Familial Clustering of the Serum Cytokine Profile

Comparison Between the Asymptomatic First-Degree Relatives of RA Patients and Controls with No Family History of Autoimmune Disease

Hani S. El-Gabalawy¹, David B. Robinson¹, Donna M. Hart¹, Irene Smolik², Charles N. Bernstein¹, Marianna M. Newkirk³, Marvin J. Fritzler³

1. University of Manitoba, Winnipeg, MB
2. McGill University Health Centre, Montreal, QC
3. University of Calgary, Calgary, AB

Disclosures

• Funding for the study provided by the Canadian Institutes of Health Research MOP7770

Background

RA in North American Natives (NAN)

• RA is prevalent in NAN; 2-3% in Cree-Ojibway ¹-³
• frequent familial clustering of RA in NAN ⁴
• severe, mostly seropositive disease ⁵
• one third of unaffected first-degree relatives (FDR) of NAN RA patients are RF+ or ACPA+ ⁶
• 60-70% of background population *1402 or *0404 positive ⁷
• environmental RA risk factors common


Background

serum cytokines in pre-clinical RA

• RF and anti-CCP detectable in the pre-clinical period ¹-³
• serum levels of multiple cytokines elevated in pre-clinical RA ⁴-⁶
• elevated serum cytokines associate with RA autoantibodies ⁴-⁶
• combinations of RA autoantibodies and cytokine profile may serve as predictors of imminent RA onset ⁴-⁶
• high RF levels may amplify levels of specific cytokines on multiplex platforms ⁷


Objectives

• Determine how the serum cytokine profile of unaffected FDR of NAN RA patients differs from:
  – the serum cytokine profile of the RA patients
  – the serum cytokine profile of individuals with no family history of autoimmunity
• Determine if the serum cytokine profile of autoantibody positive FDR differs from autoantibody negative FDR?

Methods

study population

• Cree, Ojiway, Ojicee people of Central Canada
• RA probands from clinics helped recruit FDR
• ethnicity by self-report: at least 3 of 4 grandparents NAN
• urban locations: Winnipeg, Saskatoon
• rural locations: Norway House, St Theresa Point, Manitoba
• NAN controls recruited at local health fairs and by advertising
• Caucasian controls, primarily from the Winnipeg area
• all controls had no self-declared personal or family history of autoimmune diseases including:
  – RA, SLE, SpA, IBD, DM T, MS, and thyroid disease
• all study subjects completed questionnaires, underwent phlebotomy, and were examined by a rheumatologist
Methods

**biomarker testing**

- anti-CCP2 by ELISA (Inova); positivity cutoff ≥ 40 units for high specificity
- RF by nephelometry; positivity ≥ 50 IU based on excluding 95% of Caucasian controls
- multiplex cytokine testing performed in Dr Fritzler’s lab, University of Calgary (Eve Technologies) using a 42-plex laser bead assay and Luminex fluorometer
- a subset of samples were tested with and without blocks
- MCP-1 levels were determined by ELISA
- high sensitivity C-reactive protein (hsCRP) was determined by ELISA

**data analysis**

- raw cytokine levels were normalized by log transformation
- log transformed cytokine levels were compared by ANOVA
- post-hoc Bonferroni adjustment corrected for multiple comparisons
- discriminant analysis classified groups based on non-overlapping contributions from cytokines using their standardized coefficients
- FDR and NAN controls compared using logistic regression
- differences in cytokine levels between autoantibody positive and negative FDR tested by ANOVA with Bonferroni adjustment

**Cytokines tested**

- General activation
  - IL-1 beta
  - IL-1ra
  - sIL-2R alpha
  - TNF alpha
  - IL-6
  - IL-2
  - IL-15
  - IFN alpha
  - IL-1 alpha
- Th1 related
  - IL-12 (p40)
  - IL-12 (p70)
  - IFN gamma
- Th2 related
  - IL-4
  - IL-5
  - IL-9
  - IL-13
  - Eotaxin
- Th17 related
  - IL-17
- Treg cell related
  - IL-10
- Bone marrow derived
  - IL-7
  - GM-CSF
  - G-CSF
- Stromal and angiogenic
  - FGF-2
  - PDGF-AA
  - PDGF-AB/BB
  - VEGF
  - EGF
- Chemokines
  - IL-8
  - IP-10
  - MCP-1
  - MCP-3
  - MIP-1 alpha
  - MIP-1 beta
  - GRO alpha
  - MCP-3
  - TGF alpha
  - TNF beta
- Others
  - Flt-3 Ligand
  - Fractalkine
  - G-CSF
  - CXCL12
  - TNF alpha
  - TNF beta

**Demographic characteristics**

<table>
<thead>
<tr>
<th></th>
<th>RA patients (n=105)</th>
<th>FDR (n=273)</th>
<th>NAN controls (n=200)</th>
<th>Caucasian controls (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>females (%)</strong></td>
<td>87%</td>
<td>69%</td>
<td>60%</td>
<td>75%</td>
</tr>
<tr>
<td>age (mean ± SD)</td>
<td>47 ± 15</td>
<td>35 ± 13</td>
<td>35 ± 9</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>rural (%)</td>
<td>52%</td>
<td>53%</td>
<td>32%</td>
<td>15%</td>
</tr>
<tr>
<td>ever smoker (%)</td>
<td>71%</td>
<td>81%</td>
<td>62%</td>
<td>39%</td>
</tr>
<tr>
<td>current smoker (%)</td>
<td>55%</td>
<td>65%</td>
<td>62%</td>
<td>17%</td>
</tr>
</tbody>
</table>

**Prevalence of RA autoantibodies**

<table>
<thead>
<tr>
<th></th>
<th>RA patients (n=105)</th>
<th>FDR (n=273)</th>
<th>NAN controls (n=200)</th>
<th>Caucasian controls (n=150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-CCP2 positive (≥ 40 units)</td>
<td>81%</td>
<td>9%</td>
<td>4%</td>
<td>0</td>
</tr>
<tr>
<td>RF positive (≥ 50 units)</td>
<td>88%</td>
<td>34%</td>
<td>9%</td>
<td>5%*</td>
</tr>
<tr>
<td>anti-CCP2 or RF</td>
<td>91%</td>
<td>39%</td>
<td>11%</td>
<td>5%*</td>
</tr>
<tr>
<td>anti-CCP2 and RF</td>
<td>77%</td>
<td>3%</td>
<td>2%</td>
<td>0</td>
</tr>
</tbody>
</table>

* prevalence was pre-determined at a level that excludes 95% of the Caucasian controls
hs-CRP levels in the study groups

Variables differentiating FDR from NAN controls

**logistic regression model**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Chi-square</th>
<th>Incremental correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>140</td>
<td>74%</td>
</tr>
<tr>
<td>COX4L</td>
<td>115</td>
<td>83%</td>
</tr>
<tr>
<td>IL-1</td>
<td>96</td>
<td>86%</td>
</tr>
<tr>
<td>TGF alpha</td>
<td>36</td>
<td>88%</td>
</tr>
<tr>
<td>hsCRP</td>
<td>34</td>
<td>91%</td>
</tr>
<tr>
<td>IP10</td>
<td>27</td>
<td>91%</td>
</tr>
<tr>
<td>PDGF alpha</td>
<td>22</td>
<td>91%</td>
</tr>
<tr>
<td>sIL-2Rα TGF α 1 2 3 4</td>
<td>24</td>
<td>92%</td>
</tr>
<tr>
<td>MDC</td>
<td>7</td>
<td>92%</td>
</tr>
<tr>
<td>IL-8</td>
<td>6</td>
<td>93%</td>
</tr>
<tr>
<td>age</td>
<td>6</td>
<td>94%</td>
</tr>
<tr>
<td>GRO alpha</td>
<td>5</td>
<td>94%</td>
</tr>
</tbody>
</table>

Variables differentiating RF-/CCP- FDR (n=167) from RF-/CCP- NAN controls (n=178)

**logistic regression model**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Chi-square</th>
<th>Incremental correct classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1</td>
<td>130</td>
<td>77%</td>
</tr>
<tr>
<td>CD40L</td>
<td>100</td>
<td>86%</td>
</tr>
<tr>
<td>IL-7</td>
<td>56</td>
<td>87%</td>
</tr>
<tr>
<td>hsCRP</td>
<td>34</td>
<td>91%</td>
</tr>
<tr>
<td>PDGF alpha</td>
<td>26</td>
<td>91%</td>
</tr>
<tr>
<td>TGF alpha</td>
<td>14</td>
<td>92%</td>
</tr>
</tbody>
</table>
Correlation of MCP-1 levels by multiplex and ELISA

Spearmans' rho = 0.56, p=0.000

MCP-1 levels in the study groups

Cytokines differentiating autoantibody positive and negative FDR (n=167)*

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>anti-CCP only</th>
<th>RF+ only</th>
<th>anti-CCP+/RF+</th>
</tr>
</thead>
<tbody>
<tr>
<td>EG1</td>
<td>1.6 (0.02)</td>
<td>--</td>
<td>1.6 (0.04)</td>
</tr>
<tr>
<td>GRO alpha</td>
<td>--</td>
<td>0.6 (0.03)</td>
<td>--</td>
</tr>
<tr>
<td>IL-1 alpha</td>
<td>1.1 (0.09)</td>
<td>0.1 (0.02)</td>
<td>--</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.1 (0.04)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>IFN</td>
<td>--</td>
<td>0.1 (0.03)</td>
<td>--</td>
</tr>
<tr>
<td>IL-10</td>
<td>-0.8 (0.03)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MCP-1 alpha</td>
<td>1.4 (0.04)</td>
<td>0.7 (0.02)</td>
<td>1.7 (0.01)</td>
</tr>
<tr>
<td>PDGF AB/BB</td>
<td>-1.1 (0.02)</td>
<td>-0.4 (0.05)</td>
<td>--</td>
</tr>
<tr>
<td>Rantes</td>
<td>--</td>
<td>-0.1 (0.02)</td>
<td>--</td>
</tr>
</tbody>
</table>

* p values after post-hoc Bonferroni adjustment for multiple comparisons

Summary

• Serum cytokine profile of FDR are intermediate between RA and controls
• The high prevalence of RF in FDR did not account for the elevated cytokine levels
• MCP-1 is the strongest discriminaor between FDR and NAN controls
• Autoantibody positive and negative FDR have modest differences in serum cytokine profile

Limitations

• A number of confounders were not accounted for, particularly diabetes and BMI
• Relevance to non-NAN populations needs to be established
Acknowledgements

University of Manitoba
Christine Peschken
Carol Hitchon
Denise Jacobs

Centre for Aboriginal Health Research
Brenda Elias
John O’Neil

Rheumatic Diseases Research Laboratory
Keng Wong

University of Saskatchewan
Janet Markland

Assembly of Manitoba Chiefs
Norway House, St Theresa Point Band Councils