

Dendritic cells as potential targets for mucosal immunotherapy

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Purpose of review

A recent meta-analysis confirmed that sublingual immunotherapy represents a safe and efficient alternative to traditional subcutaneous immunotherapy in the treatment of allergic rhinitis. However, the immunological mechanisms have not been elucidated in detail yet. The current concept postulates a Th2/Th1 shift along with tolerance induction. Without doubt, oral mucosal antigen-presenting cells such as dendritic cells play a central role as they process antigens to initialize and modulate T-cell activation.

Recent findings

Oral mucosal dendritic cells (oDCs) are capable of processing antigen applied on mucosal surfaces. The natural pro-tolerogenic character of oDCs has been demonstrated by the induction of tolerogenic T cells in response to oDCs activated by either externally applied antigens such as ovalbumin or antigens originating from commensal bacteria like Toll-like receptor ligands, which have been shown to be promising candidates for adjuvants. Nevertheless, the finding of relatively low numbers of oDCs within the sublingual region opens the search for different allergen application sites within the oral cavity such as the vestibular region where higher numbers of oDCs are detectable.

Summary

oDCs display a natural pro-tolerogenic character, which is suspected to be essential for successful sublingual immunotherapy. Further knowledge about oDCs behaviour might give rise to new sublingual immunotherapy strategies such as adjuvant supplementation.

Keywords

adjuvants, Langerhans cells, oral mucosa, sublingual immunotherapy, specific immunotherapy

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Introduction

Systematic reviews demonstrated safety and efficiency of sublingual immunotherapy (SLIT) as an alternative to subcutaneous immunotherapy (SCIT) [1,2,3,4]. Nevertheless, the immunological mechanisms of allergen-specific immunotherapy (AIT) have not been elucidated in detail. The current concept postulates a shift from a Th2 to a Th1 immune response along with tolerance induction mediated by FoxP3(+) T-regulatory cells (Tregs) or transforming growth factor (TGF)- β 1 and interleukin (IL)-10-producing regulatory T cells [5,6]. Most likely, oral mucosal antigen-presenting cells (APCs) such as dendritic cells play a pivotal role, as they are critical for the initiation and modulation of T-cell immune responses [7].

Oral mucosa tissue as a target organ for sublingual immunotherapy

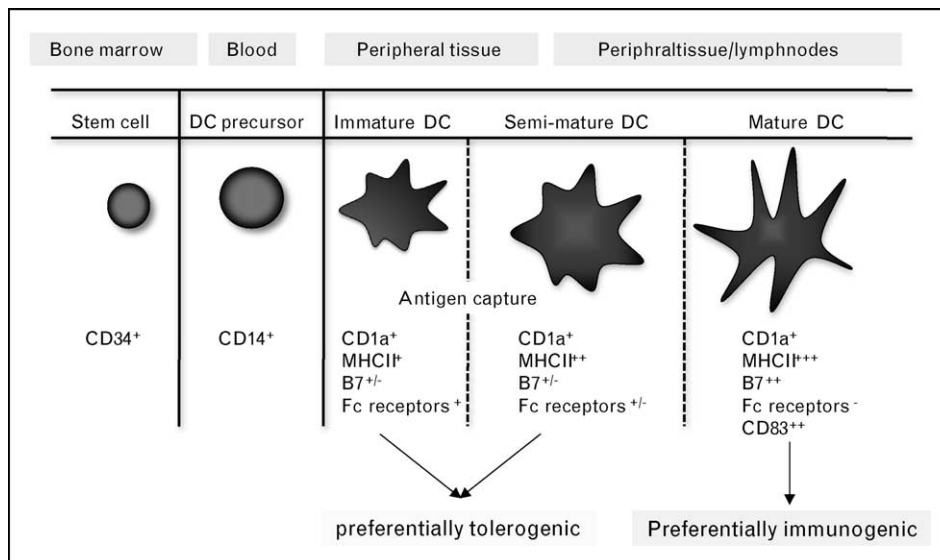
The oral mucosa belongs to the sophisticated network of mucosal immune system, where tolerance induction towards a variety of antigens from commensal bacterial and nutritional products predominates to maintain local

immunostasis [8]. A prime example of the natural tolerogenic character of oral mucosal tissue is the excellent wound healing without severe scar development and without acute inflammation in spite of high bacterial colonization [9]. In regard to SLIT, the tolerogenic potential of oral mucosal tissue is reflected by the lack of inflammatory cells within oral mucosal tissue after allergen administration in sensitized individuals [10]. Furthermore, oral mucosal tissue displays high permeability also for allergens as it has been shown that orally applied allergen as well as monomeric allergoid retains within oral tissue up to 20 h [11–13].

Together, the natural pro-tolerogenic properties combined with the high permeability of oral mucosal tissue are ideal premises for successful SLIT.

Oral mucosal dendritic cells as target cells for sublingual immunotherapy

Without much doubt, oral mucosal dendritic cells are involved in the maintenance of local immunostasis, as dendritic cells are capable of initiating and modulating an

Figure 1 Dendritic cells derive from CD34⁺ stem cells

Dendritic cells such as Langerhans cells derive from CD34⁺ stem cells found in the bone marrow and enter the blood stream as precursors to migrate into the peripheral tissue like the oral mucosa as immature dendritic cells. After antigen capture, they differentiate into semimature dendritic cells and further on into mature dendritic cells to migrate into the lymph node. Whereas immature and semimature dendritic cells preferentially induce tolerogenic immune reactions, fully matured dendritic cells are known for their immunogenic character [15–17]. DC, dendritic cell.

immune response by activating antigen-specific T cells [14,15]. Among the heterogeneous population of dendritic cells, myeloid dendritic cells (mDCs) are differentiated from plasmacytoid dendritic cells (pDCs) [16]. Further on, the maturation state of dendritic cells is critically involved in mediating tolerance. Whereas semimature dendritic cells preferentially induce tolerogenic immune responses, matured dendritic cells mediate expansion of interferon (IFN)- γ -producing T cells [17] (Fig. 1).

It has been shown recently that local dendritic cells within the murine oral mucosa are composed by CD11b(+)/CD11c(–) and CD11b(+)/CD11c(+) mDC at the mucosal/submucosal junction zone, CD207(+) Langerhans cells within the mucosal epithelium and B220(+)/120G8(+) pDCs in the submucosal region [18^{*}]. Most interestingly, sublingual application of ovalbumin (OVA) in BALB/c mice resulted in mucosal absorption and accumulation of OVA at the mucosal/submucosal junction within 15–30 min, followed by a continuous clearance of OVA suggesting its uptake and process by CD11b(+)/CD11c(–) and CD11b(+)/CD11c(+) mDC dendritic cells [18^{*}]. This is in line with another study on mice demonstrating that CD11c(+) represent the critical dendritic cell population in antigen uptake within the mouse oral mucosa [19]. In contrast to mice, Langerhans cells represent the predominating dendritic cell population within human oral mucosa, whereas pDCs are virtually absent [20,21]. Nevertheless, oral mucosal Langerhans cells (oLCs) differ from the

classical epidermal Langerhans cells (eLCs), especially by the constitutive expression of the high affinity receptor for IgE (Fc ϵ RI) [21,22]. It has been shown *in vitro* that expression of Fc ϵ RI appears in early differentiation of Langerhans cells, leading to the suggestion that oLCs represent a more immature dendritic cell subpopulation, which would further underline their pro-tolerogenic character [23]. Most interestingly, it has been reported that oLCs of allergic individuals displayed increased expression of Fc ϵ RI occupied by IgE [22]. As oLCs are located within the suprabasal epithelium layer, it is most likely that they bind and process allergens during SLIT. It is tempting to speculate that Fc ϵ RI on oLCs facilitates allergen capture via receptor-bound IgE during SLIT in humans.

The natural tolerogenic state of oral mucosal tissue is critically influenced by the microenvironment composed by commensal bacteria [8]. Bacterial products are sensed by distinct structures of the innate immune systems, the so-called pathogen-specific-pattern-recognition receptors such as the Toll-like receptor (TLR) family. Lipopolysaccharide (LPS) represents a common bacterial product frequently present within the oral mucosa. LPS recognition requires an innate immunoreceptor complex formed by TLR4 combined with the LPS receptor/CD14 (CD14) and the connecting molecule MD2 [24]. It has been reported that TLR ligation on one hand activates a complex signal transduction cascade leading to transcription of pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor (TNF)- α , but on

Table 1 Different dendritic cell subtypes found within human and murine oral mucosa

	Human	Mice	References
Major dendritic cell population	Langerhans cells	CD11b(+), CD11c(+) dendritic cells	[18*,20–22]
Location	Suprabasal layer of oral epithelium	Epidermal/dermal junction	[18*,21]
Surface markers	FcεRI, TLR4, CD1a(+), CD207(+), CD11b(+)	CD207(–),CD11c(+) and CD11c(–)	[18*,20–22,27]
Cytokines produced	IL-10	IL-10	[18*,19–22]
Tolerance induction	+	+	[27–30]
Enforcing Th1	+	+	[27–30]

IL-10, interleukin-10; TLR, Toll-like receptor.

the other hand is required to sustain the intestinal homeostasis [25,26]. It has been demonstrated that human oLCs express CD14 and TLR4. In this context, activation of TLR4 on oLC by monophosphoryl A (MPL) led to an upregulation of coinhibitory molecules B7-H1 and B7-H3 as well as to the induction of IL-10 production by oLCs [27]. Furthermore, ligation of TLR4 on human oLC induced FoxP3(+) Tregs producing IL-10 and TGF-β1 next to IFN-γ-producing Th1 cells [27]. This leads to the assumption that innate immune receptors such as TLR4 on human oLC are involved in the maintenance of natural tolerance within oral mucosal tissue. Moreover, it has been demonstrated in a BALB/c mouse model that sublingual OVA challenge results in the induction of not only Th1 cytokine IFN-γ, but also IL-10-producing Th1/regulatory T cells [18*]. Taken together, oral mucosal dendritic cells process antigens applied on mucosal surfaces and are equipped with receptors such as FcεRI, which could facilitate allergen binding in SLIT. Furthermore, the published data endorse the tolerogenic character of oral mucosal dendritic cells in response to externally applied antigens such as OVA as well as to antigens originating from local commensal bacterial products signalling via TLR.

Oral mucosal dendritic cells as targets for adjuvants in sublingual immunotherapy

This opens the questions whether TLR ligands could enhance the efficiency of SLIT. This assumption is further supported by data from mice. Recently, it has been demonstrated in a BALB/c asthma model that TLR2 activation via Pam3CSK4 together with OVA decreased airway hyperresponsiveness and Th2 responses in the cervical lymph node of mice previously sensitized to OVA [28]. Moreover, TLR2 activation on purified murine oral mucosal mDC led to the upregulation of IL-10 and the induction of IFN-γ and IL-10-producing T cells [28]. Similar results were detected by using other adjuvants such as synthetic pseudodipeptide molecule OM-294-BA-MP or a combination of 1,25-dihydroxyvitamin D3 and dexamethasone (VitD3/Dex) [29,30]. A recent study [31] reports that enhancement of tolerogenic properties of murine oral dendritic cell could also be achieved by mucoadhesive formulations such as chitosan particles, which facilitate allergen contact to oral mucosa leading to increased induction of IFN-γ and IL-10-producing T cells. Another study

[32] reported the effectiveness of mucoadhesive malto-dextrin to enhance the reduction of airway hyperresponsiveness in a murine asthma model. In view of these encouraging data, it is most likely that not only adjuvants but also mucoadhesive particles find their way into the next generation of SLIT formulations. Nevertheless, as dendritic cell populations within oral mucosa differ between murine models and humans, further studies need to investigate receptors for adjuvants on human oLC (Table 1).

Different oral mucosal regions as targets for allergen application

The oral cavity harbours different mucosal epithelium such as lining and masticatory mucosa [8]. It has been shown recently that oLCs are differentially distributed within distinct human oral mucosal sites. In this study, oLCs were more frequently located within the vestibulum, bucca, palatum and lingua, whereas the sublingual regions contained the fewest numbers of oLCs [21]. Although the sublingual mucosa represents the region with the highest permeability, this study opens the question of alternative allergen application sites within the oral cavity. A recent study [33] could demonstrate the clinical effectiveness of sublingual and supralingual grass pollen administration. However, both regions have direct connection to the oropharynx so that accidental swallowing might occur. In this context, the vestibular region appears to be an attractive site for allergen application, as it is separated from the oropharynx by teeth alignment. Moreover, the high number of Langerhans cells and relatively low number of mast cells at this site could endorse the tolerogenic effect of immunotherapy on one hand and might lead to a reduction of allergen dosage on the other hand.

Conclusion

Oral mucosal dendritic cells are capable of processing antigens applied to the mucosal surface. The natural pro-tolerogenic character of local mucosal dendritic cells is mirrored by the induction of tolerogenic T cells in response to oral mucosal dendritic cell activation by either externally applied antigens such as OVA or antigens originating from the local commensal bacterial flora via TLR ligands. This knowledge leads to the search for adjuvants endorsing tolerance during SLIT, which will

most likely find their application as the next generation of SLIT formulations. Additionally, recent data showing that the sublingual region in human harbours low numbers of dendritic cells open the question whether we apply the allergen within the optimal oral mucosal site.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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