LETTER TO THE EDITOR

Analysis of the skin of mice humanized for the immune system

Abstract

Development of new immunotherapeutic strategies relies on the ability to activate the right cells at the right place and at the right moment and on the capacity of these cells to home to the right organ(s). Skin delivery has shown high potency for immunotherapeutic administration. However, an adequate in vivo model of human skin immunity is still a critical bottleneck. We demonstrated here that the skin of human immune system mice is colonized by human hematopoietic cells, mainly human T cells and that complementation with human antigen-presenting cells at the vaccination site allowed the induction of an immune response.

1 | BACKGROUND

The skin, the largest organ of the human body, is the first line of protection against pathogens, physical and chemical injuries. It is a major immunological organ with an important density of antigen-presenting cells (APC)[1] able to capture pathogens through endocytic pathways and exhibiting cross-presentation ability. In addition, the skin tissue contains a large pool of T cells, mostly memory T cells expressing the skin-homing marker CLA (cutaneous lymphocyte antigen) and displaying long-term immune protection capacity.[2,3] These are features highly desirable for induction of vaccination and tissue maintenance of immune protection. We previously demonstrated that delivery of vaccine via the skin allows for the generation of a potent CD8 T-cell response and the induction of a humoral

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

DATA S1 Extended information on experimental methods.

FIGURE S1 Analysis of circulating platelet-derived microparticles using NAVIOS™ cytometer

TABLE S1 Plasma levels of PMPs (μL−1) and STA-PPL (s) in patients with stage III and IV melanoma

Abbreviations: APC, antigen-presenting cells; cDC, conventional dendritic cells; CLA, cutaneous lymphocyte antigen; hHPC, human hematopoietic progenitor cells; HIS, human immune system; MVA, modified vaccinia Ankara; pDC, plasmacytoid dendritic cells.

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immune response in the mucosa. However, the lack of an appropriate small animal model to study human skin immunity in vivo remains a major roadblock, which has been addressed by constructing mice humanized with human skin graft. However, this mouse model is technically challenging to routinely establish. Alternatives exist with mice humanized for the human immune system (HIS).

2 | QUESTIONS ADDRESSED

Human immune system mice represent an attractive tool to study ontogeny of cellular components of the human immune system in vivo. However, little is known about the skin compartment in these animals. Here, we investigated colonization of the skin of HIS mice by human hematopoietic cells in order to establish a preclinical model to investigate vaccination and immunotherapeutic application via the skin route.

3 | EXPERIMENTAL DESIGN

HLA-A2 transgenic NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg(HLA-A2.1)1Enge/SzJ) mice were humanized (NSG-HLA-A2-HIS) with a single intra-hepatic injection of 0.5×10^5-1.5×10^5 HLA-A2+CD34+ human hematopoietic progenitor cells (hHPC) into sublethally irradiated newborn NSG-HLA-A2 mice (<5 days old). This led to a high-level engraftment of a human immune system with long-term maintenance capacity in vivo. NSG-HLA-A2-HIS mice were used as they demonstrated improved T-cell functionality after infection/immunization.

4 | RESULTS

We first evaluated by flow cytometry the level of human hematopoietic cell (hCD45+) engraftment in the skin as compared to various organs of NSG-HLA-A2-HIS mice. We observed a high level of hCD45+ engraftment in the spleen, liver, lymph node (Figure 1A) and thymus of the animals (data not shown). The lung and bone marrow exhibited good to intermediate level of human hCD45+ cells engraftment (Figure 1A), whereas the vagina and intestine (data not shown) were moderately colonized by human hematopoietic cells. This data were confirmed by histology (Fig. S1). In comparison, the skin from the flank and the ear was moderately engrafted with human CD45+ cells, which were mostly located in the dermis (Figure 1A). The engraftment efficiency of human cells in the skin was time-dependent, with increasing human cell density in the skin of older animals (23-26 weeks old, Figure 1C) as compared to younger ones (12-13 weeks old, Figure 1B).

The human cells observed in the skin of the animals as well as in other mucosal tissues (eg lung, vagina) were mainly human T cells (Figure 2A,B), and only few human APC, in contrast to human skin explants (Fig. S2). The human T cells present in the skin were composed for two-thirds of human CD4+ T cells and one-third of human CD8+ T cells, which were both predominantly of memory phenotype (Figure 2C), similarly to what is observed in human skin explants (Fig. S2) and the HIS mice lymphoid organs (Fig. S3). A high fraction (~80%) of these human T cells expressed CLA allowing them to reside in the skin (Fig. S3). Interestingly, human CD4+ regulatory T cells (CD25+FoxP3+) were also identified in the skin of the animals at a frequency similar to the one observed in the spleen (Figure 2C).

We next immunized NSG-HLA-A2-HIS mice, intra-dermally, with MVA (Modified Vaccinia Ankara, 1 pfu/cell) or PBS control. No human T-cell response was detected by IFN-γ ELISPOT in the spleen of the animals (data not shown). Considering the size of the hAPC compartment in HIS mice and their critical role in the induction of T-cell responses, we speculated that hAPC density at the site of administration (ie the skin) is potentially too low to optimally trigger T cells. We thus tested the capacity of human DC complementation in the skin of the NSG-HLA-A2-HIS mice, deriving autologous human cDC from human CD14+ cells isolated from the same cord blood than the hHPC used to reconstitute NSG-HLA-A2-HIS mice. Autologous umbilical cord blood CD14+ monocytes were isolated by MACS and cultured for 3 days in presence of 50 ng/mL hGM-CSF and 5 ng/mL hIL-4. Autologous human monocyte-derived DC were loaded for...
LETTER TO THE EDITOR

2 hours at 37°C with MVA or PBS. Next, NSG-HLA-A2-HIS mice were immunized intra-dermally in the flank with 0.5×10^6 matched human CD14^+ derived MVA- or PBS-loaded DC loaded. A human T-cell response was observed 7 days after immunization by IFN-γ ELISPOT in the spleen of the MVA-vaccinated animals (Figure 2D), with significantly more responder animals (3/5) than in the control group (0/5) (chi-square test, P = .019).

5 | CONCLUSIONS

We demonstrated here that the skin of HIS mice is colonized by human hematopoietic cells, mainly human T cells expressing CLA and exhibiting a memory phenotype. Complementation with human APC at the vaccination site allowed the induction of an immune response. This highlighted the potential of the HIS mice as an innovative preclinical model for human skin immunity as well as for prospective analysis of vaccination via skin route, and notably DC therapies. As such, this will also generate knowledge on understanding the early events following immunotherapeutic administration and support demands for in vivo biomodelling of the interaction of immunotherapeutic with the human immune system.

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CONFLICT OF INTERESTS

The authors declare they have no conflict of interests.

Keywords

antigen-presenting cells, human immune system mice, intra-dermal vaccination, route of administration, skin T cells

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Herpes simplex virus 1 and cytomegalovirus are associated with pemphigus vulgaris but not with pemphigus foliaceus disease

Abstract

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are blistering autoimmune diseases that depend on interaction between genetic and environmental factors. Viral infections, like herpes simplex viruses 1 and 2 (HSV1/2), cytomegalovirus (CMV), Epstein-Barr virus and dengue virus, could trigger or exacerbate pemphigus. IgM and IgG antibodies against these viruses in serum from PV and PF, their relatives and controls were determined. HSV1/2 expression was evaluated by direct immunofluorescence (DIF) and qPCR in affected or not oral mucosa from PV patients compared with uninjured PF mucosa. IgG anti-HSV1 was higher in the PV group compared with all groups. IgG anti-CMV resulted higher in PV group compared with PF patients and PV relatives. HSV1 was confirmed by DIF and qPCR on oral samples from patients with PV. Lack of HSV1 expression in the oral mucosa of patients with PF corroborate that immunosuppressive therapy cannot be the main cause for HSV1 replication in PV disease.

1 | BACKGROUND

Pemphigus diseases involve production of autoantibodies against desmogleins (DSG), which cause acantholytic intra-epidermal blisters. The two main clinical forms of pemphigus are pemphigus vulgaris (PV), which affects the skin and mucous membranes by production of anti-DSG1 and anti-DSG3 antibodies, and pemphigus foliaceus (PF), which affects the skin by generation of anti-DSG1.[1–5]

The production of autoantibodies against DSG is well known, but the etiopathogenesis of pemphigus still requires elucidation, and depends on interaction between genetic and environmental factors.[6–9]

2 | ADDRESSED QUESTIONS

Studies have related viral detection to the complications of pemphigus but have not recognized viruses as triggering agents. Conflicting results about the role of HSV in triggering pemphigus exist, making additional analysis to support this possible relationship necessary.

A large number of PV and PF cases are diagnosed in the northeastern region of the state of São Paulo each year.[8,9,14] We have investigated some viruses in patients with PV and compared them in patients with PF in this prevalent area for both PV and PF. We decided to study HSV1/2, CMV and EBV to confirm that these viruses are associated with pemphigus, mainly with PV.[15] We added DENV to our study because only a single report on PF exists,[13] and because dengue disease is endemic in our region.[16]

3 | EXPERIMENTAL DESIGN

This cross-sectional study comprised a convenience sample of patients with PV and PF attended at the outpatient clinic of the University Hospital of the Ribeirão Preto Medical School, University of São Paulo, Brazil, from January 1999 to December 2013. Diagnosis of PV and PF was based on clinical, histopathological and immunofluorescence features and on determination of autoantibodies against DSG1 and DSG3 with ELISA kits (MBL, Japan). This study also included...