In vitro detection of contact (photo-) allergens:

Development of an optimized protocol and performance of an international ring study using human Peripheral Blood Monocyte Derived Dendritic Cells

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DANKE

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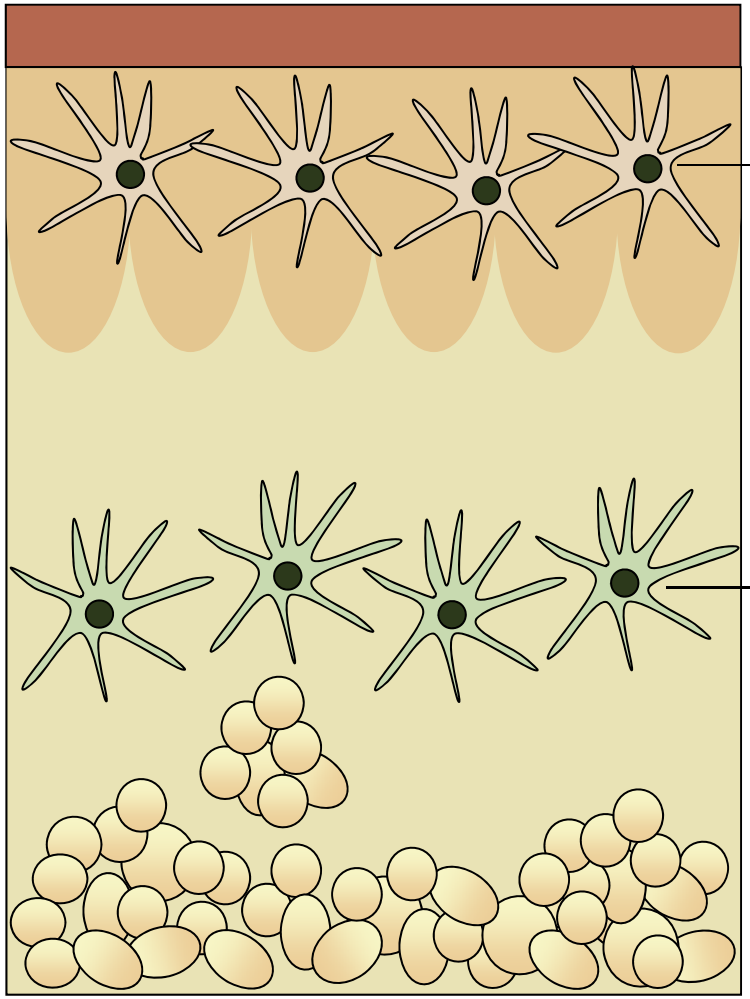
Toxikologischer Endpunkt: Sensibilisierung
– Hintergründe

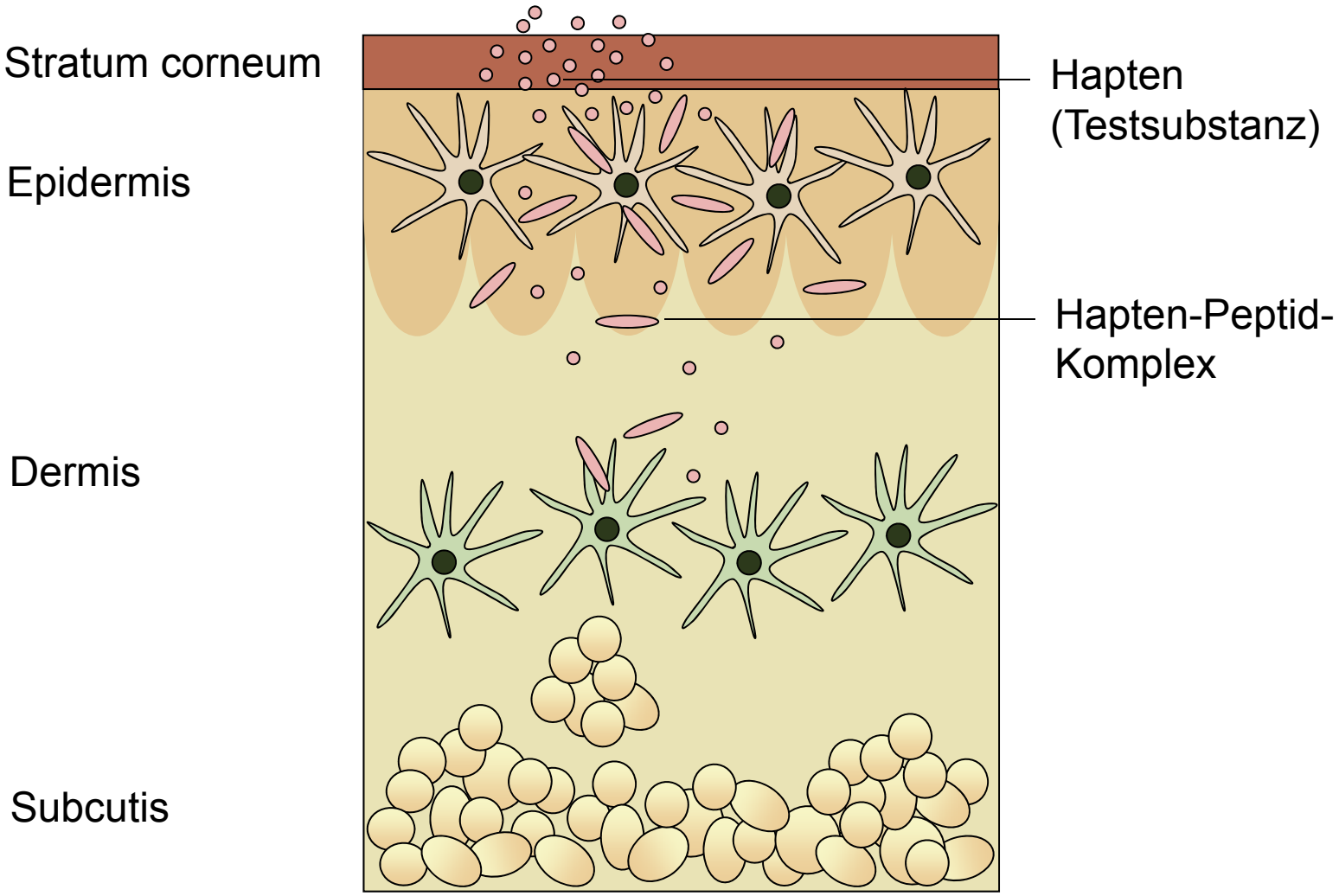
Allergische Kontaktdermatitis, Typ IV- Allergy:

- **T-Zell vermittelt**
- **Verursacht durch niedermolekulare reaktive Chemikalien (Haptene)**
- **Haptene können sein: Antibiotika, Antimykotika, Metallionen, kosmetische Inhaltsstoffe**

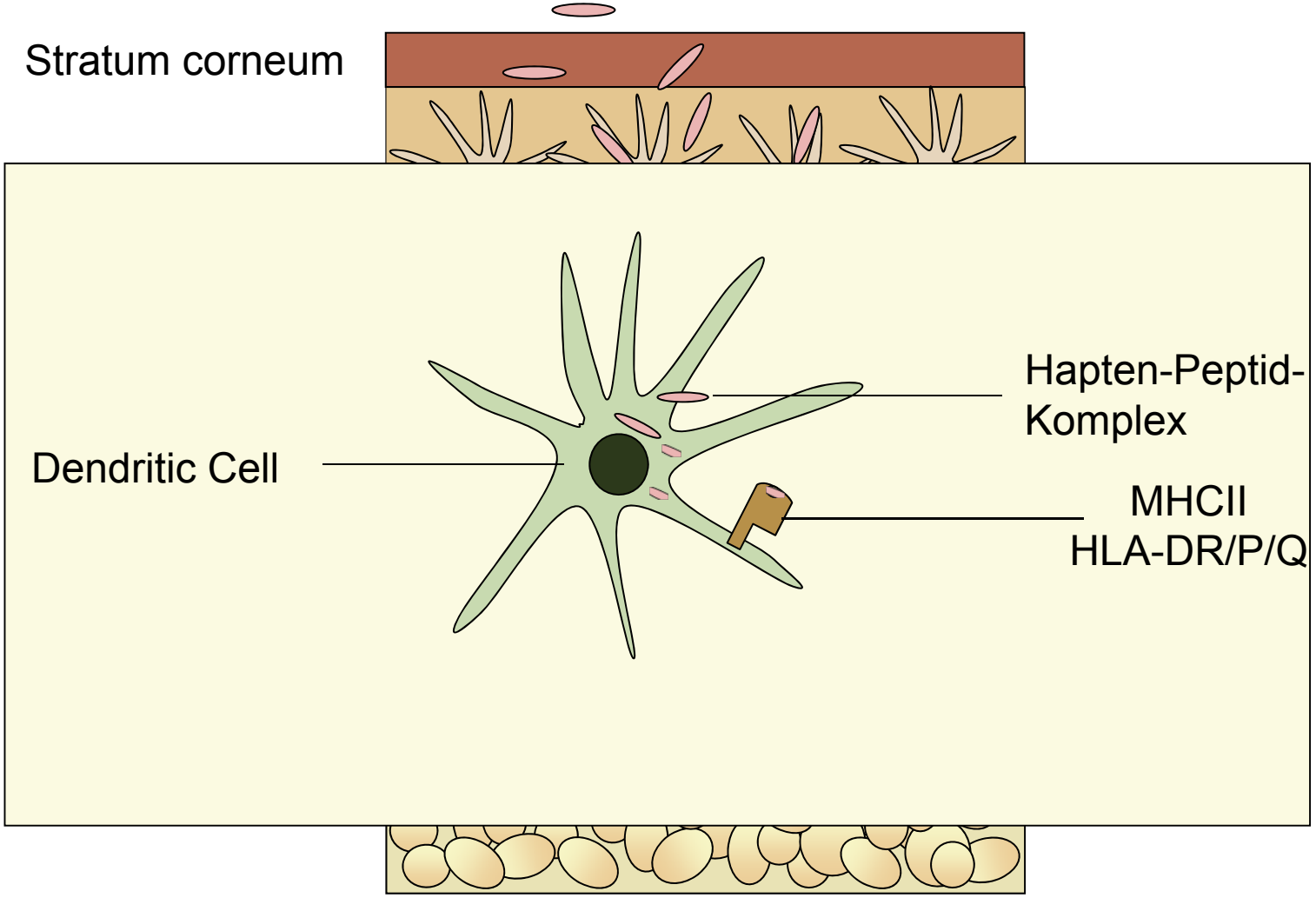
Kontaktallergie - Grundlagen



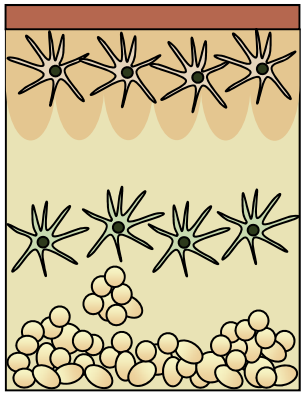
Kontaktallergie - Grundlagen



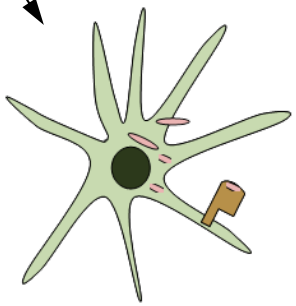
Kontaktallergie – Grundlagen: Prozessierung/MHC II

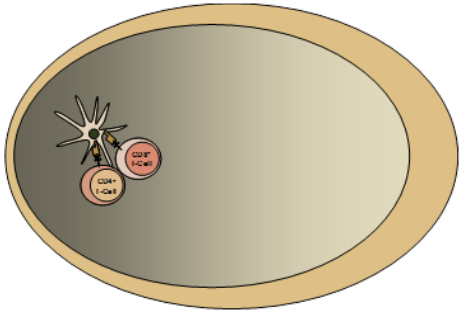


Kontaktallergie: Wanderung zum Lymphknoten

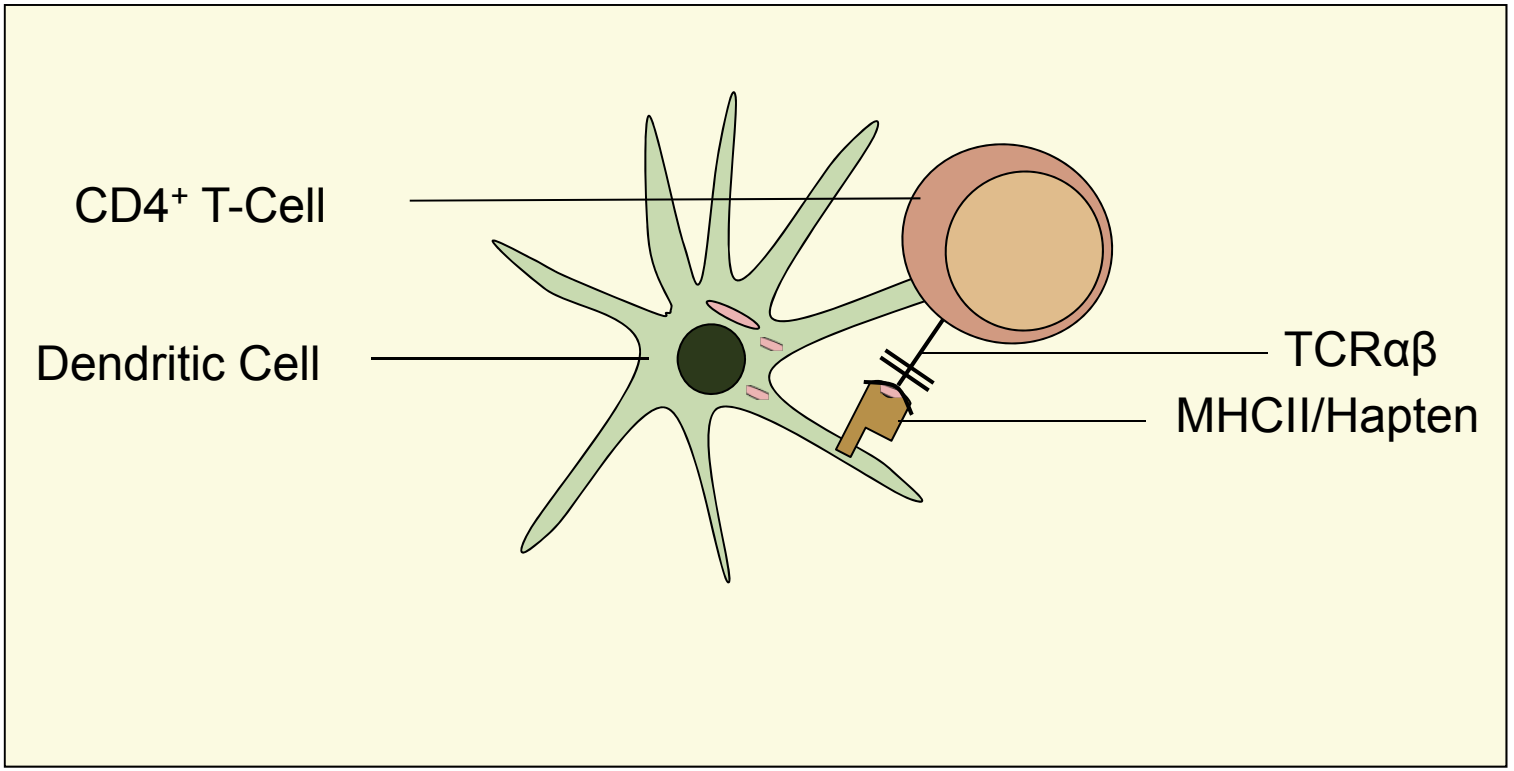


Sensibilisierung?

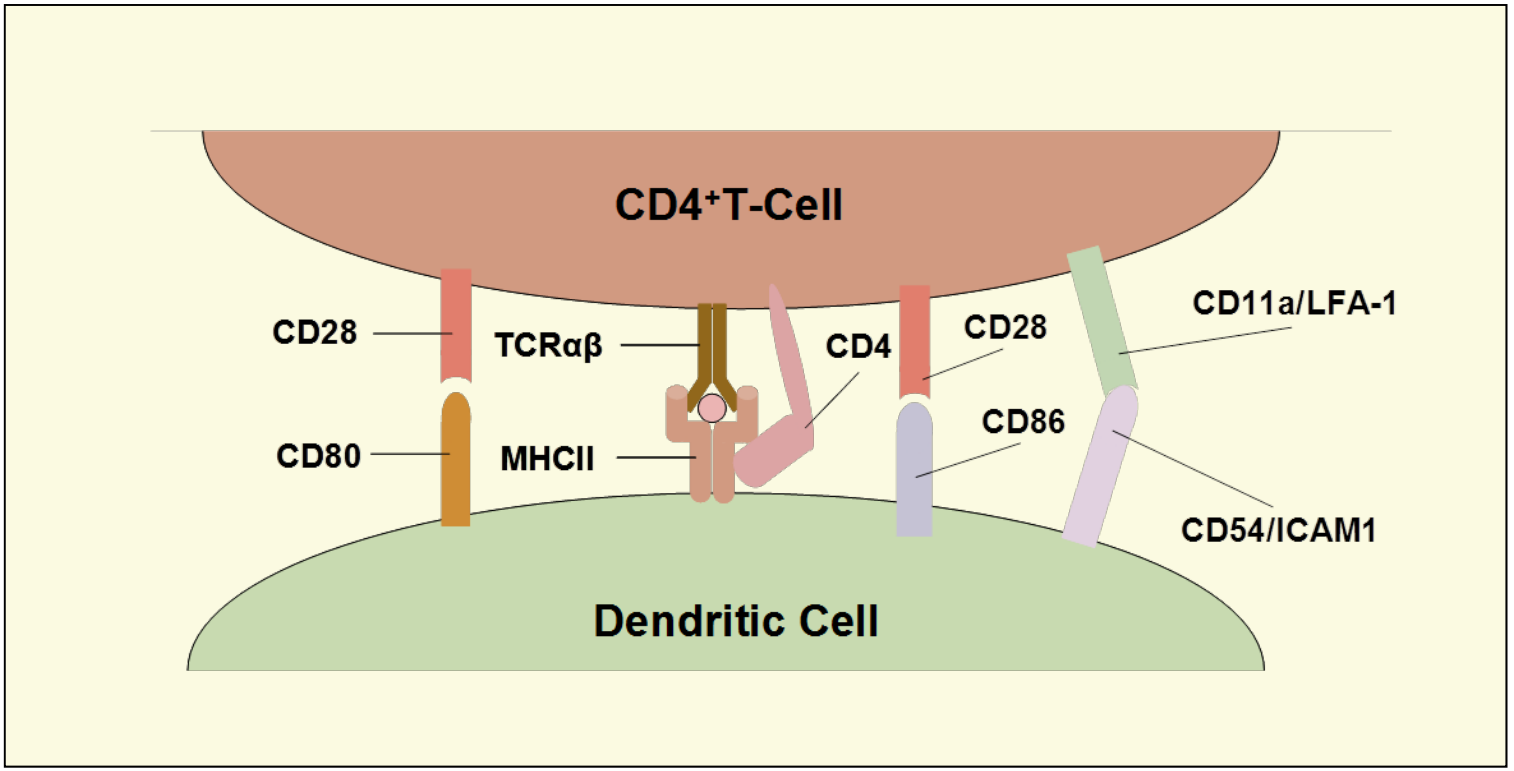




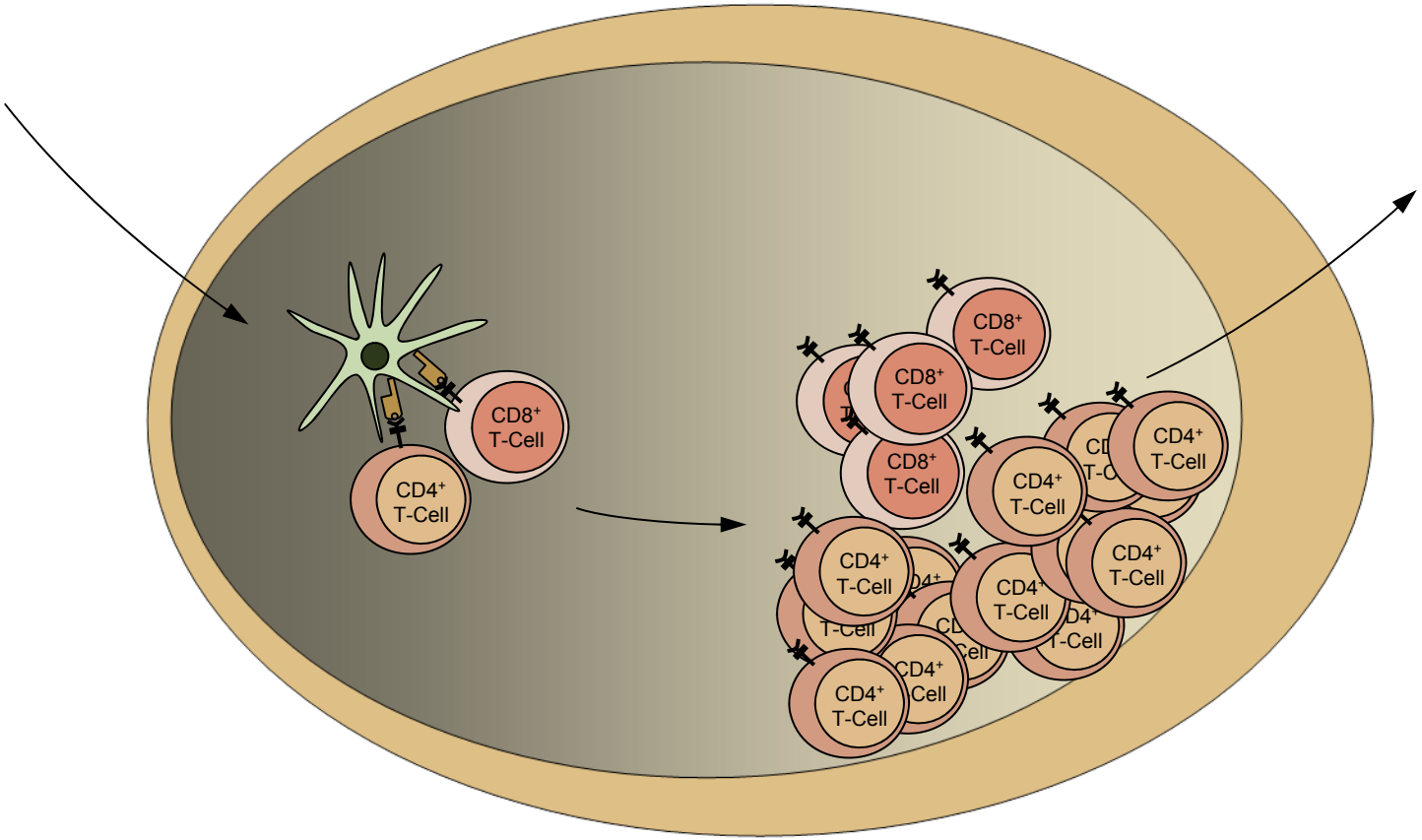
Kontaktallergie - Grundlagen



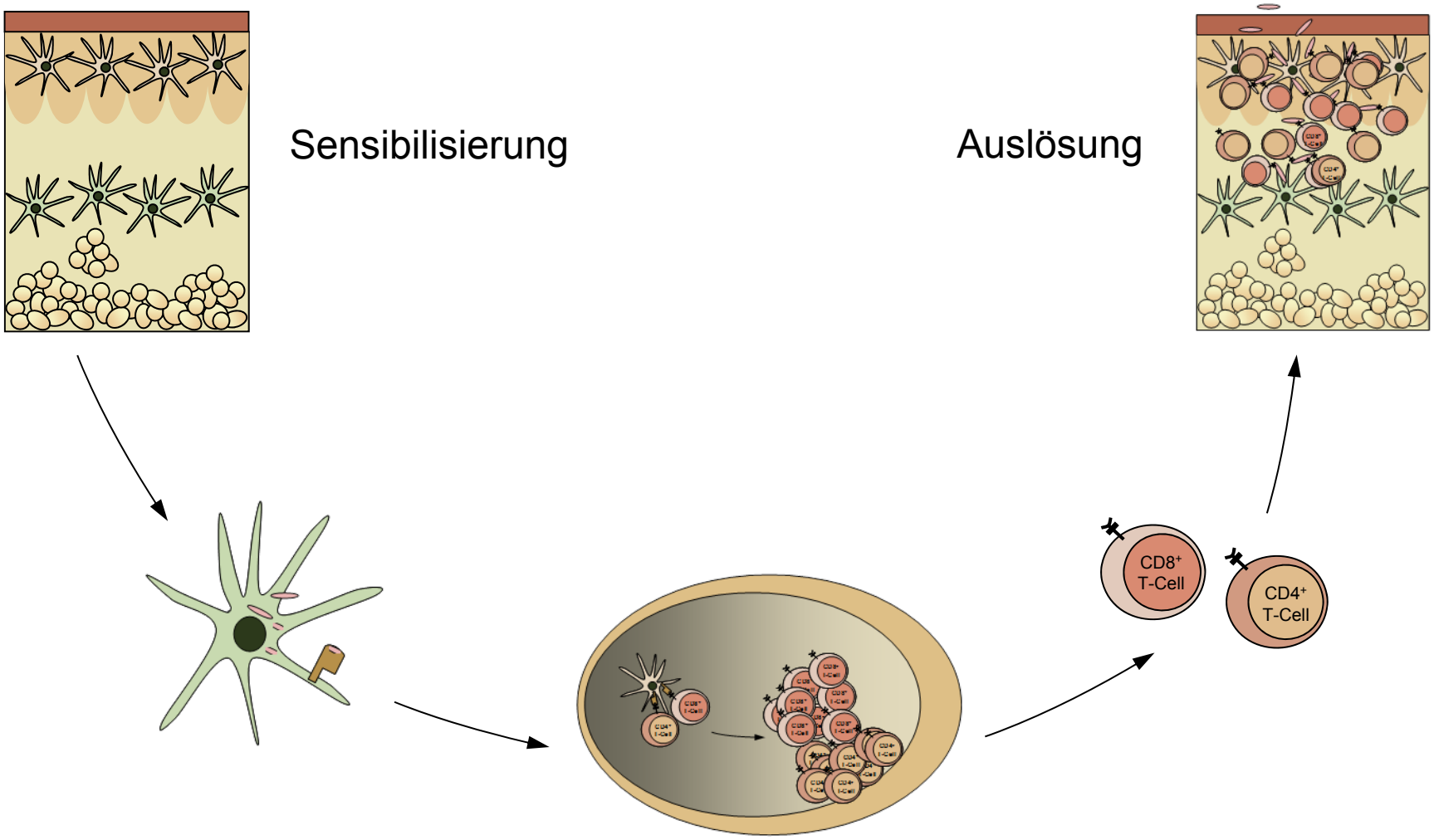
Kontaktallergie - Grundlagen



Kontaktallergie - Grundlagen



Kontaktallergie - Grundlagen



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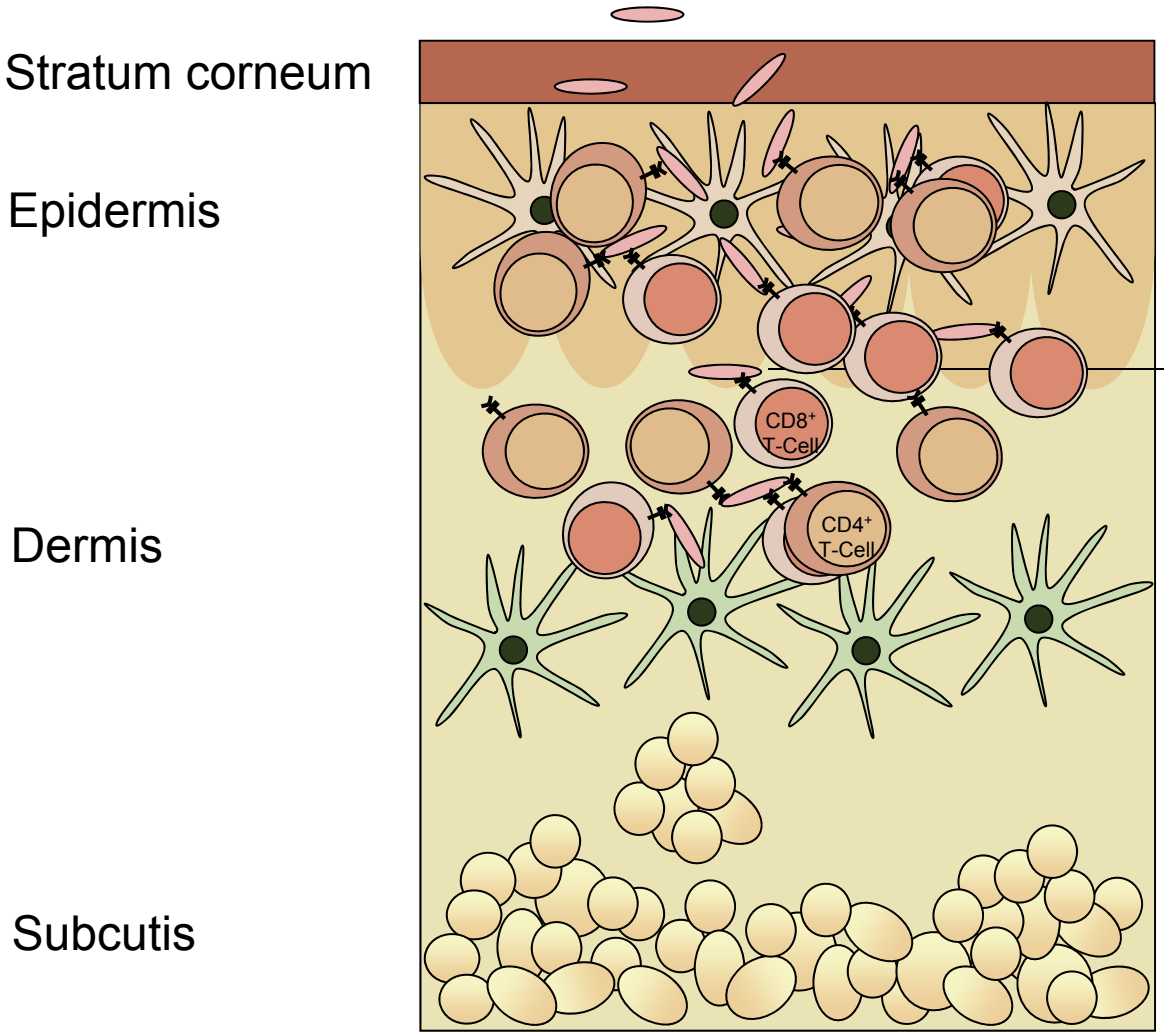


Auslösung der Allergie

Endpunkt: Sensibilisierung –
Klassische Tierversuchsmethoden gemäß Chemikalienprüfung

Klassische Methoden zur Identifizierung des Sensibilisierungspotenzials einer Substanz:

Bühler Test (normale Applikation)

Guinea Pig Maximization Test (GP MT)
(provokativer Test mit Adjunvanz)

Local Lymph Node Assay (LLNA) (mechanistisch basierter Test (Refinement))

Endpunkt Sensibilisierung - Klassische Tierversuchsmethoden

Klassische Tierversuchsmethoden, durch die eine sensibilisierende Substanz identifiziert werden kann:

Bühler Test

Guinea Pig Maximization Test (GP MT)

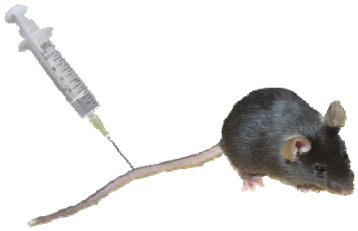
Local Lymph Node Assay (LLNA)

Local Lymph Node Assay (LLNA)

3 x Test Substance

5 days

^{3}H Methyl-Thymidin



5 hours

Lymph Node Analysis in β-Scintillation Counter

EC_3

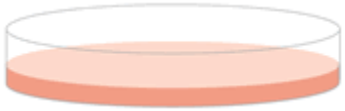
Endpunkt Sensibilisierung - Neue Alternative Methoden

An optimized protocol using human Peripheral Blood Monocyte Derived Dendritic Cells

Experimental Setup

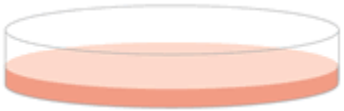
Blood Donor

Ficoll gradient and magnetic bead separation



CD14 enriched Monocytes

Differentiation with IL4 and GM-CSF

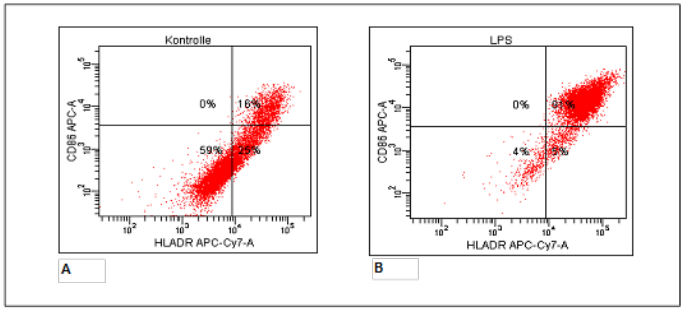


Unmature PBMDCs

Incubation with test substance

Activated PBMDCs

Antibody staining



Analysis

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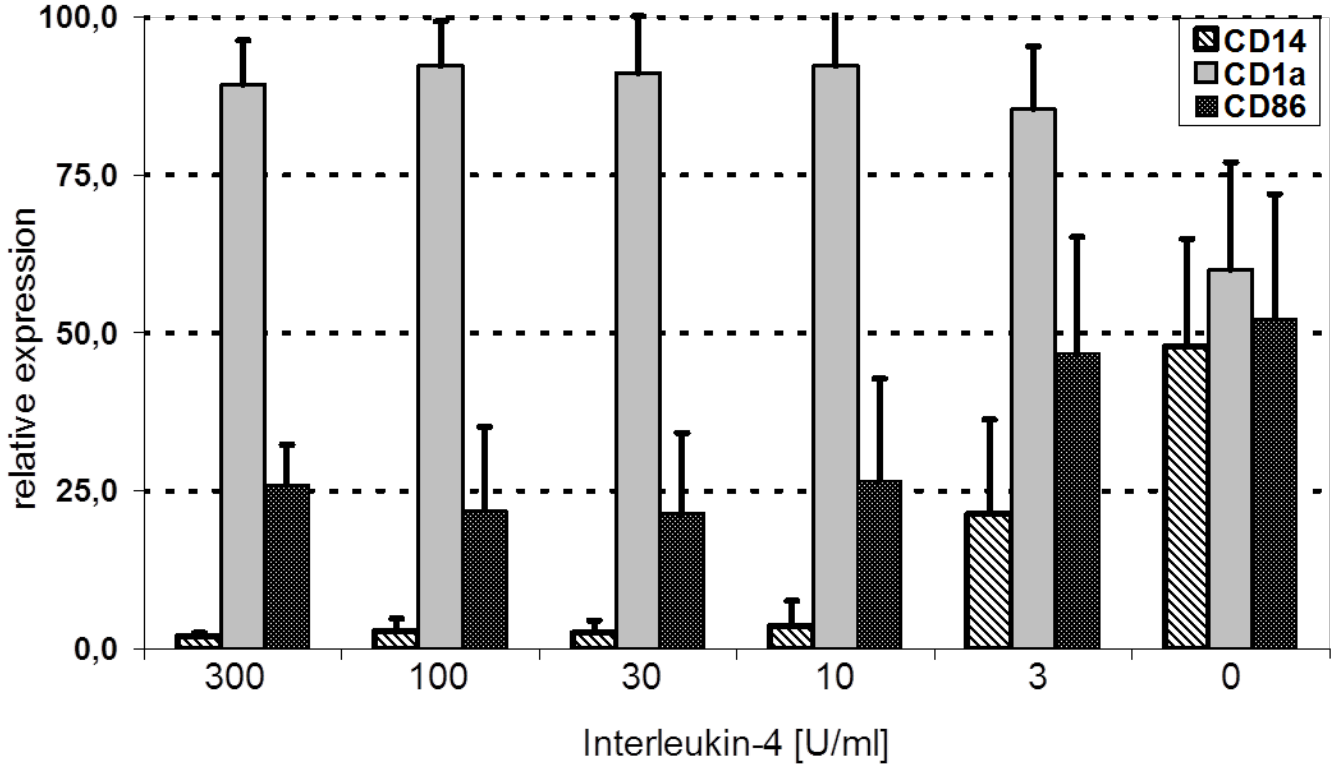
Differentiation of Monocytes to PBMDCs –influence of IL4 concentrations



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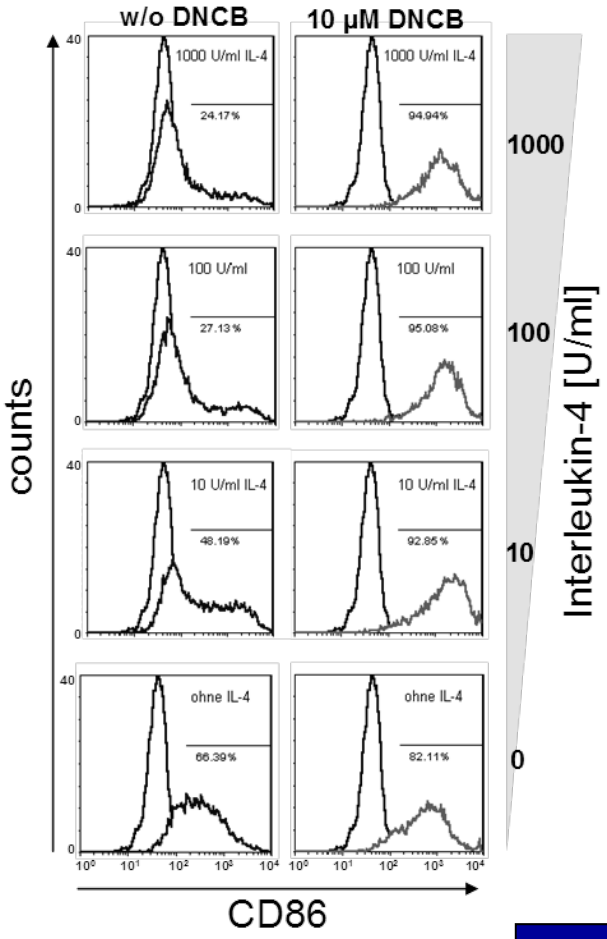
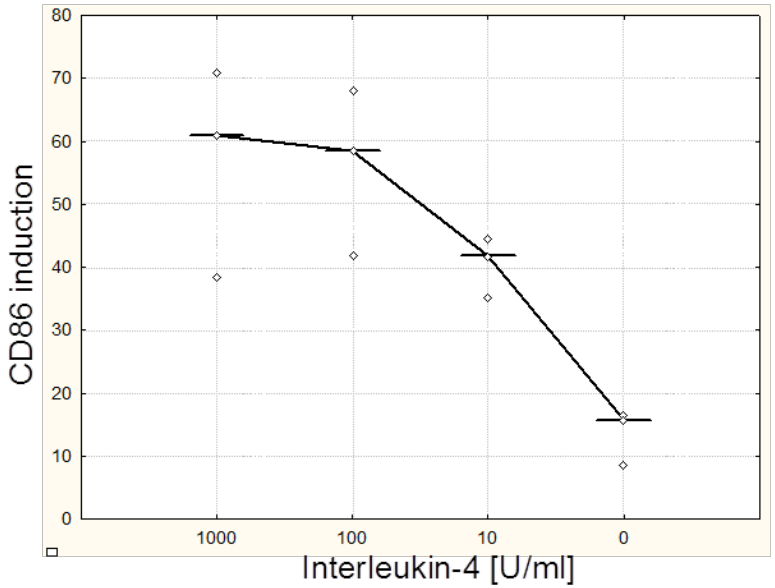
Differentiation of Monocytes to PBMDCs –influence of IL4 concentrations





CD86 induction decreases with [IL-4]

100 U/ml Interleukin-4 and 200 U/ml GM-CSF for differentiation

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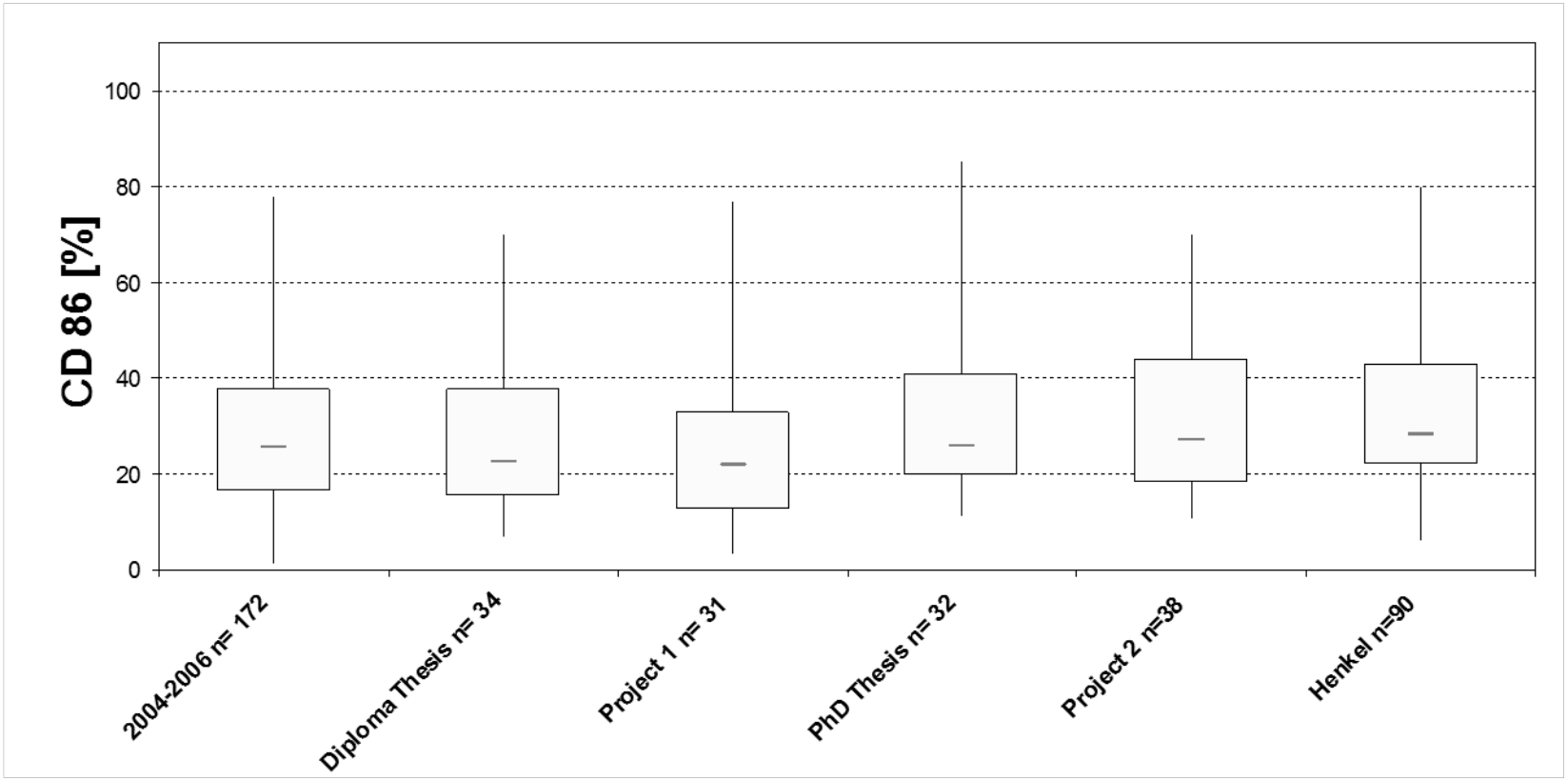
Basal CD86 expression on differentiated PBMDCs obtained from 397 donors



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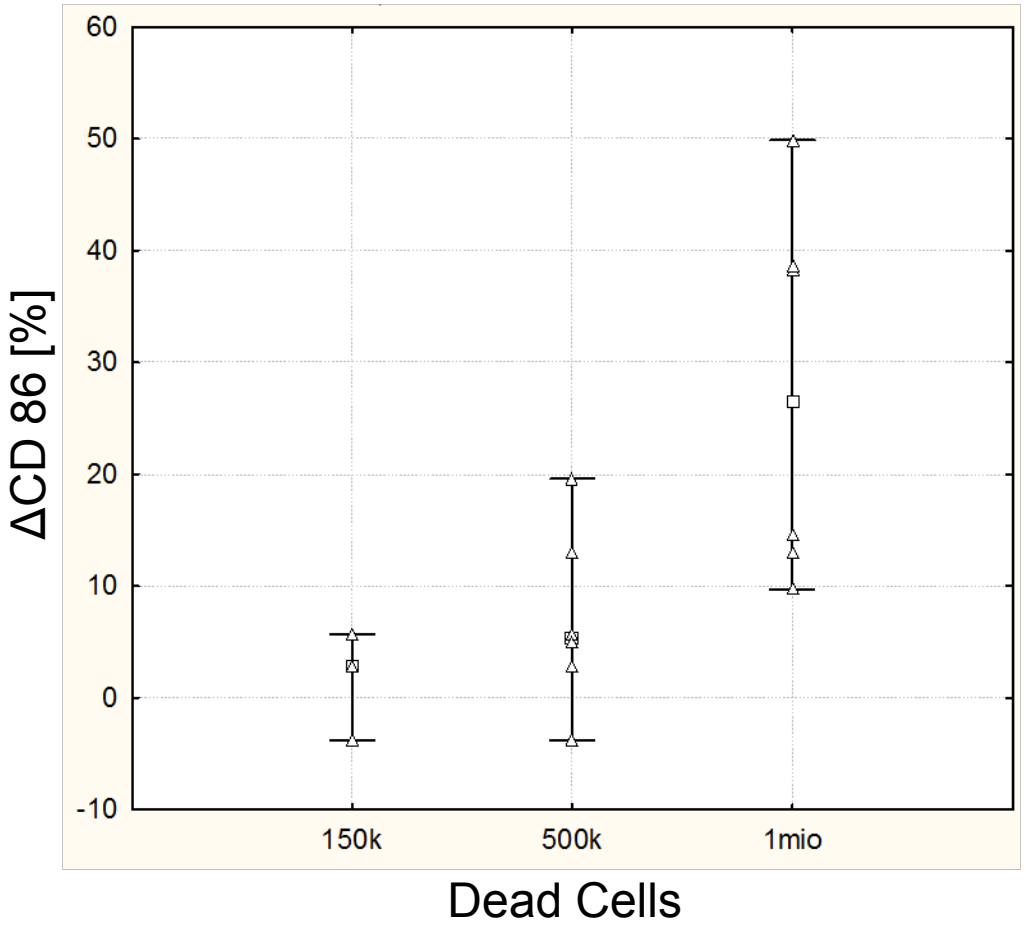
Cytotox Limit



Viability > 80%

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Experimental Setup

Optimized

Blood Donor

Ficoll gradi… …agnetic …ation

Optimized

CD14 enriched Monocytes

Differenti… …L4 and (5d)

Optimized

Unmature PBMDCs

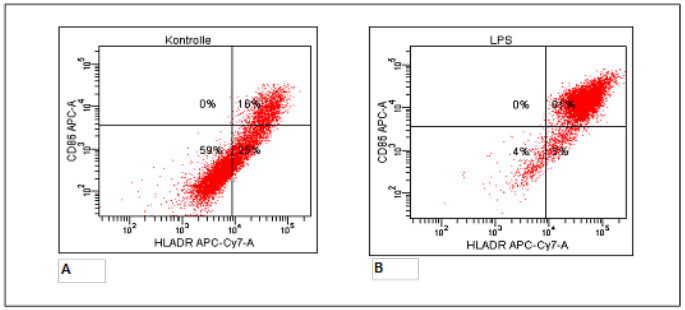
▪Incubation wit… …tance

Optimized

▪Ap… …ning

Optimized

Activated PBMDCs



Analysis

ΔCD86 = % CD86⁺ treated cells - % CD86⁺ untreated cells

ΔCD86 > 20% ➡ Sensitizer

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Versuchsaufbau Photosensibilisierungs-Assay

Blood Donor

Ficoll gradient and magnetic bead separation

CD14 enriched Monocytes

Differentiation with IL4 and GM-CSF (5d)

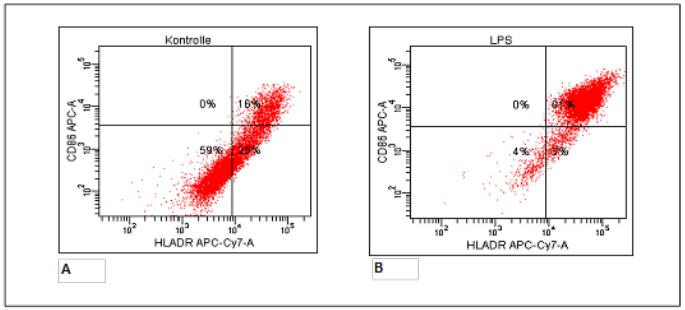
Unmature MoDCs

UV irradiation

Incubation with test substance (2d)

Activated MoDCs

Antibody staining



Analysis