Immunological Memory to Influenza Virus

• Long-Lived Plasma Cells in the Bone Marrow

Human Bone Marrow Plasma Cells

1. What is the frequency of influenza virus specific plasma cells in the bone marrow?
2. What is the correlation between flu specific plasma cells in the BM and flu specific antibody levels in the serum?
3. Do influenza specific BM plasma cell numbers increase after flu vaccination?
4. Is there any correlation between the increase in BM plasma cells and the size of the plasmablast response in the blood?
5. Is the increase in BM plasma cells following influenza vaccination sustained over time?

Frequency of antigen specific plasma cells in bone marrow

<table>
<thead>
<tr>
<th></th>
<th>Total IgG</th>
<th>Total IgA</th>
<th>Flu IgG</th>
<th>Flu IgA</th>
<th>TT IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC plated</td>
<td>60,000</td>
<td>20,000</td>
<td>6667</td>
<td>2222</td>
<td>24</td>
</tr>
</tbody>
</table>

Frequency of antigen specific plasma cells in the bone marrow

<table>
<thead>
<tr>
<th></th>
<th>Antigen specific / total plasma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu IgG</td>
<td>10.00</td>
</tr>
<tr>
<td>Flu IgA</td>
<td>1.00</td>
</tr>
<tr>
<td>TT IgG</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Total IgG: 10,000
Total IgA: 3333
Flu IgG: 1111
Flu IgA: 370
TT IgG: 123
Correlation between influenza specific bone marrow plasma cell numbers and virus specific antibody titers in blood

Influenza specific bone marrow plasma cells (% of total IgG secreting cells)

Endpoint influenza specific IgG ELISA titer

\[ R^2 = 0.3707 \]

1000
10000
100000
1000000
0.1 1 10

Influenza vaccination / Bone marrow study design

Influenza vaccine

Marrow sample

Days

Blood samples

Do influenza specific bone marrow plasma cells increase after flu vaccination?

Increase in influenza specific plasma cells in human bone marrow post vaccination

Correlation between number of influenza IgG plasmablasts in the blood and increase in influenza IgG bone marrow plasma cells?
Are the same B-cell clones which are secreting antibody in the blood migrating to the bone marrow?

The dominant plasmablast clonotypes from the blood at day 7 can be found in the bone marrow plasma cell compartment on day 28

Is the increase in BM plasma cells following influenza vaccination sustained over time?

Identifying specific BCR rearrangements in the responding plasmablasts

Identification of dominant clones and design of clonotype specific qPCR assays

Single cell sorting of day 7 ASC into PCR plates

VH 5' PCR primer

CDR3 Taqman probe

3' PCR primer

FAM quencher
Conclusions

1. There is an increase in influenza specific plasma cells in the bone marrow post vaccination.
2. There is a strong correlation between the levels of influenza specific antibody (IgG) in the blood and the number of influenza specific plasma cells in the bone marrow.
3. The same influenza specific B-cell clones which are present as plasmablasts in the blood at day 7 can be found at day 28 in the bone marrow.
4. Although influenza vaccination is able to induce new antigen-specific bone marrow plasma cells, the lifespan of these cells may be shorter than those produced by other vaccines.
   - Mechanism for reduced lifespan/persistence? Intrinsic vs. extrinsic factors.
   - Role for adjuvants in improving vaccine longevity?

Acknowledgements

- Carl Davis
- Megan McCausland
- Cathy Chang
- Jens Wrammert
- Edmund Waller
- Aneesh Mehta