European Journal of Immunology

PLZF-expressing CD4 T cells show the characteristics of terminally differentiated effector memory CD4 T cells in humans

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Handling Executive Committee member: Prof. Shimon Sakaguchi

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

04-Jan-2018

Dear Dr. Chang,

Manuscript ID eji.201747426 entitled "PLZF-expressing CD4 T cells show the characteristics of terminally differentiated effector memory CD4 T cells in human" which you submitted to the European Journal of Immunology has been reviewed. The comments of the referees are included at the bottom of this letter. We are sorry for the delay in the peer review, the reason for this is the holiday season and unavailability of the Executive Committee.

Please note that we think that your manuscript is not of sufficient priority rating for a full research article because it lacks mechanistic insights and therefore we're inviting you to re-submit a

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revised manuscript as a Letter to the Editor, a new article type that EJI has recently introduced. Letters to the editor will be discoverable on PubMed and this is the section for research dedicated to short format thought-provoking, significant, preliminary studies. This is different to our Correspondence articles, which are meant to provide feedback on articles and issues that we publish. Letters to the editor should be no longer than 1500 words in total (including references and Figure/Table and legends) and contain no more than 10 references and two display (i.e. Figure or Table) elements (please see below for more details on this format).

A revised version of your manuscript that takes into account the comments of the referees will be reconsidered for publication. Please note that further experiments are not requested but all other Referees comments must be addressed. Should you disagree with any of the referees' concerns, you should address this in your point-by-point response and provide solid scientific reasons for why you will not make the requested changes.

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. Please provide information about the number of experiments performed, as well as the gating strategy. 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 referees 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,
Nadja Bakocevic
On behalf of

European Journal of Immunology

Prof. Shimon Sakaguchi

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Reviewer: 1

Comments to the Author

This paper contains a simple message: RA+R0+CD4+ human T cells express the transcription factor PLZF. The authors draw the conclusion that these cells resemble terminally differentiated T cells but I think this is a incorrect interpretation of their data and a sub standard knowledge on the literature.

Major comments:

- CD45RA+CD45R0+CD4+ T cells have been described before (see e.g. Rentenaar et al., 2000 and references therein) and these cells emerge during acute infection and bear many features of recently activated (so not terminally differentiated) CD4+ T cells. In fact, the authors reproduce that these cells are larger, express Ki67 and CD69 and therefore bear all features of recently primed T cells.
- Having established that these Ra+R0+CD4+ cells have been described more than a decade ago, this leaves a single message: recently activated CD4+ T cells express PLZF. The authors could have addressed whether induction of PLZF can be mimicked through in vitro activation of naïve CD4+ cells.
- The function of PLZF in these cells is not experimentally addressed.

Reviewer: 2

Comments to the Author

the authors identify that the transcription factor, PLZF, is expressed by a subset of human CD4 T cells which exhibit features of terminally differentiated effector cells and are mostly in lymphoid tissue, rather than blood. Overall the results provide convincing evidence for PLZF expression in this subset. The

manuscript could be improved by some revision of the interpretation so as not to extrapolate too much on cellular origin, and also from additional statistical presentation of the data as listed below.

1. The results showing negligible PLZF cells in pediatric thymus tissues (Currently in supplemental figure 2) should be moved to the main figure. A compilation of the percent PLZF+ CD4 SP cells in fetal and pediatric thymii should be shown in a graph with statistical analysis to compare PLZF expression between the two developmental age groups, and support their conclusion that there is neglgible PLZF expression in pediatric compared ot fetal thymii. The statement that PLZF+ cells do not develop in the thymus after birth becuase their data do not support this conclusion-- there are just much fewer PLZF+ cells in the thymus-- they still could be developing and leaving the thymus-- you can't know the origin of these cells by examining human tissue.

2. Figure 1E could include PLZF expression in the CD161 and MAiT subsets, as this is mentioned in the results but not shown.

3. The interpretation provided that PLZF+ cells develop in the fetus and migrate directly to lymphoid tissues is not supported at all by the results. They would need to investigate other fetal-derived markers to suggest this. The terminal effector phenotype and co-expression of CD45RA and CD45RO suggests an transitional activation state of these cells, based on previous studies of RA+RO+ cells. The authors should also mention what PLZF could be regulating here or why PLZF expression woudl be associated with an effector or activation phenotype.

4. Please go over text for grammar and statements of novelty. "In humans" is the proper phrase for the title, and sentences within the results. Please remove statement, "for the first time", the word "unique" for the functional profile (Suggest using "Distinct" instead).

First revision – authors' response
12-Feb-2018

Dear Editor,

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We appreciate the positive and insightful comments of the reviewers and thank you for the opportunity to revise this manuscript. As suggested, we are submitting the manuscript in the format of 'Letter to the Editor', with 2 figures and supplementary information. Additionally, we have incorporated most of reviewers' suggestions to the revised version and provide below, responses to the reviewer's comments.

Reviewer: 1

This paper contains a simple message: RA+R0+CD4+ human T cells express the transcription factor PLZF. The authors draw the conclusion that these cells resemble terminally differentiated T cells but I think this is a incorrect interpretation of their data and a sub-standard knowledge on the literature.
Major comments:
- CD45RA+CD45R0+CD4+ T cells have been described before (see e.g. Rentenaar et al., 2000 and references therein) and these cells emerge during acute infection and bear many features of recently activated (so not terminally differentiated) CD4+ T cells. In fact, the authors reproduce that these cells are larger, express Ki67 and CD69 and therefore bear all features of recently primed T cells.

We respectively disagree with this reviewer that the $PLZF^{+}CD45RA^{+}CD45RO^{+}$CD4 T cells reported in this manuscript are the same cells described by Rentenaar et al., study. First, the $PLZF^{+}CD45RA^{+}CD45RO^{+}$CD4 T cells are found in human tonsil and spleen but not blood, as reported earlier. Second, unlike the virus-specific CD4 T cells which produced IFNγ but not IL-4, $PLZF^{+}CD45RA^{+}CD45RO^{+}$CD4 T cells produce different cytokines including IL-4. Moreover, they express granzyme B and perforin. A statement about how the $PLZF^{+}CD45RA^{+}CD45RO^{+}$CD4 T are different from earlier reported $CD45RA^{+}CD45RO^{+}$CD4 T cells is added to the revised manuscript and the study by Rentenaar and colleagues is cited.

Lastly, the expression of granzyme B and perforin along with CCR7 expression pattern seen in $PLZF^{+}CD45RA^{+}CD45RO^{+}$CD4 T cells are characteristics unique to Temra cells. As stated in the manuscript, it is possible that PLZF might be important for the generation of Temra cells or PLZF+CD45RA+CD45RO+CD4+ T cells could be a subset of Temra or temra-like cells.

Reviewer: 2

Comments to the Author
the authors identify that the transcription factor, PLZF, is expressed by a subset of human CD4 T cells which exhibit features of terminally differentiated effector cells and are mostly in lymphoid tissue, rather than blood. Overall the results provide convincing evidence for PLZF expression in this subset. The manuscript could be improved by some revision of the interpretation so as not to extrapolate too much on cellular origin, and also from additional statistical presentation of the data as listed below.

1. The results showing negligible PLZF cells in pediatric thymus tissues (Currently in supplemental figure 2) should be moved to the main figure. A compilation of the percent PLZF+ CD4 SP cells in fetal and

pediatric thymii should be shown in a graph with statistical analysis to compare PLZF expression between the two developmental age groups, and support their conclusion that there is neglgible PLZF expression in pediatric compared ot fetal thymii. The statement that PLZF+ cells do not develop in the thymus after birth becuase their data do not support this conclusion-- there are just much fewer PLZF+ cells in the thymus-- they still could be developing and leaving the thymus-- you can't know the origin of these cells by examining human tissue.

The revised manuscript has the fetal thymic data as part of main figures (Fig. 1A). While 3 fetal thymii were analyzed, 9 pediatric thymii from 2 days - 5.5 years of age were examined. We therefore chose to show three different pediatric thymii of a broad range of age, none of which had a detectable number of PLZF+ CD4 T cells, while the representative fetal thymus clearly shows the presence of these cells, which has been reported as well. With a compiled graph of all the pediatric thymic data as the reviewer suggested, the ages will not be evident. Secondly, we have changed the statement that 'PLZF+ cells do not develop in the thymus after birth' to 'PLZF+ cells are not detectable in the thymus after birth'.

2. Figure 1E could include PLZF expression in the CD161 and MAiT subsets, as this is mentioned in the results but not shown.

We are not showing this figure in the revised version due to constraint in space with new format.

3. The interpretation provided that PLZF+ cells develop in the fetus and migrate directly to lymphoid tissues is not supported at all by the results. They would need to investigate other fetal-derived markers to suggest this. The terminal effector phenotype and co-expression of CD45RA and CD45RO suggests an transitional activation state of these cells, based on previous studies of RA+RO+ cells. The authors should also mention what PLZF could be regulating here or why PLZF expression woudl be associated with an effector or activation phenotype.

We agree with the reviewer's comment. In the revised version, we stated that these cells can be found in and reside in the peripheral lymphoid organs in humans after birth. A statement about what PLZF could be doing is added to the manuscript.

4. Please go over text for grammar and statements of novelty. "In humans" is the proper phrase for the title, and sentences within the results. Please remove statement, "for the first time", the word "unique" for the functional profile (Suggest using "Distinct" instead).

As per reviewer's suggestion, we have made these changes.

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Second Editorial Decision

08-Mar-2018

Dear Dr. Chang,

It is a pleasure to provisionally accept your manuscript entitled "PLZF-expressing CD4 T cells show the characteristics of terminally differentiated effector memory CD4 T cells in humans" 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,
Eloho Etemire

on behalf of
Prof. Shimon Sakaguchi

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