In vitro generation of Langerhans cells useful in an immune competent *in vitro* 3D skin model

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Abstract

INTRODUCTION

Langerhans cells (LCs) are antigen-presenting immune cells in the epidermis, acting as sentinels continuously monitoring the environment and the first line of defense against foreign pathogens [1]. Despite their shared tissue-resident macrophage developmental origin, LCs adopt a dendritic cell-like phenotype upon differentiation [2]. Recent studies, based on distinct phenotypic and functional characteristics, have classified LCs into four subsets: effector LCs (LC1) and regulatory LCs (LC2) present at steady state, and activated LCs (aLC) along with migratory LCs (migLC) found during skin inflammation. This intra-populational heterogeneity has been suggested to be implicated in skin disorders like psoriasis [3].

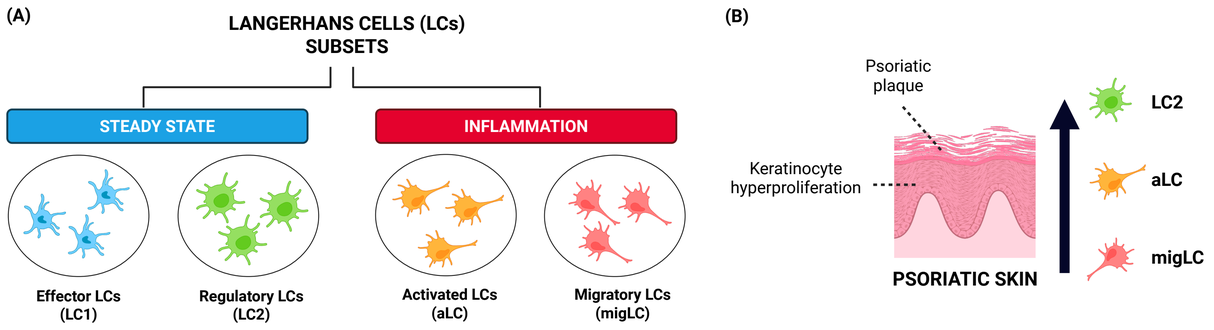

Figure 1. Graphical visualization of the introduction. **(A)** Classification of Langerhans cells (LCs) into distinct subsets present at steady state and during inflammation. **(B)** The Langerhans cell subsets exhibiting increased presence in psoriatic skin. Created with BioRender.com.

RESEARCH AIM

Developing a comprehensive toolbox for generating, identifying, and characterizing distinct subsets of Langerhans-like cells derived from *in vitro* differentiation of human peripheral monocytes.

METHODS

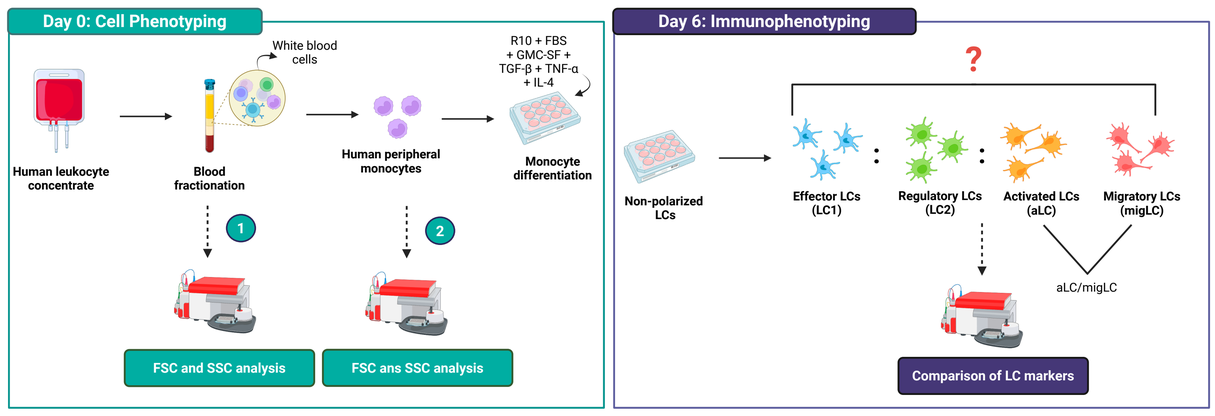

Figure 2. Shortened visualizarion of Langerhans cells (LCs) differentiation method pipeline. The process includes: cell phenotyping (day 0); complete removal of IL-4 from the culture medium (day 2; not visualized) by replacing 100% of the culture medium with IL-4-free medium; partial removal of IL-4-free medium (day 4; not visualized) performed by exchanging 50% of the culture medium with 50% of fresh IL-4-free medium; and LC immunophenotyping (day 6). IL-4 free medium contains R10, FBS, GMC-SF, TGF-β and TNF-α. Created with BioRender.com.

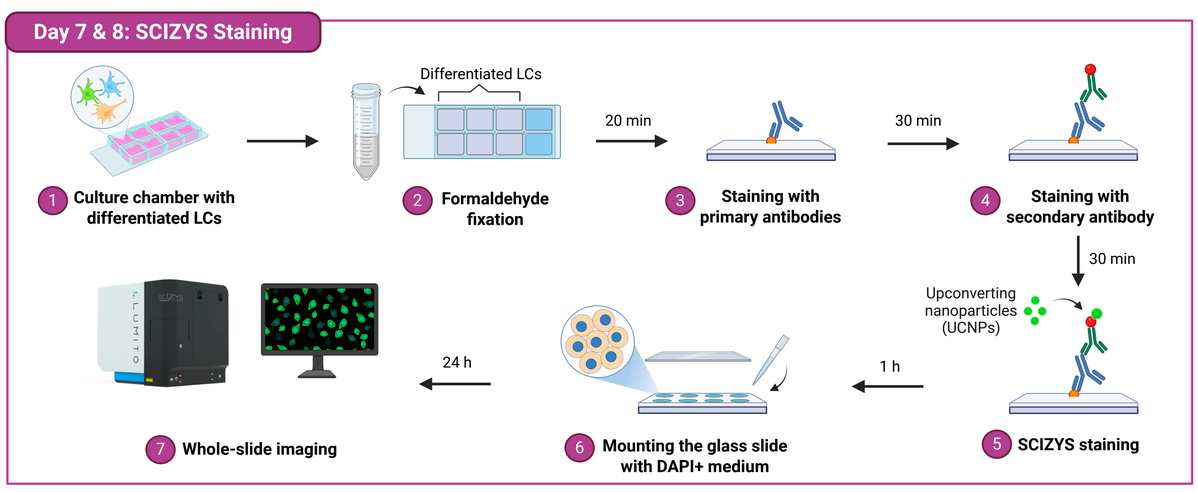

Figure 3. SCIZYS staining of the differentiated Langerhans cells (LCs) for whole-slide imaging using Lumito technology. The process includes formaldehyde fixation of the LCs; staining with primary & secondary antibodies; SCIZYS staining using upconverting nanoparticles (UCNPs); mounting the slides with DAPI-containing mounting medium (day 7); and whole-slide imaging using Lumito technology (day 8). Created with BioRender.com.

RESULTS

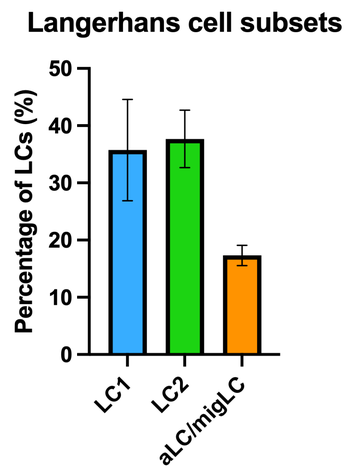

Figure 4. Proportion of different Langerhans cell (LC) subsets obtained via *in vitro* differentation of human peripheral monocytes. Cells were analyzed by flow cytometry using CD83, CD207, CD197, CD209, CD1a surface markers to identify different LC cell subsets. Data are presented as mean percentage (%) ± standard error of the mean (SEM) from six individual donors (n = 6).

Table 1. Proportion of different Langerhans cell (LC) subsets identified by flow cytometry in samples from six individual donors (n = 6). The table presents the percentages (%) of each LC subset differentiated *in vitro* from individual donors, alongside the total percentage (%) of LC population as well as the mean and standard error of the mean (SEM) for each subset across all donors.

Donor number	LC SUBSETS (%)			TOTAL LC POPULATION (%)
	LC1	LC2	aLC/migLC	
1	7.9	51.0	21.5	80.4
2	25.8	40.4	19.6	85.8
3	17.6	52.9	18.4	88.9
4	46.7	29.4	20.6	96.7
5	55.2	28.7	12.3	96.2
6	61.3	23.8	11.6	96.7
MEAN	35.8	37.7	17.3	90.8
SEM	±8.9	±5.0	±1.8	±2.8

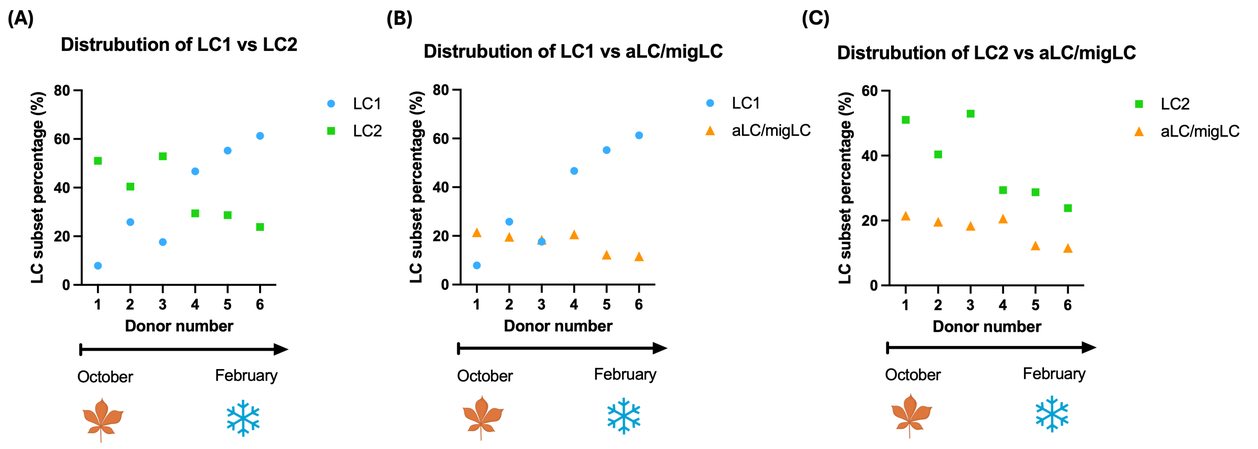

Figure 5. Distribution of Langerhans cell (LC) subsets in samples from six individual donors (n = 6) collected between October 2024 and February 2025. Comparison of **(A)** LC1 vs LC2 subsets; **(B)** LC1 vs aLC/migLC; **(C)** LC2 vs aLC/migLC. LCs from donors 1–3 were differentiated *in vitro* during October–November 2024, while those from donors 4–6 were processed between December 2024–February 2025.

To visualize Langerhans cells differentiated *in vitro* from human peripheral monocytes, we employed a novel imaging technology that combines the use of upconverting nanoparticles (UCNPs) with AI-driven image analysis, enabling high-contrast, high-resolution imaging with minimal autofluorescence and a low detection threshold.

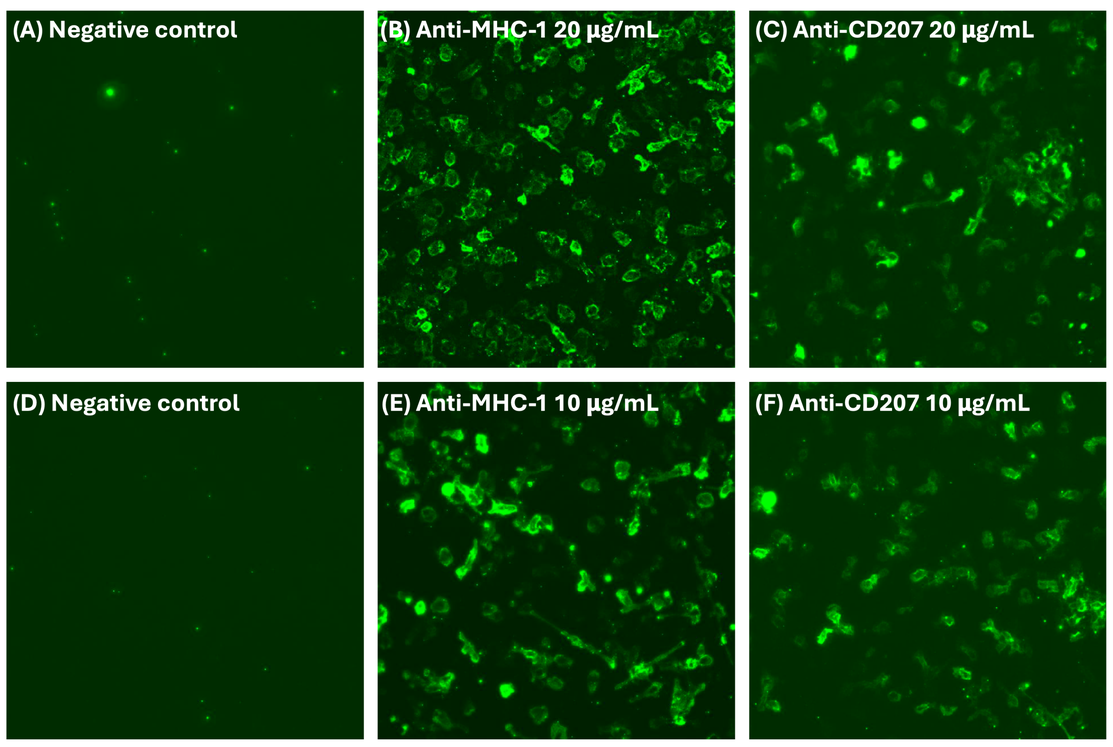

Figure 6. Visualization of Langerhans cells (LCs) via whole-slide imaging using Lumito technology. In brief, after staining the cells with primary antibodies and a biotinylated secondary antibody, fluorescent images were created using SCIZYS uponconverting nanoparticles (UCNPs) which convert low energy photons into emission in the visible region. **(A)** Negative control; **(B)** LC staining against MHC-1 (20 µg/mL); **(C)** LC staining against CD207 (20 µg/mL); **(D)** Negative control; **(E)** LC staining against MHC-1 (10 µg/mL); **(F)** LC staining against CD207 (10 µg/mL).

CONCLUSIONS

- *In vitro* differentiation of human peripheral monocytes into Langerhans-like cells enables the generation of LC1, LC2, and aLC/migLC subsets.
- The steady state subsets LC1 and LC2 are produced in higher proportions compared to the inflammation-associated aLC/migLC subset.
- The distribution of LC subsets varies between donors, reflecting individual donor-dependent variability.
- Differences in the distribution of LC1 and LC2 subsets between cells differentiated from autumn versus winter donors suggest that seasonal variation may influence the capacity of human peripheral monocytes to differentiate into steady state LC subsets.
- Whole-slide imaging using upconverting nanoparticles (UCNPs) enables clear and detailed visualization of LCs in 2D cell culture.

The *in vitro* differentiation of Langerhans cells (LCs) from human peripheral monocytes enables the generation of distinct LC subsets, providing a powerful tool for advancing research into LC biology. This approach paves the way for the development of advanced *in vitro* 3D skin models incorporating LCs, thereby enabling *ex vivo* investigations of skin disorders such as psoriasis.

ACKNOWLEDGEMENTS

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REFERENCES

1. West HC, Bennett CL. Redefining the Role of Langerhans Cells As Immune Regulators within the Skin. Front Immunol. 2018 Jan 5;8:1941. doi: 10.3389/fimmu.2017.01941. PMID: 29379502; PMCID: PMC5770803.
2. Doebel T, Voisin B, Nagao K. Langerhans Cells - The Macrophage in Dendritic Cell Clothing. Trends Immunol. 2017 Nov;38(11):817-828. doi: 10.1016/j.it.2017.06.008. Epub 2017 Jul 15. PMID: 28720426.
3. Liu X, Zhu R, Luo Y, Wang S, Zhao Y, Qiu Z, Zhang Y, Liu X, Yao X, Li X, Li W. Distinct human Langerhans cell subsets orchestrate reciprocal functions and require different developmental regulation. Immunity. 2021 Oct 12;54(10):2305-2320.e11. doi: 10.1016/j.immuni.2021.08.012. Epub 2021 Sep 10. PMID: 34508661.