

## Format for ANSWERING REVIEWERS

May 6, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO 10362-edited.docx).

**Title:** CD28/CTLA-4/B7 and CD40/CD40L costimulation and activation of regulatory T cells

**Author:** Vogel Isabel T., Van Gool Stefaan W., Ceuppens Jan L.

**Name of Journal:** *World Journal of Immunology*

**ESPS Manuscript NO:** 10362

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

### Reviewer 1

The manuscript provides an excellent review on the mechanisms of the suppressive activity of Treg cells, and the regulation of Treg/T effector balance via the co-stimulatory CD28/CTLA-4/B7 and CD40/CD40L interactions. In addition, the authors provide valuable insights on the differential effects of blocking CD28/CTLA-4/B7 in relation to strength of the inhibition and the stage of the immune process. This is a thoroughly readable, enjoyable, and stimulating manuscript and highly recommended to readers interested in Treg cells and immunological tolerance induction, both in the preclinical and clinical perspectives. Raised below are just minor points, mainly concerning some slip-ups in English:

1) Should "several data" in Core Tip and Line 12 of Introduction be changed into "several studies"?

**This has been changed.**

2) Line 22 of the next page of 2.1.1 where "divalent" should be "divalently" or "bivalently", and "monovalent" "monovalently".

**This has been changed**

3) Line 9 of 2.2: "So called" should be "the so-called"?

**This has been changed**

4) Line 11 of 3.2 "aglycosilated" and Line 13 "thomboembolic" are misspellings.

**This has been changed**



5) Line 3 of 3.3: it is not clear what the "alternative mechanisms of activation" refers to. Is it referring to the mechanisms mentioned in Section 3.4?

Alternative mechanisms of activation mean that in the absence of CD40L or CD28 triggering, the T cell might depend on activation signals coming from other costimulatory signals. E.g. if CD28 is absent or blocked, CD40L and/or ICOS triggering is in some situations sufficient to activate T cells (Howland et al. 2000 J Immunol/Kopf et al. 2000 J Exp Med).

This has been re-phrased in the text.

## Reviewer 2

The paper reviews an important issue concerning the regulation of effector and regulatory cells through costimulatory signals. The authors give us a precise review of current understandings on this subject. I will suggest some minor points to improve the quality of the paper:

- the title could be more precise and restrict to B7 and CD40 molecules

**"CD28/CTLA-4/B7 and CD40/CD40L costimulation and activation of regulatory T cells"**

- a brief summary of B7 family could be included

CD28, CTLA-4 and B7 are already extensively discussed in section 2.1.1. A summary of other members of the B7 and the CD28 superfamily has been included (section 2.1.2).

- since both molecules belong to TNF and TNFR families, other member could be included partially to summary difference and similarity between them in terms of regulation and effector of immune response

A summary of other members of the TNF and TNFR families has been added (section 2.1.4).

- new function for IDO following crosslink with CTLA-4 could be added

The possibility that the binding of CTLA-4 expressed on Treg cells to B7 receptors triggers IDO production has been discussed in section 2.2. Also, the production of IDO as one of the potential mechanisms of CTLA-4Ig mediated immune suppression has been discussed in section 3.4.

- difference in thymus and periphery and in induced and natural Tregs could be more detailed and received and special attention for readers

The difference between thymus derived (tTreg) and induced Treg (iTreg) has been described in section 2.2. We have also summarized what is known about the differential requirements of tTreg versus iTreg regarding costimulation in section 4.1. However, it is difficult to examine the effect of CD28/CTLA-4/B7 and/or CD40/CD40L blockade on tTreg versus iTreg as there is no reliable marker to differentiate between the two populations. Therefore, only very limited data are available on the effect of costimulation blockade on the two Treg cell types.

- finally, many typing errors appear throughout the text.

This has been corrected.

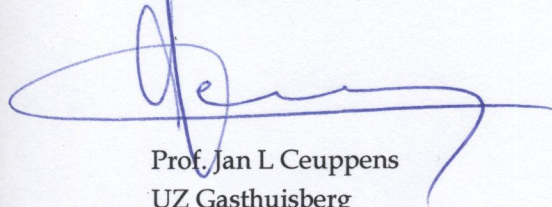


Reviewer 3

This is a nice review that talks about the specific role of costimulatory signals and their differential effects on blocking effector T vs. regulatory T (Treg) cells. The authors suggest that blocking CD40/CD40L pathway may provide a target to manipulate effector vs. regulatory T cell balance which may ultimately favor Treg activity. They also talk about other relevant factors that may affect these T cell subpopulations in the host. The information provided should help further understanding of the role of costimulatory signals in differential regulation of regulatory T cells in disease situations.

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

Sincerely yours,



Prof. Jan L Ceuppens  
UZ Gasthuisberg  
Laboratory of Clinical Immunology (bus 811)  
Herestraat 49  
3000 Leuven  
Belgium  
Phone: +32 (0)16 343804  
E-mail: jan.ceuppens@uzleuven.be