New evidences

Biomarkers to explore immunoparalysis: what future for immunostimulation?

B. François – CHU Limoges (France)
Background
New understanding of response in sepsis

Immunologic Progression in Sepsis

Co-morbidities

- innate immunity
- adaptive immunity

homeostasis

recovery

death

TIME (days)

0 1 2 3 4 5 6 7
PICS

- Persistent
- Inflammation
- Immuno-suppression
- Catabolism
- Syndrome
## Secondary Infections in Sepsis

**Effect of Eritoran, an Antagonist of MD2-TLR4, on Mortality in Patients With Severe Sepsis**

The ACCESS Randomized Trial

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=657)</th>
<th>Eritoran (n=1305)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>New infection</strong></td>
<td>183 (27.9)</td>
<td>361 (27.7)</td>
</tr>
<tr>
<td><strong>Relapse of infection</strong></td>
<td>27 (4.1)</td>
<td>65 (5.0)</td>
</tr>
<tr>
<td><strong>Superinfection</strong></td>
<td>93 (14.2)</td>
<td>192 (14.7)</td>
</tr>
</tbody>
</table>

*Opal, JAMA 2013*
Right stimulation at the right time

No recovery = death / nosocomial infections

Recovery = Survival

Sepsis onset
Compensatory mechanisms

Days

Immune functions (arbitrary scale)

Immune competence
Gray zone
Immune failure

Slide courtesy of G. Monneret
New drugs for innovative approach
Potential Immuno-adjuvant agents

- Anti-PD-1 / anti-PD-L1
- IL-7
- GM-CSF
- IFN-γ
- Thymosin alpha 1 – Critical Care 17:R8 2013
- Anti-IL-10 antibody
- Inhibition of T regulatory cells
Anti-PD-1 & Anti-PD-L1

Anti-PD-1 or anti-PD-L1
(Two hit: CLP + Candida)

% survival

Control (n=74)
αPD-1 200ug (n=35)
αPD-L1 200ug (n=39)

* p<0.01

Day

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

CLP Candida αPD-1 or αPD-L1

Chang, Critical Care 2013
Beneficial Effects of IL-7 in Infectious Disease

- ↑ Macrophage activation
- ↑ IFN-γ
- ↑ integrins
- Improved trafficking to infected site
- Improved antigen presentation (↑ T cell activation)
- ↑ production & ↑ TCR diversity
- Pathogen-induced apoptosis
- Activated T cell
- “Exhausted” T cell
- IL-7
- LFA1
- VLA4
- Thymocytes
IL7 therapeutic use in Sepsis (I)
Ex-vivo use of IL-7 in Septic Patients

- Evaluation of IL-7 effects on lymphocyte proliferation and cytokine production in septic patients and healthy volunteers.
- Ex vivo treatment of cells with IL-7 markedly improved lymphocyte proliferation and IFN-γ production in cells from septic patients.

“To our knowledge, this constitutes the first report of IL-7 ability to restore normal lymphocyte functions in septic patients. These results support the rationale for initiating a clinical testing of IL-7 in septic shock”

Venet, J. of Immunol 2012
GM-CSF therapeutic use in Sepsis

“White blood cell growth factor” stimulating and used clinically for bone marrow transplant patients and patients with immune defects

- GM-CSF restored monocyte HLA-DR in 19/19 septic patients
- GM-CSF restored ex vivo whole blood stimulated pro-inflammatory monocyte cytokine production
IFN-γ

6 patients with candidemia

Interferon-gamma as adjunctive immunotherapy for invasive fungal infections: a case series

Delsing, BMC Infectious Disease 2014
“New techniques” for New biomarkers
Cytometry method

Analysis of leukocyte subsets using multi-Color cytometer:

- Lymphocytes
- Dendritic cells
- Monocytes including HLA-DR
- Granulocytes
Biomarkers in Sepsis diagnosis (I)

- Major modification of leukocyte subset:
  - Monocytes
  - Granulocytes
  - Lymphocytes
  - Dendritic cells

- Diagnosis:
  - Granulocytes $CD64^{pos}$ specific marker of sepsis

- Prognosis:
  - Lymphopenia
  - Monocytes $CD40^{pos}$

References:
- Dimoula, Clin Infect Dis. 2014
- Monserrat, Crit Care. 2009
- Sugimoto, Shock 2003
Biomarkers in Sepsis diagnosis (II)

Leukocyte immunophenotyping in the early phase of sepsis (184 patients)

- Decrease expression of CD10 and CD16 on granulocytes
  - Linked to classification of sepsis
  - Prognosis of deterioration at 48h

Guérin, Crit Care Med. 2014
### Biomarkers in Sepsis prognosis

**Guérin, Crit Care Med. 2014**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Alive (N = 162)</th>
<th>Sepsis-related death (N = 15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils G/L</td>
<td>12.204 [8.752-16.834]</td>
<td>5.747 [3.056-7.951]</td>
<td>0.001</td>
</tr>
<tr>
<td>Gated CD10- Neutrophils (%)</td>
<td>65.67 [28.265-91.090]</td>
<td>96.04 [92.475-98.44]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gated CD64+ Neutrophils (%)</td>
<td>13.515 [1.103-53.29]</td>
<td>18.32 [7.795-72.235]</td>
<td>0.212</td>
</tr>
<tr>
<td>Gated CD16- Neutrophils (%)</td>
<td>5.912 [1.581-16.809]</td>
<td>74.282 [28.574-86.009]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monocytes G/L</td>
<td>0.838 [0.469-1.228]</td>
<td>0.332 [0.201-0.626]</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasmocytoïd Dendritic Cells G/L</td>
<td>0.001 [0.001-0.004]</td>
<td>0.001 [0-0.001]</td>
<td>0.022</td>
</tr>
<tr>
<td>Lymphocytes G/L</td>
<td>0.86 [0.661-1.403]</td>
<td>0.868 [0.419-1.069]</td>
<td>0.269</td>
</tr>
<tr>
<td>CD4+ Lymphocytes G/L</td>
<td>0.339 [0.192-0.546]</td>
<td>0.145 [0.105-0.38]</td>
<td>0.026</td>
</tr>
<tr>
<td>CD8+ Lymphocytes G/L</td>
<td>0.16 [0.081-0.298]</td>
<td>0.178 [0.046-0.334]</td>
<td>0.602</td>
</tr>
<tr>
<td>T Cytotoxic Lymphocytes G/L</td>
<td>0.015 [0.007-0.031]</td>
<td>0.009 [0.003-0.019]</td>
<td>0.068</td>
</tr>
<tr>
<td>T Regulator Lymphocytes G/L</td>
<td>0.02 [0.012-0.034]</td>
<td>0.015 [0.008-0.025]</td>
<td>0.106</td>
</tr>
</tbody>
</table>
Biomarkers to predict infections

60 patients undergoing planned cardiac surgery

CD10_{dim}/CD16_{dim} expression could predict infectious complications after cardiac surgery

Daix, Critical Care (submitted)
CD4 and CD8 Cells

Non-septic

CD4

E

CD8

F

Septic

CD4

p<0.001

Non-septic       Septic

CD8

p<0.005

Non-septic       Septic

Boomer, JAMA 2011
Results from 3 different published studies including >400 septic patients (virtual results on 100 septic patients)

- 50% of patients are immunosuppressed at day 4
- 40% of these immunosuppressed patients will die
Lymphopenia

Drewry, Shock 2014
Increase in immunosuppressor cells

- T regulatory cells
- Myeloid derived suppressor cells
- Cells with negative co-stimulatory molecules – PD-1/PD-L1 (T cell exhaustion)

Venet, ICM 2009; Guérin, Crit Care Med 2014
Conclusions

• Mortality linked to immunosuppression in septic patients
• Several interesting biomarkers both to diagnose Sepsis, predict infection and identify immuno-suppressed patients
• Promising immuno-stimulating drugs in the clinical development pipeline
• Current available techniques to document immuno-suppression outside research

“Sepsis is not anymore an infectious disease but an immunologic disorder “