Epicutaneous Immunotherapy (EPIT): New method for the treatment of allergy

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• **Prevalence** of allergic diseases: continuously increasing – reaching up to **30%** in industrialized countries

• « the new epidemics of advanced civilization »

• **Symptomatic treatment** (antihistamines, corticosteroids and inhaled β2-adrenoreceptor agonists) can efficiently ameliorate IgE-mediated symptoms

• However, **SIT** (Specific ImmunoTherapy) is the **only** disease-modifying treatment
Immunotherapy

Developments in allergen-specific immunotherapy

SCIT: SubCutaneous ImmunoTherapy
SLIT: SubLingual ImmunoTherapy
EPIT: EPicutaneous ImmunoTherapy
ILIT: IntraLymphatic ImmunoTherapy

Senti et al, 2011. Allergy, 66: 798-809
Effective treatment for: allergic rhinitis, conjunctivitis, allergy to insect venoms

SIT confers long-term benefit

SIT may interrupt the so-called « atopic march »:
✓ the progression of allergic sensitization from a single to multiple allergens
✓ and from allergic rhinitis to asthma.

SCIT: gold standard in immunotherapy but poor compliance due to 30-80 doctor visits over 3 – 5 years and frequent allergic side effects
Skin: Anatomical Structure

Epidermis

Segre et al, 2006. JCI, 116: 1150-1158
Skin: Immunological Functions


- Keratinocytes: a pivotal role in governing/polarizing adaptative immune responses

- DCs: key players in tailoring and polarizing the adaptative immune response.

- Skin DCs: epidermal LCs + dermal DCs (dDCs)
**Skin: Immunological Functions**

- **Epidermal LCs**
  - immune cellular response/processing and cross-presentation of protein Ag
  - priming of CD4+ or CD8+ T cells by presenting immunogenic peptides
  - secretion of IL-10 and IL-4/elicitiation of Th2-type responses

- **Dermal DCs (dDCs):**
  - Regulation of B-cell responses
  - Induction of IgA
  - Pro-inflammatory cytokines/Th1-type responses
Advantages of Epicutaneous Immunotherapy

- High density of LCs: potential to be highly efficacious
- Absence of vascularisation: to be highly safe
- Non-invasive approach (needle-free)
- Less costly than other approaches
Challenges to Epicutaneous Immunotherapy

- Low permeability of the *stratum corneum*
  - Physical disruption by scratching with a needle (scarification)/adhesive tape-stripping/abrasive methods/micro-needles → increase the permeability + immune-stimulatory effect through activation of keratinocytes
  - Enhancement of skin hydration by application of an occlusive patch leading to sweat accumulation
Epicutaneous allergen-specific immunotherapy

EPIT with skin barrier disruption: method of adhesive tape stripping
EPIT using hydration to enhance permeability
• 1957, Pautrizel et al.: the first to attempt treatment of pollen and house dust mite allergy by repeatedly applying drops of allergen extracts onto heavily scarified skin, effective approach, patients did not tolerate the treatment well

• 1959, Blamoutier et al.: method applied the pollen extract drop by drop on the scarified skin
Potential Mechanism of Epicutaneous Administration on abraded skin

Deep epithelial trauma:
- Stratum corneum
- Stratum granulosum
- Stratum spinosum
- Stratum basale
- Basement membrane

Epicutaneous immunization

Superficial epithelial trauma:
- Stratum corneum
- Langerhans cell
- Activated keratinocyte
- Superficial epithelial trauma:
  - TSLP
  - IL-25
  - IL-33

Deep epithelial trauma: T reg/Th2 response

Superficial epithelial trauma: Th1 response

Senti et al, 2011. Allergy, 66: 798-809
Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

Tape-stripping 6 times before application (Tesafilm tape, Tesa, Hamburg, Germany).

- Phase I/II, randomized, placebo-controlled, double-blind trial
- Grass pollen allergy
- 21 verum (1 dose) vs 16 placebo
- 4 patches before pollen season then 8 patches

Clinical endpoints: primary outcome was the change in allergic response measured by nasal provocation tests (NPTs).

Senti et al., 2009. JACI, 124:997-1002
Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

Adverse events

**TABLE III. Overview of adverse events**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Placebo (n = 16)</th>
<th>Allergen-specific immunotherapy (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema under the patch</td>
<td>23/5</td>
<td>160/15</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>4/1</td>
<td>3/2</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>4/2</td>
<td>3/2</td>
</tr>
<tr>
<td>Sneezing</td>
<td>4/2</td>
<td></td>
</tr>
<tr>
<td>Redness, swelling, wetness of upper arm</td>
<td>12/1</td>
<td></td>
</tr>
<tr>
<td>Pruritic and inflamed mammilla</td>
<td>3/1</td>
<td></td>
</tr>
<tr>
<td>Wheal</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Vertigo</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Prickle in arms and hands</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Scars</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>35/5</strong></td>
<td><strong>186/16</strong></td>
</tr>
</tbody>
</table>

**Eczema score**

- Placebo: Δ
- Allergen: ΔΔΔΔΔΔΔΔΔΔΔΔΔΔ

Treatment
Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

Primary Outcomes

Verum (filled boxes) better than placebo (open boxes): 2006, P .02; 2007, P .005)

Senti et al., 2009. JACI, 124:997-1002
Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

- Phase I/II, randomized, placebo-controlled, double-blind trial (safety, efficacy, sustained effect)
- Grass pollen allergy
- 99 verum (3 doses) vs 33 placebo
- 4 patches before pollen season then 5 patches
- Mix of 6 grass-pollen extracts

- Clinical endpoints: primary outcome was the change in allergic response measured by nasal provocation tests (NPTs).

Tape-stripping 6 times before application (Tesafilm tape, Tesa, Hamburg, Germany).

Senti et al., 2011. JACI, in press
Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

Primary Outcomes

Systemic side-effects

Senti et al., 2011. JACI, in press
Summary on EPIT with skin barrier disruption

- Efficiency was proven at the clinical level (pollen allergy/hay fever, rhinoconjunctivitis)

- Safety was validated but large local side effects were described

- This epicutaneous approach seems not to be applicable to food allergy $\rightarrow$ high risk of anaphylaxis
Epicutaneous allergen-specific immunotherapy

EPIT with skin barrier disruption: method of adhesive tape stripping

EPIT using hydration to enhance permeability
EPIT using Viaskin® technology

- EPIT using VIASKIN®: original method adapted to food allergy
- VIASKIN® technology delivers allergens to the epidermal layer of the skin only
Children allergic to milk proteins

Dupont et al., 2010. JACI, 125:1165-1167
EPIT using Viaskin® technology
First Clinical Proof of Concept

Oral Food Challenge (OFC)

Active group

Placebo group

Dupont et al., 2010. JACI, 125:1165-1167
EPIT: Absence of Free Passage to Lymphatics

- Allergen conjugated to fluorescent tracer Alexxa488 (A488)
- Application with VIASKIN®

Naive Balb/c mice (n=5/group) x2

2hr Lymph node cell isolation and flow cytometry analysis
EPIT: Absence of Free Passage to Lymphatics

- No passive passage of allergen through intact skin
EPIT: Absence of Free Passage to Lymphatics

- No passive passage of allergen through intact skin
- Specific capture by Antigen presenting Cells

Capture of allergen by non APC

- epidermis
- dermis

A468+ (%)
Allergen conjugated to fluorescent tracer Alexxa488 (A488)
Application with VIASKIN®
**EPIT**: Specific Capture by Antigen Presenting Cell (APC)

Epidermis

**Dermis**

Lymph nodes

Mechanisms of allergen delivery to skin with VIASKIN® after one application

- Antigens
- VIASKIN®
- Stratum Corneum
- Epidermis
- Basement membrane
- Dermis
- Skin

Cell migration

Langerhans Cell
Dermal Dendritic Cell
Keratinocytes

Lymph node

T cell
B cell

Cell activation ➤ Cytokine production
**Absence of free passage to lymphatics: intact skin vs stripped skin**

*Pior, J Invest Dermatol 1999:*

Small compound, able to pass through skin, reacted with lymph node cells in draining LN peaking between 1h and 3h after exposure

- Allergen conjugated to fluorescent tracer Alexa488 (A488)

**Naïve Balb/c mice (n=5/group) x2**

**Skin analysis by fluorescent microscopy**

**2hr 24hr**

Lymph node cell isolation and flow cytometry analysis

**Control**

VIASKIN®OVA-A488

VIASKIN®OVA-A488 on stripped skin

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**Figure:**

- Median of fluorescence over time (control, 15min, 1h, 3h, 12h, 24h)
- Retroauricular, mesenteric, axillary, inguinal

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Comparison of allergen passage across intact and stripped skin after 2hr

VIASKIN®-OVA-A488 on intact skin

VIASKIN®-OVA-A488 on stripped skin
Skin stripping slightly changed the phenotype of migrating DC after a unique application.
Phenotype of migrating dendritic cells

A488+ CD11c+ gated

F4/80  CD11b  MHC class II  CD80  CD205  CD86  CD83

### Phenotype difference of the two populations of migrating DC

<table>
<thead>
<tr>
<th></th>
<th>MHC-II</th>
<th>CD86</th>
<th>CD83</th>
<th>CD40</th>
<th>PIR-B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact skin</strong></td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
</tr>
<tr>
<td><strong>CD205hi</strong></td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
</tr>
<tr>
<td><strong>CD205lo</strong></td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
</tr>
<tr>
<td><strong>Stripped skin</strong></td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
</tr>
</tbody>
</table>
Capacity of DC to induce Treg in CD4 cell in vitro

Migrating DC from intact skin induced Treg in vitro more efficiently than from stripped skin.
Difference of Treg induction between $\text{CD205}^{hi}$ and $\text{CD205}^{low}$ DCs

CD205$^{hi}$ cells induced regulatory T cells in vitro only when they migrated from intact skin
EPIT : Preclinical proof of concept

EPIT is efficient in a model of mice sensitized to OVA, pollen, HDM, peanut

**EPIT is efficient in a model of mice sensitized to OVA, pollen, HDM, peanut**

Eosinophil recruitment in BAL

**Pollen**

**HDM**

**Peanut**

**EPIT vs SCIT in a model of mice sensitized to peanut: the same efficacy**

**Sensitization**
- 6 weeks

**Immunotherapy**
- 8 weeks

**Hyperresponsiveness**
- 8 weeks

- **D45**
  - PPE + CT
  - per os
  - n=20

- **D105**
  - Aerosol challenge
  - n=10

- **W8**
  - Epicutaneous 100µg (EPIT)
  - Subcutaneous 100µg (SCIT)
  - Sham treated
  - naive

- **D120**
  - Resistance
  - BAL
  - n=10

- **D121**
  - Plethysmography
  - BAL
  - n=10

- **D122**
  - Validation of sensitization

- **D123**
  - Blood samples
  - n=10

- **D124**
  - Blood samples
  - n=10

**EPIT vs SCIT in a model of mice sensitized to peanut: the same efficacy**

### Serological Responses

#### Specific IgE

<table>
<thead>
<tr>
<th></th>
<th>EPIT</th>
<th>SCIT</th>
<th>sham</th>
<th>naive</th>
</tr>
</thead>
<tbody>
<tr>
<td>D45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D105</td>
<td></td>
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</tbody>
</table>

#### Specific IgG2a

<table>
<thead>
<tr>
<th></th>
<th>EPIT</th>
<th>SCIT</th>
<th>sham</th>
<th>naive</th>
</tr>
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<tbody>
<tr>
<td>D45</td>
<td></td>
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<td></td>
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<tr>
<td>D105</td>
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</tbody>
</table>

EPIT vs SCIT in a model of mice sensitized to peanut: the same efficacy

Plasma histamine release after oral challenge

**EPIT vs SCIT in a model of mice sensitized to peanut: the same efficacy**

**BAL analysis: Eotaxin and eosinophil recruitment**

**Eotaxin**

- **Naive**
- **Sham**
- **SCIT**
- **EPIT**

**Eosinophils**

- **Naive**
- **Sham**
- **SCIT**
- **EPIT**

**EPIT vs SCIT in a model of mice sensitized to peanut: the same efficacy**

**Plethysmography**

- **Penh**
  - **EPIT**
  - **SCIT**
  - **sham**
  - **naive**

- Methacholine (mg/ml)

- AUC: Area Under the Curve

**Resistance**

- **Methacholine (mg/ml)**
- **Resistance (cmH\textsubscript{2}O.s/ml)**

- Methacholine (mg/ml)

- AUC: Area Under the Curve
Efficacy of EPIT in a New Model of Induction of Digestive Injuries

Mice sensitized to PPE

Naive mice (n=10)
EPIT (n=10) Sham (n=10)

10-day-oral exposure to peanut

Measurement of sIgE, sIgG2a, sIgG1 in blood samples

Tissue sampling
Esophagus, jejunum
• mRNA analysis
IL-4, IL-5, IL-10, TGF-β, Foxp3
• Histology
Eosinophil counts

Mondoulet et al, 2011. submitted
Efficacy of EPIT in a New Model of Induction of Digestive Injuries

Humoral Response

\[ s\text{IgE} \]

\[ s\text{IgG2a} \]

Mondoulet et al, 2011. submitted
Efficacy of EPIT in a New Model of Induction of Digestive Injuries

Cytokines Secreted by Reactivated Splenocytes

IL-4

IL-5

IL-13

IL-10

IFN-γ

Mondoulet et al, 2011. submitted
Eosinophilic infiltration in Esophagus

- No injury (mean eosinophil 0.4)
- Acanthosis
- Inflammation of the Chorion
- Eosinophilia (mean 7.6)
- Decrease of inflammation and eosinophilia (mean 2.8)
**Efficacy of EPIT in a New Model of Induction of Digestive Injuries**

**Eosinophil Quantification in Esophagus**

**Expressed as eosinophils/field**

- **naive**
- **Sham**
- **EPIT**

**Expressed as eosinophils/mm²**

- **naive**
- **Sham**
- **EPIT**

- unpaired t test with Welch’s corrections
- Mann Whitney test

**Efficacy of EPIT in a New Model of Induction of Digestive Injuries**

*mRNA Expression of Cytokines in Esophagus*

**eotaxin**

- **naive**
- **Sham**
- **EPIT**

**IL-5**

- **naive**
- **Sham**
- **EPIT**

**IL-13**

- **naive**
- **Sham**
- **EPIT**
Efficacy of EPIT in a New Model of Induction of Digestive Injuries

mRNA Expression of Cytokines in Esophagus

**Th1 transcription factor**

- Tbet
- GATA-3

**Treg transcription factor**

- Foxp3

*Significant differences (*) compared to naive group.
**EPIT Efficacy: aTreg Mechanism?**

**Sensitization**

- PPE+CT per os for 6 wks

**Sham**

- No Ab
- Anti-CD4
- Anti-CD8
- Anti-CD25

**EPIT**

- No Ab
- Anti-CD4
- Anti-CD8
- Anti-CD25

**Control**

Peripheral blood cell staining

**10-day-oral challenge**

- Histology
  - Eosinophil counts in esophagus and jejunum

- mRNA analysis in esophagus
  - IL-5, eotaxin, IFNγ, IL10 and FoxP3

- Splenocytes cell culture
  - IL-5, IFNγ in supernatants

**Serologic response**

(sIgE, sIgG1, sIgG2a)

**Serologic response**

(sIgE, sIgG1, sIgG2a)
EPIT Efficacy: aTreg Mechanism?

Cell depletion

**CD4+**
- Sham
- αCD4
- EPIT
- αCD4

**CD8+**
- Sham
- αCD8
- EPIT
- αCD8

**CD25+**
- Sham
- αCD25
- EPIT
- αCD25
EPIT Efficacy: aTreg Mechanism?

**sIgE**

**sIgG2a**
EPIT Efficacy: aTreg Mechanism?

**Eosinophil infiltration**

- C
- Sham
- No Ab
- αCD4
- αCD8

**IL-5**

- C
- Sham
- No Ab
- αCD4
- αCD8

**Eotaxin**

- C
- Sham
- No Ab
- αCD4
- αCD8

**FoxP3**

- C
- Sham
- No Ab
- αCD4
- αCD8
Effect of CD4 or CD8 depletion: Splenocytes response to PPE

**EPIT Efficacy: αTreg Mechanism?**

Effect of CD4 or CD8 depletion: Splenocytes response to PPE

**IL-5**

**IFNγ**
EPIT Efficacy: αTreg Mechanism

Naive

Sham

EPIT

EPIT + anti-CD25

Eosinophilic infiltration

Dioszeghy et al, 2011. EAACI, Istanbul, PS1340
EPIT Efficacy: aTreg Mechanism

Eosinophil infiltration

IL-5

Eotaxin

FoxP3

IFNγ

Dioszeghy et al, 2011. EAACI, Istanbul, PS1340
Regulatory T cell (CD4+CD25+) from Sham or EPIT Sensitization PPE+CT per os for 6 wks

Epicutaneous Immunotherapy VIASKIN® 100µg PPE 48h once a week for 8wks

Sensitization PPE+CT per os for 6 wks

Transfer of EPIT Treg (n=10)

Transfer of ShamTreg (n=10)

No Transfer (n=18)

oral peanut for 10 days

Esophagus Histology mRNA

Dioszeghy et al, 2011. EAACI, Istanbul, PS1340
EPIT Efficacy: aTreg Mechanism Transfer Experiment

No transfer

Transfer of Sham Treg

Transfer of EPIT Treg

- Acanthosis
- Inflammation of the Chorion
- Eosinophilia

- No decrease of inflammation nor eosinophilia

- Decrease of inflammation and eosinophilia

Dioszeghy et al, 2011. EAACI, Istanbul, PS1340
Efficacy of EPIT: intact skin vs stripped skin

- **Sensitization**
  - PPE + CT
  - 6 ig./for 6 weeks

- **Immunotherapy**
  - EPIT (n=8) (intact skin)
  - Stripping + EPIT (n=8) (tape-stripped skin)
  - Sham (n=8)

- **Naive mice (n=8)**

**10-day peanut exclusive diet**

- **Tissue sampling**
  - Esophagus:
    - mRNA analysis
    - IL-4, IL-5, IL-10, Foxp3
    - Histology
    - Eosinophil counts
  - Jejunum:
    - Histology
    - Eosinophil counts
    - Villus/crypt ratio

**Measurement of sIgE, sIgG2a, sIgG1 in blood samples**

**Blind reading**

- D42
- D106
Efficacy of EPIT: intact skin vs stripped skin

**Specific IgE**

**Specific IgG2a**
Efficacy of EPIT: intact skin vs stripped skin

EPIT on stripped skin did not induce regulatory T cells
Efficacy of EPIT: intact skin vs stripped skin

**Naive**
- No injury (mean eosinophil 0.4)

**Sham**
- Acanthosis
- Inflammation of the Chorion
- Eosinophilia (mean 7.6)

**Intact-EPIT**
- Decrease of inflammation and eosinophilia (mean 2.8)

**Stripping-EPIT**
- Acanthosis
- Inflammation of the Chorion
- Eosinophilia (mean 8.6)
Efficacy of EPIT: intact skin vs stripped skin
Efficacy of EPIT: intact skin vs stripped skin

**eotaxin**

- Relative Expression
  - mRNA CCL-11/β-actin+SDHA
  - naive: low, Sham: high, EPIT: high, stripping-EPIT: low

**IL-5**

- Relative Expression
  - mRNA IL-5/β-actin+SDHA
  - naive: low, Sham: high, EPIT: high, stripping-EPIT: low

**IL-13**

- Relative Expression
  - mRNA IL-13/β-actin+SDHA
  - naive: low, Sham: high, EPIT: high, stripping-EPIT: low
Efficacy of EPIT: intact skin vs stripped skin

**Th1 transcription factor**
- GATA-3
- Tbet

**Th2 transcription factor**
- Foxp3

**Treg transcription factor**
Conclusions
Conclusions

• Safety of EPIT administration: done

• Proof of efficacy of EPIT
  ✓ Preclinical levels: done
  ✓ Clinical stages: done for aeroallergens and milk, ongoing for peanut

• Two different approaches: abraded skin vs intact skin (high hydration is required)
  ✓ More data illustrating mechanisms of action were available for intact skin
• Points to be developed:

✓ EPIT as a preventive treatment (allergic march) (other routes of treatment: Pajno et al., 1997, 2004, PAT study (finished), PAT study (ongoing)

✓ Concepts of efficacy and antigen delivery in filaggrin-deficient mice (filaggrin is a protein involved in EDC: epidermal differentiation complex)

✓ Mechanism of action: Treg function (nTreg vs iTreg)