IAI Accepted Manuscript Posted Online 27 July 2015
Infect. Immun. doi:10.1128/IAI.00037-15

Intestinal microbiota of mice influences resistance to *Staphylococcus aureus* pneumonia

Running title: Microbiota and *S. aureus* pneumonia

Stefanie Gauguet[1, 2,#]; Samantha D'Ortona[1]; Kathryn Ahnger-Pier[1]; Biyan Duan[1], Neeraj K. Surana[3,] , Roger Lu[1], Colette Cywes-Bentley[1]; Mihaela Gadjeva[1], Qiang Shan[1], Gregory P. Priebe[1, 2, 3,*]; Gerald B. Pier[1,*]

Institutions: [1]Division of Infectious Diseases, Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; [2]Division of Critical Care Medicine, Department of Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, Boston, MA; and [3]Division of Infectious Diseases (Department of Medicine), Boston Children's Hospital, Harvard Medical School, Boston, MA, USA

*Equal contributions

Corresponding Author:
Stefanie Gauguet, M.D., Ph.D., Boston Children's Hospital, Bader 6, Division of Critical Care Medicine, 300 Longwood Avenue, Boston, MA 02115
Phone: 617-355-6363; Fax: 617-730-0453
Email: Stefanie.Gauguet@childrens.harvard.edu

25 **Conflicts of Interest:** The authors have no conflicts of interest relevant to this

26 article to disclose.

ABSTRACT

Background

Th17-immunity in the gastrointestinal tract is regulated by intestinal microbiota composition, particularly the presence of segmented filamentous bacteria (sfb), but the role of intestinal microbiota in pulmonary host defense is not well explored. We tested whether altering gut microbiota by acquiring sfb influences susceptibility to staphylococcal pneumonia via induction of type 17 immunity.

Methods

Groups of C57BL/6 mice, which differed in their intestinal colonization with sfb, were challenged with methicillin-resistant *Staphylococcus aureus* in an acute lung infection model. Bacterial burden, bronchoalveolar lavage fluid (BALF) cell counts, cell types, and cytokine levels were compared between mice from different vendors, mice from both vendors after co-housing, mice given sfb orally prior to infection, and mice with and without exogenous IL-22 or anti-IL-22 antibodies.

Results

Mice lacking sfb developed more severe *S. aureus* pneumonia than mice colonized with sfb, indicated by higher bacterial burden in lungs, lung inflammation, and mortality. This difference was reduced when sfb-negative mice acquired sfb in their gut microbiota through co-housing with sfb-positive mice or when given sfb orally. Type 17 immune effectors in the lung were higher after infection in sfb-positive mice and increased in sfb-negative mice after acquiring sfb, as demonstrated by higher levels of IL-22, higher numbers of IL-22+ TCRβ+ cells, and of neutrophils in BALF. Exogenous IL-22 protected mice from *S. aureus* pneumonia.

Conclusion

The murine gut microbiota, particularly the presence of sfb, promotes pulmonary type 17 immunity and resistance to *S. aureus* pneumonia, and IL-22 protects against severe pulmonary staphylococcal infection.

Keywords

Staphylococcus aureus

MRSA

Pneumonia

Segmented filamentous bacteria (sfb)

Gut microbiota

Th17 immunity

Type 17 immunity

IL-17

IL-22

RORγt

Neutrophils

Innate lymphoid cells type 3 (ILC3s)

INTRODUCTION

Staphylococcus aureus continues to be one of the most common pathogens causing invasive life-threatening infections (1). Methicillin-resistant *S. aureus* (MRSA) currently accounts for 20-40% of hospital-acquired and ventilator-associated pneumonias (2) and

9% of community-acquired pneumonias (3), and MRSA pneumonia is associated with very high mortality rates (3, 4).

The Th17 pathway plays an important role in mucosal host defense against a wide range of bacterial pathogens (reviewed in (5)). Defects in human Th17 signaling (e.g. in hyper-IgE or Job's syndrome) are associated with immunodeficiency syndromes characterized by increased susceptibility to staphylococcal infections of the lung and skin, suggesting a specific role for Th17 immunity in host defense against *S. aureus* (6, 7). Additionally, mice with defects in Th17 signaling have impaired bacterial clearance from the lung after infection with *Klebsiella pneumoniae* (8). More recently, the Th17 pathway has been implicated in the defense against *S. aureus* pneumonia as well (9-11). Mice lacking the IL-17 receptor or IL-22, or mice that are coinfected with influenza A and thereby deficient in type 17 immunity, displayed impaired bacterial clearance of *S. aureus* compared to wild type or influenza-free mice (10). Type 17 immunity has also been reported to contribute to mucosal vaccine responses against *P. aeruginosa* and *M. tuberculosis* (12-14).

The gastrointestinal (GI) tract of mammals is inhabited by thousands of species of commensal microorganisms that exist in a mutualistic relationship with the host. How the commensal microbiota influences the host immune system is poorly understood, but it appears clear that microbiota is a major regulator of the immune system and that bacterial signals have profound influences on antibacterial defenses in the GI tract and also in other organs (15, 16). Ivanov et al. showed that colonization of the GI tract of

mice with a commensal microbe, segmented filamentous bacterium (sfb), was sufficient to induce the appearance of Th17 cells in the small intestine, leading to increased expression of genes associated with inflammation and antimicrobial defenses, and resulted in enhanced resistance to the murine intestinal pathogen *Citrobacter rodentium* (17-19). The influence of the GI microbiota on lung immunity, the so-called gut-lung axis, has recently become the focus of more interest, but underlying mechanisms are still incompletely understood (20). Commensal organisms of the GI tract contribute to host defense in *Escherichia coli* pneumonia via toll-like receptor signaling (21), and germ-free mice have a strikingly higher mortality rate following *P. aeruginosa* pneumonia compared to conventional mice (22). Little is known regarding the role of specific organisms in modulating pulmonary immunity and whether the gastrointestinal microbiota has any influence on Gram-positive lung pathogens, or *S. aureus* in particular. We hypothesized that the intestinal microbiota can affect S. *aureus* pneumonia and that the presence of sfb in the mouse intestine specifically, influences type 17 immunity in the lung and increases resistance to *S. aureus* pneumonia. To test this hypothesis, we compared mice with differing intestinal microbiota in a murine staphylococcal pneumonia model.

C57BL/6 mice from Jackson Laboratory and Taconic Biosciences differ in their gastrointestinal microbiota and most notably in colonization with the commensal sfb, (with Jackson mice being sfb-negative and Taconic mice generally sfb-positive) (18). We demonstrate that mice from Jackson are more susceptible to intranasal challenge with the MRSA strain LAC compared to mice from Taconic. This difference in resistance

diminishes when mice from both vendors are co-housed, indicating an environmental rather than a genetic or inherent factor influencing the variation in pulmonary phenotype. Following co-housing, sfb are passed from sfb-positive mice to their sfb-negative cage-mates, leading to increased resistance to *S. aureus* pneumonia. Furthermore, when sfb-negative mice are given sfb via gavage two weeks prior to infection, they also become more resistant to *S. aureus* pneumonia. BALF from the more resistant sfb-positive mice as well as initially sfb-negative mice that acquired sfb via GI colonization contained more IL-22, more IL-22+ TCRβ+ cells, and more neutrophils, all indicators for increased type 17 immunity activation. We also show that neutralization of the Th17 cytokine IL-22 with anti-IL-22 antibody prior to challenge with *S. aureus* leads to increased susceptibility and conversely, intranasal administration of IL-22 at the time of infection with *S. aureus* renders mice more resistant to infection, suggesting that IL-22 is protective against *S. aureus* pneumonia.

MATERIALS AND METHODS

Bacterial strains.

Staphylococcus aureus strain LAC (a USA300 methicillin-resistant MRSA), initially isolated from a patient suffering from necrotizing pneumonia), was obtained from the Network on Antimicrobial Resistance in *S. aureus* (NARSA). To prepare bacterial inocula for in vivo challenge studies, frozen bacterial stocks of *S. aureus* were plated on Columbia agar (CBA) supplemented with 2% sodium chloride (Columbia salt agar, CSA) and grown at 37°C overnight. Subsequently, colonies were grown in Columbia salt broth (CSB, Columbia broth supplemented with 2% sodium chloride) at 37°C with

shaking at 200 rpm to an optical density at 650nm of 0.5. Bacteria were washed and resuspended in phosphate-buffered saline (PBS) to yield the intended calculated inoculum (2-4x10^8 per mouse for experiments measuring bacterial burdens and 7.5-8x10^8 per mouse for survival experiments). The inoculum was verified after serial dilution in TSB supplemented with 0.05% Tween and enumeration of growth on 5% sheep blood agar plates (BAP) after overnight incubation at 37°C. 20 μL of bacterial suspensions were used for intranasal (IN) challenge in the mouse experiments.

Mice

All animal experiments were approved by the Harvard Medical Area Institutional Animal Care and Use Committee. Four to six week old C57BL/6 mice were purchased from Jackson Laboratories or Taconic Biosciences. All groups of mice were age-, gender-, and weight-matched for comparison experiments. Mice were housed under virus-free conditions in microisolator-topped cages.

Co-housing and sfb detection

For co-housing experiments, two sfb-negative mice each and two sfb-positive mice were transferred into one common cage after ear tagging sfb-negative mice to enable identification. Prior to co-housing and 12-18 days into co-housing, fecal pellets (freshly produced after temporary transfer of mice to a new cage) were collected from each mouse group. DNA from feces was purified using the QIAamp DNA Stool Kit following manufacturer's instructions (Qiagen, Valencia, CA). The presence of sfb was examined by PCR using the primers 736F (GACGCTGAGGCATGAGAGCAT) and 844R

(GACGGCACGGATTGTTATTCA) and the following PCR conditions: 94° x 5 min, 30 cycles of 94° x 30 sec, 52° x 40 sec, 72° x 90 sec, and 72° x 7 min. Sfb presence resulted in a ~150bp amplicon.

Sfb Acquisition

Sfb-containing stool was obtained from initially germ-free mice that were mono-colonized with sfb (kindly provided by Dr. Neil Surana), suspended in PBS, and passed through a cell strainer to remove particulate matter. Fifty μL of this suspension was given orogastrically via gavage to four-week-old female C57BL/6 mice sedated with isoflurane. Control mice were given PBS. Prior to challenge with *S. aureus* two weeks later, stool from these mice was collected, bacterial DNA extracted, and the presence or absence of sfb confirmed by PCR.

Murine pneumonia model

Mice were anesthetized per intraperitoneal (IP) injection with ketamine and xylazine. After inoculation with 10 μL of the *S. aureus* suspension into each nostril, mice were observed until recovered from anesthesia. For survival studies, mice were observed closely for signs of illness and impending death (i.e., a moribund state) at least three times daily. If appearing moribund (based on hunched posture, inability to move, eat, or drink, ruffled fur, labored breathing, and crusted eyes), mice were euthanized by carbon dioxide inhalation followed by cervical dislocation and considered non-survivors. For quantification of bacteria and analysis of cells and cytokines in bronchoalveolar lavage fluid (BALF), mice were euthanized by IP injection of pentobarbital at indicated time

points. For experiments with IL-22 administration, recombinant IL-22 (R&D systems) suspended in PBS+0.1% BSA was mixed with the bacterial inoculum and administered to anesthetized mice via intranasal (IN) inhalation. Control mice were given the bacterial inoculum mixed with PBS+0.1% BSA.

Mice given anti-IL-22 antibody IN were sedated with ketamine and xylazine and then given 25 μL containing 25 μg polyclonal anti-IL-22 goat IgG or polyclonal goat IgG (all from R&D systems) as a control 4 hours prior to bacterial challenge.

Bronchoalveolar lavage (BAL)

The trachea of euthanized mice was cannulated with a 20-gauge angiocatheter and BALF was obtained following two instillations of 350 μL of ice-cold PBS containing 0.5 mM EDTA. BALF was centrifuged and the supernatant stored at -80°C before measurement of cytokines using the Magnetic Luminex Assay with the mouse cytokine 20-plex panel (Life Technologies, Grand Island, NY) or, for IL-17 and IL-22 concentration, by ELISA (R&D systems, Minneapolis, MN). Erythrocytes in the cell pellets were lysed using a mouse erythrocyte lysis kit (R&D systems) according to the manufacturer's instructions. Total cell counts were determined with trypan blue staining in a Countess Automated Cell Counter (Life Technologies, Grand Island, NY).

Determination of viable bacterial counts

The right lung and the spleen were removed, weighed, and homogenized using an Omni TissueMaster homogenizer-125 (Omni International, Marietta, GA) in TSB with

0.05% Tween. Serial 10-fold dilutions of the lung and spleen homogenates were then plated onto 5% sheep agar plates and incubated overnight at 37°C prior to counting CFU and calculating CFU per gram tissue.

Flow cytometry

BALF cells were washed with flow cytometry buffer (2% FCS (fetal calf serum) in PBS) and adjusted to a concentration of $5x10^5$/mL. Cells were then treated with FcγR blocker (anti-CD16/32, clone 2.4G2) in flow cytometry buffer for 20 min on ice to block nonspecific staining, then stained with appropriate isotype-matched antibodies to the following cell surface markers: CD45 (30-F11), Ly6G (1A8) (all from Biolegend, San Diego, CA), and TCRβ (H57-597, eBioscience, San Diego, CA). For intracellular cytokine staining of IL-17A (using mAb TC11-18H10.1, Biolegend, San Diego, CA) and IL-22 (using mAb 1H8PWSR, eBioscience, San Diego, CA), cells were stimulated ex vivo for 6 hours with PMA (phorbol 12-myristate 13-acetate) and ionomycin in the presence of brefeldin A followed by fixation, permeabilization with saponin, and staining (all using a Cytofix/Cytoperm Fixation/Permeabilization Kit, BD Biosciences, San Jose, CA) and then analysis on a FACSCalibur flow cytometer.

Confocal microscopy

Paraffin-embedded lung sections were de-paraffinized using EZ-Dewax as per manufacturer's protocol. De-paraffinized samples were blocked with 1% BSA/PBS overnight at 4°C followed by labeling with a dilution of 1:200 of rabbit anti-IL-22 antibody (Abcam), FITC-conjugated CD3e antibody (eBioscience), APC-conjugated RORγt

Infection and Immunity

antibody (eBioscience) or the appropriate isotype control antibodies (Biolegend or eBioscience) in 0.5% BSA/PBS overnight in a humidified chamber at 4°C. The samples were washed and 1:400 of the secondary antibody, donkey anti-rabbit IgG Alexa Fluor 568-conjugated (Invitrogen), was added to the relevant samples described above for 2 hours at room temperature. Samples were washed, mounted, and analyzed by confocal microscopy with a Zeiss LSM5 Pascal instrument equipped with a krypton/argon laser.

Histology

The trachea was cannulated with an angiocatheter and Bouin's fixative solution was directly injected into the lung. The left lung was then removed and placed in Bouin's fixative solution prior to paraffin embedding, thin sectioning, and hematoxylin-eosin staining by the Harvard Rodent Histopathology Core. The lung tissue sections were scored for percentage of lung tissue affected by inflammation by a veterinary pathologist who was blinded to the experimental conditions.

Data analysis and interpretation

The significance of differences in lethal outcomes and bacterial burdens in lungs and spleens between groups of mice was analyzed by parametric or non-parametric ANOVA or t tests, as appropriate depending on whether the data were normally distributed, followed by post-hoc analyses for significant differences between paired groups. We considered $p<0.05$ as statistically significant and used the label * for $p < 0.05$ and ** for $p < 0.01$. We performed survival analysis using the Kaplan-Meier method and the log-rank test. All analyses were performed using GraphPad's Prism software.

RESULTS

Sfb-negative C57BL/6 mice are more susceptible to *S. aureus* pneumonia than sfb-positive C57BL/6 mice.

Following intranasal challenge with the MRSA strain LAC, sfb-negative mice were found to have greater infectious bacterial burdens compared with age- and gender-matched sfb-positive mice. Sfb-negative mice had 21-fold higher bacterial burdens in their lungs 18 hours after infection compared to sfb-positive mice (mean of $1.4x10^9$ CFU/gram lung tissue vs. $6.6x10^7$ CFU/gram lung tissue, respectively, **Figure 1a**). This was accompanied by higher rates of translocated bacteria, as the spleens of sfb-negative mice had about 70 times more bacteria compared to spleens of sfb-positive mice (**Figure 1b**).

Analysis of the lung histology in MRSA infected mice showed dense inflammatory cell infiltration as visualized by H+E staining, which was more pronounced in sfb-negative mice compared to sfb-positive mice (**Figures 1c and d and 1e and f,** respectively). These results indicate a higher susceptibility of sfb-negative mice to *S. aureus* pneumonia compared to sfb-positive mice.

Resistance to *S. aureus* pneumonia is influenced by an environmental factor.

To examine whether sfb-negative mice are more susceptible to *S. aureus* lung infection due to an environmental or genetic factor, we co-housed female age-matched sfb-negative (purchased from Jackson) and sfb-positive mice (from Taconic) in the same

cages for two weeks, from 4 to 6 weeks of age, prior to respiratory challenge with *S. aureus*. While the median levels of *S. aureus* in the lungs of the initially sfb-negative mice co-housed with sfb-positive mice were somewhat higher than bacterial levels in the continuously sfb-positive mice, this difference was no longer statistically significant (**Figure 2**). In addition, mice that became sfb-positive during co-housing were significantly more resistant to *S. aureus* lung infection than sfb-negative mice that were not co-housed. Co-housing of sfb-positive mice with sfb-negative mice did not alter their response to *S. aureus* lung infection as they showed the same bacterial levels, as did mice that were sfb-positive and not co-housed. To confirm the presence of sfb in the mice in the different groups, we examined the feces of the co-housed mice as well as mice from Taconic Biosciences reportedly supplied as sfb-positive for the presence of sfb by PCR. Initially sfb-negative mice sharing a cage with sfb-positive mice became positive for sfb, as were the mice from the supplier known to carry sfb (**Supplemental Fig. S1**). Stool samples from mice from Jackson Laboratories, reportedly negative for sfb, were confirmed by PCR to be sfb-negative.

Protective effect of sfb gut colonization on susceptibility to *S. aureus* pneumonia

To determine whether the difference in susceptibility to *S. aureus* pneumonia was due to the presence of sfb in the murine intestine, we administered sfb-containing stool (obtained from initially germ-free mice that had been mono-colonized with sfb (23)) or PBS via gavage to 4-week-old sfb-negative mice 2 weeks prior to challenge with *S. aureus*. Sfb was detected by PCR in the stools of mice two weeks after the gavage with sfb. The PBS-gavaged mice were negative for sfb. When challenged with *S. aureus*,

mice that had acquired sfb were more resistant to infection, as demonstrated by significantly lower bacterial counts in lungs (4.9-fold difference**, Figure 3a**) and a trend (P=0.08) towards lower bacterial counts in spleens (11-fold difference, **Figure 3b**). When challenged with a higher bacterial inoculum in a survival assay, initially sfb-negative mice that had become sfb-positive were also at much lower risk of succumbing to their MRSA lung infection compared to continuously sfb-negative mice. All sfb-negative mice died by 36 hours, while 70% of mice rendered sfb-positive survived (**Figure 3c**).

Sfb gut colonization is associated with differential pulmonary type 17 immunity. We measured levels of type 17-associated cytokines in the BALF after *S. aureus* challenge and tested whether acquisition of sfb via gut colonization alters type 17 immunity in the lung comparing initially sfb-negative mice that became sfb-positive, either after co-housing with sfb-positive Taconic mice or after gavage with sfb-containing stool. As shown in **Figure 4a-4d**, we found that BALF levels of IL-22, one of the main Th17 effector cytokines, differed significantly between groups of mice with and without sfb-colonization. At 8 hours after intranasal challenge with MRSA, both sfb-negative and sfb-positive mice had similar IL-22 levels (**Figure 4a**), but by 18 hours post infection, all sfb-positive mice (whether initially sfb-positive or becoming sfb-positive mice after co-housing with sfb-positive mice or after sfb-gavage) had significantly higher IL-22 levels than sfb-negative mice (**Figure 4b-4d**).

We did not observe significant differences in IL-17 levels, another key cytokine in Th17 immunity, between any group of mice (**Figures 4e-4h; and S2b and S2c**), except for a small increase in IL-17 in BALF of mice initially sfb-negative after becoming sfb-positive through co-housing **(Figure 4g)**, indicating that this cytokine likely does not play a very important role in *S. aureus* pneumonia or at least not at the time points we examined.

Consistent with the known role of IL-6 in promoting IL-22 expression (24), we also found that sfb-negative mice had significantly less IL-6 in BALF at an early time point (8 hours) after challenge with *S. aureus* (**Figure 4i**). At 18 hours, sfb-negative mice had significantly more IL-6 in BALF (**Figure 4j**), possibly due to the very high bacterial levels driving IL-6 production in the sfb-negative mice at this time point, while the sfb-positive mice were beginning to clear the infection.

Th17 immunity leads to neutrophil attraction (25) and production of antimicrobial peptides (11). Sfb-positive mice (both initially colonized mice or initially sfb-negative mice after co-housing with sfb-positive mice or gavage with sfb) had higher numbers of Ly6G-positive cells (neutrophils) in BALF (**Figures 4k and 4l**), supporting their propensity towards Th17 pulmonary responses.

In order to identify the types of cells capable of producing IL-22 in the lungs after infection with MRSA, we stained lung tissue sections of infected sfb-positive mice for RORγt, IL-22, and CD3e **(Figure 4m-o).** We found two predominant populations of cells that were positive for IL-22. One group of cells was positive for RORγt, IL-22, and CD3

and likely represents Th17 or Th22 cells or γδ T cells. Another population of cells was positive for RORγt and IL-22, but negative for CD3, and possibly represents innate lymphoid cells type 3 (ILC3s). Although these images are not meant to quantify the exact number of cells present in lung tissue of sfb-negative and sfb-positive mice, IL-22-producing cells were qualitatively more prevalent in sfb-positive mice **(Figure 4m)** versus sfb-negative mice that recently acquired sfb **(Figure 4n)** and sfb-negative mice **(Figure 4o)**. For control stains of images, please see **Supplemental Figure S2**. Since studies by other groups have reported that γδ T cells are the primary source of IL-17 in a similar murine model of *S. aureus* pneumonia (26) and we observed no difference in IL-17 levels, we predicted that γδ T cells were not likely to be involved in the sfb-induced responses and focused instead on αβ T cells (Th17 cells). Therefore, we quantified the numbers of IL-22-producing Th17 cells using intracellular staining with flow cytometry using cells isolated from BALF 8 hours after infection and found significantly higher numbers of IL-22+ TCRβ+ cells in sfb-positive versus sfb-negative mice **(Figure 4p).**

IL-22 is critical for host defense against severe *S. aureus* pneumonia.

To further define the role of IL-22 in *S. aureus* pneumonia, we tested whether antibody-mediated neutralization of IL-22 or administration of recombinant IL-22 impacted the clearance of *S. aureus* from the murine lung. Sfb-positive mice given monoclonal antibody to IL-22 intranasally 4 hours prior to infection with *S. aureus* subsequently exhibited a near 2-log increase in bacterial burdens in the lung compared to mice

receiving control IgG (**Figure 5a**), indicating significantly higher susceptibility to infection in the absence of IL-22.

Alternatively, administration of recombinant IL-22 intranasally simultaneously with challenge with *S. aureus* protected sfb-negative mice from development of severe pneumonia as indicated by significantly lower bacterial burdens in their lungs and spleens (**Figure 5b-5c**). Of note, intranasal administration of IL-22 led to an about 60-fold increase in BALF IL-22 concentrations (**Supplemental Figure S3a**). Exogenous administration of anti-IL-22 antibody did not lead to significant measurable differences in BALF IL-22 and IL-17 concentrations (**Supplemental Figure S3b and S3c,** respectively). Exogenous rIL-22 administration to sfb-positive mice simultaneously to challenge with MRSA strain LAC had no significant protective effect on susceptibility to MRSA pneumonia (**Supplemental Figure S4**), suggesting that there is no additional benefit of adding IL-22 (at least at this dose) in an sfb-positive setting.

DISCUSSION

Understanding the immune responses important for host defense against *S. aureus* pneumonia, which is a leading cause of death worldwide, is critical for the development of vaccines and immunotherapies. In this study, we examined whether the GI microbiota, particularly the presence of sfb has an effect on susceptibility to *S. aureus* pneumonia. Our findings indicate that sfb-positive mice are more resistant than sfb-negative mice when challenged in a *S. aureus* pneumonia model and this resistance was correlated with a type 17 innate immune response. Sfb-negative mice were much

more susceptible to *S. aureus* pneumonia with higher bacterial burdens in lungs and spleens and higher mortality after challenge with *S. aureus*. Co-housing sfb-negative mice with sfb-positive mice for 14 days, and thereby transferring sfb and likely other GI microbiota, significantly decreased susceptibility to *S. aureus* pneumonia and improved survival. Furthermore, we demonstrate that sfb accounts for most of this change in phenotype by showing that transfer of sfb to sfb-negative mice via orogastric lavage increases resistance to *S. aureus* lung infection. Overall, a strong case is made for a critical role of sfb-driven type 17 immunity in the ability to clear *S. aureus* from infected lungs, and, in particular, a role for IL-22, whose production is closely linked to the presence of sfb microbiota in the GI tract. Thus, even though the lung does not contain many GI organisms, these clearly influence innate immunity in this tissue, indicating a far-reaching role of GI microbiota-driven immune effectors in tissues beyond the GI tract.

While the results implicate the sfb-component of the GI microbiota in susceptibility of C57Bl/6 mice to *S. aureus* lung infection, there may still be some genetic variability contributing as co-factors in the experiments comparing mice from different vendors (i.e. Jackson and Taconic). The inbred mouse line C57BL/6 is widely used in animal models, and, often little attention is paid to the breeding facility from which mice are purchased for experiments. Although all C57BL/6 mouse strains originated from the same ancestor, separation in different laboratories over multiple decades and generations has resulted in the emergence of genetic and phenotypic differences. A recent study compared in detail the genomic and phenotypic differences of C57BL/6J (established at

the Jackson Laboratories in 1948) and C57BL/6N (passed on to the National Institutes of Health (NIH) in 1951 and later transferred to Taconic Biosciences in 1991) strains, finding 34 SNPs, 2 indels, and 15 structural variants, as well as a range of phenotypic differences (27), including physiologic, metabolic, and behavioral. Simon et al. found that male C57BL/6N mice, compared to male C57BL/6J mice, showed enhanced resistance to *Listeria monocytogenes* and an increased pro-inflammatory response (represented by increased plasma levels of IL-6, interferon-inducible protein (IP)-10, and chemokine ligand (CCL)2) (27). Whether this difference was due to a genetic or an environmental factor (e.g. the GI tract microbiota) is not known. Nonetheless, it is possible that some of these genetic differences also contributed to the innate immune response of C57Bl/6 mice to *S. aureus* in our experiments comparing mice from different sources. However, genetic variation is very unlikely to play a role in our experiments comparing mice from the same source with and without sfb-gavaging.

The alteration of susceptibility to *S. aureus* pneumonia in our study is in accordance with a study by Fagundes et al. who demonstrated that colonization by indigenous microbiota alters the way a host reacts to infectious stimuli through activation of toll-like receptor (TLR)-dependent pathways (28). They showed that germ-free mice were more susceptible to *Klebsiella pneumoniae* pneumonia and that priming germ-free mice with LPS or other TLR agonists increased their inflammatory responsiveness and led to better bacterial clearance and survival (28). Two studies have recently demonstrated that the GI tract microbiota also regulates immune defenses in the respiratory tract against influenza infection via the TLR7 pathway (29) or inflammasome activation (30).

Gut microbiota are likely not only important in infections in the lung, as microbiota modulate tumoral immune surveillance in this tissue via the Th17 pathway, as well (31). In Cheng's study, mice treated with antibiotics had increased susceptibility to lung tumor development, which could be rescued by administration of γδT17 cells or IL-17(31). It is generally well described that mice without a microbiota have a vastly immature immune system, which likely contributes to differences in susceptibility to infection. Our work is unique in regards to examining a specific commensal organism affecting susceptibility to a non-enteric infection.

The Th17 pathway has been shown to play an important role in mucosal host defense against several respiratory pathogens, including *Klebsiella pneumoniae* (8), *Pseudomonas aeruginosa(32)*, *Chlamydia pneumonia (33, 34), Mycoplasma pneumonia (35),* and *Mycobacterium tuberculosis* (36) (reviewed in(5)). Type 17 immunity has been described to be important in defense to *S. aureus* pneumonia in several other studies, although some with conflicting results (9-11). The *S. aureus* virulence factor α-hemolysin leads to increased transcription of host cytokine and chemokine genes, including the p19 subunit of IL-23, producing a prominent cellular Th17 response (37). Mice lacking the IL-17 receptor or IL-22, or which are coinfected with influenza A and thereby have an inhibited Th17 immunity, display impaired clearance of *S. aureus* compared to wild type or influenza-free mice (10). These results contrast to those of Choi et al. who reported that IL-17$^{-/-}$ and IL-22$^{-/-}$ mice showed no difference in MRSA clearance compared to wild type mice (9). Whether these

conflicting results are related to variable GI tract microbiota among mouse strains, or to different bacterial challenge strains, or other experimental variables, is unclear.

IL-22 is a member of the IL-10 family of immune mediators that is expressed by a several lymphocytes, including those of the innate and adaptive immune system (38). It has multiple functions, among which is modulation of tissue response during inflammation and anti-microbial molecule production (i.e. beta-defensin, Reg3γ, and Lipocalin 2)(39-41). IL-22 has been shown to mediate early host defense against bacterial pathogens (41) and appears to prevent bacterial dissemination in the GI tract, e.g. after infection with *Citrobacter rodentium* (41) or *Salmonella enterica* serotype *Typhimurium* (39). In the lung, IL-22 has been shown to mediate mucosal host defense against pneumonia with *Klebsiella pneumoniae* (8), and IL-22 expressing γδT cells were protective in a model of *Bacillus subtilis*-induced pulmonary fibrosis (42).

Given our observation that increased resistance to *S. aureus* pneumonia was associated with higher IL-22 levels, we tested its role directly and found that antibody-mediated neutralization of IL-22 led to increased susceptibility to *S. aureus* pneumonia in sfb-positive mice. Importantly, we also found that exogenous administration of IL-22 to sfb-negative mice rescued their defense against *S. aureus* pneumonia to a resistance level similar to sfb-positive mice, which further supports the protective role of IL-22 in *S. aureus* pneumonia. These findings suggest that therapeutic administration of recombinant IL-22 is a potential new approach to treatment of *S. aureus* pneumonia.

We did not observe significant differences in IL-17 levels, another key cytokine in Th17 immunity, between any group of mice, except for a slight increase in MRSA-infected mice after sfb-acquisition through co-housing, indicating that this cytokine likely does not play a very important role in *S. aureus* pneumonia or at least not at the time points we examined. It is possible that modulation of the pulmonary immune response by the enteric microbiota only affects specific cytokines. We observed at least two populations of cells capable of producing IL-22 in the lung after challenge with MRSA. The first population was comprised of cells positive for IL-22, CD3, and RORγt likely representing Th17 or Th22 cells (43), while the second group was negative for CD3, but positive for IL-22 and RORγt, possibly representing ILC3s (44).

Our data also highlight the importance of choosing an appropriate control mouse strain in these types of experiments and might explain the conflicting results seen in prior studies. Another explanation for reported conflicting results might relate to the finding that different pathogens can induce different cytokine production and effector function in Th17 cells (45).

Protective responses to S. *aureus* pneumonia were associated with IL-6 increase, a Th17-associated cytokine, at an early time point. Previous studies have shown that IL-6 is a pleiotropic cytokine involved in the cross talk between immune cells and stromal cells, activates the STAT3 signaling axis, is one of the main regulators of Th17 cell differentiation (46), and promotes IL-22 expression (24). IL-6 deficient mice showed impaired defense against pneumococcal pneumonia (47). Interestingly, IL-6 and IL-1b

levels are elevated in mice orally treated with antibiotics resulting in commensal depletion compared to untreated mice and are associated with more severe illness (21). This is in accordance to our findings that sfb-negative mice have lower IL-6 levels compared to sfb-positive mice at 8 hours after infection and that sfb-negative mice after acquisition of sfb had markedly increased IL-6 expression. Sfb-negative mice in our study had higher IL-6 levels at 18 hours post-infection, possibly reflecting a delayed response or a response due to the continuing high levels of *S. aureus* in the lungs. IL-6 produced by macrophages during MRSA infection stimulates the pulmonary epithelium via STAT3 signaling to produce the antimicrobial protein Reg3γ, which has bacteriostatic and bactericidal effects against MRSA (9). Further studies are needed to examine the link between GI microbiota and production of certain antimicrobial proteins in the lung.

We found increased susceptibility of sfb-negative mice to *S. aureus* lung infection and could demonstrate this was related to IL-22 production. In human disease *S. aureus*, and particularly MRSA, can rapidly progress towards a fatal infection over a short time period, indicative of the importance of the innate response to infection. We do not know at this time whether humans who get these invasive *S. aureus* pneumonias have or lack organisms in their GI microbiota predisposing them to increased susceptibility, but as progress in understanding the interaction of the microbiota and the immune system ensues, it might be possible to insure that individuals carry organisms that can maximize their innate resistance to infection. Our findings also suggest exogenous IL-22

529 could be a therapy for *S. aureus* pneumonia, a disease process in desperate need for
530 improved therapies.
531

532 **ACKNOWLEDGEMENTS**

533 This work was supported by grants from the National Institutes of Health, NICHD
534 K12HD047349-07 (to S. Gauguet), K08AI108690 (to N. Surana), R01 EY022054 (to M.
535 Gadjeva), R01 HL092515 (to G. Priebe), and National Institute of Allergy and Infectious
536 Diseases (NIAID) grant numbers AI46706 and AI057159, a component of Award
537 Number U54 AI057159 (to G. Pier).
538 We are greatly thankful to James Lederer for performing the BALF cytokine Luminex
539 assays, Dr. Roderick Bronson for interpreting the histological images, and Michael
540 Coyne for helpful advice.
541

Figure 1. Sfb-negative mice exhibit increased susceptibility to MRSA pneumonia compared to sfb-positive mice.

C57BL/6 mice from Jackson (sfb-negative) and Taconic (sfb-positive) were challenged with $5x10^8$ CFU of MRSA intranasally and sacrificed after 18 hours for determination of bacterial CFU/gram lung **(A)** and spleen **(B)** tissue. Symbols represent individual animals; bars represent the medians. *P* values were determined by Mann Whitney U tests.

Histological appearance of lung tissue of a representative sfb-negative **(C and D)** and sfb-positive **(E and F)** mouse 18 h after infection with MRSA Lac. The sfb-negative mice showed higher percentages of lung tissue involved by inflammation (60% affected **(C and D)** vs. 10% affected **(E and F)**). 40-fold magnification **(C and E)** and 100-fold magnification **(D and F).** The black bar represents 1mm.

Figure 2. Sfb-negative mice acquire increased resistance to *S. aureus* pneumonia after co-housing with sfb-positive mice.

Female sfb-negative C57BL/6 mice and age-matched, female sfb-positive C57BL/6 mice were housed together in isolated cages for 2 weeks prior to bacterial challenge. All of the co-housed mice were sfb-positive at the time of bacterial challenge. Eight additional sfb-negative or sfb-positive mice were not co-housed for the same time period, and remained sfb-negative and sfb-positive, respectively. After 2 weeks, mice were challenged with $5x10^8$ CFU of MRSA IN and sacrificed after 18 hours for determination of bacterial CFU/gram lung tissue. Symbols represent individual animals; bars represent the medians. *P* values were determined by Kruskal-Wallis test (overall ANOVA, $P< 0.01$) and P values depict the Dunn's multiple pair-wise comparisons for nonparametric distributions.

Figure 3. Sfb-negative mice acquire increased resistance to *S. aureus* pneumonia after colonization with sfb in their GI tract.

Sfb-negative C57BL/6 mice were either gavaged with PBS (sfb-negative+PBS, n=5) or with sfb–containing stool (sfb-negative + sfb, n=6) 2 weeks prior to intranasal challenge with MRSA strain LAC at a dose of $5x10^8$ CFU **(A and B)** or $2x10^9$ CFU for a survival experiment (n=10 in each group) **(C)**. Bacterial CFU/gram lung tissue **(A)** and spleen tissue **(B)** at 18 h post-infection. **(C)** Kaplan-Meier survival curves. Symbols represent individual animals; bars represent the medians. *P* values were determined by Mann Whitney U tests **(A and B)** or by logrank test **(C)**.

582 **Figure 4. Type 17 immunity differs in the lungs of sfb-negative and sfb-positive**
583 **mice.**
584
585 At 8 and 18 h post-infection with MRSA, mice were euthanized and BALF was obtained
586 for (removed: cellular and) cytokine analysis. Bars represent the means and error bars
587 represent standard deviations (SD). *P* values were determined by Mann Whitney U
588 tests for 2-group comparisons or Kruskal-Wallis test with Dunn's multiple comparison.
589 **A)** BALF IL-22 8 h post-infection; **B)** BALF IL-22 18 h post-infection; **C)** BALF IL-22 18 h
590 post-infection after co-housing; **D)** BALF IL-22 18 h post-infection in Jackson (sfb-
591 negative) mice acquiring sfb after gavage two weeks prior to infection; **E)** BALF IL-17 8h
592 post-infection; **F)** BALF IL-17 18h post-infection; **G)** BALF IL-17 18 h post-infection after
593 co-housing; **H)** BALF IL-17 18 h post-infection in Jackson mice acquiring sfb after
594 gavage two weeks prior to infection; **I)** BALF IL-6 8 h post-infection; **J)** BALF IL-6 18 h
595 post-infection; **K-L)** Ly6G-positive cells (neutrophils) in BALF 18 h post-infection of co-
596 housed mice **(K)** or sfb-colonized Jackson mice **(L)**. **(M-P)** Mouse lung sections were
597 stained with anti-**RORγt** antibody (left upper squares, blue), anti-**IL-22** (middle upper
598 squares, red), and anti-**CD3e** (right upper square, green) and examined by confocal
599 microscopy. Left lower squares depict phase contrast microscopy and right lower
600 squares show merged confocal images. **(M)** Lung tissue sections of an sfb-positive
601 mouse, showing many cells positive for RORγt, IL-22, and CD3 (whitish-yellow cells in
602 merged confocal image) likely representing Th17 or Th22 cells, as well as RORγt and
603 IL-22 positive, but CD3 negative cells (purple), possibly representing ILC3s. **(N)** Lung
604 tissue sections of a previously sfb-negative mouse after acquisition of sfb 2 weeks prior

by nasogastric lavage showing also both RORγt+ IL-22+ CD3+ and RORγt+ IL-22+ CD3- cell populations, but overall in less quantities, and very few CD3-positive cells. **(O)** Lung tissue sections of an sfb-negative mouse showing even less RORγt+ or IL-22+ cells despite a denser cell infiltrate and no RORγt+ IL-22+ CD3- cells. Isotype control stains for images **Figure 4m-o** are shown in supplemental **Figure S2a-c**. **(P)** Number of dually IL-22-and TCRβ-positive cells in BALF of mice at 8 hours after infection using intracellular staining by flow cytometry showed a significantly more IL-22+ TCRβ-positive cells in sfb-positive versus sfb-negative mice.

Color key:

RORγt – blue

IL-22 – red

CD3 – green

Figure 5. Neutralization of IL-22 leads to more severe *S. aureus* pneumonia while exogenous IL-22 administration increases resistance of sfb-negative mice to infection.

C57BL/6 mice from Taconic (sfb-positive) received monoclonal anti-IL-22 antibody or control IgG 4 h prior to challenge with $5x10^8$ CFU of MRSA intranasally. **(A)** Bacterial CFU/gram lung tissue 18 h post infection. **(B and C)** C57BL/6 mice from Jackson (sfb-negative) received exogenous recombinant IL-22 or control protein BSA during challenge with $5x10^8$ CFU of MRSA LAC intranasally. Bacterial CFU/gram lung **(B)** and spleen **(C)** at 18 h post-infection. Symbols represent individual animals; bars represent the medians. *P* values were determined by Mann Whitney U tests for 2-group comparisons or Kruskal-Wallis test with Dunn's multiple comparison.

630 **REFERENCES**

631

632 1. **Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, Lessa FC,**
633 **Lynfield R, Nadle J, Petit S, Ray SM, Schaffner W, Townes J, Fridkin S,**
634 **Emerging Infections Program-Active Bacterial Core Surveillance MSI.** 2013.
635 National burden of invasive methicillin-resistant Staphylococcus aureus infections,
636 United States, 2011. JAMA Intern Med **173:**1970-1978.
637 2. **Rubinstein E, Kollef MH, Nathwani D.** 2008. Pneumonia caused by methicillin-
638 resistant Staphylococcus aureus. Clin Infect Dis **46 Suppl 5:**S378-385.
639 3. **Vardakas KZ, Matthaiou DK, Falagas ME.** 2009. Incidence, characteristics and
640 outcomes of patients with severe community acquired-MRSA pneumonia. Eur
641 Respir J **34:**1148-1158.
642 4. **Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH,**
643 **Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal**
644 **LK, Carey RB, Fridkin SK, Active Bacterial Core surveillance MI.** 2007. Invasive
645 methicillin-resistant Staphylococcus aureus infections in the United States. JAMA
646 **298:**1763-1771.
647 5. **Tsai HC, Velichko S, Hung LY, Wu R.** 2013. IL-17A and Th17 cells in lung
648 inflammation: an update on the role of Th17 cell differentiation and IL-17R signaling
649 in host defense against infection. Clin Dev Immunol **2013:**267971.
650 6. **de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M,**
651 **Feinberg J, von Bernuth H, Samarina A, Janniere L, Fieschi C, Stephan JL,**
652 **Boileau C, Lyonnet S, Jondeau G, Cormier-Daire V, Le Merrer M, Hoarau C,**

Lebranchu Y, Lortholary O, Chandesris MO, Tron F, Gambineri E, Bianchi L, Rodriguez-Gallego C, Zitnik SE, Vasconcelos J, Guedes M, Vitor AB, Marodi L, Chapel H, Reid B, Roifman C, Nadal D, Reichenbach J, Caragol I, Garty BZ, Dogu F, Camcioglu Y, Gulle S, Sanal O, Fischer A, Abel L, Stockinger B, Picard C, Casanova JL. 2008. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med **205:**1543-1550.

7. **Minegishi Y, Saito M, Nagasawa M, Takada H, Hara T, Tsuchiya S, Agematsu K, Yamada M, Kawamura N, Ariga T, Tsuge I, Karasuyama H.** 2009. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. J Exp Med **206:**1291-1301.

8. **Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S, Kreindler JL, Dubin PJ, Pilewski JM, Myerburg MM, Mason CA, Iwakura Y, Kolls JK.** 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med **14:**275-281.

9. **Choi SM, McAleer JP, Zheng M, Pociask DA, Kaplan MH, Qin S, Reinhart TA, Kolls JK.** 2013. Innate Stat3-mediated induction of the antimicrobial protein Reg3gamma is required for host defense against MRSA pneumonia. J Exp Med **210:**551-561.

10. **Kudva A, Scheller EV, Robinson KM, Crowe CR, Choi SM, Slight SR, Khader SA, Dubin PJ, Enelow RI, Kolls JK, Alcorn JF.** 2011. Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. J Immunol **186:**1666-1674.

11. **Robinson KM, McHugh KJ, Mandalapu S, Clay ME, Lee B, Scheller EV, Enelow RI, Chan YR, Kolls JK, Alcorn JF.** 2014. Influenza A virus exacerbates Staphylococcus aureus pneumonia in mice by attenuating antimicrobial peptide production. The Journal of infectious diseases **209:**865-875.

12. **Priebe GP, Walsh RL, Cederroth TA, Kamei A, Coutinho-Sledge YS, Goldberg JB, Pier GB.** 2008. IL-17 is a critical component of vaccine-induced protection against lung infection by lipopolysaccharide-heterologous strains of Pseudomonas aeruginosa. J Immunol **181:**4965-4975.

13. **Gopal R, Rangel-Moreno J, Slight S, Lin Y, Nawar HF, Fallert Junecko BA, Reinhart TA, Kolls J, Randall TD, Connell TD, Khader SA.** 2013. Interleukin-17-dependent CXCL13 mediates mucosal vaccine-induced immunity against tuberculosis. Mucosal Immunol **6:**972-984.

14. **Wu W, Huang J, Duan B, Traficante DC, Hong H, Risech M, Lory S, Priebe GP.** 2012. Th17-stimulating protein vaccines confer protection against Pseudomonas aeruginosa pneumonia. Am J Respir Crit Care Med **186:**420-427.

15. **Chewning JH, Weaver CT.** 2014. Development and Survival of Th17 Cells within the Intestines: The Influence of Microbiome- and Diet-Derived Signals. J Immunol **193:**4769-4777.

16. **Clarke TB.** 2014. Early Innate Immunity to Bacterial Infection in the Lung Is Regulated Systemically by the Commensal Microbiota via Nod-Like Receptor Ligands. Infect Immun **82:**4596-4606.

17. **Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y,**

Honda K, Littman DR. 2009. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell **139:**485-498.

18. **Ivanov, II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR.** 2008. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe **4:**337-349.
19. **Ivanov, II, Littman DR.** 2010. Segmented filamentous bacteria take the stage. Mucosal Immunol **3:**209-212.
20. **Cooke KR, Hill GR, Gerbitz A, Kobzik L, Martin TR, Crawford JM, Brewer JP, Ferrara JL.** 2000. Hyporesponsiveness of donor cells to lipopolysaccharide stimulation reduces the severity of experimental idiopathic pneumonia syndrome: potential role for a gut-lung axis of inflammation. J Immunol **165:**6612-6619.
21. **Chen LW, Chen PH, Hsu CM.** 2011. Commensal microflora contribute to host defense against Escherichia coli pneumonia through Toll-like receptors. Shock **36:**67-75.
22. **Fox AC, McConnell KW, Yoseph BP, Breed E, Liang Z, Clark AT, O'Donnell D, Zee-Cheng B, Jung E, Dominguez JA, Dunne WM, Burd EM, Coopersmith CM.** 2012. The endogenous bacteria alter gut epithelial apoptosis and decrease mortality following Pseudomonas aeruginosa pneumonia. Shock **38:**508-514.
23. **Umesaki Y, Okada Y, Matsumoto S, Imaoka A, Setoyama H.** 1995. Segmented filamentous bacteria are indigenous intestinal bacteria that activate intraepithelial lymphocytes and induce MHC class II molecules and fucosyl asialo GM1 glycolipids

on the small intestinal epithelial cells in the ex-germ-free mouse. Microbiology and immunology **39:**555-562.

24. **Li L, Shi QG, Lin F, Liang YG, Sun LJ, Mu JS, Wang YG, Su HB, Xu B, Ji CC, Huang HH, Li K, Wang HF.** 2014. Cytokine IL-6 is required in Citrobacter rodentium infection-induced intestinal Th17 responses and promotes IL-22 expression in inflammatory bowel disease. Mol Med Rep **9:**831-836.
25. **Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE, Bagby GJ, Nelson S, Charrier K, Peschon JJ, Kolls JK.** 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J Exp Med **194:**519-527.
26. **Cheng P, Liu T, Zhou WY, Zhuang Y, Peng LS, Zhang JY, Yin ZN, Mao XH, Guo G, Shi Y, Zou QM.** 2012. Role of gamma-delta T cells in host response against Staphylococcus aureus-induced pneumonia. BMC immunology **13:**38.
27. **Simon MM, Greenaway S, White JK, Fuchs H, Gailus-Durner V, Wells S, Sorg T, Wong K, Bedu E, Cartwright EJ, Dacquin R, Djebali S, Estabel J, Graw J, Ingham NJ, Jackson IJ, Lengeling A, Mandillo S, Marvel J, Meziane H, Preitner F, Puk O, Roux M, Adams DJ, Atkins S, Ayadi A, Becker L, Blake A, Brooker D, Cater H, Champy MF, Combe R, Danecek P, di Fenza A, Gates H, Gerdin AK, Golini E, Hancock JM, Hans W, Holter SM, Hough T, Jurdic P, Keane TM, Morgan H, Muller W, Neff F, Nicholson G, Pasche B, Roberson LA, Rozman J, Sanderson M, Santos L, Selloum M, Shannon C, Southwell A, Tocchini-Valentini GP, Vancollie VE, Westerberg H, Wurst W, Zi M, Yalcin B, Ramirez-**

Solis R, Steel KP, Mallon AM, de Angelis MH, Herault Y, Brown SD. 2013. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. Genome Biol **14:**R82.

28. **Fagundes CT, Amaral FA, Vieira AT, Soares AC, Pinho V, Nicoli JR, Vieira LQ, Teixeira MM, Souza DG.** 2012. Transient TLR activation restores inflammatory response and ability to control pulmonary bacterial infection in germfree mice. J Immunol **188:**1411-1420.
29. **Wu S, Jiang ZY, Sun YF, Yu B, Chen J, Dai CQ, Wu XL, Tang XL, Chen XY.** 2013. Microbiota regulates the TLR7 signaling pathway against respiratory tract influenza A virus infection. Curr Microbiol **67:**414-422.
30. **Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A.** 2011. Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A **108:**5354-5359.
31. **Cheng M, Qian L, Shen G, Bian G, Xu T, Xu W, Shen G, Hu S.** 2014. Microbiota modulate tumoral immune surveillance in lung through a gammadeltaT17 immune cell-dependent mechanism. Cancer Res **74:**4030-4041.
32. **Liu J, Feng Y, Yang K, Li Q, Ye L, Han L, Wan H.** 2011. Early production of IL-17 protects against acute pulmonary Pseudomonas aeruginosa infection in mice. FEMS Immunol Med Microbiol **61:**179-188.
33. **Wolf K, Plano GV, Fields KA.** 2009. A protein secreted by the respiratory pathogen Chlamydia pneumoniae impairs IL-17 signalling via interaction with human Act1. Cell Microbiol **11:**769-779.

765 34. **Zhou X, Chen Q, Moore J, Kolls JK, Halperin S, Wang J.** 2009. Critical role of the
766 interleukin-17/interleukin-17 receptor axis in regulating host susceptibility to
767 respiratory infection with Chlamydia species. Infect Immun **77:**5059-5070.
768 35. **Wu Q, Martin RJ, Rino JG, Breed R, Torres RM, Chu HW.** 2007. IL-23-dependent
769 IL-17 production is essential in neutrophil recruitment and activity in mouse lung
770 defense against respiratory Mycoplasma pneumoniae infection. Microbes Infect
771 **9:**78-86.
772 36. **Lockhart E, Green AM, Flynn JL.** 2006. IL-17 production is dominated by
773 gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis
774 infection. J Immunol **177:**4662-4669.
775 37. **Frank KM, Zhou T, Moreno-Vinasco L, Hollett B, Garcia JG, Bubeck**
776 **Wardenburg J.** 2012. Host response signature to Staphylococcus aureus alpha-
777 hemolysin implicates pulmonary Th17 response. Infect Immun **80:**3161-3169.
778 38. **Zenewicz LA, Flavell RA.** 2011. Recent advances in IL-22 biology. Int Immunol
779 **23:**159-163.
780 39. **Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio SP, Paixao TA,**
781 **Butler BP, Chu H, Santos RL, Berger T, Mak TW, Tsolis RM, Bevins CL,**
782 **Solnick JV, Dandekar S, Baumler AJ.** 2009. Lipocalin-2 resistance confers an
783 advantage to Salmonella enterica serotype Typhimurium for growth and survival in
784 the inflamed intestine. Cell Host Microbe **5:**476-486.
785 40. **Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R.** 2004. IL-22
786 increases the innate immunity of tissues. Immunity **21:**241-254.

41. **Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, Ouyang W.** 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med **14:**282-289.

42. **Simonian PL, Wehrmann F, Roark CL, Born WK, O'Brien RL, Fontenot AP.** 2010. gammadelta T cells protect against lung fibrosis via IL-22. J Exp Med **207:**2239-2253.

43. **Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfarani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, Durham SR, Schmidt-Weber CB, Cavani A.** 2009. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. The Journal of clinical investigation **119:**3573-3585.

44. **Van Maele L, Carnoy C, Cayet D, Ivanov S, Porte R, Deruy E, Chabalgoity JA, Renauld JC, Eberl G, Benecke AG, Trottein F, Faveeuw C, Sirard JC.** 2014. Activation of Type 3 innate lymphoid cells and interleukin 22 secretion in the lungs during Streptococcus pneumoniae infection. The Journal of infectious diseases **210:**493-503.

45. **Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, Monticelli S, Lanzavecchia A, Sallusto F.** 2012. Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. Nature **484:**514-518.

46. **Camporeale A, Poli V.** 2012. IL-6, IL-17 and STAT3: a holy trinity in auto-immunity? Front Biosci (Landmark Ed) **17:**2306-2326.

47. **van der Poll T, Keogh CV, Guirao X, Buurman WA, Kopf M, Lowry SF.** 1997. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. The Journal of infectious diseases **176:**439-444.

Figure 1. Sfb-negative mice exhibit increased susceptibility to MRSA pneumonia compared to sfb-positive mice.

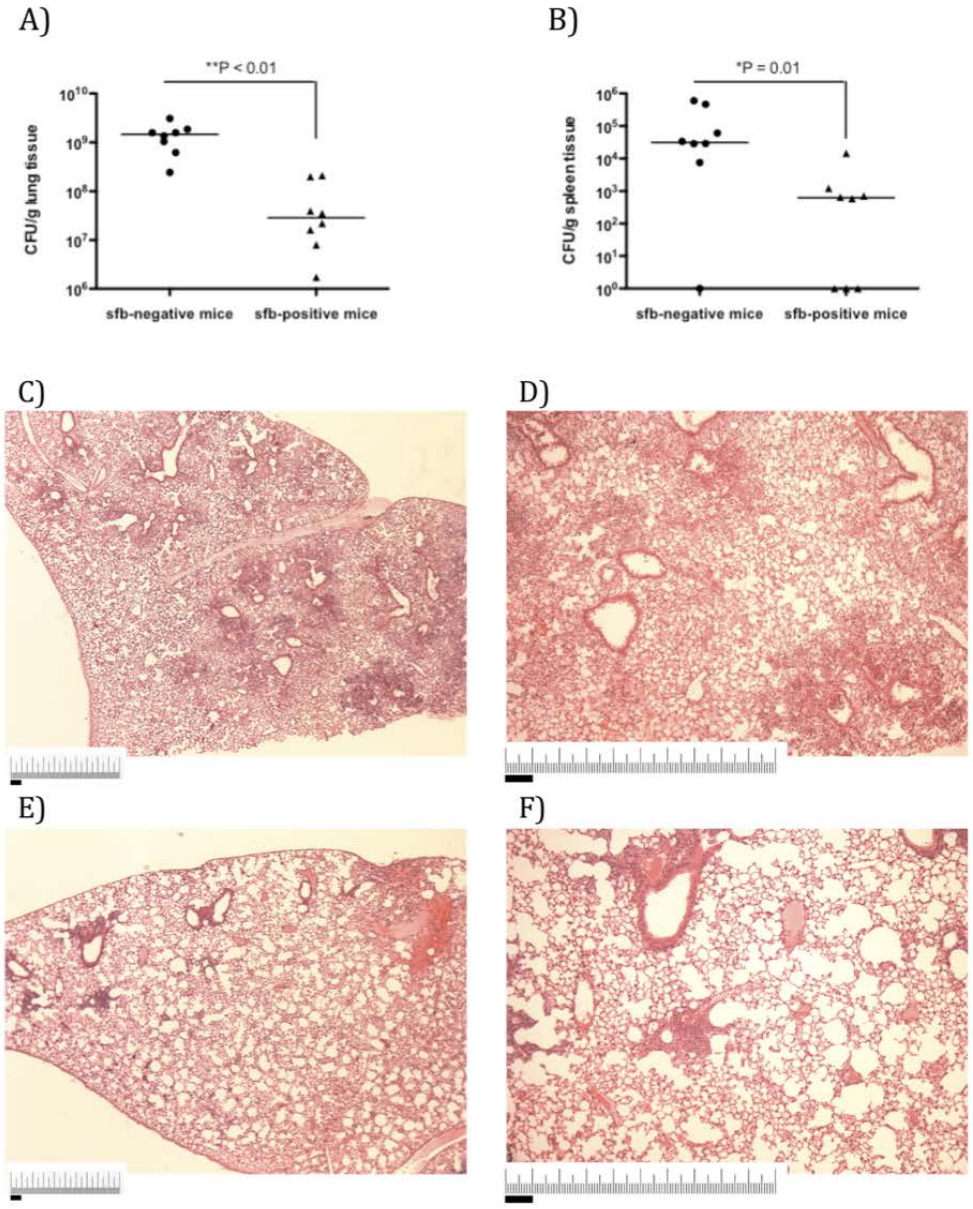


Figure 2. Sfb-negative mice acquire increased resistance to *S. aureus* pneumonia after co-housing with sfb-positive mice.

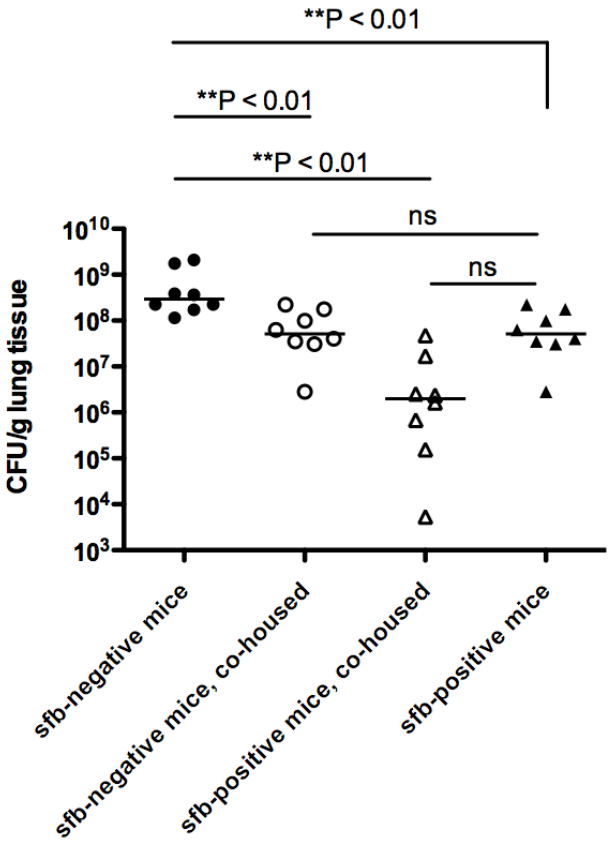


Figure 3. Sfb-negative mice acquire increased resistance to *S. aureus* pneumonia after colonization with sfb in their GI tract.

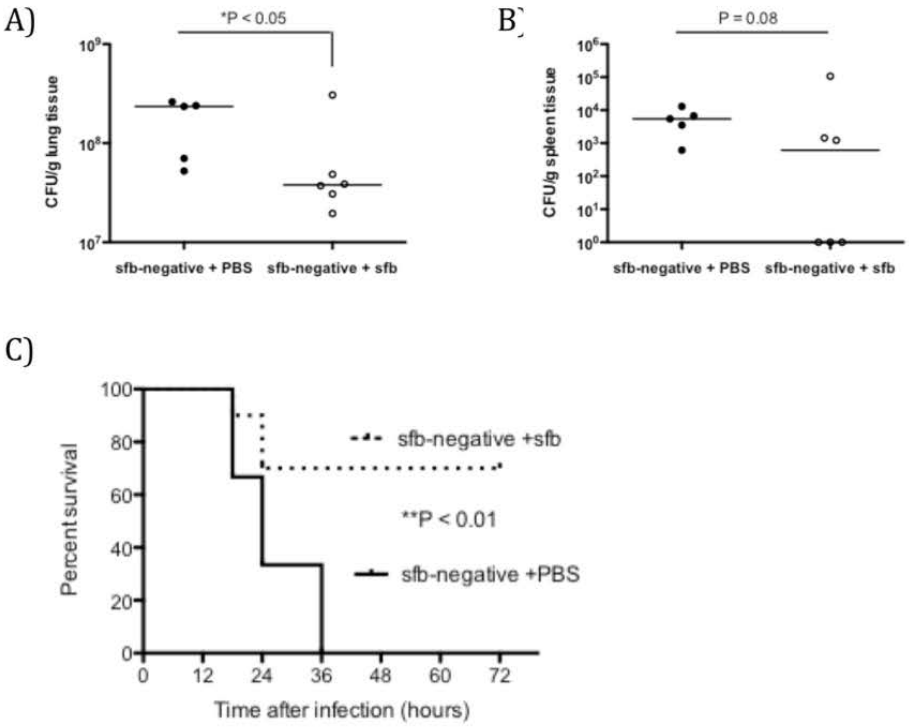
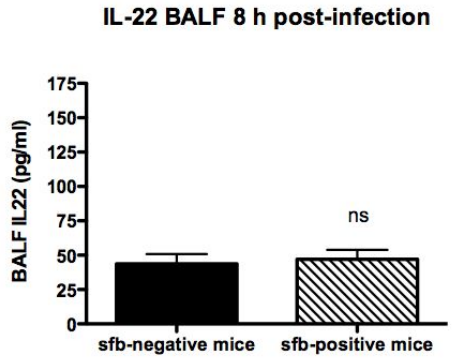
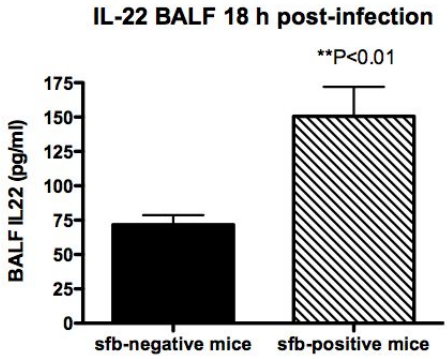


Figure 4. Type 17 immunity differs in the lungs of sfb-negative and sfb-positive mice.

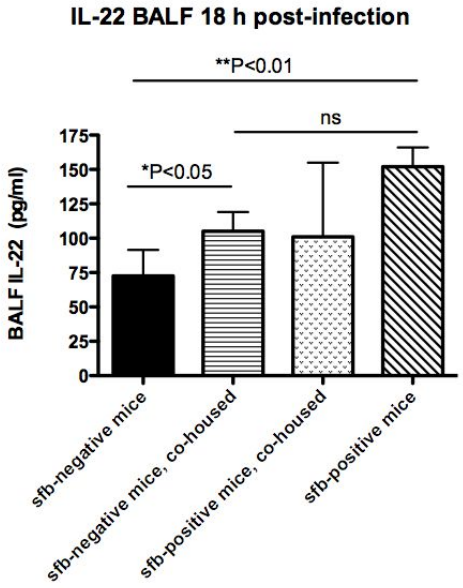
A)



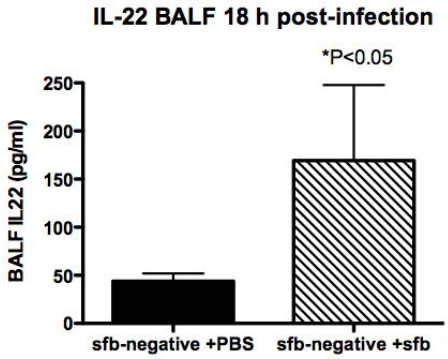
B)



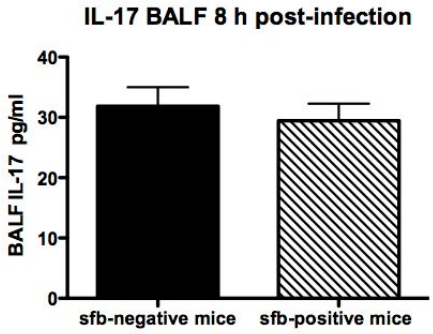
C)



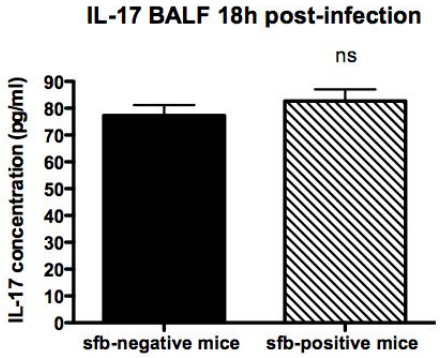
D)



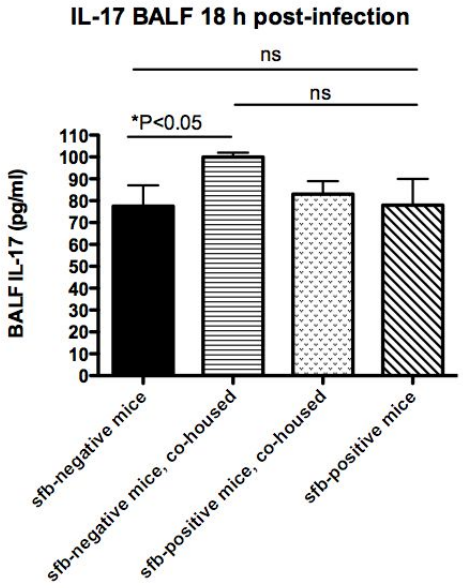
E)



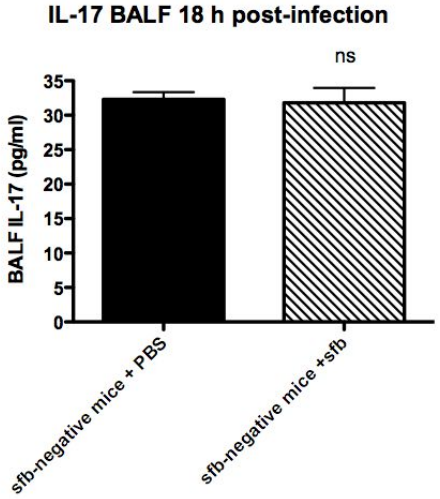
F)



G)

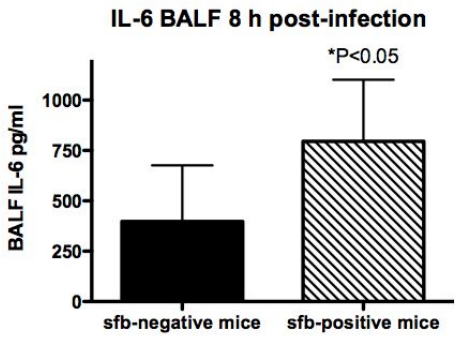


H)

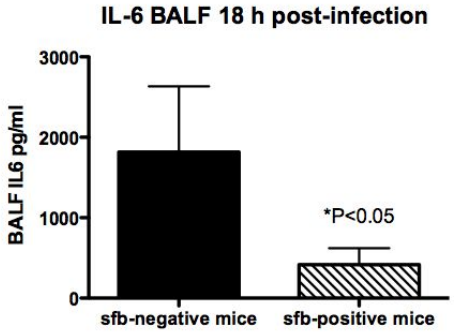


Infection and Immunity

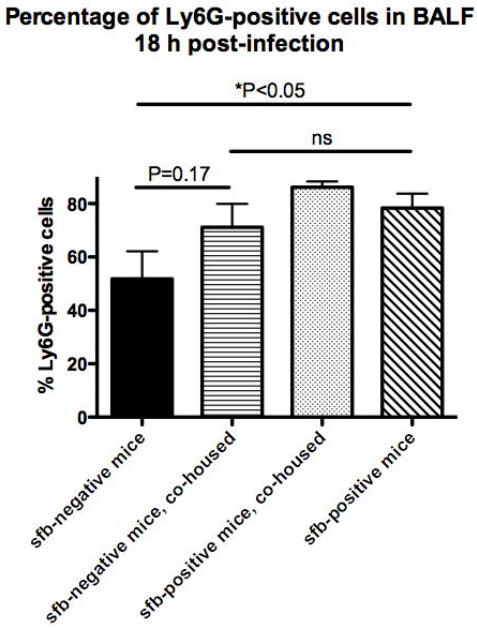
I)



J)



K)



L)

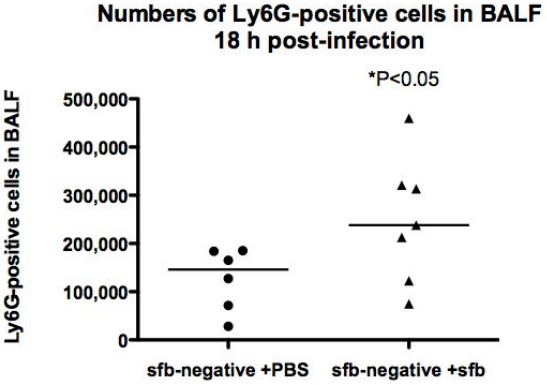
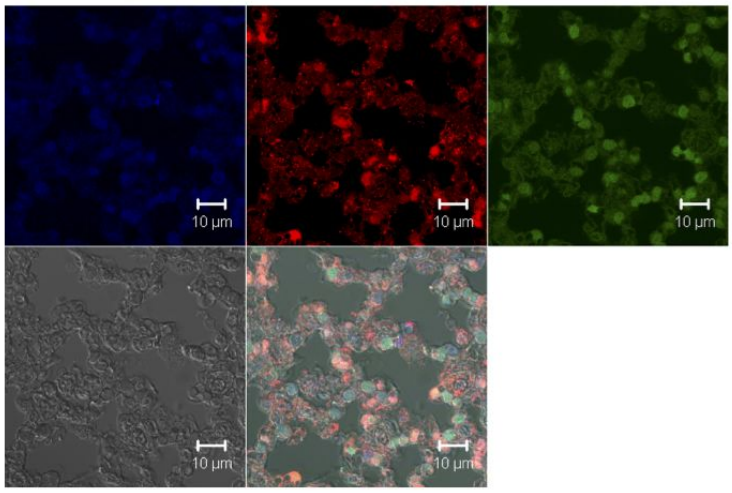
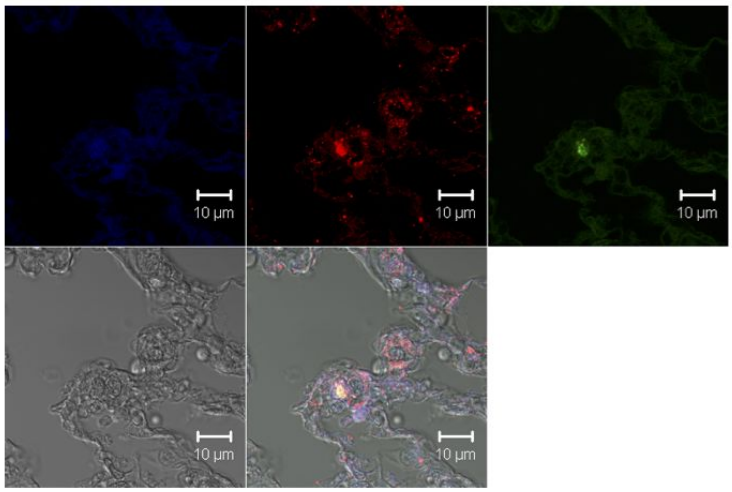


Figure 4. Type 17 immunity differs in the lungs of sfb-negative and sfb-positive mice.

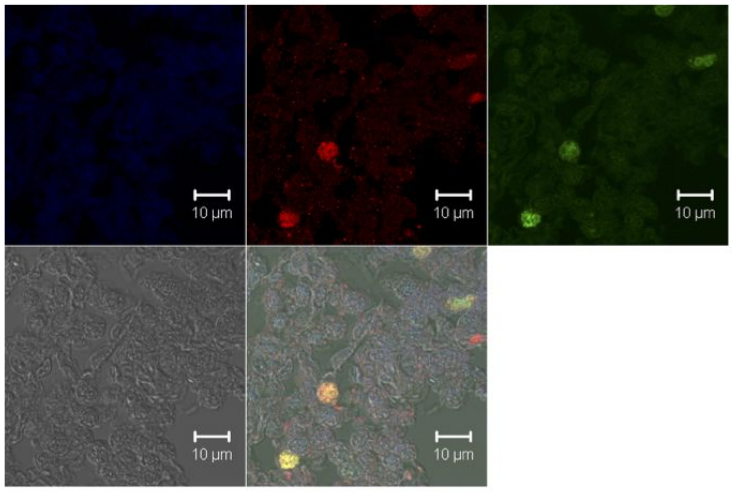
M)



N)



O)



P)

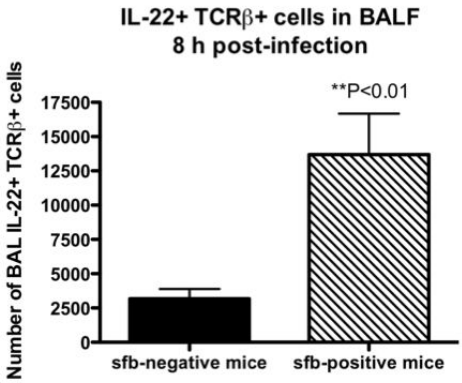
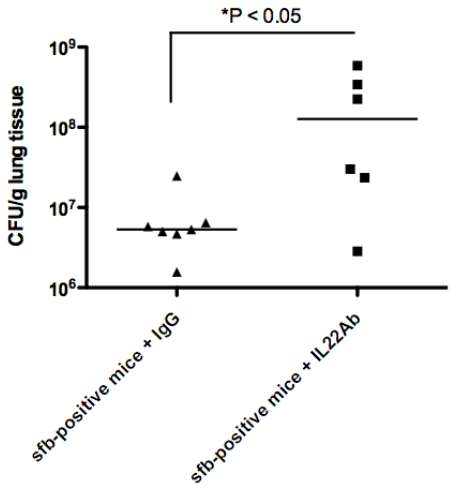
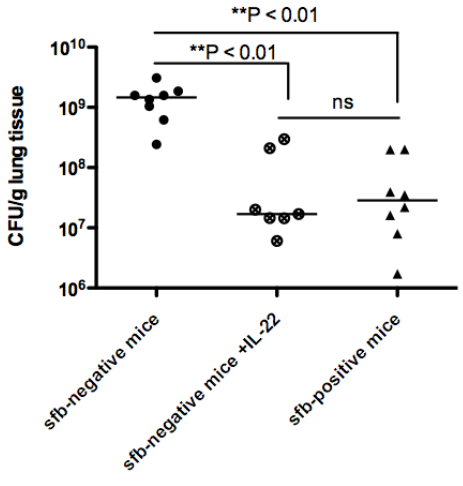


Figure 5. Neutralization of IL-22 leads to more severe *S. aureus* pneumonia while exogenous IL-22 administration increases resistance of sfb-negative mice to infection.

A)



B)



C)