Étude de la coopération entre les cellules dendritiques et les lymphocytes T dans les allergies aux produits chimiques – FPH42

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Chemical allergy: major public health concern

Chemical allergy

- 15-20 % of the general population suffer from allergic contact dermatitis.

- Major cause of occupational skin disease → estimated newly reported annual incident of 0.5-1.9 ‰ in Europe.

- **Nickel** is the most frequent contact allergen (18.1%) followed by **cobalt** (5.9%) and chromium (3%) as diagnosed by patch testing.

- In Europe, an estimated 65 million people may be allergic to **nickel**.
  - Diepgen et al. Contact dermatitis, 2013
Varying degrees of erythema, edema and vesiculation

ACD caused by nickel / cobalt

ACD caused by chromium
Drug and chemical allergy: complex mechanism

Maculopapular exanthema

Induced by β-lactams

Pustulosis exanthema

Second exposure

T-cell mediated reactions

Maculopapular exanthema

Induced by β-lactams

Pustulosis exanthema

Allergic contact dermatitis
Dendritic cells and T-cells interaction

Immature dendritic cell

Mature dendritic cell

Danger signals

MHC molecules

TCR

T-cell

CD80/86

CD28

IL-12p70

IL-23

Th1

Th17

IFN-γ

IL-17

T-cell polarization

IL-12p70

IL-23

p35

p40

p19

p40
Dendritic cells and T-cells interaction

**IL-12p70**
- p35
- p40
- TH1
- IFN-γ

**Ni²⁺**
- JAK/STAT-1
- IRF-1
- NF-κB

**IL-23**
- p19
- p40
- Th17
- IL-17A

**Antonios et al., J Immunol. 2010**
Background

- **Nickel-specific Th1 and Th17 cells were found in the blood and skin of nickel allergic patients.**
  
  Peiser, Clin Dev Immunol, 2013  
  Dyring-Andersen B et al. Contact dermatitis, 2013  
  Pennino et al. J immunol, 2010  
  Larsen et al. J Allergy Clin Immunol, 2009

- **IL-17 amplifies allergic contact dermatitis:**
  
  • **ACD clinical score correlated with the epidermal accumulation of IL-17A- producing T-cells.**
    
    Schmidt et al. Contact dermatitis, 2017
  
  • **IL-17 deficient mice demonstrated strongly reduced ear swelling response (CHS) to contact allergens**
    
    Nakae et al. Immunity, 2002  
    He et al. J Immunol, 2006
  
  • **Induction of chemokines (CXCL-8, CXCL1) and cytokines (IL-6, IL-1) release from keratinocytes**
    
    Peiser, Clin Dev Immunol, 2013
  
  • **Intensification of the ICAM-1–dependent keratinocyte-T cell interaction → promoting nonspecific T cell-induced keratinocyte apoptosis.**
    
    Pennino et al. J immunol, 2010

What are the mechanisms behind nickel-induced Th17 cells development?
Generation of human monocyte-derived dendritic cells (MoDC)

- Buffy coats
- Ficoll gradient
- Peripheral blood mononucleated cell
- Monocytes CD14+
- Culture media + GM-CSF and IL-4
- Culture for 5 days
- Day 5
- Immature dendritic cells (iDC)
Ni²⁺ induces the production of IL-23, IL-12p40 and IL-12p70 by human MoDC

ELISA-24hrs (NiSO₄ 500 µM; NiCl₂ 500 µM; CoCl₂ 500 µM; K₂Cr₂O₇ 5 µM; LPS 25 ng/ml)

N=4; *p ≤ 0.05, Mann-Whitney

Ni\textsuperscript{2+} induces the production of IL-23, IL-12p40 and IL-12p70 by human MoDC

**A**

ELISA-24hrs (NiSO\textsubscript{4} 500 µM; NiCl\textsubscript{2} 500 µM; CoCl\textsubscript{2} 500 µM; K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7} 5 µM; LPS 25 ng/ml)

- **IL-23**
  - Control
  - NiSO\textsubscript{4}
  - NiCl\textsubscript{2}
  - CoCl\textsubscript{2}
  - K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}
  - LPS

- **IL-12p40**
  - Control
  - NiSO\textsubscript{4}
  - NiCl\textsubscript{2}
  - CoCl\textsubscript{2}
  - K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}
  - LPS

- **IL-12p70**
  - Control
  - NiSO\textsubscript{4}
  - NiCl\textsubscript{2}
  - CoCl\textsubscript{2}
  - K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}
  - LPS

**B**

ELISA (NiSO\textsubscript{4} 500 µM)

- **IL-23**
  - 4h
  - 8h
  - 12h
  - 18h
  - 24h

*N=4; *p ≤ 0.05, Mann-Whitney*

Bechara R. *et al.* J Invest Dermatol. 2017
Ni²⁺ induces the production of IL-23, IL-12p40 and IL-12p70 by human MoDC

A

ELISA-24hrs (NiSO₄ 500 µM; NiCl₂ 500 µM; CoCl₂ 500 µM; K₂Cr₂O₇ 5 µM; LPS 25 ng/ml)

IL-23

IL-12p40

IL-12p70

0 500

1000

1500

(pg/ml)

Control NiSO₄ NiCl₂ CoCl₂ K₂Cr₂O₇ LPS

0 1000

2000

3000

(pg/ml)

Control NiSO₄ NiCl₂ CoCl₂ K₂Cr₂O₇ LPS

0 30

60

90

(pg/ml)

Control NiSO₄ NiCl₂ CoCl₂ K₂Cr₂O₇ LPS

B

ELISA (NiSO₄ 500 µM)

C

RT-qPCR (NiSO₄ 500 µM)

N=4; * p ≤ 0.05, Mann-Whitney

NiSO₄-treated MoDCs promote IL-17A producing CD4+ T-cells via IL-23 production

Co-culture of MoDCs and allogeneic CD4⁺ T-cells (R=1/10) → Restimulation at Day 6 → Intracellular cytokine production at Day 9 after Ionomycin/PMA activation

Healthy Donor 1
PBMC ➔ NiSO₄ ➔ iDC ➔ mDC ➔ DC-T co-culture (Ratio 1/10) ➔ 1 Restimulation ➔ D6 ➔ Intracellular staining ➔ D9 ➔ Live/Dead AmCyan ➔ CD4 Alexa fluor ➔ visible CD4⁺ T-cells 87.3

Healthy Donor 2
PBMC ➔ CD4⁺ T-cells

NiSO₄-treated MoDCs promote IL-17A producing CD4+ T-cells via IL-23 production

\[\text{N}=6; \; * \; p \leq 0.05, \; \text{Wilcoxon}\]
NiSO₄-treated MoDCs promote IL-17A producing CD4+ T-cells via IL-23 production

Unloaded MoDCs

NiSO₄ – treated MoDCs

% of IL-17A+ CD4+ T-cells

% of IL-17A+ IFN-γ+ CD4+ T-cells

% of IL-17A+ IFN-γ- CD4+ T-cells

N=6; * p ≤ 0.05, Wilcoxon

TLR4 pathway is implicated in NiSO₄-induced IL-23, IL-12p40 and IL-12p70 production.

**IL-23**

![Diagram showing ELISA-24hrs (NiSO₄ 500 µM)]

N=4; * p ≤0.05, Mann-Whitney

**IL-12p40**

![Bar chart showing IL-12p40 levels](chart)

**IL-12p70**

![Bar chart showing IL-12p70 levels](chart)

Bechara R. *et al.* J Invest Dermatol. 2017
The Jak-STAT pathway regulates the IL-23/IL-12p70 balance in NiSO₄-treated MoDCs

Ni²⁺ → STAT-1 → IRF-1 → IL-12p35

Antonios et al. J immunol. 2010

JAK Inhibitor I (0.5 µM)

ATP-competitive inhibitor of Janus protein tyrosine kinases (JAKs).
The Jak-STAT pathway regulates the IL-23/IL-12p70 balance in NiSO₄-treated MoDCs

A  ELISA-24hrs (NiSO₄ 500 µM; Jak Inhibitor I 0.5 µM)

<table>
<thead>
<tr>
<th></th>
<th>IL-23</th>
<th>IL-12p40</th>
<th>IL-12p70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control DMSO Jak Inhibitor I</td>
<td>+ NiSO₄</td>
<td>+ NiSO₄</td>
<td>+ NiSO₄</td>
</tr>
</tbody>
</table>

B  RT-qPCR-4hrs (NiSO₄ 500 µM; Jak Inhibitor I 0.5 µM)

- **il-23p19 mRNA**:
  - Fold induction
  - Control DMSO Jak Inhibitor I | + NiSO₄ |

- **il-12p40 mRNA**:
  - Fold induction
  - Control DMSO Jak Inhibitor I | + NiSO₄ |

- **il-12p35 mRNA**:
  - Fold induction
  - Control DMSO Jak Inhibitor I | + NiSO₄ |

N=4; * p ≤ 0.05, Mann-Whitney

What are the mechanisms mediating the increase in NiSO$_4$-induced IL-23 and IL-12p40 production following Jak-STAT inhibition?

- NFIL-3 (Nuclear Factor, Interleukin 3 Regulated) is a basic leucine zipper transcription factor.
- NFIL-3 serves as a key regulator in the development and functions of immune cells.
- NFIL-3 plays a crucial role in various immune-mediated diseases.

NFIL-3 is a transcriptional repressor of IL-12p40 in macrophages and mucosal immunity.

- NFIL-3-deficient macrophages expressed higher IL-12p40 and IL-23p19 compared to WT.

Kobayashi et al. J immunol. 2011
Smith et al. J Biol Chem 2011
Kobayashi et al. J immunol. 2014
What are the mechanisms mediating the increase in NiSO₄-induced IL-23 and IL-12p40 production following Jak-STAT inhibition?

1- NiSO₄ induces NFIL-3 expression in human MoDCs
What are the mechanisms mediating the increase in NiSO₄-induced IL-23 and IL-12p40 production following Jak-STAT inhibition?

1- NiSO₄ induces NFIL-3 expression in human MoDCs

2- NFIL-3 expression is mediated by the Jak-STAT pathway

N=4; * p ≤ 0.05, Mann-Whitney
-Conclusions:

-NiSO$_4$ induces the production of IL-23 and the expression of $il$-$23p19$ and $il$-$12p40$ mRNA,

-p38MAPK, NF-$\kappa$B and TLR4 were involved in IL-23 production induced by nickel.

-Nickel contributes to NFIL-3 expression in human MoDCs and JAK/STAT limit the production of IL-23 by promoting Th1 polarization

-Publications:


-2 publications in preparation.
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