The concept of resetting the immune balance

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Autologous HSCT suppresses brain inflammation in MS (Mancardi et al Neurology, 2001; Saccardi et al Blood 2005)

Mean number /month
Gd+areas: 13.5

Mean number /month
Gd+areas: zero

Time (months)

Courtesy of Prof. Mancardi
What is the therapeutic mechanism?

Rationale of HSCT as treatment for autoimmune disorders

- Abrogation of pathogenic T cell response (via immunoablation) regardless of specificity
- Assumption that environmental triggering factors are no longer present or active
- Reconstitution of a tolerant immune system
Resetting the immunological clock?

Environmental factors (viruses, bacteria)

Population at risk

Genetically susceptible

Clinically affected

Persistent tolerance

Latency

HSCT

Autoimmune disease

Abrahamsson and Muraro, Autoimmunity 2008

HSCT: Immune...

Resetting

Rebooting

Rejuvenation

Rejuvenation

Recapitulation

Renewal

Recapitulation

Reformatting

Re-education

Reformatting

Reconditioning

What do we mean by that?
How do we define immune resetting?

Replacement with new immune repertoire?

Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients

Paolo A Muraro,1 Daniel C Domnik,1 Amy Packer,1 Katherine Chang,1 Francisco J. Gennaro,1 Ricardo Cavani-Inganni,1 Catherine Campbell,1 Saritaz Memen,1 James W. Nagle,1 Frances T. Hakin,2 Ronald E. Gress,3 Henry E. McFarland,1 Richard K. Burt,1 and Roland Martin1

1. TREC assay

2. Phenotype-based Enumeration of Recent Thymic Emigrants

Thymic rebound

- Thymic rebound: the volumetric enlargement and functional reactivation of the thymus following lymphoid depletion
- Rapid and prominent in children
- Delayed and reduced in older adults
- Naïve T cell recovery in the peripheral circulation after HSCT is correlated with thymic rebound

Figure 1 Recovery of CD4+ T-cell subsets over time in SLE patients treated by immunoablation and ASCT versus levels in age-matched healthy controls

Two pathways mediate immune reconstitution after lymphodepletion

Hakim and Gress, Eur J Immunol 2005

Immune reconstitution after AHSCT

Muraro and Douek, 2006
The spectrum of thymus-independent T cell reconstitution

"True" homeostatic proliferation

Main T cell subset

Initial activation status

Oligoclonal T cell expansion

CD4+

CD8+

Naive

Memory

Diversity of TCR repertoire

Avidity and affinity of Ag-MHC stimulus

Peptide/MHC stimulus

Commensal microflora

Foreign (viral) pathogens

Self Ag

IL-7 (or IL-4 or IL-15)

IL-15, IL-7, or IL-2

Slow

Fast

Proliferation rate

Cytokine requirement

Muraro and Douek, 2006
Diversification and renewal of T cell repertoire following auto-HSCT in MS patients

**CDR3 spectratyping**

Pre-transplant

6 months

1 year

2 years


**Nucleotide sequencing**

- ~90% new T cell clones

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5. T CELL REPERTOIRE and TREC values AFTER ABMT IN SSC

<table>
<thead>
<tr>
<th>A (CR, PR) vs B (NR, relapse) (n=14 CY alone)</th>
<th>Farge Arthr Rheum 2005; 52: 1555</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal values</td>
<td>At inclusion</td>
</tr>
<tr>
<td>Polyclonal BV families, %</td>
<td>Group A</td>
</tr>
<tr>
<td>Polyclonal</td>
<td>70.3 ± 29.0</td>
</tr>
<tr>
<td>SKEWED BV families, %</td>
<td>22.8 ± 30.2</td>
</tr>
<tr>
<td>Negative BV families, %</td>
<td>6.9 ± 7.4</td>
</tr>
<tr>
<td>TREC/μg DNA</td>
<td>494 ± 776.6</td>
</tr>
<tr>
<td>TREC/CRP: r = - 0.41, p &lt; 0.001, TREC / CD19+: r = 0.35, p &lt; 0.001 (RA , SEP)</td>
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</tbody>
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**Sustained altered T cell homeostasis and abnormal Repertoire** (Crit Rev Immunol 1995)

Persistence of underlying disease mechanism after HSCT?maintenance immunosuppression

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Nov 2012 | Paris, France
How do we define immune resetting?

Replacement with new immune repertoire? ✔

Eradication of existing immune system?

Early recovery of CD4 T cell receptor diversity after “lymphoablative” conditioning and autologous CD34 cell transplantation

Jan Storek1,2, Zhao Zhao3, Ying Liu2, Richard Nash1, Peter McSweeney1,3, and David G. Maloney1

<table>
<thead>
<tr>
<th>HLA-Tetramer</th>
<th>TCR Transplant</th>
<th>HLA-Tetramer</th>
<th>TCR Original</th>
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HLA-tetramer and TCR analysis: persistence of pre-existing Ag-specific clones either carried over from autologous graft or surviving conditioning chemo

Storek et al BBMT 2008
Treatment protocol: a high-intensity myeloablative conditioning regimen

**Peripheral CD34 selected (Isolex, Baxter) Autologous graft**

- **Mobilization**
  - Peripheral CD34 selected (Isolex, Baxter)

- **Conditioning**
  - CY 2.0 g/m²
  - G-CSF 5 µg/kg per day
  - CY 60 mg/kg/day for 6 days
  - TBI 150 cGy, twice daily, for 4 consecutive days yielding a total dosage of 1200 cGy

**Post Transplant**

Dubinsky et al. BMT 2009

T-cell clones persisting in the circulation after autologous hematopoietic SCT are undetectable in the peripheral CD34+ selected graft

AN Dubinsky1,2, R K Burt3, R Martin1,4 and PA Muraro1,2

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How do we define immune resetting?

Replacement with new immune repertoire? ✓

Eradication of existing immune system? partial ✓

Re-instatement of immune regulation?
Figure 7. Induction of CD4+CD25bright regulatory T cells in rats undergoing BMT


Recovery of CD4+CD25bright T-cell frequency after ASCT

Figure 2 Phenotypic analysis of FoxP3+ Treg levels in 5 patients after ASCT compared with those in healthy controls and conventionally treated SLE patients

Zhang et al. JI 2009 (Nov. 15)

Regulatory T Cell (Treg) Subsets Return in Patients with Refractory Lupus following Stem Cell Transplantation, and TGF-β-Producing CD8+ Treg Cells Are Associated with Immunological Remission of Lupus

Li Zhang, Anne M. Bertucci, Rosalind Ramsey-Goldman, Richard K. Bart, and Nymal K. Dutta

Zhang et al. JI 2009 (Nov. 15)
Paolo Muraro | The concept of resetting the immune balance

**How do we define immune resetting?**

- **Replacement** with new immune repertoire?
- **Eradication** of existing immune system? (partial)
- **Re-instatement** of immune regulation?

**Shifting the balance**

- **Auto-reactive memory T** and **B cells**
- **IL-1**, **TNF**
- **IFN-g**, **IL-17**
- **Impaired immune regulation**
- **AHSCT**
- **Naïve cells**
- **Regulatory cells**
- **TGF-b**
- **IL-10**
- **Apoptosis**
Conclusions

**Autologous hematopoietic stem cell transplantation**
has the potential to stop MS inflammation through
reset of immune system

**We have identified 3 main mechanisms of immune resetting**
- Influx of naïve cells from thymus
- “Debulking” of mature memory lymphocyte repertoire
- Boost of number of regulatory cells

Perspective

- The 3 identified mechanisms of immune resetting may be complementary or synergistic
- Their relative contribution may depend on transplantation regime
- Their importance in determining the clinical outcome remains to be established