Mapping of the lingual immune system reveals the presence of both regulatory and effector CD4^+ T cells

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Summary

Background Sublingual immunotherapy (SLIT) is safe and reduces both symptoms and medication requirements in patients with type I respiratory allergies. Nonetheless, immune mechanisms underlying SLIT need to be further documented.

Objective A detailed characterization of the lingual immune system was undertaken in mice, to investigate the presence of tolerogenic and pro-inflammatory mechanisms.

Methods Immune cells were characterized in lingual tissues from BALB/c mice using immunohistology and flow cytometry. Resident CD4^+ T cells were sorted and toll-like receptor (TLR) expression profiles as well as functional characterization were assessed by RT-PCR, T cell suppressive assays and cytokine gene expression, respectively.

Results Eosinophils and mast cells were only detected in submucosal tissues. No NK, NK-T, γ/δ, CD8^+ T cells, nor B-lymphocytes were detected. Potential antigen presenting cells include various subsets of dendritic cells (CD207^+ Langerhans cells, CD11b^-CD11c^- myeloid cells and 120G8^+ plasmacytoid DCs) together with F4/80^+ macrophages. Noteworthy, both CD103^- and CD103^+ CD4^+ T cells expressing TLR2 and TLR4 receptors are present along the lamina propria, in vicinity of myeloid CD11b^-CD11c^- dendritic cells. Such resident lingual CD4^+ T lymphocytes comprise both suppressive T cells as well as cells with memory/effector functions (i.e. expressing IFNγ, IL4, IL10 and IL17 genes following stimulation), irrespective of the presence of the mucosal addressing marker CD103.

Conclusion The sublingual route is pertinent to induce antigen-specific tolerance, due to (i) limited numbers of pro-inflammatory cells, rather located in submucosal tissues, (ii) co-localization of APCs and resident CD4^+ T cells with regulatory functions. Since the oral immune system can also elicit pro-inflammatory effector responses, the cytokine milieu in which allergens are presented by sublingual APCs needs to be controlled during immunotherapy (e.g. with adjuvants) in order to favour tolerance over inflammation.

Keywords antigen presenting cells, CD103, mucosal CD4^+ T cell, mucosal immunity, sublingual immunotherapy

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Introduction

Allergen-specific sublingual immunotherapy (SLIT) is established as a safe and efficient treatment for type I respiratory allergies, both in adults and children [1–7]. Although, SLIT-induced allergen-specific T cell responses are thought to play a critical role in tolerance induction, immune mechanisms involved at the site of administration are still elusive [8]. Improving our understanding of the latter is critical in order to better target the allergen to appropriate immune cells, thus favouring tolerance induction as opposed to pro-inflammatory responses. Interestingly, all subsets of oral antigen presenting cells (APCs) described so far in mice or in humans were shown to support antigen-specific tolerance, following induction of IFNγ and/or IL10 producing CD4^+ T cells with a suppressive function [9–11]. In contrast, little is known regarding the presence of resident regulatory or effector lymphoid cells in oral tissues.

In this study, we conducted a comprehensive analysis of the lingual immune system in BALB/c mice. Few pro-inflammatory cells [i.e. eosinophils, mast cells (MC)] cells
and no lymphoid cells, besides resident CD4^+ T lymphocytes, are detected within lingual tissues. The latter co-localize along the lamina propria with CD11b^+ myeloid APCs, and comprise both regulatory and effector/memory T cells.

**Materials and methods**

**Mice, reagents and antibodies**

Six- to eight-week-old BALB/c female mice were obtained from Charles River (L’Arbresle, France). DO11.10 female mice transgenic for the ovalbumin (OVA)_{323-329} peptide-specific T cell receptor (TCR) [12] were bred in the Centre d’Exploration et de Recherche Fonctionnel (CERFE, Evry, France). International levels of ethical standards were applied for animal handling.

Phosphate-buffered saline (PBS) and RPMI 1640 were purchased from Invitrogen (Carlsbad, CA, USA). The following monoclonal antibodies (mAbs) were used, either as purified antibodies for immunohistology or labelled with fluorescein isothiocyanate (FITC), phycoerythrin (PE) or allophycocyanin (APC) for flow cytometry analysis: anti-MHC Class II (I-A/I-E) IgG2b, anti-CD86 IgG2a, anti-CD103 IgG, anti-CD25 IgG1, anti-CD62L IgG2a, anti-CD44 or allophycocyanin (APC) for flow cytometry analysis: anti-CD4 IgG2b, anti-CD3 IgG1, anti-CD28 IgG (all from eBiosciences, San Diego, CA, USA), anti-CD205 IgG, anti-CD117 IgG2b, anti-F4/80 IgG2a, anti-pan NK IgM, anti-CD40 IgG, anti-CD80 IgG, anti-GR-1 IgG2b, anti CD19 IgA, anti-CD207 IgG2a, anti-CD3 IgG2b, anti-CD28 IgG (all from eBiosciences, San Diego, CA, USA), anti-CD3 IgG2b, anti-CD3 IgG1, antifollicular dendritic cells IgG2c (clone FDC-M1), anti-γδ T cells IgG2a, anti-CD11b IgG2b, anti-CD11c IgG1 (all from BD Biosciences, San Jose, CA, USA), anti-CD205 IgG (NLDC-145 clone; Cedarlane Laboratories Ltd, Burlington, ON, Canada). Corresponding isotype-matched mAbs were used as controls in all experiments.

**Animal sensitization**

Mice sensitization was performed by two intraperitoneal (i.p.) injections at 14-day intervals of 10 µg OVA adsorbed on 2 mg Al(OH)_3, administered in a volume of 100 µL. This was followed by a 20 min aerosol challenge with 1% w/v OVA on 4 consecutive days using an aerosol delivery system (Buxco Europe Ltd, Winchester, UK). Such, OVA-sensitized mice exhibit airway hyperresponsiveness (AHR) in response to metacholine in a dose-dependent manner (from 12.5 to 100 mg/mL) as well as OVA-specific IgEs.

**Immunohistology and fluorescence-activated cell sorter analysis of lingual immune cells**

For immunohistology, spleen, thymus and lingual tissues were recovered from naïve or OVA-sensitized mice and frozen at −80 °C. In our analyses, the buccal floor is also included and considered as part of the ventral side of the tongue. Tissue sections (4–6 µm wide) were serially cut, air-dried for at least 30 min, fixed in acetone for 1–2 min, and incubated for 10 min in 3% hydrogen peroxide (Sigma, St Louis, MO, USA) to block endogenous peroxidase activity. After washing in Tris-buffered saline (TBS: 0.05 m Tris, 0.15 m NaCl, pH 7.4), primary antibodies (diluted 1/100 in TBS) were added onto samples and incubated for 1 h at room temperature. Tissue sections were washed in TBS and incubated with biotinylated rabbit anti-goat IgG secondary antibodies (Sigma, 1/400) for 30 min before adding streptavidin–biotin horseradish peroxidase (SA-HRP, Sigma). After 30 min, samples were washed and specific staining was visualized using diaminobenzidine (DAB, Sigma) as a substrate. Tissue sections processed in the absence of primary antibodies were included as negative controls.

To analyse the phenotype and function of immune cells, lingual tissues removed from BALB/c mice were treated for 45 min at 37 °C with 400 U/mL collagenase type IV, 50 µg/mL DNAse I (Roche diagnostic, Mannheim, Germany) and 2 U/mL dispase (Invitrogen) in RPMI 1640. After blocking residual enzymatic activity with 5 mM EDTA in PBS, lingual tissues were dissociated in PBS. Cells were recovered and stained with various antibodies for 15 min at 4 °C as described above. Results were expressed as a mean percentage of positive cells ± SD (n = 3), after subtracting background staining obtained with corresponding isotype-matched mAbs.

**RNA isolation and real-time polymerase chain reaction analysis**

Total RNA (RNeasy Mini kit, Qiagen) was isolated from 10^4 CD4^+ T cells purified by cell sorting from lingual tissues or spleen; cDNAs were synthesized using random hexamers (Taquin Reverse Transcription Reagents, Applied Biosystems, Foster city, CA, USA) according to the manufacturer’s protocol. Real-time PCR analysis of IFNγ (Mn00801778_m1), IL4 (Mn00445259_m1), IL10 (Mn00439616_m1), IL17 (Mn00439619_m1), TLR 1–7 (Mn00441868_s1; Mn00442346_m1; Mn00446577_g1; Mn00445274_m1; Mn00454288_s1; Mn00446590_m1, respectively) and TLR9 (Mn00446193_m1) gene expression was conducted with Predefined Taqman Gene Expression Assays and reagents (Applied Biosystems) according to the manufacturer’s specifications, using a 7300 Real-time PCR system (Applied Biosystems). Forty cycles of amplification were performed as follows: 95 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s. Levels of mRNA for each gene were normalized to the amount of β-actin mRNA (Mn00801778_m1), which is an appropriate reference gene to allow the quantification of cytokine gene expression in lingual T cells [see supporting information, table showing
the expression of various housekeeping genes i.e. glyceraldehyde-3-phosphate dehydrogenase [GAPDH; Mn999-99915_g1], β-microglobulin [Mn00437762_m1]) and hypoxanthine-guanine phosphoribosyltransferase [HPRT; Mn01324427_m1]. For TLR gene expression, PCR products were visualized after electrophoresis on 1.5% agarose gels, using a transilluminator.

Functional characterization of lingual CD4+ T cells

Both splenic and lingual T cells were stained at 4 °C for 15 min, with anti-CD3-APC, anti-CD4-PE and anti-CD103-FITC mAbs. CD3+CD4+CD103− and CD3+CD4+CD103+ pure T cell populations were isolated with a MoFlo (Dako, Glostrup, Denmark) cell sorter. Cells within each subset were more than 99% pure, as confirmed by flow cytometry analysis.

For T cell activation, both CD3+CD4+CD103− and CD3+CD4+CD103+ T cells sorted from spleen and lingual tissues (2×10^6/well) were stimulated using a combination of anti-CD3 and anti-CD28 (1 μg/mL, each) antibodies in RPMI 1640, supplemented with 10% foetal calf serum and antibiotics (all from invitrogen) in 96-well round-bottom plates. After 24 h, T cells were recovered for gene expression analysis.

For suppressive assays, naïve CD4+ T cells were purified from spleens of D011.10 mice and CFSE-labelled as previously described [13]. 2×10^4 of such CFSE-labelled OVA-specific CD4+ T cells were co-cultured with each of the sorted CD3+CD4+CD103− and CD3+CD4+CD103+ T cell populations in presence of murine BM–DCs and OVA_{323–339} peptide (10 μg/mL) for 4 days using 1/2–1/8 (suppressive/responder) cell ratios. As a control, 2×10^4 CFSE-labelled OVA-specific CD4+ T cells were cultured with or without the OVA_{323–339} peptide (10 μg/mL) for 4 days. OVA-specific T cells were stained with the PE-KJ1.26 anti clonotypic mAb and proliferating cells were evaluated by fluorescence-activated cell sorter (FACS), based on a decrease in CFSE-associated fluorescence.

Results

Mapping and distribution of immune markers in lingual tissues from BALB/c mice

The phenotype and tissue distribution of immune cells was assessed by immunohistology in lingual tissues from naïve and OVA-sensitized BALB/c mice, using a panel of specific antibodies, as described in ‘Materials and methods’. Frequencies of immune cells expressing a given marker were further evaluated in parallel from tissues digested by collagenase and dispase, after antibody staining and flow cytometry analysis. Staining in the absence of primary antibodies was always negative in those experiments (data not shown). Qualitative and quantita-

tive results obtained in three independent experiments conducted in both naïve and sensitized animals are summarized in Table 1. Collectively, those results revealed the presence of APCs, pro-inflammatory cells and resident CD3+CD4+ T lymphocytes, as will be discussed below.

Identification of antigen presenting cells in lingual tissues from BALB/c mice

In agreement with our previous study [11], we detected surface markers associated with at least four subsets of APCs, including CD207+ Langerhans cells, 120G8+ plasmacytoid DCs, as well as CD11b+CD11c+ and CD11b+CD11c− myeloid APCs. While the three first subsets represent each <0.1% total cells, CD11b+CD11c− cells (Fig. 1 and Table 1) are the most abundant APCs (representing 1.44±0.42% and 0.78±0.10% of total cells in naïve and OVA-sensitized mice, respectively, mean±SD, n = 3). Furthermore, we also detected F4/80+ macrophages located in submucosal tissues (Fig. 1 and Table 1). In contrast, follicular dendritic cells were missing, consistent with the absence of structured lymphoid tissues in the mouth of mice (Table 1). Although not shown, no further recruitment of such APCs was observed in tissue sections of naïve mice sublingually treated with the OVA protein for 4, 24 or 48 h.

Other surface markers classically associated with APCs, such as MHC-II (1.17±0.64% and 0.62±0.12% in naïve and OVA-sensitized mice, respectively, mean±SD, n = 3) and CD86 (0.43±0.20% and 0.12±0.04% in naïve and OVA-sensitized mice, respectively, mean±SD, n = 3) molecules were mostly detected on cells located in both ventral and dorsal tissue sections of the lingua, as well as along the lamina propria together with CD11b+ APCs (Fig. 1 and Table 1). Other co-stimulatory molecules such as CD40 and CD80 were not detected by immunohistology in any of the tissue sections analysed (Table 1). Noteworthy, we did not detect any differences between dorsal and ventral sides of lingual tissues in term of distribution of those potential APCs (Table 1). Collectively, those results suggest that (i) lingual tissues contain multiple potential APCs, including LCs, myeloid and plasmacytoid DCs, together with macrophages, (ii) such cells do not express high levels of co-stimulatory molecules [11].

Characterization of pro-inflammatory and lymphoid cells in lingual tissues from BALB/c mice

Tissue sections stained with haematoxylin, eosin and safran revealed the presence of pro-inflammatory cells, i.e. eosinophils and mast cells (MCs), deep within submucosal tissues in naïve mice (Fig. 2a). The presence of MCs (0.65±0.35% and 0.48±0.29% in naïve and OVA-sensitized mice, respectively, mean±SD, n = 3) and granulocytes (0.27±0.12% and 1.11±0.29% in naïve
and OVA-sensitized mice, respectively, mean±SD, n = 3) in muscular tissues was confirmed using anti-CD117 and anti-GR-1 antibodies, respectively (Fig. 2b and Table 1). Beyond the noticeable influx of GR-1+ granulocytes in OVA-sensitized mice, such pro-inflammatory cells were also found closer to the mucosal/submucosal interface when compared with naïve animals (Table 1).

We failed to detect B lymphocytes (CD19+) in lingual tissues. Also, no natural killer (CD49b+), γ/δ nor CD8+ cells were detected by immunohistology (Table 1). In contrast, we detected CD4+ T cells (0.20±0.08% and 0.11±0.04% in naïve and OVA-sensitized mice, respectively, mean±SD, n = 3) in lingual tissues (Fig. 3a, right panels and Table 1), using spleen cells as positive controls (Fig. 3a; left panels). Interestingly, lingual CD4+ T cells are predominantly located along the lamina propria, i.e. in close vicinity with APCs (Fig. 3a). Such cells were confirmed by cytofluorometry to express CD25, CD44 (high), CD45RB (low), CD62L (low), CD69, as well as for 25% of them, the ζβ7 integrin CD103 acting as a mucosal addressing receptor (Figs 3a and b). In addition, Foxp3+ cells were not detected by FACS nor by immunochemistry (data not shown), suggesting that natural regulatory T cells are not present in significant amounts in lingual tissues.

### Resident lingual CD4+ T cells exhibit both regulatory and effector properties

We further characterized resident CD4+ T cells (both CD103- and CD103+) from lingual tissues in comparison with their splenic counterparts. Among TLR genes, TLR2 and TLR4 were significantly expressed in all subsets, most particularly in CD4+ CD103+ T cells (Fig. 4). The presence of those receptors at the cell surface was confirmed by cytofluorometry (data not shown). CD4+ T cells were sorted from corresponding tissues and tested in parallel for their potential regulatory activity, using a third party T cell suppressive assay. As shown in Fig. 5, both CD4+CD103- and CD4+CD103+ T cell subsets exhibit a suppressive activity (Fig. 5). No quantitative differences are observed in this regard between T cells obtained from the spleen or lingual tissues. Although not shown, triggering TLR2 or TLR4 (using Pam3CSK4 or LPS, respectively) did not further enhance T cell suppression in this in vitro assay. Patterns of cytokine expression were also analysed in both CD103- and CD103- lingual CD4+ T cells after polyclonal activation in five independent experiments (Fig. 6). As their splenic counterparts, the two subsets of resident lingual CD4+ T cells express IFNγ, IL4 and IL17

### Table 1. Mapping and distribution of cells expressing various markers in lingual tissues from naïve or OVA-sensitized mice

<table>
<thead>
<tr>
<th>Markers</th>
<th>Percentage of total cells</th>
<th>Distribution</th>
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<tbody>
<tr>
<td></td>
<td>Naïve mice (%)</td>
<td>OVA-sensitized mice (%)</td>
</tr>
<tr>
<td>CMH-II</td>
<td>1.17±0.64</td>
<td>0.62±0.12</td>
</tr>
<tr>
<td>CD40</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD80</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD86</td>
<td>0.43±0.20</td>
<td>0.12±0.04</td>
</tr>
<tr>
<td>CD11b</td>
<td>1.44±0.42</td>
<td>0.78±0.10</td>
</tr>
<tr>
<td>CD11c</td>
<td>0.10±0.01</td>
<td>0.12±0.03</td>
</tr>
<tr>
<td>120G8</td>
<td>0.11±0.08</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CD205</td>
<td>&lt;0.1</td>
<td>ND</td>
</tr>
<tr>
<td>CD207</td>
<td>&lt;0.1</td>
<td>ND</td>
</tr>
<tr>
<td>F4/80</td>
<td>0.15±0.07</td>
<td>0.20±0.10</td>
</tr>
<tr>
<td>FDC</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GR-1</td>
<td>0.27±0.12</td>
<td>1.11±0.29*</td>
</tr>
<tr>
<td>CD117</td>
<td>0.65±0.35</td>
<td>0.48±0.29*</td>
</tr>
<tr>
<td>CD3</td>
<td>0.23±0.15</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>CD4</td>
<td>0.20±0.08</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>CD8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>γδ TCR</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD19</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD103</td>
<td>&lt;0.1</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>CD49b</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Both dorsal/ventral and muscle tissue sections of the tongue of BALB/c mice were analyzed by immunohistology, using a panel of specific antibodies, as described in methods. Frequencies of immune cells expressing a given marker were evaluated by flow cytometry and expressed as mean percentages of total cells±SD, out of three independent experiments conducted in both naïve and sensitized animals. ND = not detected. For distribution, results are expressed as presence (+) or absence (−) of cells expressing a given marker depending upon tongue area. *Refer to cells located in muscular tissue but also close to the mucosal/submucosal interface.

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genes following activation (Fig. 6) suggesting that they both contain cells mediating effector functions associated with Th1, Th2 and Th17 polarization. The functional relevance of quantitative differences in patterns of cytokines (e.g. IL4, IL10) elicited in CD103⁺ and CD103⁻ lingual CD4⁺ T cells remains unclear.

**Discussion**

Successful allergen-specific immunotherapy down-regulates established Th2 responses [14, 15] together with the induction of Th1 [16–26], as well as IL10 producing regulatory T cells [27–30]. Nonetheless, immune mechanisms underlying SLIT remain to be fully elucidated, most particularly at the site of allergen(s) administration. Specifically, one issue to improve current vaccination protocols is to better target the allergen to appropriate APCs in order to enhance tolerance induction, while limiting exposure to pro-inflammatory cells, including MCs and effector T cells. All APCs described thus far in the oral mucosa of mice and human, including LCs, myeloid and
plasmacytoid DCs, exhibit tolerogenic properties [9–11]. Specifically, such APCs drive naïve T cells to produce IFNγ and/or IL-10. Also, cross-linking of high affinity receptors for IgE (FcεRI) on human LCs induces the expression of regulatory cytokines such as TGFβ or IL-10 [9, 31]. Whereas the oral immune system appears to be prone to elicit antigen-specific tolerance due to the peculiar biology of resident APCs, the presence of pro-inflammatory immune cells remains to be characterized.

Herein, we undertook a detailed characterization of dorsal/ventral lingual immune cells in naïve BALB/c mice.
We detected pro-inflammatory cells, i.e. MCs and eosinophils, which are located in submucosal tissues, in agreement with previous studies conducted in humans [32, 33]. One inference is that in the absence of mucosal effraction, allergen contact with such cells should be limited after SLIT. In line with our previous study we confirmed the presence of several subsets of APCs, located at the mucosal/submucosal junction, likely involved in antigen capture during SLIT [11]. These APCs include myeloid CD11b<sup>+</sup>CD11c<sup>−</sup>, CD11b<sup>−</sup>CD11c<sup>+</sup>, with a semi-mature phenotype (CD40<sup>+</sup>CD80<sup>−</sup>CD86<sup>+</sup>), consistent with their tolerogenic function [34, 35]. In addition, we further detected in submucosal tissues plasmacytoid dendritic cells but also F4/80<sup>+</sup> macrophages, whose potential APC function is currently under investigation.

Although we did not see any significant differences in immune cell distribution in mice depending upon the site, Allam et al. [33] reported such differences in humans in terms of distribution of LCs and MCs within the oral cavity, with the vestibular region identified as the most favourable site for allergen application during SLIT, given its high LC and low MC density.

No B, NK, NK-T, γδ or CD8<sup>+</sup> cells were detected in lingual tissues from naïve BALB/c mice, establishing that they are not prime effectors in tolerance induction via the sublingual route. Interestingly, resident mucosal CD4<sup>+</sup> T cells were detected in lingual tissues from naïve BALB/c mice, establishing that they are not prime effectors in tolerance induction via the sublingual route. Interestingly, resident mucosal CD4<sup>+</sup> T cells were found along the lamina propria, i.e. in the vicinity of the main subset of CD11b<sup>+</sup> oral APCs, suggesting that such T cells may initiate adaptive immune responses to antigens. Such T cells exhibit an effector/memory phenotype (CD44 high, CD45RB low, CD62L low and CD69<sup>+</sup>). Also, they express the αEβ7 integrin CD103, a mucosal homing receptor proposed by others as a marker for regulatory CD4<sup>+</sup> T cells [36, 37]. Interestingly, TLR2 and TLR4 genes are significantly expressed in both CD103<sup>+</sup> and CD103<sup>+</sup> T cells suggesting that appropriate

Fig. 5. Suppressive capacity of lingual CD4<sup>+</sup> T cells. Sorted splenic and lingual CD3<sup>+</sup>CD4<sup>+</sup>CD103<sup>−</sup> or CD3<sup>+</sup>CD4<sup>+</sup>CD103<sup>+</sup> T cells from DO11.10 mice were cultured with naïve CFSE-labeled ovalbumin (OVA)-specific CD4<sup>+</sup> T cells in the presence of murine BM–DCs and the OVA<sub>323–339</sub> peptide (10 μg/mL) for 4 days. Ratios (suppressiv/responder cells) between 1/2 and 1/8 have been tested. As controls, 2 × 10<sup>5</sup> CFSE-labelled OVA-specific CD4<sup>+</sup> T cells were cultured with or without the OVA<sub>323–339</sub> peptide (10 μg/mL) for 4 days. After staining with the PE-KJ1.26 anti clonotypic mAb, proliferating cells were evaluated by FACS based on a decrease in CFSE-associated fluorescence. CTR, control.
targeting of those receptors (e.g. with an adjuvant) might modulate the properties of such T cells. We confirmed that lingual resident CD4\(^+\) T cells exhibit a regulatory T cell activity, although no differences were found in this regard between CD103\(^-\) and CD103\(^+\) cells. Interestingly, this organization is reminiscent of another critical site for tolerance induction, namely the intestinal mucosa, with CD11b\(^+\) APCs similarly co-localized with CD4\(^+\) T cells along the lamina propria [38].

Whereas the default pathway for oral immune responses to antigen seems to be tolerance induction [11, 39, 40], we confirm that the oral immune system also comprises local effector mechanisms. Following polyclonal activation of both CD103\(^+\) and CD103\(^-\) T cells, cytokine genes associated with Th1, Th2 and Th17 patterns of polarization are induced. In agreement with our observation, recent data emphasize that the oral immune system retains the capacity to promote effector responses [41]. In particular, sublingual administration of inactivated influenza viruses induces specific cytotoxic T lymphocytes as well as mucosal antibody responses [41, 42]. Appropriate targeting of oral immune cells using a mucosal adjuvant, i.e. mCTA-LTB, further enhances those responses, resulting in a complete protection against respiratory viral challenge [41, 42]. Furthermore, resident IL17-producing T cells were recently confirmed to be critical in protecting against oropharyngeal candidiasis [43].

Given such a dual capacity of the lingual immune system, appropriate signalling to APCs, e.g. with adjuvants [13, 44, 45] is needed to promote tolerance over inflammation in the course of allergen-specific immunotherapy.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Relative quantity of mRNAs corresponding to each housekeeping genes normalized to distinct potential endogenous controls (Mean of RQ±SD in activated versus non activated CD4⁺CD103⁻ and CD4⁺CD103⁺ T cells from lingual tissues).

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