Effect of anti-CD20 antibody-induced B-cell depletion on the susceptibility to *Streptococcus pneumoniae* infections

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Disclosures

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Background and objective

B-cell depletion with anti-CD20 therapies

- Anti-CD20 therapy results in depletion of B cells and is effective in the treatment of B-cell malignancies and autoimmune disorders

Mechanism of action of anti-CD20 therapies

Anti-CD20 antibody binds to CD20 antigens expressed on the surface of B cells

Objective

To investigate the effect of B-cell depletion on antibody-mediated immunity to *Streptococcus pneumoniae* in mice
Methods

• In this analysis, a single dose of the anti-CD20 antibody (mIgG1) was administered to mice via two different routes of administration (i.v. or s.c.) to investigate the effect of B-cell depletion on the antibody-mediated immunity to *Streptococcus pneumoniae*

<table>
<thead>
<tr>
<th>Animal</th>
<th>C57BL/6 female mice, aged 6 weeks</th>
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| B-cell depletion | • B cells were depleted by administration of 50 μg/mouse of anti-CD20 (mIgG1, n=8 per group) either via i.v. or s.c. route of administration  
• Mice without B-cell depletion received the same concentration of isotype control antibody (s.c.) |
| Vaccination | • Mice were vaccinated with pneumococcal 13-valent conjugate vaccine Prevnar13® (20 µL/mouse i.p.)  
  – **One-dose vaccination study:** One dose of Prevnar13  
  – **Two-dose vaccination study:** Two doses of Prevnar13  
• Control animals (neither depleted nor vaccinated) received PBS (i.p.) |

**Treatment groups**

- **Depleted (i.v.)** (N=8)
  - Anti-CD20 antibody (i.v.) + Vaccinated (i.p.)
- **Depleted (s.c.)** (N=8)
  - Anti-CD20 antibody (s.c.) + Vaccinated (i.p.)
- **Non-depleted** (N=8)
  - Isotype control antibody (s.c.) + Vaccinated (i.p.)
- **Control** (N=4)
  - Isotype control antibody (s.c.) + Non-vaccinated (PBS; i.p.)
**Methods**

**B-cell depletion and vaccination study design**

**One-dose vaccination study**
- Day 0: B-cell depletion
- Day 3: Vaccination
- Day 14: Endpoint

*Anti-CD20 (mlG1) dose: 50 µg/mouse
Prevnar dose: 20 µL/mouse*

**Two-dose vaccination study**
- Day 0: B-cell depletion
- Day 6: First vaccination
- Day 16: Sera collection
- Day 17: Second vaccination
- Day 29: Endpoint

*S. pneumoniae-specific IgG levels via ELISA
B-cell repertoire and S. pneumoniae-specific IgG and IgM levels via ELISA and FACS*

**Assessments**
- Serum pneumococcal-specific IgG levels were measured at Day 16 (after the first dose of the vaccine) and Day 29 (endpoint) by whole-cell ELISA on pneumococcus (TIGR4 strain)-coated plates.
- Pneumococcal bacteria were incubated with mice sera (Day 29) and the antibody binding on the Pneumococcal surface was measured by flow cytometry (FACS); pneumococcal-specific IgG and IgM levels were measured by a serum deposition assay.

ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; Ig, immunoglobulin; i.p., intraperitoneal; i.v., intravenous; PBS, phosphate buffer saline; s.c., subcutaneous
One-dose vaccination study

Total B-cell population at Day 14

- Blood, the spleen and lymph nodes were analyzed to observe the effect on the B-cell repertoire levels at Day 14.

**B-cells gating:**
- CD19+
- CD3-
- CD11b-
- Ly6G/C-

After 14 days of anti-CD20 antibody treatment, the number of remaining B cells was around 20% compared to untreated groups.
One-dose vaccination study

B-cell subtypes at Day 14

B cells subtypes gating:
- Marginal zone: CD23-CD21+
- Follicular: CD23+IgD+
- Germinal center: PNA+IgD-

A marked decrease in the follicular B cell-subtypes was observed in both i.v. and s.c. anti-CD20 treatment groups.

Ig: immunoglobulin; i.v.: intravenous; s.c.: subcutaneous
Two-dose vaccination study

B-cell depletion at Day 29

Spleen homogenates

Flow cytometric gating strategy

<table>
<thead>
<tr>
<th>Lymphocyte subset</th>
<th>Gating strategy</th>
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<tbody>
<tr>
<td>B cells</td>
<td>CD19+</td>
</tr>
<tr>
<td>Marginal zone</td>
<td>CD19<em>CD23</em>CD21/CD35++</td>
</tr>
<tr>
<td>Germinal center</td>
<td>CD19<em>PNA</em>CD95++</td>
</tr>
<tr>
<td>Follicular</td>
<td>CD19*CD23++CD21/CD35+</td>
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- Only 60% of the B-cell population was reconstituted after 4 weeks of anti-CD20 treatment
- No significant differences in the B-cell subtypes were observed between the s.c. and i.v. anti-CD20 treatment groups
Two-dose vaccination study
Pneumococcal-specific immunoglobulin levels (IgG/IgM)

**Pneumococcal-specific IgG levels (ELISA)**

- The level of IgG against pneumococcus in anti-CD20 treated mice (i.v. and s.c.) was comparable to the vaccinated group after the first dose of the vaccine (Day 16)
- No significant difference in the IgG level was observed between the s.c. and i.v. anti-CD20 treatment groups

**Pneumococcal-specific IgG and IgM levels at Day 29 (FACS)**

- Antibody deposition on the bacterial surface suggested a lower level of IgG binding to pneumococcus in the anti-CD20 treated groups (depleted samples) compared to the vaccinated samples; the IgM levels, however, were comparable

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**p<0.01; ***p<0.0001; ELISA, enzyme linked immunosorbent assay; FACS, fluorescence-activated cell sorting; Ig, immunoglobulin; i.v., intravenous; OD, optical density; MFI, mean fluorescence intensity; ns, not significant; s.c., subcutaneous**
Conclusions

- A marked decrease in the follicular B-cell subtypes was observed with anti-CD20 treatment, while the marginal zone and germinal center B-cell subtypes appeared to be less affected
  - The preservation of marginal zone and germinal center B-cell subtypes may play a role in preserving a rapid first-line and selective/diverse immune response to infections, respectively
- When administered at the same dose, the route of administration of anti-CD20 antibody does not influence the non-depleted B-cell populations
- The B-cell population was not fully reconstituted after 4 weeks of anti-CD20 treatment, demonstrating a prolonged pharmacodynamics effect
- B-cell depletion reduced the pneumococcal-specific IgG levels, while a reduction in IgM levels was much lower
  - Further clinical validation is warranted to understand the impact of B-cell depletion on the production of antigen specific IgG and IgM in response to vaccinations or infections

Thank you for your attention!