Cytokines in lupus nephritis – IL-17 and IL-23 in association to histopathology and response to treatment

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Background

- The pathogenesis for lupus nephritis (LN) involves multiple components of the immune system

- Cytokines – originating from both the innate and adaptive immune system
  - "Disease drivers" involved in every step of the pathogenesis

- Several studies on cytokines in LN
  - None with major influence on clinical practice
  - Effects of treatment – not much known

I have no financial relationships to disclose.

IL-17 in SLE

- SLE patients
  1. Increased IL-17-producing cells in peripheral blood
     (CD4+Th17 cells and DN T-cells)
  2. Increased serum levels of IL-17, IL-23

- Lupus nephritis
  1. IL-17 producing T-cells in renal tissue in LN
  2. High IL-17 expression in glomerular and interstitial infiltrates

Aim

- To study cytokines, previously indicated in LN, in association to
  - histopathology
  - response to therapy
- Potential biomarkers

Patients & Methods

- 52 patients with active lupus nephritis
- Renal biopsies at baseline and after standard induction treatment
  - Renal biopsy 1
  - Renal biopsy 2 ≥ 6 months
- Clinical and laboratory data at both occasions
- TNFα, IFNγ, IL-2, IL-4, IL-6, IL-6r, IL-10, IL-21, IL-23 and TGF-β at both occasions
  - (ELISA (R&D) and Cytometric Bead Array – CBA)
- 13 healthy controls

Definitions of response

1. Histopathological response
   - Renal biopsies - WHO classification
   - Response; WHO class I-II at follow up

2. BILAG response
   - Renal disease activity - renal BILAG
   - Complete response; improvement ≥ 2 levels BILAG
   - Partial response; improvement 1 level
   - No response; no improvement

Results; Response

Cytokine levels at baseline in patients vs. controls

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Patients</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
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<tr>
<td>IL-10</td>
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<tr>
<td>IFN</td>
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<tr>
<td>IL-6R</td>
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</table>
Cytokine levels at baseline in patients vs. controls

IL-17

[Graph showing IL-17 levels with p-values]

IL-23

[Graph showing IL-23 levels with p-values]

TGF beta

[Graph showing TGF beta levels with p-value]

Results, cytokines

- No difference in cytokine levels at baseline -
  - proliferative vs. membraneous LN
- Cytokines decreased following therapy
  - except IFNγ and TGF (increased)
- No difference between treatments

Cytokines in relation to response; Histopathology....

Baseline levels of IL-17 in relation to histopathological response

IL-17 was significantly higher in patients with a persisting active nephritis in follow-up biopsies (=non-responders)

Baseline levels of IL-17 in relation to histopathology at follow-up

IL-17 at baseline and follow-up

Baseline vs. Follow-up vs. Controls
Baseline high levels of IL-17

13 patients with the highest levels of IL-17 (>165 pg/ml) at baseline
- Had higher levels of IL-23, TNF-α and IFN-γ
- 11/13 (85%) were histopathological non-responders

Follow-up high levels of IL-17

- 8 patients with persisting high levels of IL-17 at follow-up (>165 pg/ml)
  - Had higher levels of IFN-γ and TNF-α
  - 8/8 patients were histopathological non-responders

...and BILAG response...

IL-23 at follow-up & BILAG response

- BILAG non-responders had high levels of IL-23
  - Most pronounced among non-responders WHO V

Summary

- High baseline levels of IL-17 predicted unfavourable histopathological response
  - Patients with high levels of IL-17 may represent a subgroup of patients with more severe disease

Conclusions

- This study indicates a role for IL-23/IL-17 axis in LN regarding response to treatment
  - These cytokines may be used as biomarkers
  - Targets for new therapies in SLE/LN?
Acknowledgements

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IL-23 was higher in patients with persisting proteinuria (>500 mg/d at follow-up)

Correlations – cytokines and clinical parameters

- Proteinuria sign correlation to IL-23 and IL-6 rec at follow up
- C3 sign inverse correlation to IL-23, TNF, IFN

What about IL-4, IL-2 and IL-21?

- IL-4 and IL-2; undetectable in >75 % cases
  → not included in the statistical analyses for response
  → IL-2 detected in 23% of cases vs. 62 % of controls

- IL-21; problems with the assay and results not reliable
  → not included in analyses

IL-2 was lower in patients vs. controls

\[ p = 0.05 \]

\[ p = 0.01 \]
## Baseline cytokines (pg/ml) in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>10.02 (0.62-81.8)</td>
<td>2.18 (0.62-4.6)</td>
<td><strong>0.000027</strong></td>
</tr>
<tr>
<td>IL-10</td>
<td>9.78 (0.54-81.0)</td>
<td>0.54 (0.54-3.9)</td>
<td><strong>0.000001</strong></td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.24 (0.55-12.5)</td>
<td>0.55 (0.55-4.3)</td>
<td><strong>0.099621</strong></td>
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<tr>
<td>IFN-γ</td>
<td>7.05 (0.52-338.9)</td>
<td>0.52 (0.52-9.0)</td>
<td><strong>0.031007</strong></td>
</tr>
<tr>
<td>IL-17</td>
<td>97.43 (3.30-381.6)</td>
<td>3.30 (3.30-62.7)</td>
<td><strong>0.000296</strong></td>
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<tr>
<td>IL-23</td>
<td>5.52 (0.66-121.5)</td>
<td>0.66 (0.66-0.7)</td>
<td><strong>0.000055</strong></td>
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<tr>
<td>IL-7</td>
<td>19.64 (2.90-27.9)</td>
<td>14.19 (3.71-21.9)</td>
<td><strong>0.073843</strong></td>
</tr>
<tr>
<td>IL-6-rec</td>
<td>51638.9 (30631.5-95346.8)</td>
<td>37325.7 (36794.1-59469.2)</td>
<td><strong>0.000472</strong></td>
</tr>
<tr>
<td>TGF-β</td>
<td>42990.8 (10219.5-85939.4)</td>
<td>82710.5 (57800.6-119055.9)</td>
<td><strong>0.000001</strong></td>
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