Application Note

Enhanced T cell proliferation & activation in InflammaSkin®: A flow cytometry perspective

GENOSKIN

Abstract

This application note highlights new comparative data on T cell dynamics in HypoSkin and InflammaSkin, generated using flow cytometric analysis. The analysis reveals increased expression of **CD69**, a T cell activation marker, and proliferation marker **Ki-67**, along with differences in **CD8+** and **CD4+ T cell populations** and the presence of double-positive T cells. These findings emphasize InflammaSkin's ability to replicate an inflammatory environment characteristic of psoriasis, offering a robust platform for evaluating T cell-mediated immune responses and potential therapeutic interventions.

Introduction

The InflammaSkin model provides an innovative tool for studying T cell-mediated inflammation, designed to mimic the Th17/Th1 polarized immune environment typical of psoriasis. In contrast, HypoSkin represents a baseline, non-inflammatory skin model. This application note presents a comparative analysis of T cell activation, proliferation, and subset dynamics using flow cytometry and our proprietary spatial biology platform, MANTIS. The results shown here further validate InflammaSkin as a relevant system for investigating psoriasis-related immune mechanisms and drug efficacy.

Methods

Model preparation

- **HypoSkin:** *Ex vivo* human skin model comprising preserved epidermis, dermis, and hypodermis. The models are produced using Genoskin's standard culturing methods with daily medium renewal.
- **InflammaSkin:** *Ex vivo* human skin model comprising preserved epidermis, dermis, and hypodermis. *In situ* T cell activation and Th17/Th1 polarization are induced using a proprietary cocktail, with the models otherwise cultured under identical conditions than the HypoSkin models.

Flow cytometry analysis

- Single cells were identified using forward and side scatter properties, then live and dead cells were distinguished using a viability dye.
- CD45+ cells were gated to identify immune cells, followed by CD3+ T cell identification.
- Within CD3+ cells, CD69 (activation) and Ki-67 (proliferation) markers were analyzed for relative expression levels.

MANTIS® (Multiplex ANnotated Tissue Imaging System) Spatial Biology

The MANTIS spatial biology platform is a cutting-edge 3D multiplex imaging technology that delivers detailed insights into human skin's immune landscape. By combining fluorescence-based imaging with AI-driven analysis, MANTIS enables spatially resolved phenotyping and simultaneous visualization of over 12 markers in a single acquisition. This platform provides data on immune cell interactions and responses, offering preclinical researchers a useful tool for understanding the effects of tested compounds on real human skin.
Here, MANTIS provided immune cell subset identification and spatial localization, offering insights into the tissue distribution of T cells, including double-positive (CD4+CD8+) T cells.

Results

CD69 as a Marker of Enhanced T cell Activation in InflammaSkin® vs. HypoSkin®

Understanding immune cell activation in different tissue environments is critical for evaluating the immunological impact of therapeutic candidates. In this study, we compared T cell activation markers in InflammaSkin and HypoSkin. Flow cytometry revealed a significant increase in CD69 expression among CD3+ T cells in InflammaSkin compared to HypoSkin **(Figure 2)**. Elevated CD69 levels reflect increased T cell activation, consistent with the inflammatory microenvironment induced in InflammaSkin.

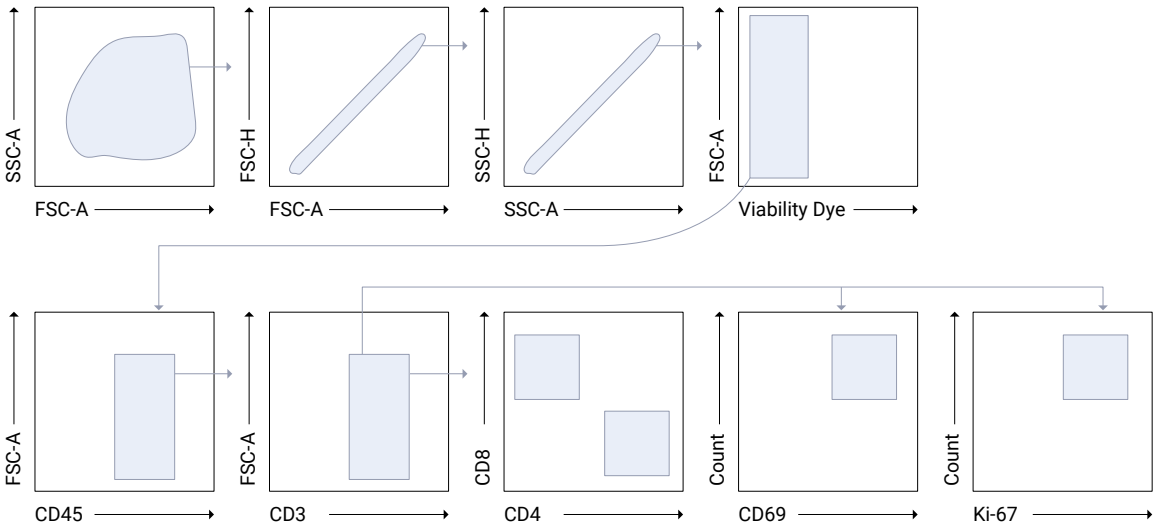

Figure 1. Gating strategy for flow analysis of Ki-67 and CD69 on skin-resident T cells.

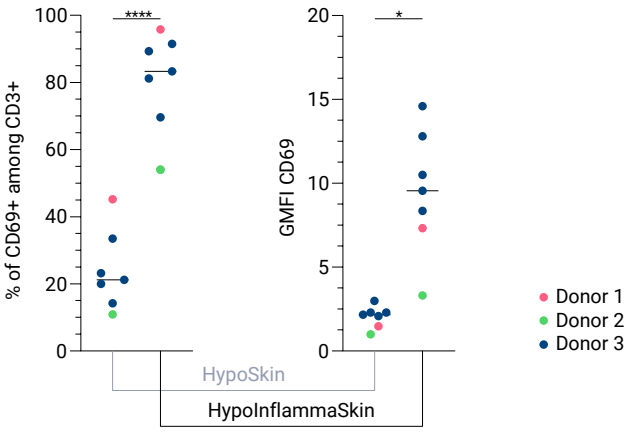

Figure 2. Histogram comparing CD69 expression in T cells from HypoSkin and InflammaSkin models produced from three separate donors. Each dot of the same color represents a biological replicate.

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Ki-67 Expression Highlights Enhanced T cell Proliferation in InflammaSkin®

To investigate cellular proliferation within distinct skin environments, we assessed the expression of Ki-67, a key marker of active cell division, in InflammaSkin and HypoSkin models. Analysis of Ki-67 revealed substantially higher expression in the T cells derived from InflammaSkin models compared to HypoSkin **(Figure 3)**. This suggests that the InflammaSkin models have increased numbers of proliferating T cells, replicating the hyperproliferative state observed in psoriasis.

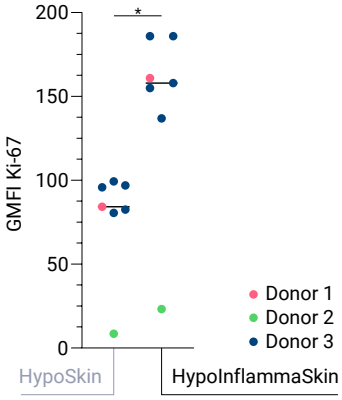

Figure 3. Histogram illustrating Ki-67 expression in T cells between the two models produced from three separate donors. Each dot of the same color represents a biological replicate.

T cell Subset Dynamics

CD8+/CD4+ Proportions: Flow cytometry data revealed shifts in the proportions of CD8+ and CD4+ T cells in InflammaSkin compared to HypoSkin **(Figure 4A)**, indicating changes in T cell populations associated with an inflammatory environment.
Immune Cell Populations: Spatial analysis using MANTIS highlighted a marked presence of activated immune cells, including double-positive (CD4+CD8+) T cells, in InflammaSkin versus HypoSkin **(Figure 4B)**.

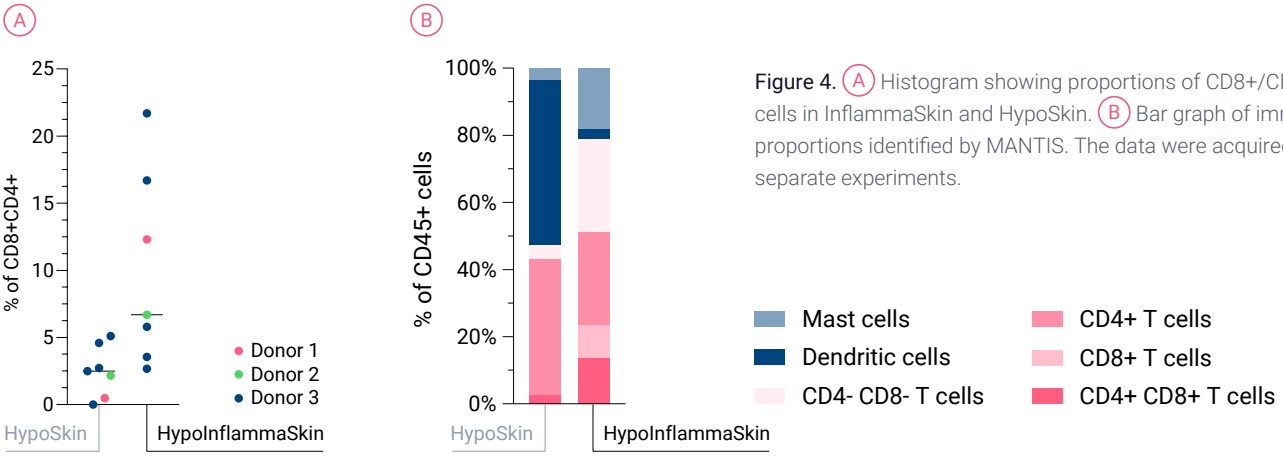

Figure 4. (A) Histogram showing proportions of CD8+/CD4+ T cells in InflammaSkin and HypoSkin. (B) Bar graph of immune cell proportions identified by MANTIS. The data were acquired from 2 separate experiments.

Discussion

The enhanced activation and proliferation of T cells in InflammaSkin highlight its ability to replicate psoriasis-like inflammation at the cellular level. Elevated CD69 expression underscores increased T cell activation, while increased Ki-67 expression reflects the robust proliferative environment characteristic of psoriasis. These findings emphasize InflammaSkin as a biologically relevant model, bridging preclinical data with clinical relevance for psoriasis research.

Notably, both flow cytometry and the MANTIS spatial biology platform confirmed the presence of CD4+CD8+ double-positive T cells in InflammaSkin, with lower quantities observed in HypoSkin. Double-positive T cells represent a memory-like subset associated with advanced differentiation and enhanced functional capacity, including cytokine production (e.g., IFNγ, TNFα, IL-17A) and cytotoxic activity (e.g., granzyme B, perforin). Their presence correlates with inflammatory severity and further validates InflammaSkin as a platform for modeling complex immune interactions in psoriasis[1].

Additionally, spatial analysis using MANTIS revealed distinct tissue localization patterns of activated immune cells, enhancing the model's ability to mimic *in vivo*-like conditions (Data not presented). This dual-layered insight—from cellular activity to tissue architecture—underscores InflammaSkin's relevance for drug discovery targeting T cell-mediated pathways.

Conclusion

The data presented here further demonstrate the unique capabilities of InflammaSkin as an advanced platform for psoriasis research. The flow cytometry and spatial biology data demonstrate that InflammaSkin is a powerful platform for psoriasis research. Elevated T cell activation (CD69) and proliferation (Ki-67) highlight its ability to replicate an inflammatory environment, while the presence of **CD4+CD8+ double-positive T cells**, confirmed by both flow cytometry and spatial analysis, underscores its relevance for modeling complex immune responses. The integration of cellular activity with spatial insights further enhances InflammaSkin's value for evaluating T cell-targeted therapies and advancing our understanding of psoriasis-related inflammation.

[1]Hagen, M. et al., *Biomedicines 2023*