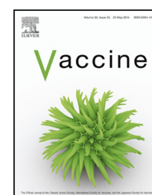
Vaccine 32 (2014) 5669–5676

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Repeated antigen painting and sublingual immunotherapy in mice convert sublingual dendritic cell subsets

Chenyang Zhang[a], Tatsukuni Ohno[a], Siwen Kang[a], Toshiro Takai[b], Miyuki Azuma[a,*]

[a] Department of Molecular Immunology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8547, Japan

[b] Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

ARTICLE INFO

Article history:
Received 26 April 2014
Received in revised form 21 July 2014
Accepted 8 August 2014
Available online 29 August 2014

Keywords:
Dendritic cells
Oral mucosa
Pollinosis
Sublingual immunotherapy
Sublingual mucosa

ABSTRACT

The sublingual mucosa (SLM) is utilized as the site for sublingual immunotherapy (SLIT) to induce tolerance against allergens. The contribution of SLM-dendritic cells (SLM-DCs) has not been clarified. The aim of this study was to examine the dynamics and phenotype of SLM-DCs after topical antigen painting and SLIT. SLM-DCs were histologically evaluated after FITC painting. A novel murine Japanese cedar pollinosis (JCP) model was generated and change in SLM-DCs after SLIT was examined. The density of SLM-DCs was clearly lower compared with the buccal mucosa and dorsal surface of the tongue. Topical FITC painting on the SLM induced maximal recruitment of submucosal DCs (smDCs) at 6 h, but most smDCs had vanished at 24 h. Repeated painting on the SLM induced exhaustion and conversion of the smDC phenotype. $CD206^{high}CD11c^{low}$ round-type cells with fewer dendrites and less lymph node migration capacity became dominant. In the murine model of JCP, SLIT efficiently inhibited clinical symptoms and allergen-mediated immunological responses. SLIT markedly reduced the number of SLM-DCs, converted to the round-type dominant phenotype and inhibited the activation of regional lymph node DCs. Topical antigen painting on the SLM induced rapid exhaustion and conversion of smDCs. The unique dynamics of SLM-DCs may contribute to tolerance induction in SLIT.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The oral mucosa is a type II mucosal surface covered by a stratified squamous epithelium, which share many features with the skin and serves as a convenient route of low-molecular-weight drug delivery due to its high permeability and easy accessibility [1,2]. The oral mucosa also represents a natural barrier and sensor against microbial pathogens. The sublingual mucosa (SLM) has long been used as a route for sublingual immunotherapy (SLIT), which induces tolerance against allergens [3,4]. The SLM has also been considered as a mucosal vaccine delivery route, and sublingual vaccination induced systemic and mucosal immune responses for protection against infectious pathogens [5–7].

Dendritic cells (DCs) are the most potent initiators of innate and adaptive immune responses and regulate immunity and tolerance by integrating various signals [8,9]. DC populations are highly heterogeneous and local microenvironments greatly affect their differentiation, maturation, and function. Oral mucosal DCs (OMDCs) play a pivotal role in induction of immune responses in concert with epithelial cells [3,10,11]. In the steady state, at least two types of DCs, Langerhans cells (LCs) and interstitial/submucosal DCs (smDCs), exist as resident DCs. However, little is known about the physiological features of OMDCs after antigen (Ag) exposure. OMDCs have been shown to possess tolerogenic properties reflected in production of IL-10, upregulation of the co-inhibitory molecule CD274, generation of regulatory T cells (Tregs), or T helper cell deviation [3,4,12,13]. Among the various OMDC subsets, $CD11b^{+}CD11c^{-}$ macrophage-like cells play a key role in tolerance induction following SLIT in asthmatic mice [13].

We previously examined the local distribution and dynamics of buccal mucosa (BM)-DCs after Ag application [14,15]. After FITC painting, MHC class II $(MHC\ II)^{+}CD207^{-}$ DC numbers in the submucosal layer increased markedly, peaking at 6 h [14]. In the regional LNs (RLNs), four Ag-captured migrating DC subsets were identified: resident $CD11b^{hi}$ smDCs, newly recruited blood-derived smDCs, resident $CD207^{+}$ smDC, and resident LCs [15]. Resident $CD11b^{hi}$ smDCs migrated rapidly and expressed high levels of the potent

* Corresponding author. Department of Molecular Immunology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan. Tel.: +81 3 5803 5935; fax: +81 3 5803 5935.
E-mail address: miyuki.mim@tmd.ac.jp (M. Azuma).

http://dx.doi.org/10.1016/j.vaccine.2014.08.013
0264-410X/© 2014 Elsevier Ltd. All rights reserved.

costimulators, CD86 and CD273 at 24 h; newly recruited smDCs represented a major subset at all time points; and LCs migrated at the late time point.

Japanese cedar (*Cryptomeria japonica*) pollinosis (JCP) is the most common allergic disease in Japan and is considered a national affliction [16]. More than one-third of the Japanese populations have JCP, and its prevalence is continuously increasing. Clinical trials showed that SLIT ameliorated JCP symptoms and improved QOL scores [17–19]. However, the immunological mechanisms of SLIT and the contribution of SLM-DCs remain unclear.

To understand biological features of SLM-DCs, we focused on their distribution and dynamics after topical Ag application and examined SLM-DCs histologically in comparison to BM-DCs. We further examined SLM-DC status after SLIT in a newly established murine JCP model.

2. Materials and methods

2.1. Mice

Female BALB/c mice were purchased from Japan SLC (Hamamatsu). All mice were maintained under SPF conditions at the Tokyo Medical and Dental University animal facility. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

2.2. Topical painting on oral mucosae

Under anesthesia with a ketamine/xylazine mixture, 5 μl of 3% FITC (dissolved in a 1:1 (*v*/*v*) acetone: dibutyl phthalate) was applied onto the right side of the BM or the ventral side of the posterior tongue (SLM) (Fig. S1). After painting, mice were maintained with their heads in an anteflexion position for 30 min to avoid swallowing effects and to keep the Ag solutions on the exact mucosal surface.

2.3. Immunohistochemistry

Painted regions of mucosal tissues were surgically dissected at the indicated time points, embedded in Tissue-Tek, frozen, and stored at −80 °C until use. The painted tongue region was coronally sectioned (Fig. S1). Serial cryostat sections were fixed in cold absolute acetone and subjected to enzymatic immunohistochemistry as described previously [14] (Supplementary Materials and Methods).

2.4. Murine model of JCP and SLIT

Under inhalation anesthesia using isoflurane, BALB/c mice received nasal instillation of 10 μl each of Japanese cedar (*C. japonica*) pollen grains (Wako 168-20911, 5 mg/ml, resuspended in PBS) onto both nostrils twice a week (12–13 times in total) as sensitization. Unsensitized mice received similar anesthesia and nasal inhalation of PBS alone. After measurement of serum total IgE and pollen-specific IgG1 (pollen-IgG1) at 7–9 weeks, the sensitized mice were divided into two groups as the mean IgE and pollen-IgG1 titers are comparable, and SLIT was started. Based on the therapeutic schedule of SLIT for JCP [20], gradually escalated doses of cedar pollen extract (kindly provided by Torii Pharmaceutical Co.) were applied onto the ventral surface of the tongue for continuous 4 weeks. The dose escalation protocol is shown in Supplementary Table. Control mice received similar application of PBS. Three days after the final SLIT application, challenge was performed by intranasal instillation for 7–8 days.

Following awakening from the final challenge anesthesia, frequency of sneezing and duration of rubbing were recorded. Sixteen hours after the final challenge, blood, spleens, RLNs (submandibular and cervical LNs), bronchoalveolar lavage fluid (BALF), and craniofacial tissues were collected for immunological assessment. Serum IgE was measured by sandwich ELISA [21]. Pollen-IgG1 in sera and pollen-specific cytokine production by splenocytes were measured

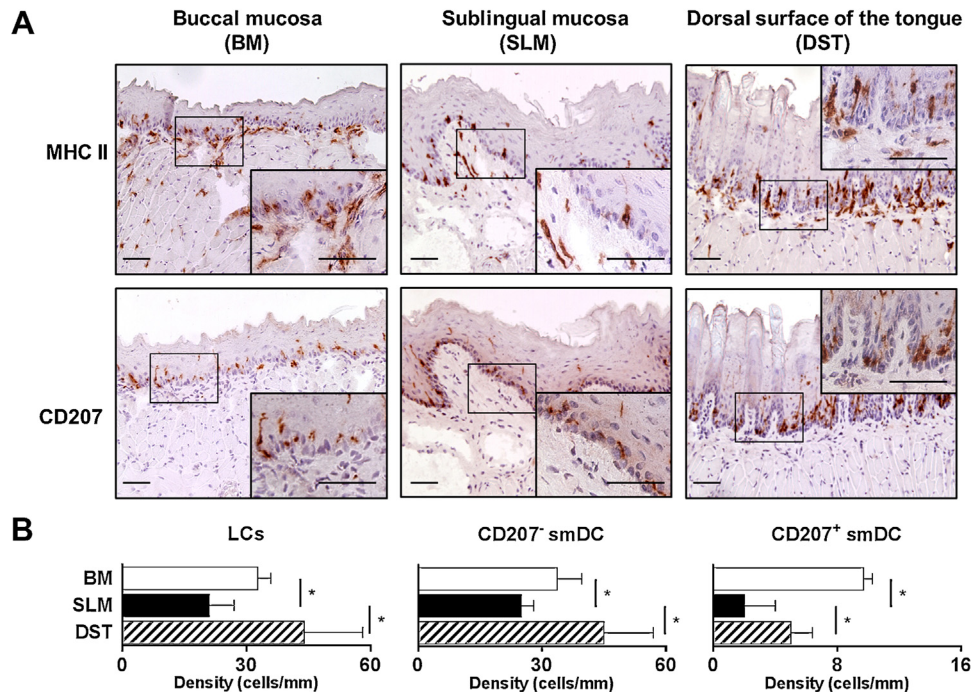


Fig. 1. Distribution of MHC class II⁺ and/or CD207⁺ DCs in three sites of oral mucosae.
Serial cryostat sections of buccal mucosa (BM) and the posterior part of the tongue from intact mice were stained with anti-MHC class II or anti-CD207 mAb. (A) Representative images are shown. Ventral surfaces of the tongue are shown as sublingual mucosa (SLM). Scale bars = 50 μm. (B) Numbers of MHC class II⁺CD207⁺ LCs (LCs), CD207⁺MHC class II⁺ smDCs (CD207⁺ smDC) and CD207⁻ MHC class II⁺ smDCs (CD207⁻ smDC) were evaluated as described in the Materials and methods section. The values shown are the means ± SD from 3–5 mice. $^{*}p < 0.05$.

Download English Version:

https://daneshyari.com/en/article/10964313

Download Persian Version:

https://daneshyari.com/article/10964313

Daneshyari.com