Patients with chronic bone and joint infection due to staphylococci expressing small-colony-variant phenotype do not have any deficiency in natural killer cell function

S. Viel,1, 2, 3 P. Rouzaire,1, 2, 3 F. Laurent,2, 3, 4, 5 T. Walzer,3 J. Bienvenu,1, 2, 3 D. Peyramond,2, 6, 7 C. Chidiac,2, 3, 6, 7 T. Ferry*, 2, 3, 5, 6, 7
On behalf the Lyon BJI Study Group

1 Department of Cellular Immunology, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, France
2 Université Claude Bernard Lyon 1, Lyon France
3 INSERM 851 Pathogénie Bactérienne et Immunité Innée, Lyon, France
4 Laboratory of Bacteriology, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, France
5 Centre National de Référence des Staphylocoques, Lyon, France
6 Department of Infectious and Tropical Diseases, Hôpital de la Croix-Rousse, Hospices Civils de Lyon, France
7 Centre Interrégional de Référence Rhône-Alpes Auvergne des IOA complexes, Lyon, France
**Background**

- Staphylococci (S. aureus, coagulase-negative staphylococci) are frequently involved in BJI
  

- Staphylococci may produce small colony variant (SCV) related to intracellular persistence
  

- SCV are associated with recurrence and relapse
  
  Sendi P, *Clin Infect Dis*; 2006;43:961-7

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*Host or bacterial factors that lead to in vivo production of SCV are unknown*
Background

• NK cells (CD3-C56+) are innate lymphocytes specialized in the recognition and killing of intracellular pathogens

Vivier E, Science 2011;331:44–49

NK cell activity deficiency might be a risk factor for BJI due to staphylococci expressing the SCV phenotype.
Patients and methods

• Cross-sectionnal study for 7 months
• **Patients with chronic BJI due to:**
  - Staphylococci expressing SCV (SCV+)
  - Staphylococci not expressing SCV (SCV-)
  - Other pathogen(s)

• ≥1 bacterial culture for *S. aureus*
• ≥3 bacterial cultures for CNS

• **SCV was defined by:**
  - Typical phenotypic aspect of colonies from peroperative specimen cultures
  - Lack of hemolysin activity on blood agar (*S. aureus*)

Phenotypic analysis of circulating NK cells

Blood

Ficoll

NK cell

CD16

CD56

perforin

granzyme

**Number of circulating NK cells**

(\(\text{CD3-CD56}^{\text{dim}}\))

% of activated circulating NK cells (CD69+)

Level of intracellular perforin in circulating NK cells

% of circulating NK cells expressing CD16+
Functional assay of circulating NK cells

Blood

Ficoll

K562 cells (CMHI deficient cells)

NK cell

CD16

CD56

IFNγ

perforin

granzyme

Measurement of NK cell activity

% of NK cells with Intracellular expression of IFNγ

% of NK cells CD107a+

CD107a (LAMP-1, Lysosomal-Associated Membrane Protein 1)

anti-CD107a

Activating signal

Inhibitory signal

% of NK cells

CD107a+

Functional assay of circulating NK cells

Measurement of NK cell activity

% of NK cells with Intracellular expression of IFNγ

% of NK cells CD107a+
Results

- **SCV+ (n=10)**
  - 8 with an orthopaedic implant
  - 7 due to *S. aureus*, 3 to CNS

- **SCV- (n=10)**
  - All with implant
  - 5 due to *S. aureus*, 5 to CNS

- **Other BJI (n=6)**
  - 3 with an orthopaedic implant
  - due to *P. acnes*, *Enterobacteriaceae*, or *P. aeruginosa*

- **19 healthy volunteers (HV)**
<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Patients with SCV+ (n=10)</th>
<th>Patients with SCV- (n=10)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interval between symptoms and bacterial diagnosis (median, days)</td>
<td>133 (61-209)</td>
<td>129 (13-88)</td>
<td>0.082</td>
</tr>
<tr>
<td>Interval between bacterial diagnosis and blood sampling (median, days)</td>
<td>93 (20-248)</td>
<td>52 (15-121)</td>
<td>0.356</td>
</tr>
<tr>
<td>Age (median, years)</td>
<td>61 (52-79)</td>
<td>57 (47-69)</td>
<td>0.247</td>
</tr>
<tr>
<td>Male sex (n, %)</td>
<td>6 (60)</td>
<td>5 (50)</td>
<td>0.653</td>
</tr>
<tr>
<td>Charlson comorbidity index &gt;2 (n, %)</td>
<td>6 (60)</td>
<td>5 (50)</td>
<td>0.656</td>
</tr>
<tr>
<td>Implant-associated infection (n, %)</td>
<td>9 (90)</td>
<td>10 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Recurrence (n, %)</td>
<td>7 (70)</td>
<td>0 (0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Multimicrobial infection (n, %)</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td>1</td>
</tr>
</tbody>
</table>
Phenotypic analyses

Equal number of circulating NK cells
NK cells are activated \textit{in vivo}.

NK cells displayed reduced CD16 and perforin expression that may suggest \textit{in vivo} cytotoxicity.
Degranulation and IFN-γ secretion by NK cells in response to K562 cells were similar.
Discussion

• Limitations:
  – Circulating NK cells
  – SCV are easy to overlook
  – SCV could be expressed at the site of infection without being detected in culture
  – SCV phenotype has not been described only with staphylococci

• Intracellular persistence may be a common characteristic of bacteria responsible for chronic BJI

• NK cells could have a role in the immune response during chronic BJI, by killing infected host cells
Conclusion

• NK cells could have a role in the immune response during BJI, regardless of the involved bacteria

• Gram-positive and negative bacteria might produce SCV at the site of infection during chronic BJI

• Expression of SCV phenotype during staphylococcal BJI:
  – could be independent of host factors
  – might be due to strain-related characteristics.
Lyon BJI study group


Surgeons – S. Lustig, G. Demey, P. Neyret, JP. Carret, G. Vaz, MH. Fessy

Microbiologists – F. Laurent, F. Vandenesch, JP. Rasigade

Nuclear Medicine – I. Morelec, E. Deshayes, M. Janier, F. Giammarile

PK/PD specialists – M. Tod, MC. Gagnieu, S. Goutelle

Immunologists – S. Viel, P. Rouzaire, T. Walzer