Therapeutic strategies to boost the ageing immune system

Dr Sian Henson
Challenges of the ageing immune system

**Developmental defect**

- HSC → ELP → ETP → thymus

**Post-thymic acquired defect**

- RTE → New naive → Old naive → Effector → Memory

**Cellular ‘age’**

- Function decreases with age

**Factors that -**

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Cellular age</th>
<th>Homeostatic division</th>
<th>Differentiation to memory cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extrinsic</td>
<td>Exposure to oxidative and other stress</td>
<td>Thymic atrophy</td>
<td>Stromal factors</td>
</tr>
</tbody>
</table>

**Preserve or enhance function**
Challenges of the ageing immune system

Developmental defect

- Gene therapy?
- Stem cell therapy?

Min et al. 2004 J. Immunol
Formation of a functional thymus by a epithelial progenitor cells

- Addition of FoxN1$^+$ ETPs
- Formation of thymic lobules containing both cortical and medullary areas
- Can support normal T cell development

Bleul et al. 2006 Nature
Challenges of the ageing immune system

Post-thymic acquired defect

- RTE
- New naive
- Old naive
- Effector
- Memory

OLD

- % CD27-CD28-CD4+ T cells

Fletcher et al. 2006 J. Immunol

- Treat the CMV infection?
- Vaccination against CMV?
Currently no licensed vaccine against CMV

- Many CMV vaccines have been tested in human trials
- Recipients who were originally CMV +ve do not benefit from vaccination
- Vaccination of CMV -ve renal transplant recipients results in a reduction of disease severity but not a prevention in infection
Challenges of the ageing immune system

• Castration
• Sex steroid ablation
• Survival cytokines
Thymic regeneration in mice following castration

- Restoration of thymic architecture following castration

Sutherland et al. 2006 J. Immunol
Thymic regeneration in humans following androgen blockade

• Prostate cancer patients given LHRH

• After 4 months showed increased T cell numbers

• These cells are coming from the thymus

Sutherland et al. 2006 J. Immunol
T cell development in the thymus

HSC → ETP → CD3⁺CD4⁻CD8⁺ → CD4⁺CD8⁺

TCRβ chain rearrangement and selection → TCRβlo TCRαlo TCRαβ high

CD4⁺CD8⁻αβ high

CD8⁺CD4⁻αβ high

Positive and Negative Selection
## Effect of age on the T cell numbers

![Diagram showing the effect of age on T cell numbers](image)

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Total</th>
<th>CD4⁺CD8⁺</th>
<th>CD4⁺CD8⁻</th>
<th>CD4⁻CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo</td>
<td>9</td>
<td>1.64±0.3x10⁸ (100%)</td>
<td>1.4±0.3x10⁸ (86±3%)</td>
<td>1.2±0.4x10⁷ (7±2%)</td>
<td>8.5±4.7x10⁶ (5±2%)</td>
</tr>
<tr>
<td>20 mo</td>
<td>8</td>
<td>2.75±0.6x10⁷ (100%)</td>
<td>2.2±0.5x10⁷ (83±19%)</td>
<td>2.6±0.89x10⁶ (10±3%)</td>
<td>1.1±0.4x10⁶ (4±1%)</td>
</tr>
<tr>
<td>difference</td>
<td></td>
<td>1.36x10⁸</td>
<td>1.17x10⁸</td>
<td>9.32x10⁶</td>
<td>7.38x10⁶</td>
</tr>
</tbody>
</table>
Ageing and intrathymic differentiation

CD44+CD25−

CD44−CD25−

CD44+CD25+

CD44−CD25+
Analysis of triple negative thymocytes

CD44^+CD25^-

CD44^+CD25^+

CD44^-CD25^+

CD44^-CD25^-

young

old

7AAD

Annexin V
T cell development in the thymus

Location of Bottleneck in Aged Thymus
Possibly causes for this atrophy

• Hypothesis

cells seeding from the bone marrow fail to differentiate and survive because of the environment.
T cell development in the thymus

Location of Bottleneck in Aged Thymus
IL-7 production in the thymus declines with age.
Benefits of IL-7 therapy - mouse

- Increased thymic output

- 24 month old mice, treated with IL-7 rested for 3 weeks, then $0.5 \times 10^6$ spleen cells used to measure $\delta$EC levels
Benefits of IL-7 therapy - mouse

- Increased function
- 24 month old mice, treated with IL-7 rested for 3 weeks, then spleen cells tested for ability to proliferate to anti-CD3
Benefits of IL-7 therapy - rhesus macaque

- Increased T cell numbers
- 20 year old rhesus macaques, treated with IL-7, blood taken and T cell numbers assessed

![Graph showing CD3+ T cell numbers over weeks of study](chart.png)

- **IL-7 treatment period**, 60 µg/Kg alternate days
- Open symbols = saline controls
- Closed symbols = IL-7 treated
Benefits of IL-7 therapy - rhesus macaque

- Increased thymic output
- 20 year old rhesus macaques, treated with IL-7, blood taken and thymic output determined

![Graph showing Log₁₀ No. TRECS per ul of blood over weeks of study with annotations for IL-7 treatment period, 60 μ/Kg alternate days. Open symbols = saline controls, Closed symbols = IL-7 treated]
### Benefits of IL-7 therapy - rhesus macaque

<table>
<thead>
<tr>
<th>T=IL-7</th>
<th>Animal</th>
<th>Week 10</th>
<th>Week 12</th>
<th>Week 14</th>
<th>Week 16</th>
<th>Week 18</th>
<th>Week 20</th>
<th>Week 22</th>
<th>Week 26</th>
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<tbody>
<tr>
<td>S=saline</td>
<td>1FV</td>
<td>&lt;10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>T</td>
<td>1VQ</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20</td>
<td>10</td>
<td>&lt;10</td>
</tr>
<tr>
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<td>1JW</td>
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<td>&lt;10</td>
<td>80</td>
<td>80</td>
<td>160</td>
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<td>30</td>
</tr>
<tr>
<td>T</td>
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<td>&lt;10</td>
<td>&lt;10</td>
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<td>10</td>
<td>10</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

- A protective serum anti-HI titre of ≥40 can be found in about 80% of individuals after a natural bout of influenza and for vaccines
- European Agency for the Evaluation of Medical Products states that for HI tests seroconversion corresponds to a pre vaccination/post vaccination titre ratio of ≥40
- After the first vaccination shot, 50% of the treated animals show a titre ratio of ≥40
Human trials involving IL-7

- **Recruiting:** IL-7 in treating patients with metastatic melanoma or locally advanced or metastatic kidney cancer
- **Recruiting:** Safety study of IL-7 in HIV-infected patients
- **Recruitment ended:** Safety of IL-7 in HIV infected people currently taking anti-HIV
- **Recruitment ended:** IL-7 in treating patients with refractory solid tumors
- **Completed:** IL-7 and vaccine therapy in treating patients with metastatic melanoma
- **Completed:** IL-7 to treat HIV-infected people receiving antiretroviral therapy
Benefits of IL-7 therapy - human

- Increased numbers of CD4 and CD8 T cells over the 21 day treatment period
- These increases were seen in the naïve T cell compartment

Rosenberg et al. 2006 J. Immunother
Problems with IL-7 therapy

- Half life of IL-7 is short, 115 minutes in vivo

- Frequent injection at a dose high enough to reach the thymus in sufficient quantity to have an effect
Modification of IL-7 to improve effectiveness

• Targeting

create a fusion protein between IL-7 and a chemokine receptor whose ligand is organ specific
CCR9/IL-7 fusion clone

atgatgccccacagaactcacaagccttatcctgtgcatgtttagatgtctcagctatgac
M M P T E L T S L I P G M F D D F S Y D
tccacgtcctccacagatgactacatgaatttgaatccagtacctctctgtataagaaa
S T A S T D D Y M N L N F S S F F C K K
aataatgctagggcagttgtgacacggtcgtgctccagttttttagatatatacttt
N N V R Q F A R G R M F H V S F R Y I F
ngaattcctccactgatatctttgtgctgctgtcactcatctgtagtgccacattaaa
G I P P L I L V L L P V T S S E C H I K
gacaaaaaaggttaagcatatgagagtactgtatgactagcatcagcatgaattgacaaa
D K E G K A Y E S V L M I S I D E L D K
atgcacaggaacctagtaatgtcgcgaataatgaaaccaaatatttttagaaaacatgta
M T G T D S N C P N N E P N F F R K H V
tgtgtgatataaaaaggaagctgcttttttctaatctgtgctgctccggaagttgaagcaattt
C D D T K E A A F L N R A A R K L Q F
dtttaaatgatatcagtaaggaattcagatgtcctacttaacagtacacaagcaca
L K M N I S E F N V H L L T V S Q G T
cmaactctgtgagctcacaagtaaggaagaaaaaacgtaaaggacagaaaaagaat
Q T L V S C T S K E E K N V K E Q K K N
gatgcagtttctttaagagactactgtgaaatataaaacactttgtgataaatttttg
D A C F L K R L L R E I K T C W N K I L
aaggcagtata
K G S I
Improved benefits of giving the CCR9/IL-7 Fusion Protein

- Increased amounts of IL-7 in the thymus
- 24 month old mice, treated with CCR9/IL-7 rested for 3 weeks, then thymus removed and IL-7 content measured
Improved benefits of giving the CCR9/IL-7 Fusion Protein

- Increased thymic output
- 24 month old mice, treated with IL-7 rested for 3 weeks, then $5 \times 10^5$ spleen cells used to measure $\delta$EC levels
Restoration of thymic architecture after treatment with the fusion protein

- Rejuvenation of thymic medulla and cortex

- 24 month old mice, treated with fusion protein or IL-7 rested for 3 weeks, then the thymus removed sectioned and stained
Increased thymocyte numbers after treatment with the fusion protein

- Increased numbers of CD44$^+$CD25$^-$ thymocytes

- 24 month old mice, treated with fusion protein or IL-7 rested for 7 days, the thymus removed and thymocyte numbers determined
Fusion treatment causes reduced influenza disease progression

- Reduced vial load
- 24 month old mice infected with influenza, treated with fusion protein or IL-7 rested for 3 weeks, then spleen cells used to measure viral load
Fusion treatment causes reduced influenza disease progression

- Reduced amounts of TNF$_{\alpha}$
- 24 month old mice infected with influenza, treated with fusion protein or IL-7 rested for 3 weeks, then the TNF$_{\alpha}$ concentration measured
Fusion treatment increases the amount of cells fighting the influenza infection

- Increased CD45RB_{low} expression in CD8 T cells
- 24 month old mice infected with influenza, treated with fusion protein or IL-7 rested for 3 weeks, then the amount of CD45RB cells measured
Fusion treatment increases the amount of cells fighting the influenza infection

- Increased expression of influenza specific CD8 T cells
- 24 month old mice infected with influenza, treated with fusion protein or IL-7 rested for 3 weeks, then the amount of influenza specific cells measured
Conclusions

• There are therapies in development to boost the ageing immune system

• IL-7 therapy is yielding the most promising results
Acknowledgments

IL-7 Work
Imperial College
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