European Journal of Immunology

Supporting Information

for

DOI 10.1002/eji.201445284

Romain Vuillefroy de Silly, Laura Ducimetière, Céline Yacoub Maroun, Pierre-Yves Dietrich, Madiha Derouazi and Paul R. Walker

Phenotypic switch of CD8$^+$ T cells reactivated under hypoxia toward IL-10 secreting, poorly proliferative effector cells

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Vuillefroy de Silly, Romain; Ducimetière, Laura; Yacoub Maroun, Céline; Dietrich, Pierre-Yves; Derouazi, Madiha; Walker, Paul

Correspondence: Dr. Paul Walker, 24 rue Micheli-du-Crest, Geneva 14, CH-1211, Switzerland

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Review Timeline:

Submission date:	23-Oct-2014
First Editorial decision:	08-Dec-2014
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Second Revision received:	13-Apr-2015
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Handling Executive Committee member: Dr. Steffen Jung

Please note that the correspondence below does not include the standard editorial instructions regarding preparation and submission of revised manuscripts, only the scientific revisions requested and addressed.

First Editorial Decision - 08-Dec-2014

Dear Dr. Walker,

Please accept my sincere apologies for the prolonged delay in processing the review of your manuscript ID eji.201445284 entitled "Phenotypic switch of CD8+ T cells reactivated under hypoxia towards IL-10 secreting, poorly proliferative effector cells." which you submitted to the European Journal of Immunology. There was a severely delayed report and a difference in opinion for which we sought additional assessment from the Executive Editor.

The comments of the referees are included at the bottom of this letter. Even though referee #1 thinks that the study does not provide any mechanistic or biological significance and suggests rejection, the other two referees have suggested major revisions, and the Executive editor would like to see a revised version of your manuscript that takes into account all the comments of the referees. The revised manuscript will be reconsidered for publication.

European Journal of Immunology

You should also pay close attention to the editorial comments included below. **In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Failure to do this will result in delays in the re-review process.**

Please note that submitting a revision of your manuscript does not guarantee eventual acceptance, and that your revision will be re-reviewed by the referees before a decision is rendered.

If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referee(s) to ensure the relevance and timeliness of the data.

Once again, thank you for submitting your manuscript to European Journal of Immunology and we look forward to receiving your revision.

Yours sincerely,
Laura Soto Vazquez

On behalf of
Dr. Steffen Jung

Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu
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Reviewer: 1

Comments to the Author
Most in vitro cell culture is conducted at ambient oxygen tension; however, cells in the body never experience such a “hyperoxic” environment. Since oxygen levels have been reported to critically affect immune responses, it is quite understandable that the regular cell culture condition in ambient oxygen tension may be artificial to the cells. In this study, the authors defined 5% oxygen, the average concentration expected in peripheral blood, as “normoxic” to the cells, and they examined functional changes in CD8+ T cells when cultured at more physiological 5% oxygen instead of the regular 21% oxygen. CD8+ T cell activation at 5% oxygen enhanced cytotoxicity as well as the expression of activation-related cell surface

markers. Culture in hypoxic atmosphere strongly induced IL-10 production from CTLs.

The cell culture system used in this study is closer to physiological conditions in terms of oxygen levels, but it is still different from in vivo conditions in many ways. Although this in vitro system should provide very useful information, it will be important to validate the observation in vivo. For instance, hypoxic induction of IL-10-producing CD8+ T cells and its biological significance are yet to be determined in vivo. In addition, relatively small but significant changes in mRNA levels of diverse molecules have been noted, but the analysis lacks depth. In its current form, the manuscript remains descriptive because these observations were not extended to study in vivo significance or mechanisms.

Minor comments:

The term “normoxia” has been used often to describe oxygen levels in the atmosphere at sea level (21% oxygen). The authors define it as tissue oxygen levels that can be found in animals breathing normoxic atmosphere. However, it is still confusing whether “normoxia” means 5% or 21% oxygen. Different names are recommended for clarity.

In this experiment, hypoxia suppressed cell proliferation but not cytotoxicity. However, the duration of hypoxic exposure in the cytotoxicity assay is only 4 h, while the difference in cell number became significant after 3 days. How will cytotoxicity of CTLs look after culturing in the hypoxic atmosphere for 3 days?

Reviewer: 2

Comments to the Author
The manuscript by Vuillefroy de Silly and colleagues examines the effects of various oxygen tensions on CD8+ T cell function in vitro. They rightfully point out the physiologic oxygen levels in secondary lymphoid structures in vivo is closer to 5% than ambient 21%. They also compare to hypoxic conditions of 1% O2. They find priming of CD8+ T cells in vitro under 5% O2 yields a modest increase in the effector phenotype. However, stimulating T cells under 1% O2 conditions gives rise to high IL-10-producing T cells. These are interesting studies that have implications for CD8+ T cell function within the tumor microenvironment, which is expected to be hypoxic. However, the paper should go farther and actually test this notion.

Specific comments:
1. Some of the older literature on this topic should be cited. For example, MacDonald et al. JEM 1977 and Cham et al EJI 2008.

2. All the experiments have been performed in vitro. However, it would be very straightforward to test relevance for T cell function within the tumor microenvironment in vitro. Flow cytometric analysis and functional testing of CD8+ T cells infiltrating tumors could be performed, to determine the expression of 4-1BB and CD25. That subset could also be sorted and stimulated to determine whether IL-10 is being produced.

3. Since Pmel TCR Tg T cells were used for the in vitro studies, it also would be straightforward to see whether T cells primed under 21%, 5%, or 1% O2 are superior at rejection of B16 melanoma tumors after adoptive transfer in vivo. The implication is that the high IL-10 production by T cells primed under hypoxia should render inferior tumor control in vivo.

4. The Discussion should be shortened given the amount of data presented. In fact, it would be less speculative if the above experiments were included.

Reviewer: 3

Comments to the Author
In this manuscript, Vuillefroy de Silly et al. explore the role of physiologic oxygen tensions on differentiation of effector CD8+ T cells. They demonstrate through in vitro activation and restimulation experiments that CD8+ T cells experiencing physiologic oxygen tensions (5% O2) exhibit alterations to their expression of a wide range of effector and memory molecules in comparison to CD8+ T cells in atmospheric oxygen tensions (21% O2). They also conclude that CD8+ T cells activated in 5% O2 that then experience 1% O2 exhibit few differences upon reactivation in the lower oxygen tension. We agree with the authors on the importance of recapitulating in vivo conditions while performing in vitro experiments and agree that determining the impact of physiologic O2 concentrations will be essential for understanding the differentiation of CD8+ T cells during infectious responses. However, we have a fundamental concern with the conclusions drawn regarding the second major conclusion of this manuscript.

Our primary concern is the conclusions drawn regarding the impact of hypoxia on reactivation of CTL. The authors show that while large differences can be seen in terms of genes upregulated and downregulated when comparing reactivation in 21% O2 to 1% O2, many of these changes are lost upon initial activation in 5% O2 and reactivation in 1% O2. We agree that examining gene expression changes in the more physiologically relevant scenario of activation at 5% O2 and reactivation at 5%/1% O2 is necessary to truly identify genes that are impacted by oxygen tension on T cell differentiation, however at 48 hrs post activation, when the authors perform their gene expression analysis, it is difficult to parse out the impact of oxygen tension from TCR induced stabilization of HIFs. It has been shown by several groups that within the

first 60 hrs, HIFs can be stabilized through TCR engagement regardless of oxygen tension, providing an additional variable to consider when analyzing the lack of gene expression changes seen in their data (Wang et al. Immunity 2011, Finlay et al J Exp Med 2012, Doedens et al. Nat Immunology 2013). Parsing out oxygen dependent and TCR dependent alterations in gene expression at the oxygen tensions tested will be necessary to truly conclude whether physiologic oxygen tensions make little difference in T cell differentiation and function. Similar analysis should be performed with and without TCR restimulation and attention to changes in expression in the first 24/48 are also relevant but not measured in many cases. HIF protein levels for each condition should be shown to understand relevant comparisons.

Minor points:

- The inconsistent use of normoxia, physiologic normoxia, and atmospheric O2 is confusing at times.

- In Figure 1D it would be useful to have an comparison of naive CD8 T cells for the CD127 staining as activation of CD8 T cells drives downregulation of CD127 and it may be more relevant than comparing to isotype control in this situation.

- As previous papers have compared early phenotypic changes (phenotype before restimulation) of CTL it would be interesting to see how activation in 5% O2 is driving dramatic changes in differentiation of CTL. Additional phenotyping in Figure 1 before the CTL have rested would be interesting.

- Protein validation of changes the authors see in gene expression would be informative.

- Consistent usage of either Tnfrsf9 or 4-1BB and CD137 would be helpful.

First Revision – authors' response - 08-Mar-2015

Replies to Reviewers' comments.

Reviewer: 1

The cell culture system used in this study is closer to physiological conditions in terms of oxygen levels, but it is still different from in vivo conditions in many ways.

----------REPLY----------

We agree that in vivo conditions cannot be fully recapitulated in vitro. However, we believe that using physiological oxygen fraction better mimics what could happen in vivo, and is still more appropriate in order to extrapolate in vivo from in vitro data.

Although this in vitro system should provide very useful information, it will be important to validate the observation in vivo. For instance, hypoxic induction of IL-10-producing CD8+ T cells and its biological significance are yet to be determined in vivo. In addition, relatively small but significant changes in mRNA levels of diverse molecules have been noted, but the analysis lacks depth. In its current form, the manuscript remains descriptive because these observations were not extended to study in vivo significance or mechanisms.

----------REPLY----------

The reviewer rightfully highlights the importance of confirming our data in vivo. Therefore, we included an in vivo/ex vivo part (now Fig. 4) that strengthen the fact that CTLs overexpress IL-10, CD25 and CD137 under low oxygen fractions and that it happens in vivo. Indeed, we purified CD8+ TILs from EG.7-OVA –bearing mice (that expressed more hif2a than CD8+ T cells from the spleen), and we show that they express IL-10 (il10 mRNA presence in CD8+ TILs was not due to contaminating cells (Supporting Information Fig. 8C)), CD25 and CD137, and that expression of these molecules is enhanced when they are reactivated ex vivo under low oxygen fractions, while their expansion was limited.

The biological significance of IL-10 produced by CTLs in vivo is a major issue that warrants further study and that is prioritized as the next step of our work. However, we believe this should be the subject of another publication.

Minor comments:

The term “normoxia” has been used often to describe oxygen levels in the atmosphere at sea level (21% oxygen). The authors define it as tissue oxygen levels that can be found in animals breathing normoxic atmosphere. However, it is still confusing whether “normoxia” means 5% or 21% oxygen. Different names are recommended for clarity.

----------REPLY----------

In order to clarify the conditions, we used the term “physioxia” for what we consider as “tissue normoxia”/”physiological normoxia” (that we modeled as 5% O2). This term has already been used in literature (Carreau et al., J Cell Mol Med 2011).

In this experiment, hypoxia suppressed cell proliferation but not cytotoxicity. However, the duration of hypoxic exposure in the cytotoxicity assay is only 4 h, while the difference in cell number became significant after 3 days. How will cytotoxicity of CTLs look after culturing in the hypoxic atmosphere for 3 days?

----------REPLY----------

This remark is very relevant, as hypoxia could impact CTLs cytotoxicity in a chronic way, and as the assay only lasts 4h. As suggested, we preconditioned CTLs for 3 days under 21%, 5 and 1% O2 and then looked at their lysis capacities (now added in Fig. 1D). Of interest, CTLs that were generated under 21% and preconditioned under 1% showed increased cytotoxicity (either from OT-I or Pmel-1). However, when CTLs were generated under 5% O2, hypoxia conditioning had no impact. We also looked at Granzyme B content by flow cytometry and did not observe any modification when CTLs were preconditioned under hypoxia (Supporting Information Fig. 2F-G). Nevertheless, confirming our in vitro data showing an increase of the effector profile and in the killing capacities of CTLs generated under 5%, GrB was increased in CTLs generated under 5% as compared to those generated under 21%. We also looked at Perforin expression by flow cytometry, but the staining profiles were not clear enough to support any conclusion.

Reviewer: 2

1. Some of the older literature on this topic should be cited. For example, MacDonald et al. JEM 1977 and Cham et al EJI 2008.

----------REPLY----------

As MacDonald et al and Cham et al show that low oxygen concentration does not modify cytotoxicity, they are now cited in the Introduction after the following sentence: “The impact of hypoxia and oxygen tensions on CD8+ T cells is restricted to a few interesting studies” and in the Discussion: “In agreement with previous studies (citation), we observed that hypoxia did not modify CTL killing capacities in a short term assay, regardless of the oxygen tension used for CTL generation”.

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2. All the experiments have been performed in vitro. However, it would be very straightforward to test relevance for T cell function within the tumor microenvironment in vitro. Flow cytometric analysis and functional testing of CD8+ T cells infiltrating tumors could be performed, to determine the expression of 4-1BB and CD25. That subset could also be sorted and stimulated to determine whether IL-10 is being produced.

----------REPLY----------

The reviewer is entirely right, as confirming our data in vivo would strengthen our study and would prove that IL-10 production is not an artifact of CTL generation and reactivation in vitro. As advised, we purified CD8+ TILs from E.G7-OVA –bearing mice and compared them to CD8+ from their spleen (subcutaneous tumors are expected to contain less oxygen that spleen). We confirmed that CD8+TILs were expressing more IL-10, CD25 and CD137 than CD8+T cells from the spleen (now Figure 4; we also observed more hif2a).As observed in scatter graphs showing correlation between CD8+ purity and il10 expression (Supporting Information Fig. 8C), il10 mRNA presence in CD8+TILs was not due to contaminating cells. Finally, we restimulated these cells ex vivo with antiCD3/CD28 -coated beads and observed that expression of all these molecules was negatively correlated to oxygen fraction.

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3. Since Pmel TCR Tg T cells were used for the in vitro studies, it also would be straightforward to see whether T cells primed under 21%, 5%, or 1% O2 are superior at rejection of B16 melanoma tumors after adoptive transfer in vivo. The implication is that the high IL-10 production by T cells primed under hypoxia should render inferior tumor control in vivo.

----------REPLY----------

The impact of IL-10 produced by CTLs is a major issue. However, as we describe in our manuscript, oxygen fraction during the priming of CD8+ T cells does not predict whether they will release more IL-10 in vivo; rather, we show that IL-10 secretion is dependent on oxygen fraction during reactivation of already primed CTLs. Also, priming of CD8+ T cell under 1% does not yield sufficient number of T cells. Even if the impact of IL-10 produced by CTLs is of importance, we think that it should be the subject of further studies using different approaches.

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4. The Discussion should be shortened given the amount of data presented. In fact, it would be less speculative if the above experiments were included.

----------REPLY----------

As we added a significant number of experiments in the manuscript, we believe that the discussion is less speculative and reflects the quantity of data obtained.

European Journal of Immunology

Reviewer: 3

Our primary concern is the conclusions drawn regarding the impact of hypoxia on reactivation of CTL. The authors show that while large differences can be seen in terms of genes upregulated and downregulated when comparing reactivation in 21% O2 to 1% O2, many of these changes are lost upon initial activation in 5% O2 and reactivation in 1% O2. We agree that examining gene expression changes in the more physiologically relevant scenario of activation at 5% O2 and reactivation at 5%/1% O2 is necessary to truly identify genes that are impacted by oxygen tension on T cell differentiation, however at 48 hrs post activation, when the authors perform their gene expression analysis, it is difficult to parse out the impact of oxygen tension from TCR induced stabilization of HIFs. It has been shown by several groups that within the first 60 hrs, HIFs can be stabilized through TCR engagement regardless of oxygen tension, providing an additional variable to consider when analyzing the lack of gene expression changes seen in their data (Wang et al. Immunity 2011, Finlay et al J Exp Med 2012, Doedens et al. Nat Immunology 2013). Parsing out oxygen dependent and TCR dependent alterations in gene expression at the oxygen tensions tested will be necessary to truly conclude whether physiologic oxygen tensions make little difference in T cell differentiation and function. Similar analysis should be performed with and without TCR restimulation and attention to changes in expression in the first 24/48 are also relevant but not measured in many cases.

----------REPLY----------

The reviewer highlights an important point: whether il10, cd25 and tnfrsf9 are only induced by hypoxia itself, or through a combination of TCR stimulation plus hypoxia. As advised we analyzed the same panel of genes on CD8+ T cells that were left in culture for 48h without activation (Supporting information Fig. 6). Interestingly, il10 was not expressed, while cd25 was not modulated. tnfrsf9 was not modulated when comparing 21to1% vs 21to21%, while it was when comparing 5to1 vs 5to5%. However, at the protein level, when we used CD8+ TILs purified from EG.7-OVA –bearing mice that were left in culture for three days without reactivation, we did not observe any modulation of this molecule at the protein level under 1% oxygen (Fig.4 D); the same observation was done for CD25 and IL-10 (Fig.4 D-E). As such, all the molecules that were modulated by reactivated CTLs under hypoxia seem to be overexpressed by a mechanism involving a combination of TCR activation plus hypoxia.

HIF protein levels for each condition should be shown to understand relevant comparisons.

----------REPLY----------

As HIF-1a is described to be a major regulator to hypoxia response, we performed Western blot (Supporting Information Fig.3). Interestingly, while at 24h post reactivation HIF-1a levels were correlated negatively to oxygen fractions, its level seemed to decrease at 48h under 5 and 1% of oxygen, while it started to be

stabilized under 21% of oxygen (and even more stabilized than under 5 and 1%); thus confirming previous studies that HIF-1a is involved in TCR signaling.

Minor points:

- The inconsistent use of normoxia, physiologic normoxia, and atmospheric O2 is confusing at times.

----------REPLY----------

As advised, we now use consistently the same terms all throughout the manuscript. In order to avoid confusion, physiological normoxia (5% O2) has now been replaced by “physioxia”; a term that has already been used in literature (Carreau et al., J Cell Mol Med 2011).

- In Figure 1D it would be useful to have an comparison of naive CD8 T cells for the CD127 staining as activation of CD8 T cells drives downregulation of CD127 and it may be more relevant than comparing to isotype control in this situation.

----------REPLY----------

As advised, we performed a staining on naïve CD8+ T cells, but also at day 3 post priming. This has been done for Pmel-1 and OT-I CD8+ T cells and is now included in the manuscript (Supporting Information Fig.2A and C).

- As previous papers have compared early phenotypic changes (phenotype before restimulation) of CTL it would be interesting to see how activation in 5% O2 is driving dramatic changes in differentiation of CTL. Additional phenotyping in Figure 1 before the CTL have rested would be interesting.

----------REPLY----------

This is indeed of interest. We thus did a time course during generation of CTLs at day 0, day 3 and day 6 for the CD44+ CD62L- fraction and for CD127 expression on Pmel-1 and OT-I CD8+ T cells (Fig.1C and Supporting Information 2A-C).

- Protein validation of changes the authors see in gene expression would be informative.

----------REPLY----------

We agree that protein validation would be of interest. However, the panel of genes analyzed was composed of more than 40 different genes. We thus decided to look at the two specific genes that were modulated between CTLs reactivated under 5to1% vs 5to5%: i.e. Bcl-2 and HIF-2a. Bcl-2 was analyzed by flow cytometry (Supporting Information Fig. 5G). Whereas we confirmed that it was upregulated at 21to1% vs 21to21%, we did not confirm the upregulation that we observed at the RNA level at 5to1% vs 5to5%. Unfortunately, despite efforts, we were not able to detect any specific band by Western blot for HIF-2a using 2 commercially available antibodies.

- Consistent usage of either Tnfrsf9 or 4-1BB and CD137 would be helpful.

----------REPLY----------

We agree that we did not use consistently CD137 or 4-1BB. So the protein is now consistently called CD137. However, as tnfrsf9 is the correct gene nomenclature, we retain this for the RNA in the manuscript.

Second Editorial Decision - 02-Apr-2015

Dear Dr. Walker,

Thank you for submitting your revised manuscript ID eji.201445284.R1 entitled "Phenotypic switch of CD8+ T cells reactivated under hypoxia towards IL-10 secreting, poorly proliferative effector cells." to the European Journal of Immunology.

Your manuscript has been re-reviewed and the comments of the referees are included at the bottom of this letter. Although the referees have recommended publication, some revisions to your manuscript have been requested by Ref#1. Therefore, I invite you to respond to the comments of the referee and revise your manuscript accordingly.

You should also pay close attention to the editorial comments included below.

If the revision of the paper is expected to take more than three months, please inform the editorial office. Revisions taking longer than six months may be assessed by new referee(s) to ensure the relevance and timeliness of the data.

European Journal of Immunology

Once again, thank you for submitting your manuscript to European Journal of Immunology. We look forward to receiving your revision.

Yours sincerely,
Laura Soto Vazquez

on behalf of
Dr. Steffen Jung

Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
www.eji-journal.eu

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Reviewer: 1

Comments to the Author
The manuscript has been revised vigorously. Cytotoxicity assay after chronic exposure to lower oxygen tension revealed an augmentation of cytotoxic activity (Fig. 1D) corresponding to the cytokine data. Figures are now organized better and are easier to follow. CD8+ T cells isolated from solid tumor resembled CTLs activated in vitro in the hypoxic/physioxic culture condition. Although tumors are known to be potentially hypoxic, tumor microenvironment might be different from normal lymphoid tissues in many ways. It should be noted that the differences found between TIL and splenocytes are not necessarily attributable to tissue hypoxia. The recent paper by Hatfield et al. (J Mol Med 92, 1283 (2014)) discusses about oxygen-dependent immunoregulation in tumor microenvironment.

Page 7 line 3: normoxia -> physioxia
Page 9 line 19 Fig. 4D -> Fig. 3D
Page 9 line 21 Fig. 4E -> Fig. 3E
Page 10 line 3 Fig. 4F -> Fig. 3F

Reviewer: 2

Comments to the Author

Although I had my doubts with the original version, I like tis paper and its implications since TIL were analyzed as an in vivo confirmation. So I would support a decision to accept at this point.

Reviewer: 3

Comments to the Author
The authors have done a very thorough job addressing all of the concerns raised by all three reviewers, adding extensive new data and revising the manuscript. This is an important contribution to the field and is of broad interest. Publication is warranted and encouraged.

Second Revision – authors' response- 13-Apr-2015

Reviewer: 1

Comments to the Author
The manuscript has been revised vigorously. Cytotoxicity assay after chronic exposure to lower oxygen tension revealed an augmentation of cytotoxic activity (Fig. 1D) corresponding to the cytokine data. Figures are now organized better and are easier to follow. CD8+ T cells isolated from solid tumor resembled CTLs activated in vitro in the hypoxic/physioxic culture condition. Although tumors are known to be potentially hypoxic, tumor microenvironment might be different from normal lymphoid tissues in many ways. It should be noted that the differences found between TIL and splenocytes are not necessarily attributable to tissue hypoxia.

That is true that differences between TILs and splenocytes cannot be entirely attributable to tissue hypoxia. That is why we did *ex vivo* reactivation under 21%, 5% and 1% O_2 of $CD8^+$ TILs and show that there was indeed an increase in IL-10 secretion (Fig. 4E) but also in CD25 and CD137 expression (Fig. 4D) when oxygen fractions were decreased.

The recent paper by Hatfield et al. (J Mol Med 92, 1283 (2014)) discusses about oxygen-dependent immunoregulation in tumor microenvironment.

This paper is indeed of interest, but as we are limited by the word count limit (that we already exceeded), we cannot cite all the papers. Nevertheless, we already discuss about the possible role of the adenosinergic pathway in our system in the discussion where we cite other interesting papers from this group.

Page 7 line 3: normoxia -> physioxia

Page 9 line 19 Fig. 4D -> Fig. 3D

Page 9 line 21 Fig. 4E -> Fig. 3E

Page 10 line 3 Fig. 4F -> Fig. 3F

Changes have been applied accordingly.

European Journal of Immunology

Third Editorial Decision -15-Apr-2015

Dear Dr. Walker,

It is a pleasure to provisionally accept your manuscript entitled "Phenotypic switch of CD8+ T cells reactivated under hypoxia towards IL-10 secreting, poorly proliferative effector cells." for publication in the European Journal of Immunology.

For final acceptance, please follow the instructions below and return the requested items as soon as possible as we cannot process your manuscript further until all items listed below are dealt with.

Please note that EJI articles are now published online a few days after final acceptance (see Accepted Articles: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1521-4141/accepted). The files used for the Accepted Articles are the final files and information supplied by you in Manuscript Central. You should therefore check that all the information (including author names) is correct as changes will NOT be permitted until the proofs stage.

We look forward to hearing from you and thank you for submitting your manuscript to the European Journal of Immunology.

Yours sincerely,
Laura Soto Vazquez

on behalf of
Dr. Steffen Jung

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