction to discussing the regulation of DcR3? I found this to abruptly come into the review and wonder whether regulation of functions should be a separate section.

Reply: in the revised manuscript we have separated the sections of "regulation of expression" and "function".

Line 4 Define HVEN

Reply: we have corrected accordingly

Line 15 disease severity?

Reply: we do not understand the comment

Line 8 ankylosing spondylitis (AS)

Reply: we have changed the text accordingly

Last sentence re-write "↑Although DcR3 regulation of cell death has been primarily emphasised for neoplastic conditions, it also has relevance to regulating pathogenesis…"Indeed inhibition of apoptosis of leukocytes may lead to perpetuation of chronic tissue injury.'

Reply: we have changed the text accordingly

Page 6: Please give supporting references to statements made in the first paragraph.

Reply: we have added the relevant references

Line 8-9: I do not understand this statement why is it "on the other hand …." Doesn't immunomodulatory refer also to what you are saying in the first paragraph? Define what you mean by immunomodulatory as you continue to use it?

Reply: we agree with the reviewer. We have re-wrote the sentence and state that immunomodulatory function also includes inhibition of apoptosis

Line 18: "Nevertheless" does not seem an appropriate word here. You say "There is also recent evidence".

Reply: we have changed the text accordingly

Page 7, Line 4: Delete "inflammatory" and change to "homing of cells to inflammatory foci".

Reply: Reply: we have changed the text accordingly

Line 5: Replace "Taken together" with "collectively". Delete "may" change to "exerts". Change also "multiple" to 'pleotropic'.

Reply: we have changed the text accordingly

Line 7: Change “highly upregulated” to detectable and/or highly elevated in a

Reply: we have changed the text accordingly

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Immunology*.

Sincerely yours,

Giorgos Bamias, MD,
Consultant in Gastroenterology,
Academic Department of Gastroenterology,
Ethnikon and Kapodistriakon University,
Laikon Hospital,
17 Agiou Thoma Str., 11527,
Athens, Greece.
email: gbamias@gmail.com
Tel: +30-210-7456504
Fax: +30-210-7791839