The role of C-type lectin receptors in human skin immunity: immunological interactions between dendritic cells, Langerhans cells and keratinocytes
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**SUMMARY**

**Introduction**

Langerhans cells (LCs) and dendritic cells (DCs) are antigen presenting cells that reside in tissues at the interface between the human body and the outside world, such as skin, the airways and mucosal tissues. LCs reside in the epidermis of skin in close proximity to keratinocytes (KCs), while DCs reside in the collagen-containing dermis underneath the epidermis. LCs and DCs express pattern recognition receptors, such as C-type lectin receptors, to sense invading pathogens. Upon weakening or wounding of the protective skin barrier, pathogens such as bacteria, fungi and viruses can invade the body. C-type lectin receptors have been implicated in pathogenic carbohydrate recognition, pathogen uptake and subsequent activation of LCs and DCs. Activated LCs and DCs will migrate to lymph nodes to activate adaptive T cell responses in order to eradicate pathogens.

The balance between the immune response and the intensity of inflammation determines the quality of wound healing and the efficiency of pathogen killing. Especially during wound healing control is needed, since too much immune activation might lead to poor wound healing and too less immune activation might lead to infection. However, some pathogens have evolved to evade the immune system inducing chronic infection. Once HIV-1 infection has been established, the human immune system is unable to clear the virus from the body. LCs express the C-type lectin receptor langerin, which recognizes and internalizes HIV-1 and prevents subsequent transmission to T cells. In this thesis we investigated the role of keratinocytes, Langerhans cells and dendritic cells in burn wound healing, inflammation and infection.

**Results**

Burn injuries are caused by fire, scalding or hot water and induce tissue damage and excessive inflammation, which is the cause of hypertrophic scar formation. Although the burn wound locally inflames, the systemic adaptive immune system is suppressed in patients. Therefore, the risk of bacterial co-infection and complications resulting in sepsis is high.

In chapter 3 we investigated the functionality of LCs and DCs after burn injury. Human skin was burned with the Human Ex vivo Temperature Regulating-Machine (HEAT-M), and we observe that LCs and DCs from burned skin are dysfunctional. LCs and DCs from burned skin do not activate T cells in contrast to LCs and DCs derived from healthy skin. Moreover, a burn factor released in the medium decreases the activity of healthy DCs to stimulate T cells. These are strong indications that immune responses and wound healing are dysregulated after burn injury. The results we obtained regarding dysfunctional LCs and DCs after burn could possibly explain the observed suppressed systemic immune system in burn patients.

In chapter 4 we set out to improve wound healing after burn injury. KCs, LCs and DCs all express the C-type lectin receptor dectin-1. Dectin-1 recognizes specific fungal beta-glucan carbohydrates and it is known that beta-glucans induce DC and LC maturation. Here we investigate the effect of beta-glucans on KCs. Burn wounds were applied to skin in culture with the HEAT-M and the effect of beta-glucans was...
investigated by culturing skin for 14 days. We show that beta-glucans induce enhanced KC proliferation and migration. Next, we investigate the effect of beta-glucans on a wound healing \textit{in vivo} in a porcine wound healing model, but beta-glucans do not improve porcine wound healing. Thus, the results of the human \textit{in vitro} model are promising, however, the effects of beta-glucans on \textit{in vivo} wound healing and scar-tissue formation need to be further investigated.

In \textit{chapter 5} we have unravelled the mechanisms behind the cellular interaction between LCs and DCs. In activated skin, LCs migrate to the dermis and reside in close proximity of DCs in the dermis. \textit{In vitro} LCs indeed cluster very efficiently with DCs and these data suggest that LCs and DCs interact and communicate. The C-type lectin receptor \textit{langerin} on LCs strongly binds to the carbohydrate \textit{hyaluronic acid} on DCs. Next to skin, both LCs and DCs are present in foreskin and vaginal epithelium and are therefore the first immune cells encountering HIV-1 upon sexual intercourse. We hypothesized that clustering could enable antigen transfer from LCs to DCs for T cell stimulation. We show that LCs take up HIV-1, but are not able to activate cytotoxic T cells. Notably, when LCs are co-incubated with DCs, antigens are transferred from LCs to DCs and DCs subsequently activate T cells. These data show that LCs and DCs interact via langerin and hyaluronic acid and that this interaction enables antigen transfer from LCs to DCs for T cell activation. These data provide clues to design new vaccination strategies against HIV-1 and other pathogens.

In \textit{chapter 6} we show that LCs also interact with KCs via langerin and hyaluronic acid. KCs are present in the epidermis and abundantly express hyaluronic acid on their cell surface. We show that activation of LCs leads to upregulation of the enzymes hyaluronidase-1 and -2, which trim HA and control migration out of the epidermis. LC-DC clustering is also regulated via hyaluronidase expression by both LCs and DCs. Thus, we describe how langerin-hyaluronic acid binding and LC migration is regulated in epidermis and dermis.

In \textit{chapter 7} we have identified the nature of Birbeck granules in LCs. Dr. Birbeck discovered tennis-racquet-shaped organelles in LCs in 1961, which were named ‘Birbeck granules’, that are langerin-positive. LCs mediate HIV-1 uptake via langerin and the virus is taken up into Birbeck granules. We identified that Birbeck granules are caveolin-1positive and show that HIV-1 is taken up via the caveolar internalization route. By blocking this endocytic route, HIV-1 DNA integration increases in the host genome. Therefore we conclude that caveolin-1 and langerin are important in HIV-1 uptake and degradation via the caveolar endocytic route. These data are important since they provide insight in the mechanisms underlying HIV-1 infection and can be used for improving HIV-1 prevention strategies.

**General implications**

In this thesis I have described the role of LCs, DCs and KCs in wound healing, inflammation and infection. Restoring the function of LCs and DCs after burn could prevent infections and could possibly result in better wound healing. In addition, I have shown that activation of dectin-1 on KCs improves wound healing, thus the C-type lectin receptor dectin-1 present on KCs can be targeted to improve (burn)
wound healing by inducing KC proliferation and migration. Carbohydrate-structures, such as beta-glucans, could be added to creams that are topically applied onto the wound. Moreover, dectin-1 activation on LCs and DCs could possibly overcome the immune suppression observed after burn injury. This knowledge could be used to improve wound healing and to improve skin disease treatments, such as eczema, psoriasis or skin allergies.

I have identified a cellular ligand for langerin, and show that this ligand -hyaluronic acid- is important in cellular interactions between LCs and DCs, and between LCs and KCs. These interactions are important in immune responses as well as migration of LCs. These data provide new insights for epidermal vaccination, since targeting LCs in epidermis also targets DCs in dermis. Furthermore, hyaluronic acid is present in the extracellular matrix as well as expressed by other cell-types. Further studies are necessary but these data suggest that LC interactions might be governed primarily by langerin as a specific adhesion receptor on LCs. The fundamental knowledge of LC and DC immuno-biology could be used in strategies to combat not only HIV-1, but also other viral, bacterial or fungal infections, cancer and auto-immune diseases.