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Efficient antigen delivery to the draining lymph nodes is a key component in the immunogenic pathway of the intradermal vaccine

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ABSTRACT

Background: It has been clinically demonstrated that intradermal (ID) vaccines have a potential to confer a superior immunogenic profile compared to intramuscular (IM) or subcutaneous (SC) vaccines. In terms of distribution of a vaccine antigen depending on the administration routes, at least two independent immunogenic pathways of the vaccines have been proposed: (1) the antigen recognition by the immune cells present at the vaccine-administered site and (2) the antigen recognition by the lymph node (LN)-resident immune cells through the lymphatic flow from the vaccine-administered site after the antigen is directly delivered into the draining LNs.

Objective: In order to clarify the key components for the immunogenic pathway of the ID vaccine, the correlation between the kinetics of the antigen distribution to the draining LNs and antibody responses to the antigen were evaluated.

Methods: We compared the antibody responses in the groups with by surgical removal of the administration site immediately after the ID administration, and by surgical removal of the draining LNs before the ID administration.

Results: The results suggested that the efficient and direct antigen delivery to the draining LNs plays an important role in the antibody responses to the ID vaccine. Indeed, it was confirmed that the direct administration into the draining LNs with the antigen elicited comparable levels of the antibody responses with the ID vaccine. At the cellular level, it was shown that the LN-resident immune cells such as B cells, dendritic cells, and macrophages including medullary macrophages and subcapsular sinus macrophages interacting with the antigens following the ID administration. Finally, we demonstrated by immunofluorescence analysis that the lymphatic vessels are more diffusely distributed in the dermis as compared with the subcutaneous area and muscle.

Conclusion: The results of the present study suggested that the skin is an optimal tissue to facilitate the vaccine antigen access to the draining LNs, which is an important immunogenic pathway of the ID vaccine. Further elucidation of regulatory mechanisms underlying such an immunogenic pathway of the ID vaccine would provide us with elements for the development of novel adjuvants and devices to enhance the immunogenicity of the ID vaccines.

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1. Introduction

Most of the injection-type vaccines currently used in clinics are administered via the intramuscular (IM) or subcutaneous (SC) route, whereas the results of recent clinical studies have suggested that a variety of intradermal (ID) vaccines, whose formulation contains antigens of an inactivated-type, live-attenuated-type, or recombinant proteins, have a potential to confer a superior immunogenic profile compared to an IM or SC vaccine [1–4]. In

Abbreviation: ID, intradermal; SC, subcutaneous; IM, intramuscular; LN, lymph node; LV, lymphatic vessel; HA, haemagglutinin; EBD, Evans Blue dye; iLN, intra lymph node; DC, dendritic cell; ROI, region of interest; MFI, mean fluorescent intensity; SSM, subcapsular sinus macrophage; MM, medullary macrophage.

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fact, the World Health Organization recommend the ID administration of a reduced dosage of the rabies vaccine originally used for the IM injection with an expectation to improve vaccination coverage for post-exposure prophylaxis in low-income countries [5]. The only ID vaccine currently marketed was first launched in 2007 as a vaccination for seasonal influenza, and has been used in the markets of several developed countries [6].

Because the dermis in humans is a thin tissue located at a depth of less than 1 mm from the skin surface and the thickness of the dermis is approximately 1–3 mm depending on the local site of the body, both training and experience are generally required for practitioners to perform an accurate and consistent ID injection by using a standard needle and syringe, i.e., Mantoux technique. In order to offer an easy and reliable ID vaccination, several different types of ID administration devices have been developed, and their excellent performance has been demonstrated in clinical studies [7–9]. In fact, the results of the clinical studies have demonstrated that the immunogenic profile of the ID vaccine using such an ID administration system is better than that of the SC vaccine, while the safety profile of the ID vaccine is similar to that of the SC vaccine [7].

Recent scientific evidence has suggested that the skin has functionally developed for recognition of invading foreign antigens, while the immunogenic pathways of the ID vaccines remain to be elucidated. Anatomically, the skin consists of two distinct regions, i.e., the epidermis and dermis. The epidermis is derived from ectoderm and is an epithelial layer that is primarily composed of keratinocytes. Langerhans cells, known to have an antigen-presenting function, are present primarily in the epidermis under the steady-state condition. In contrast, the dermis is derived from mesoderm and is a relatively cell-sparse tissue, forming a stromal layer underneath the epidermis. It is populated primarily by fibroblasts that secrete components of the complex extracellular matrix. In the steady-state condition, a variety of immune cells, such as mast cells and dendritic cells (DCs), are present in the dermis and play a pivotal role in the skin immune responses to antigens [10]. The skin-resident DCs are believed to be responsible for the first-line surveillance of foreign antigens. After capture of the antigens in the skin, they become differentiated and migrate into the draining lymph node (LN), where they present antigenic epitopes to cells, accounting for the acquired immune responses [11–13].

In addition to such immunological properties, the skin has also unique micro-vascular structures. The rich vascularization of the dermis is assured by terminal arterioles forming the plexus of 65–75 capillary loops/mm^2 [14]. The extensive dermal lymphatic network originates in the dermis close to the epidermis (papillary dermis) as small lymphatic vessels (LVs), merging and forming major channels in the deep layers of the dermis (reticular dermis), which drain to the regional draining LNs. Such a vascular system facilitates antigen migration via LVs and subsequent stimulation of LN-resident immune cells [15].

The LNs are the secondary lymphoid tissue to facilitate the cross-talk of innate and acquired immune responses. Two different pathways have been proposed for the antigen presentation in LNs. In addition to the pathway, and depending on migratory antigen-presenting DCs as described above, the LNs have an independent pathway to quarantine foreign antigens flowing through afferent LVs by LN-resident DCs and macrophages. After trapping antigens in the LNs, such cells are also capable of activating antigen-specific T cells, and induction of memory B cells, independent of antigen-presenting DCs migrating from outside of LNs [16,17].

In the present study, the components of the skin immune system and the skin lymphatic system required for the immunogenic pathway of the ID vaccine were examined by using mouse models. The results suggested that efficient antigen delivery to draining LNs and uptake of the antigen by LN-resident DCs and macrophages, rather than skin-resident immune cells migrating into the draining LNs from the skin, are a key to the immunogenicity of the ID vaccine.

2. Materials and methods

2.1. Animals

BALB/c mice 6–10 weeks old were purchased from CLEA Japan or Charles River Japan. Mice were maintained under a specific pathogen free condition. All immunization and treatments were performed under ketamine-xylazine anesthesia. All experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of National Institutes of Biomedical Innovation, Daiichi Sankyo and Kitasato Daiichi Sankyo vaccine.

2.2. Distribution of Evans Blue dye (EBD) and the antigen into draining LN and LVs after ID administration

EBD (Sigma) was dissolved at 0.5% (weight/volume) in PBS. EBD solution was injected into the ear pinna by using a 35 G needle (React System). Within 5 s or 30 min after injection, the color changes in the ear and LNs were recorded using a digital camera (RICOH, CX6). Next, 20 μL of 0.15 mg/mL Alexa 647-labeled OVA (Life technologies) in PBS was administered into the right ear pinna or administered SC at right ear base near the auricular LNs. Thirty minutes after injection, mice were euthanized, their LNs of interest were collected and washed softly with PBS, and then their fluorescent intensities were examined by using an IVIS system (Summit Pharmaceuticals international Corporation). The results were shown as total fluorescent intensity in the region of interest (ROI). The equal-sized circular ROI was applied to the image of each LN using Living Image® software. In the same experiment, mice were injected into ear pinna with 0.2 μg of Alexa 488-labeled OVA in 2 μL of PBS. Immediately after injection, the injected ear was removed and then the distribution of the fluorescent signal was examined under TCS SP5 confocal system (Leica, Wetzlar, Germany) and processed with Leica LAS software.

2.3. Vaccination and measurement of antibody response

Influenza A H1N1 (A/Puerto Rico/8/34) hemagglutinin (HA) protein (Sino biological) was used as the vaccine antigen and prepared in different concentrations after either concentrated by using Vivaspin 500, 10 kDa (GE healthcare) or dissolved in PBS. Three micrograms of HA antigen was administered into the ear pinna or administered SC at the ear base near the auricular LNs or administered ID or SC at the back. Three weeks after vaccination, antibody response specific to the HA antigen was measured by ELISA. Ninety-six well plates were coated with 0.5 μg/mL of HA antigen or with different concentrations (3-fold serial dilutions from 0.25 μg/mL) of purified mouse IgG (Southern Biotech). Plates were washed three times with PBS with 0.05% Tween 20 (Wash buffer), and then treated with PBS with 0.05% Tween 20 containing 1% BSA (Blocking buffer). The sera from immunized mice were diluted with blocking buffer. The diluted sera were added to plates and then the plates were incubated for an hour at 25 °C. After washing, the plates were incubated with either horseradish peroxidase-conjugated goat anti-mouse IgG, IgG1, or IgG2a (Southern Biotech) for an hour. After washing, TMB microwell peroxidase substrate (KPL) was added to the plates. Five to ten minutes after incubation at 25 °C, 2N sulfuric acid (Wako) was added and finally absorbance at 450–540 nm was measured by plate reader (Power wave HT, BioTek). The antibody concentration in each serum sample was analyzed using Gen 5® software.

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