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Keio University

Thesis Abstract

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Thesis Title

Langerhans Cells Prevent Autoimmunity *via* Expansion of Keratinocyte Antigen-Specific Regulatory T Cells

（ランゲルハンス細胞はケラチノサイト抗原特異的な制御性 T 細胞の増殖を介して自己免疫反応を抑制する）

Thesis Summary

Langerhans cells (LCs) are antigen-presenting cells (APC) in the epidermis whose roles in antigen-specific immune regulation remain poorly understood. Desmoglein 3 (Dsg3) is a desmosomal glycoprotein expressed by keratinocytes and is the autoantigen targeted in pemphigus vulgaris. We hypothesized that LCs, given their proximity to keratinocyte-associated autoantigens, are the major APC to regulate torelogenic immune responses against Dsg3.

To address our question, we first sought to determine via immunofluorescence microscopy whether LCs were capable of acquiring Dsg3 from surrounding keratinocytes. Detection of non-conformational epitopes of Dsg3 with LC cytoplasm suggested that LCs captured Dsg3 from surrounding keratinocytes. To confirm this, we generated mice that express a Dsg3-eGFP fusion protein driven by the keratin 5 promoter, which enabled us to detect Dgs3 by eGFP signals. Flow cytometry analysis of epidermal cells suspension revealed that up to 5% of LCs were $eGFP^{+}$, indicating that a subset of LCs had taken up Dsg3 from surrounding keratinocytes.

To determine the immunological consequence of Dsg3 uptake by LCs, we co-cultured LCs from unmanipulated WT (LC^{WT}) mice as APC and Dsg3-reactive $CD4^{+}$ T ($H1^{rag}$) cells as responders. The responder cells underwent robust proliferation, which was completely blocked by antibodies against MHC class II (MHC II). Moreover, LCs from $Dsg3^{-/-}$ mice failed to activate $H1^{rag}$ cells, demonstrating that LCs acquire Dsg3 and present it in the context of MHC II in a Dsg3-specific manner. We hypothesized that the uptake of Dsg3, a highly glycosylated protein, is mediated by langerin. Langerin is a c-type lectin required for the formation of Birbeck granules and was demonstrated to be an endocytic receptor in vitro, but its roles in immune response remain unknown. When we co-stained langerin and Dsg3 in epidermal sheets prepared from WT mice, we observed co-localization of Dsg3 and langerin signals in LC cytoplasm. To explore the role of langerin in immune response, we utilized LCs from mice that lack functional langerin ($LC^{DTR/DTR}$). $LC^{DTR/DTR}$ were impaired in their ability in vitro to activate $H1^{rag}$ cells, indicating that langerin mediated Dsg3 uptake prior to antigen presentation. Additional evaluation of phenotype of $H1^{rag}$ cells revealed that LC expanded both regulatory T (T_{reg}) and effector T (T_{eff}) cells.

Because of LC^{WT} expanded both T_{reg} and T_{eff} cells *in vitro*, we sought to confirm the role of LCs in Experimental Autoimmune Dermatitis (EAD) model, a $CD4^{+}$ T cell-mediated autoimmune response against Dsg3. In this model, when H1 cells are adoptively transferred into $Rag2^{-/-}$ mice, they readily infiltrated the skin and elicit INF-γ-dependent response directed against keratinocytes, causing

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apoptosis and obscuring the dermo-epidermal junction. Interestingly, EAD in mice that lack LCs exhibited an aggravated phenotype with significantly elevated clinical score and more prominent interface dermatitis. Moreover, re-stimulation of $CD4^+$ T cells from lesional epidermis *in vitro* revealed an increase production of IL-17 as well as trend toward increased INF-γ. In addition, flow cytometry analysis of skin-draining lymph nodes revealed a decreased expansion of T_{reg} cells in mice that lacked LCs. In aggregate, the results suggest that LCs suppress autoimmunity *in vivo* by expanding T_{reg} cells.

To explore the mechanism by which LCs expand T_{reg} cells, we utilized WT $CD4^+$ T cells as responder cells rather than $H1^{rag}$ cells because the activation of T_{eff} and cytokines that they produce may have secondary effects on T_{reg} cell expansion. Consistent with a previous report, LCs induced significant proliferation of T_{reg}, but not T_{eff}, demonstrating that LCs preferentially expanded T_{reg} cells in the absence of autoimmune T cells. The T_{reg} cells that proliferated may be specific for various epidermal self-antigens that were acquired by LCs. We added neutralizing antibodies against molecules that are involved in T_{reg} cell induction and found that only anti-MHC II and anti-IL-2 antibodies had notable effects on T_{reg} cell expansion. Blockage of IL-2 receptor α (CD25) had an effect similar to IL-2 neutralization. Furthermore, thymus derived T_{reg} cell markers Helios and Neuropilin-1 were expressed by LC-expanded T_{reg} cells. These data suggest that LCs mediate the expansion of naturally-occuring T_{reg} cells, rather than induced T_{reg} cells.

The effect of IL-2 and CD25 blockage on T_{reg} cells could be explained by direct effects on T cells. However, LCs highly upregulated CD25 upon emigration from epidermis, suggesting that IL-2 might directly affect LCs. Indeed, signal transducer and activation of transcription 5 (STAT5) underwent phosphorylation (pSTAT5) in LC both the epidermis and lymph nodes of WT mice, indicating that IL-2 signaling takes place in LCs. To further characterize the effect of IL-2 on LCs, LCs were exposed to rIL-2 in vitro. Global transcripts analysis by Nanostring showed decreased expression of *Citta*, a regulator of MHC II, and increased *tgfb1* and *smad3*, both of which are associated with TGF- β signaling and LC migration. To predict the biological significance of rIL-2-induced overall gene expression change in LCs, we performed Ingenuity Pathway Analysis and found that there were upregulation of pathways that were involved in cell motility and migration. Thus, IL-2 signaling in LC evokes a less activated but motile phenotype that may represent homeostatic migration of LCs that carry autoantigens. Finally, to determine the consequence of impaired IL-2 signaling in LCs during T_{reg} cell proliferation, we utilized LCs from $CD25^{-/-}$ mice and found that CD25 on LCs was required for optimal T_{reg} cell expansion and that the IL2-IL2 receptor axis in LCs conditioned them to expand T_{reg} cells.

In conclusion, these data establish that LCs take up Dsg3 via langerin and suppress autoimmunity by expanding regulatory T cells. In addition, IL-2 receptor signaling occurs in LCs conditioning them to mediate peripheral tolerance. Exploring environmental cues that govern LCs' immunological polarity should enable new strategies to control skin immunity and to achieve tolerogenic responses in autoimmune blistering diseases such as phemphigus.