Effect of anti-CD20 antibody-mediated B-cell depletion on susceptibility to *Pneumocystis* infection in mice

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Background and objective

- *Pneumocystis* species are heterogeneous atypical microscopic fungi\(^1\)
- Immune response against *Pneumocystis* infection is thought to be mediated by B and T cells\(^1,2\)

**Objective**

To investigate the effect of subcutaneous (s.c.) anti-CD20 antibody-induced B-cell depletion on T-cell responses and antibody generation against primary and secondary *Pneumocystis* infection in mice

**Methods**

**Experimental design**

- **Group 1; Primary infection** (5 mice per group)
  - Female C57BL/6 mice
  - Day -3: Injection of anti-CD20 or isotype: 30 or 150 µg s.c.
  - Day 0: Mice infected with 2x10⁵ cyst of live *P. murina* by oropharyngeal administration
  - Day 14: Injection of anti-CD20 or isotype control
  - Day 28: Injection of anti-CD20 or isotype control

- **Group 2; Secondary infection** (4-5 mice per group)
  - Mice infected with 2x10⁵ cyst of live *P. murina* by oropharyngeal administration
  - Day -3, 4, 11, 18, 25: Injection of anti-CD20 or isotype control
  - Day 0: Mice infected with 2x10⁵ cyst of live *P. murina* by oropharyngeal administration
  - Day 14: Injection of anti-CD20 or isotype control
  - Day 28: Injection of anti-CD20 or isotype control

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*P. murina, Pneumocystis murina; s.c., subcutaneous*
Methods
Assessments and statistics

Assessments

- Flow cytometry was used to assay T and B cells in the lung at Days 14 and 28 after infection
- Quantitative PCR was used to determine lung fungal burden
- Serum IgG, IgE, and IgM levels were measured by ELISA

Statistics

- Graphs were generated and statistical significances were analyzed using GraphPad Prism software
- $P$ values of pairwise comparisons between groups of 2 were performed by a simple 2-tailed unpaired Student’s $t$ test, while groups of 3 or more used 1-way ANOVA with Tukey’s multiple comparisons
Effect of anti-CD20 antibody treatment on B cells in lungs

Primary *Pneumocystis* infection

Anti-CD20 antibody treatment depleted both CD19+ and CD27+CD19+ cells, in the lung at Days 14 and 28

ISO, isotype control
Effect of anti-CD20 antibody treatment on T cells at Day 14
Primary *Pneumocystis* infection

No significant differences in the number of lung CD4+, IFNg+CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after infection at Day 14

IFN, interferon; IL, interleukin; ISO, isotype control; n.s., non-significant
No significant differences in the number of lung CD4+, IFNγ+CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after infection at Day 28.
Effect of anti-CD20 antibody treatment on IgG in sera
Primary *Pneumocystis* infection

Anti-CD20 antibody treatment did not alter antigen-specific serum immunoglobulin levels compared with control mice

IgG, immunoglobulin G; ISO, isotype control; n.s., non-significant; OD, optical density
**Effect of anti-CD20 antibody treatment on lung fungal burden**

Primary *Pneumocystis* infection

**Day 14**

![Graph showing fungal burden at day 14](image)

- Anti-CD20-30µg
- Anti-CD20-150µg
- ISO-30µg
- ISO-150µg

*P* = 0.004

**Day 28**

![Graph showing fungal burden at day 28](image)

- Anti-CD20-30µg
- Anti-CD20-150µg
- ISO-30µg
- ISO-150µg

*P* < 0.001

Although anti-CD20 antibody treatment impaired fungal clearance at Day 14 post-infection, fungal burden in the lungs was substantially reduced at Day 28 in both B-cell depleted and control mice.
Effect of anti-CD20 antibody treatment on B cells
Secondary *Pneumocystis* infection

- Anti-CD20 antibody treatment partially depleted CD19+ but not other measured cell subsets including CD27+CD19+

- No significant differences in the number of lung CD4+, IFNγ+CD4+, IL-4+CD4+, IL-5+CD4+ and IL-17A+CD4+ cells between depleted and control mice after secondary infection

- Anti-CD20 antibody treatment did not alter antigen-specific serum immunoglobulin levels compared with control mice 14 days after re-infection

- The lung fungal burden was comparable between depleted and control mice 14 days after re-infection

ISO, isotype control; n.s., non significant
Subcutaneous anti-CD20 antibody treatment may delay fungal clearance but it does not impair the ability of the host to clear *Pneumocystis* infection, irrespective of primary or secondary infection.

Thank you