European Journal of Immunology

Tissue-resident MAIT cell populations in human oral mucosa

Michal Sobkowiak, Haleh Davanian, Robert Heymann, Anna Gibbs, Johanna Emgård, Joana Dias, Soo Aleman, Carina Kruger-Weiner, Markus Moll, Annelie Tjernlund, Edwin Leeansyah, Margaret Chen, Johan Sandberg

Correspondence: Prof. Dr. Johan K. Sandberg, CIM, Department of Medicine, F59, Karolinska Institutet, Karolinska University Hospital Huddinge, 14186 Stockholm

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Review Timeline:
Submission date: 19-Jun-2018
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Revision/s received: 21-Sep-2018
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Handling Executive Committee member: Prof. James Di Santo

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 - 23-Jul-2018

Dear Prof. Sandberg,

Manuscript ID eji.201847759 entitled "Functionally specialized tissue-resident MAIT cell populations in human oral mucosa" 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.

A revised version of your manuscript that takes into account the comments of the referees will be reconsidered for publication. 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 state the exact number of donors per experiment and if the data shown are representative or rather pooled. Please

show fluorochrome axis labels and scaling for all flow cytometry data. 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 Prof. James Di Santo

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

This paper studies MAIT cells in the oral mucosae. The authors quantify and characterize MAIT cells in the oral mucosae using multiparametric cytometry and histology to precise the exact location of the cells. In vitro restimulation assays provide lymphokine secretion potential information.
Altogether this study represents a nice addition to the description of MAIT cells in different tissues in humans. On a whole, the study is correctly performed. However, two points deserve a deeper analysis:
1. In fig. 1d-f, examples of staining are provided and it is concluded that MAIT cells are in the epithelium near the basal membrane. From the images, this is not clear and no quantification is provided.

It is important to determine whether MAIT cells are in the epithelium layer or in the laminar propria. Indeed, in the gut MAIT cells are mostly in the lamina propria but the oral epithelium structure is different.

2. The repertoire analysis does not support the conclusions of the authors about a putative higher diversity in the oral mucosae as the number of cells studied from the oral mucosae is probably much lower than from the blood. From the method section, it is not clear whether Jalpha usage of the Va7.2 TCR was normalized to Calpha or to Gapdh. Only the former would be correct. In any case, the approximate number of cells (T cells or Va7.2) studied in each sample should be provided. In fact, although not solving the cell number issue, deep sequencing of the amplicons could be useful since the CDR3 of Va7.2 rearrangements are polymorphic in humans: Deep sequencing of the Va7.2-Calpha amplicons would allow a focus on the CDR3s using Ja33, Ja12 and Ja20 of the canonical CDR3 length (12 AA) allowing a better accuracy. This would either strengthen or dismiss the author conclusion that MAIT cells from the oral mucosae are "partly distinct" from blood MAIT cells. The abstract should also be modified

3. On page 10, the existence of a CD103neg subset harboring distinct phenotype characteristics is clearly demonstrated. Whether this population is "non-resident" is not demonstrated by the data that are static. The conclusion of this paragraph as well as the abstract should be modified.

Reviewer: 2

Comments to the Author

This study describes for the first time MAIT cell populations in humans in the oral mucosal. The study is technically well done, includes a fairly large number of healthy volunteers with paired mucosal and blood samples and a detailed evaluation of MAIT populations within oral mucosal tissues. The authors document phenotypic characteristics, “tissue residency status”, tissue localization, Ja-TCR usage and ex-vivo cytokine secretion in oral vs blood MAIT from the same individual. Although functionality of oral MAITs is difficult to decipher and cannot be conclusively commented on, this study provides novel insights for oral immunity.

A few comments to address:

1. Given that functional specialization is not well documented and uncertain in vivo, the title should be modified to read “tissue resident MAIT cell population sin the human oral mucosa”

2. Similarly, from the abstract, please remove speculative comment that “low in perforin is indicative of subdued cytolytic potential”- one can just note that perforin was low

3. Figure 5D is not very informative, unless the subsets are classified as co-expressors of specific cytokines, whether cells have 1 or 2 /3 functions is not necessarily informative unless the functions are described

European Journal of Immunology

First Revision – authors' response - 21-Sep-2018

Reviewer 1

Overall comment: "This paper studies MAIT cells in the oral mucosae. The authors quantify and characterize MAIT cells in the oral mucosae using multiparametric cytometry and histology to precise the exact location of the cells. In vitro restimulation assays provide lymphokine secretion potential information. Altogether this study represents a nice addition to the description of MAIT cells in different tissues in humans. On a whole, the study is correctly performed."

Response: We thank the reviewer for the overall positive assessment of our manuscript.

Critique 1: "In fig. 1d-f, examples of staining are provided and it is concluded that MAIT cells are in the epithelium near the basal membrane. From the images, this is not clear and no quantification is provided. It is important to determine whether MAIT cells are in the epithelium layer or in the laminar propria. Indeed, in the gut MAIT cells are mostly in the lamina propria but the oral epithelium structure is different. "

Response: The tissue stainings we have done suggest that MAIT cells can be found located on both sides of the basal membrane, i.e. both in the epithelium and in the lamina propria. In fact, this was the result we presented already in the first submitted version of the manuscript. From visual inspection of microscopy images, it appears that most of the MAIT cells are located relatively close to the basal membrane. To look into this in some more detail, the distance and numbers of MAIT cells were quantified in tissue sections from four subjects using image analysis software (RFig 1). The emerging pattern support the notion that MAIT cells are primarily located relatively close to the basal membrane, and that they can be found on both the epithelial and lamina propria sides of the membrane. A sentence to highlight this has been added to the results section of the manuscript.

RFig 1. The distance measurement between the MAIT cells and the basal membrane. A) Distance measurement between the Vα7.2+IL-18Rα+ (MAIT) cells and the basal membrane using the digital application CaseViewer, where the distance from the surface of the double-positive cells to the basal membrane was measured. Epithelium (EP),connective tissue (CT), dashed line indicates the basal membrane, scale bar represents 10 μm. B) MAIT cell location and distance from the basal membrane in buccal mucosal tissue sections from four human subjects. Each dot represents a single MAIT cell (the number above represents the number of cells per image).

Critique 2: "The repertoire analysis does not support the conclusions of the authors about a putative higher diversity in the oral mucosae as the number of cells studied from the oral mucosae is probably much lower than from the blood. From the method section, it is not clear whether Jalpha usage of the Va7.2 TCR was normalized to Calpha or to Gapdh. Only the former would be correct. In any case, the approximate number of cells (T cells or Va7.2) studied in

each sample should be provided. In fact, although not solving the cell number issue, deep sequencing of the amplicons could be useful since the CDR3 of Va7.2 rearrangements are polymorphic in humans: Deep sequencing of the Va7.2-Calpha amplicons would allow a focus on the CDR3s using Ja33, Ja12 and Ja20 of the canonical CDR3 length (12 AA) allowing a better accuracy. This would either strengthen or dismiss the author conclusion that MAIT cells from the oral mucosae are "partly distinct" from blood MAIT cells. The abstract should also be modified"

Response: We can confirm that the data on Jalpha usage was normalized against Calpha. Regarding cell numbers, we do not have exact information on T cell numbers in the biopsies. Based on data on the GAPDH qPCR of both mucosal biopsies and PBMC, and percentages seen in FACS straining, it is probably so that there are fewer MAIT cells in mucosal samples than in PBMC. However, we believe that the numbers in mucosa are still sufficient (in the 100-200 cells range) for us to be comfortable with the conclusion that the higher variability in Jalpha usage in matched mucosal samples is real and not an artifact. Nevertheless, we have toned down the interpretation of this data in the results, and completely removed mentioning of this data in the abstract.

Critique 3: "On page 10, the existence of a CD103neg subset harboring distinct phenotype characteristics is clearly demonstrated. Whether this population is "non-resident" is not demonstrated by the data that are static. The conclusion of this paragraph as well as the abstract should be modified."

Response: There is a fairly comprehensive literature indicating that the combination of CD69 and CD103 on T cells in tissue identifies a resident population. To firmly demonstrate that this applies in human oral mucosa in vivo is extremely difficult and way beyond the scope of this study. Nevertheless, we feel that it is useful for the reader that we try to interpret this data within the scope of current models of tissue T cell residency. Nevertheless, we have toned down the conclusion presented in the paragraph on page 10 to accommodate the reviewer's concern.

Reviewer 2

Overall comment: "This study describes for the first time MAIT cell populations in humans in the oral mucosal. The study is technically well done, includes a fairly large number of healthy volunteers with paired mucosal and blood samples and a detailed evaluation of MAIT populations within oral mucosal tissues. The authors document phenotypic characteristics, "tissue residency status", tissue localization, Ja-TCR usage and ex-vivo cytokine secretion in oral vs blood MAIT from the same individual. Although functionality of oral MAITs is difficult to decipher and cannot be conclusively commented on, this study provides novel insights for

oral immunity."
Response: We thank the reviewer for the overall very positive assessment of our manuscript.
Critique 1: "Given that functional specialization is not well documented and uncertain in vivo, the title should be modified to read "tissue resident MAIT cell population sin the human oral mucosa."
Response: We agree that the expression "functional specialization" can be interpreted in different ways, and we have revised the title as suggested by the reviewer.
Critique 2: "Similarly, from the abstract, please remove speculative comment that "low in perforin is indicative of subdued cytolytic potential"- one can just note that perforin was low"
Response: We have now revised the abstract and removed the comment about "subdued cytolytic potential" as suggested by the reviewer.
Critique 3: "Figure 5D is not very informative, unless the subsets are classified as coexpressors of specific cytokines, whether cells have 1 or 2 /3 functions is not necessarily informative unless the functions are described."
Response: We believe that the different analytical approaches to the MAIT cell functional dataset presented in the different parts of Figure 5 complement each other to give a comprehensive view of the functional capacity and profile in oral mucosa and blood. Figure 5D is one way of looking at the data, and the in-depth analysis of cytokine co-expression patterns requested by the reviewer is presented in Figure 5E.

Editor's comment: "In particular, please edit your figure legends to follow Journal standards as outlined in the editorial comments. Please state the exact number of donors per experiment and if the data shown are representative or rather pooled. Please show fluorochrome axis labels and scaling for all flow cytometry data."
Response: We have done the updates of the figures and legends as requested.

Second Editorial Decision - 09-Oct-2018

Dear Prof. Sandberg,

It is a pleasure to provisionally accept your manuscript entitled "Tissue-resident MAIT cell populations in human oral mucosa" 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: https://onlinelibrary.wiley.com/toc/15214141/0/ja). 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
Prof. James Di Santo

Editorial Office
European Journal of Immunology
e-mail: ejied@wiley.com
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