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Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   The frequency of Klebsiella O-antigen-reactive B cells in humans has not been reported. To accomodate for inter-donor variablility we used at least 3-11 donors. Low-frequent O1 and O3 O-antigen-reactive B cells were identified from every donor (11/11) analyzed by flow cytometry. Cross-specific antibodies were cloned from 5 of 5 donors. Since all donors showed these cells and cross-specific antibodies were cloned from all donors we considered the sample size sufficient.

   For the mouse experiments, sample size was chosen according to former studies by Szijarto et al.

2. Data exclusions

   Describe any data exclusions.

   No data was excluded from analysis.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   All experiments were internally controlled to ensure reproducibility of the findings. The findings were reproduced in independent experiments as stated.
   Fig 6a was controlled by including clonally-related antibodies and non-O3 reactive controls.
   Fig. S7a could only be performed once due to limited material.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   For in vivo experiments, female 6–8¬week-old BALB/cJRj mice were randomly allocated into groups.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   For in vivo experiments, the investigators were not blinded to group allocation during data collection and analysis. We chose an objective readout as a measure (survival or no survival) and therefore didn't consider blinding necessary, because the outcome and results are not prone to be influenced by subjective evaluation.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Statistics were performed using Prism 6 (Graphpad) and R version 3.2.2. Single cell sort index data was recorded using BD FACSDIVA 7 V8.0.1 software and extracted using the flowCore package for R. Plots were produced using FlowJo V10.4.2 (FlowJO, LLC), Prism 6, Illustrator CS v16.0.3 (Adobe), Photoshop CS 6 (Adobe) and R (The R foundation) using the gplots, ggplot2 and circlize packages. Paired reads from Miseq250 sequencing were assembled using PANDAseq v2.11 and analyzed using the QIIME 1 suite v1.9.1.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All unique materials are available from the authors upon reasonable request under a material transfer agreement.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies used in this study are commercially available, were titrated and tested onto a known target population (human peripheral blood memory B cells or LP plasmablasts) and/or the clone was previously established as shown in Benckert et al, JCI, 2011 ; Muellenbeck et al, J Exp Med, 2013 and Triller et al, Immunity, 2017.
Mouse anti-human CD19-APC-H7 BD Biosciences SJ25C1, Cat.No.560727: , Lot: 3144891
Mouse anti-human CD27-BV605 BD Biosciences L128, Cat.No.: 562655 , Lot: 5085687
Mouse anti-human CD27-PE BD Biosciences M-T271, Cat.No.: 555441, Lot: 3331644
Mouse anti-human IgG-V450 BD Biosciences G18-145, Cat.No.: 561299, Lot: 4143658
Mouse anti-human IgA-PE Miltenyi IS11-8E10, Cat.No.: 130-093-128, Lot: 5151022069
Goat anti-human IgA-FITC Life technologies Cat#62-7411, Cat.No.: , Lot: 886049B
Mouse anti-human IgG-BV510 BD Biosciences G18-145, Cat.No.: 563247, Lot: 4184545
Mouse anti-human CD45-Viogreen Miltenyi 5B1, Cat.No.: 130-096-906, Lot: 5140925195
Goat anti-human IgG, HRP-conjugated Jackson, Cat.No.: , Lot: 132034
Goat anti-human IgG, Alexa647 Jackson, Cat.No.: 109-605-098,, Lot: 124868
Goat anti-human IgG, Alexa488 Invitrogen, Cat.No.: A-11013 , Lot: 1668688
Goat anti-human IgG, HRP-conjugated Southern Biotech, Cat.No.: 2040-05, Lot: n/a
Non-commercial isotype control: Human recombinant negative control mG053, in-house production, Wardemann et al., 2003

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK293F: Life Technologies GmbH, Karlsruhe, Germany
HEK293T: DSMZ, Braunschweig, Germany
HEK-Blue-hTLR4: Invivogen, Toulouse, France
RAW 264.7: ATCC TIB-71, USA

b. Describe the method of cell line authentication used.

No method for cell authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cells were tested negative for mycoplasma contamination in 3-mo intervals.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

Used cell lines are not listed in the ICLAC database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

In bacteremia models female 6-8 week old BALB/cJRj mice (Janvier) were used. Murine intestinal microbes were isolated from male 16-32-week old C57BL/6(J) mice.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Peripheral blood and non-inflamed healthy intestinal tissue samples were obtained from healthy male and female adults (28 - 64 years) or patients (55-64) undergoing intestinal surgery (carcinoma, localized inflammation of undiagnosed origin, diverticulitis). Human intestinal microbes were obtained from healthy controls (Fig. S7a and b) and a stool bank from patients with inflammatory bowel disease (Crohns disease, ulcerative colitis) (Fig. S7a).

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Detailed sample preparation for every flow cytometry experiment is provided in the Material and Methods section.
6. Identify the instrument used for data collection.	Flow cytometric measurements were performed using LSR II, Accuri C6 and FACSAria III instruments (all BD).
7. Describe the software used to collect and analyze the flow cytometry data.	Flow cytometric measurements were acquired using BD FACSDIVA 7 V8.0.1 software. Single cell index sorting data was acquired using BD FACSDIVA 7 V8.0.1 software and extracted using the flowCore package for R. FlowJo v10 was used for all subsequent analysis of flow cytometry data.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Sorting was performed on single cell level and therefore post-sort analysis are impossible.
9. Describe the gating strategy used.	Gating strategies are displayed in Supplementary Figure 8. O-antigen reactive B cells from peripheral blood were identified as single, 7-AAD-, CD19+, CD27+, and O-antigen+. O-antigen reactive lamina propria plasmablasts were single cell sorted as single, 7-AAD-, CD19+, CD45+, and O-antigen+. Antibody-bound intestinal microbes or bacterial strains were identified as anti-hIgG+. Boundaries between positive and negative populations were established using fluorescence-minus-one controls (for O-antigen reactive B cells) or a non-reactive control antibody (bacteria or microbiota stainings).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒