ROLE OF LANGERHANS CELLS IN THE FORMATION OF GERMINAL CENTER AND MODULATION OF HUMORAL IMMUNITY DURING EPICUTANEOUS IMMUNOTHERAPY

Dr. Vincent Dioszeghy, PhD ¹, Camille Plaquet ¹, Dr. Hugh A. Sampson, MD FAAAAI ²

(1) DBV-Technologies, Montrouge, France (2) DBV Technologies, New York, NY
MECHANISM OF ALLERGEN SPECIFIC IMMUNOTHERAPY

Allergen → Th2 → Tfh → Foxp3+ Treg → B cell

→ Mast cell

→ Allergen, IgE, IgG4

Anaphylactic reaction

Allergen specific Immunotherapy
MECHANISM OF ALLERGEN SPECIFIC IMMUNOTHERTAPY

- **Allergen**
- **Th2**
- **Tfh**
- **Treg**
- **Foxp3+**
- **B cell**
- **Mast cell**
- **Anaphylactic reaction**
- **IgE**
- **IgG4**
- **Langerhans cell**
- **Dermal cDC1**
- **Dermal cDC2**
- **skin**
- **MECHANISM OF ALLERGEN SPECIFIC IMMUNOTHERAPY**

**EPIT**
EPIT MODULATES ALLERGEN-SPECIFIC ANTIBODY RESPONSES

Mechanisms of EPIT include:

- **In mice** (Mondoulet, 2011; Dioszeghy, 2011; Mondoulet 2012):
  - Decrease of IgE
  - Increase of IgG1 and IgG2a
EPIT MODULATES ALLERGEN-SPECIFIC ANTIBODY RESPONSES

Mechanisms of EPIT include:

- **In mice** *(Mondoulet, 2011; Dioszeghy, 2011; Mondoulet 2012)*:
  - Decrease of IgE
  - Increase of IgG1 and IgG2a
  - Induction of Tregs *(Dioszeghy, 2014)*
EPIT MODULATES ALLERGEN-SPECIFIC ANTIBODY RESPONSES

Mechanisms of EPIT include:

- **In mice** *(Mondoulet, 2011; Dioszeghy, 2011; Mondoulet 2012)*:
  - Decrease of IgE
  - Increase of IgG1 and IgG2a

- **In human** *(Sampson, 2017; Koppelman, 2019)*:
  - Transient increase IgE
  - Induction of IgG4
LANGERHANS CELLS ARE REQUIRED

Allergen

Anaphylactic reaction

CD25+ Foxp3+ (% of CD4)

Naive Control Control + DT EPIT EPIT + DT

*** **

EPIT

Lang-DTReGFP mice + DT

LANGERHANS CELLS ARE REQUIRED

Allergen

Dermal cDC1
Dermal cDC2

Drop in temperature (°C)

Naive  Control  Control + DT  EPIT  EPIT + DT

***  *  *

Epitope

IgE

IgG4

Lang-DTReGFP mice + DT

ROLE OF LANGERHANS CELLS ON GERMINAL CENTER

Allergen

Th2

Tfh

Treg

Foxp3+

Tfr

Foxp3+

B cell

Mast cell

Anaphylactic reaction

Th2

Tfh

Tfh

Treg

Foxp3+

B cell

B cell

B cell

B cell

IgE

IgG4

Dermal cDC1

Dermal cDC2

Langerhans cell

skin

EPIT

Langerhans cell

Langerhans cell

Langerhans cell
ANALYSIS OF THE GERMINAL CENTER IN VIVO AFTER 2 APPLICATIONS OF VIASKIN®-OVA WHEN LCS ARE DEPLETED

**SENSITIZATION**

<table>
<thead>
<tr>
<th>D1</th>
<th>SENSITIZATION</th>
<th>VIASKIN® APPLICATION</th>
<th>D21</th>
<th>D35</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA</td>
<td>OVA</td>
<td>2 WEEKS WITH 1 VIASKIN® APPLICATION 48hrs</td>
<td>Brachial LN Flow cytometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **SC**
- **IN**

**VIASKIN® APPLICATION**

- **No Viaskin**
- **Viaskin® – OVA-100µg**

**Lang-DTR mice**

- **OVA-sIgE / IgG1**
- **Tfh / Tfr**
- **GL7+ B Cell**

**Flow cytometry**

- **Sham**
- **V-OVA**
- **V-OVA +DT**
OVA-SPECIFIC ANTIBODY RESPONSE

- 2 wks of EPIT is not enough to significantly decrease sIgE
- 2 wks of EPIT significantly increase sIgG1
- Absence of LCs reduced sIgG1 induction

*p<0.05 by Mann-Whitney test
ANALYSIS OF $T_{FH}$ / $T_{FR}$: GATING STRATEGY

Gating on Lymphocytes (FSC/SSC) / Singlet / live cells / CD4+:
EPIT significantly increased the level of TFH cells in sdLNs of non-LC depleted mice compared to Sham mice.

When EPIT was performed in LC-depleted mice, the induction of TFH was significantly lower.

*p<0.05 by Mann-Whitney test*
INDUCTION OF T<sub>FR</sub> IN SKIN DRAINING LYMPH NODES

- EPIT significantly increased level of T<sub>FR</sub> cells in sdLN of non-LC depleted mice compared to Sham mice.
- When EPIT was performed in LC-depleted mice, there was no induction of T<sub>FR</sub>.

*p<0.05 by Mann-Whitney test*
EPIT significantly increased level of GL7+ B cells in sdLN of non-LC depleted mice compared to Sham mice.

When EPIT was performed in LC-depleted mice, the induction of GL7+ cells was significantly lower.

*p<0.05 by Mann-Whitney test*
Induction of OVA-sIgG1 correlated with the number of $T_{FH}$ in sdLN after epicutaneous application of allergen.

Even if OVA-sIgE was not significantly decrease after 2wks EPIT, modification of level of IgE tend to associate inversely with the number of $T_{FH}$ in sdLN.

Simple linear regression and Spearman correlation test.
CONCLUSIONS

- EPIT significantly increased numbers of Tfh cells (CD4+CXCR5+ICOS+PD1+), Tfr (CD4+CXCR5+ICOS+PD1+Foxp3+) and GC B cells (GL7+CD19+) in sdLN s
- When EPIT was performed in LC-depleted mice, there was no induction of Tfh, Tfr, or GC B-cells
- OVA-specific IgG1 was significantly reduced in LC-depleted mice
- Formation of germinal center induced by skin-derived LCs is pivotal in the modulation of humoral immunity
PERSPECTIVES

- Kinetics of modification of germinal center during EPIT
- Modulation of Different $T_{FH}$ and B cell response by EPIT:

IMMUNOLOGY

Identification of a T follicular helper cell subset that drives anaphylactic IgE

PRELIMINARY RESULTS

EPIT down modulate $T_{FH13}$:

- Naive
- Sensitized+
- V-Excipient
- Sensitized+ V-OVA
ACKNOWLEDGEMENTS

DBV R&I Team

Hugh Sampson
Fabrice Porcheray
Vincent Dioszeghy
Pierre-Louis Hervé
Léo Laoubi
Benjamin Pelletier
Jean-Louis Labernardiere
Camille Plaquet
Nathalie Oreal
Noémie Assoun
Laetitia Gaulme
Audrey Perrin

Collaborations:

CIRI
Centre International de Recherche en Infectiologie

Inserm
La science pour la santé
From science to health

Ciml
IMMUNOLOGY

Mount Sinai

CNRGH
CENTRE NATIONAL DE RECHERCHE EN GÉNOMIQUE HUMAINE