Towards animal free testing: Human skin and gingiva organotypic models for the study of Langerhans Cell biology

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1.11 IMMUNE COMPETENT HUMAN SKIN AND GINGIVA EQUIVALENT MODELS

Although animals, in particular mice, are used extensively for skin models, misleading conclusions can be made due to very clear differences between human and animal immunology as illustrated by the fact that extensive differences have been reported between human and mouse DC subsets (Shortman and Liu, 2002;van de Ven et al., 2011). Importantly, no suitable animal models exist to study oral immunity and in particular, no oral animal models exist to identify potentially hazardous substances which come into contact with the oral cavity (e.g. ingredients of toothpastes, mouth wash, dental restorative materials). In order to investigate DC biology, in particular in the area of human safety and risk assessment of chemicals, and for testing novel drugs and therapeutic strategies, a number of *in vitro* models have been proposed. Fresh human skin explants provide a very relevant model to study LC biology *in situ* (Ouwehand et al., 2008;Ouwehand et al., 2010a;Jacobs et al., 2006;Lindenberg et al., 2013;Oosterhoff et al., 2013). However, the use of skin explants for research purposes and *in vitro* diagnostic assays requires a steady supply of large amounts of freshly excised skin, which creates a logistical bottleneck and can introduce high donor variation. To avoid the use of explant material, cultured, easily expanded cells have been used. DC (primary and immortalized cell lines, human or animal-derived) have been extensively studied (dos Santos et al., 2009). A limitation in using these DC cultures is that they lack interactions with other cell types (e.g. keratinocytes and fibroblasts) which are essential for promoting *in vivo*-like DC migration and phenotypic changes. Furthermore, the skin barrier (stratum corneum) is absent. In order to overcome the absence of the skin barrier, the group of Schmidt incorporated cord blood-derived LC into human reconstructed epidermis constructed from primary keratinocytes (Facy et al., 2005;Regnier et al., 1997). Widespread implementation of this model is limited however by its dependence on fresh cord blood which again creates a major logistical problem as well as donor variation. Furthermore, the dermal component is absent which is essential for LC migration from the epidermis to the dermis (Ouwehand et al., 2008;Ouwehand et al., 2010a). Previously we have described a full-thickness skin equivalent (SE) composed of a reconstructed epidermis on a fibroblast populated collagen gel (Spiekstra et al., 2005). Since it was not possible to distinguish allergens from irritants in this model by assessing increases in cytokine secretion, we next proceeded to incorporate LC into this *in vitro* SE model (Ouwehand et al., 2011b;Ouwehand et al., 2012). We were able to provide novel evidence that the physiologically relevant LC-like cell line MUTZ-3 indeed allowed discrimination of allergens from irritants using this integrated model (Kosten et al., 2015).

When considering the study of oral DC biology, the limitations of using explant tissues are far outweighing those for skin with regards to the logistics involved in getting fresh

tissue to the laboratory, the short viability of oral tissue *ex vivo* (48 h) and the extremely limited size of healthy oral mucosa (gingiva) biopsies.

A limited number of studies have described oral mucosa equivalents (Macneil et al., 2011;Moharamzadeh et al., 2012). Applications of engineered oral mucosa include clinical transplantation (Watkin, 2008;Izumi et al., 2013), *in vitro* investigations of the interaction of materials with oral mucosa (Moharamzadeh et al., 2008;Moharamzadeh et al., 2009), oral disease modelling (Andrian et al., 2004;Andrian et al., 2007;Claveau et al., 2004;Rouabhia and Deslauriers, 2002;Yadev et al., 2011) and evaluation of drug delivery systems (Hearnden et al., 2012). However, these reconstructed models described above do not contain DC, and therefore their application is limited. One model describes the incorporation of fresh cord blood derived DC which creates an additional major logistical problem as well as donor variation (Sivard et al., 2003).

In this thesis, as a first step to overcome these limitations and in addition to the previously described skin equivalent which contains MUTZ-3 LC (Ouwehand et al., 2008;Ouwehand et al., 2011b), we describe a technical advance in that we have developed a novel human gingiva equivalent containing MUTZ-3 LC and used it alongside our previously described human immune competent skin equivalent to investigate differences and similarities between skin and oral mucosa immunology with emphasis on LC migration after allergen exposure.

1.12 OUTLINE OF THIS THESIS

The primary aim of this thesis was to compare skin and oral immunity with a focus on DC biology in relation to allergic responses. In order to do so we needed to develop a more physiologically relevant human skin and oral mucosa model with an appreciation of the cross-talk between cells. First, we needed to study the role of the cytokines and chemokines which were already identified in skin as having a key significance in DC migration and compare these to gingiva since much is known about key innate immune events in skin, but little is known about oral mucosa. This is described in **Chapter 2** where we have deployed skin- and gingiva-derived keratinocytes and fibroblasts as well as tissue equivalents and used them as a tool to study cytokine release after a stimulus in order to study the synergistic effects in this regard of keratinocytes and fibroblasts and the cross-talk between these cells. Thus, oral mucosa and skin-equivalents were compared in their secretion of cytokines and chemokines involved in LC migration and general inflammation. Basal secretion, representative of homeostasis, and also secretion after stimulation with TNFα, an allergen or an irritant was assessed.

Since we were interested in cutaneous DC biology we have used a previously described human skin equivalent model which contains MUTZ-3 LC and we exposed it to allergens and irritants to determine the functionality of the model and test the plasticity of the MUTZ-3 Langerhans cells (in analogy to their physiological counterparts) in **Chapter 3.**

Chapter 4 describes the results of a comparative study of the phenotype and functional abilities of skin and gingiva derived migratory DC subsets. We used the crawled out cells from healthy freshly excised skin and gingiva and studied them extensively using flow cytometry and measuring their cytokine release.

In **Chapter 5** we explored the immune reactivity in a 3D gingiva equivalent model containing MUTZ-3 LC as compared to the previous described skin equivalent model, when both were exposed to the known allergen cinnamaldehyde.

Finally, in the concluding **Chapter 6** we combine all our findings into a comprehensive discussion of their significance for the field of skin and oral mucosa based allergy and discuss some probable future prospects for the use of these newly constructed *in vitro* 3D equivalent models.