CD90+ Human Dermal Stromal Cells Are Potent Inducers of FoxP3+ Regulatory T Cells

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Mesenchymal stromal cells (MSC)

- plastic-adherent, self-renewing, multipotent cells
- express a set of stem cell markers (e.g., CD90, CD105, CD73), but lack hematopoietic markers
- localized in virtually every prenatal and adult tissue, including human skin
- are immunomodulatory & suppress a great variety of lymphocytes and differentiate and expand Tregs
- immunomodulation is facilitated by cell–cell contact and the release of soluble factors (e.g., TGF-β, IL-10, HLA-G5)
- are hypoimmunogenic as they lack expression of HLA class II or costimulatory molecules
Regulatory T cells (Tregs)

- control the activation and expansion of aberrant, over- or self-reactive lymphocytes thereby preventing overwhelming pathophysiological immune response
- express CD4, CD25, and the transcription factor forkhead box P3 (FoxP3) and are mostly negative for CD127
- are generated in the thymus through presentation of self-peptides by thymus-resident stromal cells \( \rightarrow \) naturally occurring (n)Tregs
- can be generated from naive CD4\(^+\)CD45RA\(^+\) T cells \textit{in vitro} and \textit{in vivo}
- Thymic dendritic cells and/or stromal cells regulate the positive selection of self-reactive thymocytes and generate FoxP3\(^+\) Tregs via provision of costimulatory molecules (CD80, CD86) through ligation of CD28
Aim of the study

• to determine whether dermal MSC subsets have immunosuppressive capacity

• to investigate whether the dermal MSC can induce the generation of Tregs

• to explore the differentiation potential of dermal MSC toward the endothelial lineage
Experimental design

Carboxyfluoresceine succinimidyl ester (CFSE)-based division tracing coculture system with plastic-adherent dermal cells and CFSE-labelled T-cells stimulated via αCD3/CD28 beads

CFSE labeling is used to monitor distinct generations of proliferating cells by dye dilution. Live cells are covalently labeled with a very bright, stable dye. Every generation of cells appears as a different peak on a flow cytometry histogram.
Phenotype of dermal cells – characterization by CLSM
Suppressive and FoxP3-inducing potential of plastic-adherent dermal cells
Dermal cells induce FoxP3 expression in CD25−CD4+CD45RA+ T cells irrespective of CD28 costimulation.

**Generation of FoxP3+ T cells in the presence of dermal and BM cells**

**Proliferation of CD25−CD4+CD45RA+ T cells induced by dermal and BM cells**

**Dermal cells do not express CD80 or CD86 before and after co-culture with T cells**
Dermal cells induce functional forkhead box P3+(FoxP3+) Tregs

CD127 is downregulated in dermal cell-induced FoxP3+CD4+ T cells

Dermal cell-induced FoxP3+CD4+ T cells are functional as they significantly suppress the proliferation of CD25-depleted, αCD3/CD28-activated T cells
Co-expression pattern of CD25, CD127, and FoxP3 in αCD3-stimulated CFSE-labeled CD25⁺CD4⁺CD45RA⁺ T cells co-cultured with dermal cells, BM cells and HEK cells

**a**

![Flow cytometry chart showing CD25 and CD127 expression in DERMIS 1, DERMIS 2, DERMIS 3, BONE MARROW, and HEK cells.](chart.png)

CD25 and CD127 in dermal and BM stromal cells
Expression level of CD25 and CD127

b

DERMIS 1
DERMIS 2
DERMIS 3
HEK
BONE MARROW

DERMIS 1
DERMIS 2
DERMIS 3
HEK
BONE MARROW

normalized MFI

CD25
CD127
TGF-β and FoxP3 induction

d
TGF-β and FoxP3 induction

e
TGF-β1 (pg ml⁻¹)

<table>
<thead>
<tr>
<th>Sample</th>
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<tr>
<td>Bone marrow</td>
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<tr>
<td>HEK</td>
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g
Latency-associated peptide (LAP)
TGF-β is involved in dermis-induced FoxP3 expression

\( \alpha \text{TGF-\( \beta \)} \)-blocking mAb reduces the number of FoxP3-expressing T cells co-cultured with dermal cells, reduction is concentration-dependent
Ability of different stromal cell subsets to induce FoxP3 in naive CD25−CD4+CD45RA+ T cells w/o provision of costimulatory molecules
CD90^+ dermal cells induce more FoxP3 than CD90^- cells.

CD271^- dermal cells show a tendency to induce more FoxP3 compared to CD271^+ dermal cells.
Cytokine profile upon co-culture of dermal cells, BM cells (+), HEK cells (-) and CD25−CD4+CD45RA+ T cells with and w/o αCD3-stimulation
CD90+ dermal cells are predominantly localized perivascularly

CD3+ T cells are in close proximity to CD90+ cells
CD90^+ dermal cells in EC differentiation medium - expression of endothelial (progenitor) cell markers (CD133, VEGFR2, CD34) & mature endothelial cell markers (CD31, CD144)
Summary

• Plastic-adherent dermal cells suppress T-cell proliferation stimulated via αCD3/CD28 beads in a cell-density dependent manner

• Induction and maintenance of suppressive Tregs within healthy human skin is CD28-independent

• Dermal cells are able to expand natural Tregs and increase the percentage of activation-induced Tregs

• CD90⁺ dermal cells induce significantly higher percentages of FoxP3⁺ T cells compared with CD90⁻ cells

• Plastic-adherent dermal cells are able to differentiate toward the endothelial lineage suggesting that CD90⁺ cells provide a local pool of vessel precursors
Thank you!