Antibiotic-related Gut Dysbiosis Induces Lung Immunodepression and Worsens Lung Infection.

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Research

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Abstract

Background

Gut dysbiosis due to the adverse effects of antibiotics affects outcomes of lung infection. Previous murine models relied on significant depletion of both gut and lung microbiota, rendering the analysis of immune gut-lung cross-talk difficult.

Here we study the effects of antibiotic-induced gut dysbiosis without lung dysbiosis on lung immunity and the consequences on acute *P. aeruginosa* lung infection.

Methods

C57BL6 mice were administered 7 days oral vancomycin-colistin, modulated or not by fecal microbial transplant or exogenous hematopoietic cytokine, Fms-related tyrosine kinase 3 ligand (Flt3-Ligand), followed by intranasal *P. aeruginosa* strain PAO1. Gut and lung microbiota were studied by next-generation sequencing, and lung infection outcomes were studied at 24h. Effects of vancomycin-colistin on underlying immunity and bone marrow progenitors were studied in uninfected mice by flow cytometry in the lung, spleen, and bone marrow.

Results

Vancomycin-colistin administration induces widespread cellular immunosuppression in both lung and spleen, decreases circulating hematopoietic cytokine Flt3-Ligand, and depresses dendritic cell bone marrow progenitors leading to worsening of *P. aeruginosa* lung infection outcomes (bacterial loads, lung injury and survival). Reversal of these effects by fecal microbial transplant shows that these alterations are related to gut dysbiosis. Recombinant Flt3-Ligand reverses the effects of antibiotics on subsequent lung infection.

Conclusions

These results show that gut dysbiosis strongly impairs monocyte/dendritic progenitors and lung immunity, worsening outcomes of *P. aeruginosa* lung infection. Treatment with a fecal microbial transplant or immune stimulation by Flt3-Ligand both restore lung cellular responses to and outcomes of *P. aeruginosa* following antibiotic-induced gut dysbiosis.

Background

Since their discovery, antibiotics have transformed the outcomes of bacterial infectious diseases. However, antibiotics also cause adverse effects, such as the selection of resistant organisms resulting in increased individual and collective changes in the microbial ecology of infections and diseases such as antibiotic-associated diarrhea. These adverse effects result from the "collateral" antimicrobial effects exerted not only upon targeted pathogens but also upon the normal microbial flora. Advances in high-

throughput bacterial sequencing, allowing the large-scale study of the microbial communities in humans and experimental models, led to the study of the bacteria composing this flora since termed "microbiome" (or microbiota) as well as its pathologic alterations termed dysbiosis (or dysbioses).

The microbiome has been shown to shape host immunity. The disruption of this microbiome-driven immune homeostasis is considered a mechanism for the deleterious effects of antibiotics beyond the gut, such as predisposition to allergic lung diseases [1]. Non-culture-based methods have shown that the human microbiome includes bacterial communities in body compartments other than the gut, such as the lung. Therefore, the microbiome-driven immune cross-talk between the gut and the lung, or "gut-lung axis," involves both gut and lung microbiota [2]. However, these studies are based on models of antibiotic-induced dysbiosis that rely on very broad-spectrum antibiotics using associations of 4 to 5 antibiotics, many with systemic diffusion. Not only are such models far from mirroring clinical situations, but they also result in the form of dysbiosis with significant overall depletion [3], and deplete both the gut and lung microbiota [3] [4]. Therefore, it is difficult to decipher the respective roles of the lung and gut microbiota on lung immunity and its impact on lung infection.

Therefore, we aim to determine the effects of antibiotic-induced gut dysbiosis, without lung dysbiosis, on outcomes in a non-lethal murine model of acute *P. aeruginosa* lung infection and the underlying immune alterations in order to manage side effects of antibiotics.

Methods

Animals

Specific pathogen-free C57BL/6 mice of 6–8 weeks old were purchased from Janvier Labs (Le Genest-Saint-Isle, France). Mice were housed in a controlled, specific pathogen-free environment. Mice were euthanized by lethal injection of 0.2 mL of dolethal (Vétoquinol, Paris, France). The national ethics committee approved all experiments (APAFIS number 7166).

Bacterial Strain

P. aeruginosa PAO1 strain was grown overnight at 37 °C in Luria-Bertani broth, with orbital shaking, harvested by centrifugation (3500 rpm, 10 min), and washed with sterile isotonic saline. Bacteria suspensions and inoculum standardization were then determined based on optical density at 600 nm and verified by serial dilutions and plating on Luria-Bertani agar (Fisher Scientific, Hampton, NH, USA).

Experimental model

Gut dysbiosis was induced by treating mice with non-absorbable antibiotics (vancomycin 0.5 g/L and colistin 0.15 g/L) administered in drinking water *ad libitum* for seven days. After seven days, mice were anesthetized briefly with inhaled sevoflurane, and acute sub-lethal pneumonia was performed by intranasal instillation of PAO1 at 5.10^6 CFU. Lethal pneumonia was induced by intranasal instillation of PAO1 at 5.10^7 CFU. Control mice were instilled with Phosphate Buffer Saline (PBS). For all experiments,

mice were euthanized at 24 hours after PAO1 inoculation. The general design of the present experimental model is illustrated in supplemental Fig. 1.

Fecal microbiota transplantation and Flt3-Ligand treatment

Fecal pellets from untreated mice were suspended in PBS (1 fecal pellet/1 mL of PBS). For each experiment, several fecal pellets from different untreated mice were resuspended together in PBS. A total of 200µL of the resuspended pool fecal material was administered by oral gavage to gut microbiota-depleted mice over two consecutive days after antibiotic treatment was stopped; control mice were given water alone during this period. Flt3-Ligand treatment (Ozyme, Saint-Cyr L'Ecole, France) was given by intraperitoneal injection over two consecutive days after antibiotic treatment was stopped, for a total of 10 µg/mice.

Lung injury

Alveolar capillary barrier permeability was evaluated by measuring fluorescein isothiocyanate (FITC)-labeled-albumin leakage from the vascular compartment to the alveolar-interstitial compartment, as previously described [5].

Bronchoalveolar lavage (BAL)

Lungs from each experimental group were washed with a total of 1.5 mL PBS. Recovered lavage fluid was centrifuged (1500 rpm, 10 min), the cellular pellet was washed twice with PBS. BAL samples were frozen at -80 °C after collection for cytokine measurement. Cell counts were performed directly by optical microscopy.

Bacterial burden

Mouse lungs and spleens were homogenized in sterile containers with PBS. Lung and spleen homogenates were sequentially diluted and cultured on Luria-Bertani agar plates for 24 hours to assess bacterial load.

Measurement of cytokines

IL-6, IL-10, IL-17, IL-22, TNF-α, and CXCL1 were measured in BAL supernatants using enzyme-linked immunosorbent Assay (ELISA) kits (Biolegend, San Diego, CA, USA).

Flow cytometry

Antibodies and the permeation kits were purchased from BD. Cells harvested from BAL fluid and lungs were washed and incubated with appropriate dilutions of an antigen-presenting-cell antibody panel or a lymphoid-cell antibody panel for 30 min in PBS and then washed twice and resuspended in PBS 2% fetal calf serum. For each antibody, a control isotype was used for compensation. Cells were analyzed on a LSR Fortessa (BD Biosciences). Flow cytometry gating strategy to analyze AM, cDC1, cDC2, Inflammatory monocytes, monocytes, neutrophils, T lymphocytes ($CD4^+$ and $CD8^+$), NK cells, iNKT cells, NKT cells, and

Tγδ cells, is illustrated in supplementary Fig. 2. Generated data were analyzed using FlowJo 8.7 (Tree Star, Stanford, CA).

Bone marrow progenitor analysis

Cell populations ($CD45^+$ cells) were analyzed with a BD LSR Fortessa (BD Biosciences) according to the following cell surface phenotypes: (MDPs): $Lin(*)^-CD11b^-CD117^+CD135^+CD115^+$; (CDPs) $Lin(*)^-CD11b^-CD117^{low}CD135^+CD115^+CD11c^-$; (pre-DCs): $Lin(*)^-CD11b^-CD11c^+MHCII^-CD135^+Sirp\alpha^{-/low}$ and $Siglec\text{-}H^-Ly6C^-$ for cDC1-biaised pre-DCs or $Siglec\text{-}H^-Ly6C^+$ for cDC2-biaised pre-DCs; (cMoPs): $Lin(*)^-CD11b^-CD117^+CD135^-CD115^+Ly6C^+$ and (monocytes): $Lin(*)^-CD11b^+CD115^+Ly6C^{high}$ (inflammatory monocytes) or $Ly6C^{low/neg}$ (patrolling monocytes). Dead cells were excluded by propidium iodide staining. For analysis, 5×10^5-10^6 $CD45^+$ cells were acquired and the data were analyzed with FACSDiva or FlowJo software (TreeStar, US). Lin*: CD3, NK1.1, Ter119, CD45R/B220, Ly6G.

Next-generation sequencing

For impact study of FMT, we used an Illumina Miseq (Illumina, San Diego, CA). Total DNA concentration was measured using Picogreen (Invitrogen). Global 16S gene DNA copy numbers were determined by a qPCR adapted from Maeda *et al.*, allowing for inhibition effect estimation and DNA concentration adjustment. The sequence region of the 16S rRNA gene spanning variable region V3–V4 was amplified using the broad-range forward primer: Bact-0341, 5'-CCT ACG GGN GGC WGC AG-3' and reverse primer: Bact-0785, 5'-GAC TAC HVGGG TAT CTA ATC C-3' (Klindworth et al. 2013). Individual samples were barcoded, pooled to construct the sequencing library, and then sequenced using an Illumina Miseq (Illumina, San Diego, CA) generating paired-end 2 × 300 bp reads.

Microbiome data were analyzed using QIIME 2 2020.2 [6]. Raw sequence data were demultiplexed and quality filtered with the q2-demux plugin followed by denoising with DADA2 [7]. Taxonomy was assigned to amplicon sequence variants (ASVs) using the q2-feature-classifier plugin against the SILVA 132 99% database. Phylogenetic data clustered into ASVs were further analyzed using the phyloseq package. Contaminant DNA sequences were identified and removed using the decontam package [8]. Both packages were used in R software version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria).

Statistical analyses

Statistical analysis was carried out using Prism 5 software (Graph-Pad Software, San Diego, CA). Values are expressed as mean ± SD. Comparison between groups was analyzed with nonparametric ANOVA followed by Dunn's multiple comparisons posthoc tests. Survival curves were analyzed using the Log-rank (Mantel-Cox) test. Significance was accepted at $p < 0.05$

Results

Prior oral non-absorbable antibiotic-induced gut dysbiosis worsens outcomes ofP. aeruginosalung infection.

First, we assessed if prior oral non-absorbable antibiotic modified outcomes of *P. aeruginosa* acute pneumonia. Antibiotics administered in drinking water seven days before *P. aeruginosa* intra-nasal challenge were associated with worse infection outcomes (Fig. 1) as shown by increased lung bacteria load (Fig. 1A), increased extrapulmonary bacterial dissemination to the spleen (Fig. 1B) and increased lung injury (Fig. 1C) when compared to untreated controls. Finally, the survival of antibiotic-treated mice decreased compared to untreated controls (Fig. 1D). Overall, acute sublethal *P. aeruginosa* pneumonia became lethal in antibiotic-treated mice compared to untreated controls.

Second, we assessed if oral non-absorbable antibiotics induced gut dysbiosis. The gut microbiota from antibiotic treated-mice before *P. aeruginosa* intra-nasal challenge was altered compared to control (Fig. 1E). We also observed an eradication of several bacterial families like *Muribaculaceae*, *Prevotellaceae*, and *Lachnospiraceae* and an expansion of *Burkholderiaceae*, *Clostridiales*, and *Lactobacillaceae*. Likewise, gut microbiota diversity (Fig. 1F) by one log of 16srDNA compared to untreated mice (supplemental Fig. 3). As expected, we did not observe any modification of the effect of prior oral non-absorbable on the composition and the diversity of the lung microbiota before *P. aeruginosa* infection (Fig. 1G).

Finally, to demonstrate the causality of gut dysbiosis in worse outcomes of *P. aeruginosa* infected mice, we treated dysbiosis using fecal microbiota transplant (FMT). Following dysbiosis-inducing antibiotics, FMT before *P. aeruginosa* inoculation restored pneumonia outcomes to those of control mice (Fig. 1A, B, C, D). FMT also restored the composition and the diversity of the microbiota equivalent to controls (Fig. 1E, F) and did not modify the lung microbiota (Fig. 1H). Of note, *P. aeruginosa* intra-nasal challenge did not further modify the gut microbiota shift observed in antibiotic-treated mice (supplemental Fig. 4). As expected, *P. aeruginosa* intra-nasal challenge resulted in a lung microbiota dominated by *Pseudomonadaceae* (supplemental Fig. 4).

Oral non-absorbable antibiotic-induced gut dysbiosis results in widespread lung and spleen immune depression associated with altered myelopoiesis.

Next, we assessed whether antibiotic-induced gut dysbiosis had any effects on baseline lung and circulating cellular immune profiles as a potential mechanism underlying worse outcomes following *P. aeruginosa* lung infection.

Cell population analysis in lung tissue showed that antibiotics resulted in widespread depression of lung cellular immunity with a significant decrease in alveolar macrophages, cDC2, patrolling monocytes, neutrophils, γδ-T cells, NKs, and iNKT cells (Fig. 2A). FMT restored most of these alterations (Fig. 2A),

establishing a significant role of antibiotic-induced gut dysbiosis in this widespread depression of the lung immune response.

Likewise, antibiotics resulted in widespread depression of most studied spleen immune cell populations ($p < 0.05$ except for a trend in NK cells), also partially restored by FMT (Fig. 2B).

Because the spleen is a hematopoietic organ in mice, we sought to determine the effects of antibiotics on immune cell hematopoietic factors, specifically on circulating serum levels of GM-CSF, M-CSF, and Flt3-Ligand (Fig. 3A). While circulating levels of GM-CSF were at the detection threshold and remained unaltered, antibiotics induced a significant decrease in Flt3-Ligand, and only a trend in M-CSF decrease. Given the known role of Flt3-Ligand as a major hematopoietic stimulating factor, mainly for monocyte and DC progenitors [9], we studied the effects of antibiotics and FMT on bone marrow monocyte and DC progenitors (Fig. 3B). We found that antibiotics were associated with a significant decrease in bone marrow progenitors specific to resident monocytes (MonoLy6C-) and several specific to DCs (total pre-DCs, pre-DCs 1 and 2 and cCD2 biased pre-DCs). Among these, FMT following antibiotics restored levels of pre-DCs 1. FMT also stimulated the expansion of bone marrow progenitors common to monocytes/DCs (MDP), common dendritic cell progenitors (CDP), and specific to monocytes (cMoPs). Our results suggest that the effects of antibiotic-induced dysbiosis on monocyte and DC progenitors may be involved in the widespread antibiotic-induced lung immune depression associated with worse *P. aeruginosa* lung infection outcomes.

Hematopoietic cytokine Flt3-L stimulates bone marrow progenitor and lung immune cell expansion and restores outcomes ofP. aeruginosalung infection following oral non-absorbable antibiotics.

Systemic Flt3-L following antibiotics (Fig. 4A) stimulated the expansion of several progenitors (MDPs, CDPs, and cDC-1 biased pre-DCs). Flt3-Ligand administration partially restored or overstimulated the expansion of depressed alveolar macrophages, cDC2, patrolling monocytes, neutrophils, NK, and iNKT (Fig. 4B).

Finally, the effects of Flt3-Ligand administration following antibiotics on the outcomes of sublethal *P. aeruginosa* lung infection were similar to those of FMT: outcomes were restored to levels not significantly different from controls without antibiotics (Fig. 4C, 4D, 4E). Flt3-Ligand was also associated with decreased mortality in a lethal *P. aeruginosa* lung infection model similar to the effects of FMT (Fig. 4F).

Discussion

Our results show that oral non-absorbable antibiotics induce gut dysbiosis, without lung dysbiosis, leading to a widely depressed lung immune cellular response responsible for worse outcomes of

subsequent *P. aeruginosa* lung infection. These effects involve bone marrow progenitor depression and are susceptible to therapeutic immunomodulation by the hematopoietic cytokine Flt3-L as well as fecal microbial transplant.

The first description linking altered gut microbiota and outcome of bacterial lung infection used germ-free mice infected with *Klebsiella pneumoniae* [10]. Several authors described afterward that the gut microbiota protects against respiratory infection by *Streptococcus pneumoniae*, *K. pneumoniae*, and *P. aeruginosa* [4] [11] [3]. However, these studies used very broad-spectrum antibiotic regimens, which lead to a significant depletion of overall bacterial 16srDNA in the gut. We observed a reduction from 10^6 to 10^2 gene copies/ng DNA [3], which is closer to “germ-free-like” models than to clinically relevant antibiotic regimens. In comparison, our model's gut dysbiosis was mainly due to shifts in phyla, not to extreme overall depletion. We observed depletion of only 1 log copies 16 s rDNA (supplemental Fig. 3) rather than 4 log copies 16 s rDNA [3]. Therefore, our data suggest that eradicating some phyla and/or expansion of others is sufficient to alter the responses to lung infection independently of significant/complete overall gut microbiome depletion.

Conversely to lung dysbiosis associated with altered outcomes like asthma [12], our treatment did not significantly modify the lung microbiota, suggesting other links between the observed gut dysbiosis and lung outcomes. Since we used non-absorbable oral antibiotics, this treatment had no direct effect on the lung microbiota. In contrast, this may have been the case in other dysbioses induced by systemically diffusing antibiotics, as in the study of Robak *et al.* in which lung microbiota depletion was confirmed [3]. Therefore, lung dysbiosis is not a requirement for the deleterious effects of prior antibiotics, and lung dysbiosis may rather be another collateral damage when antibiotics diffuse systemically to the lung.

In previous studies in which antibiotic-induced gut dysbiosis was shown to worsen bacterial pneumonia outcomes, the study of lung immune responses was focused in scope. Indeed, assessment of lung immunity restricted to macrophages showed that macrophage function was impaired consecutively to antibiotic-induced gut dysbiosis and involved in worse outcomes in an *S. pneumoniae* pneumonia model [11]. Likewise, lung immunity assessment restricted to IgA-producing cells found that IgA production was impaired in the lung and involved in worse outcomes in a *P. aeruginosa* pneumonia model [3].

We conducted a broader assessment of the immune response after antibiotic-induced gut dysbiosis and observed widespread lung cellular immune depression. Immune depression included cells crucial to the clearance of *P. aeruginosa* from the lung, such as alveolar macrophages, non-conventional lymphocytes, and neutrophils. Bone marrow progenitors of monocytes and DC were depressed and partially corrected by FMT, suggesting the involvement of immune cell hematopoiesis. A link between antibiotic-induced dysbiosis and depressed haematopoiesis has been demonstrated. In a non-infectious model [13] and in mice infected by Flaviviridae [14].

We found that FMS-like tyrosine kinase 3 ligand (Flt3-Ligand) was significantly decreased by antibiotic-induced gut dysbiosis, and partially restored by FMT. Flt3-Ligand is a cytokine that acts as a

hematopoietic growth factor for early progenitors through its receptor, Flt3, in synergy with other cytokines [9]. When administered after antibiotics, Flt3-L: a) restored or stimulated the expansion of several monocytes and dendritic cells (DC) bone marrow progenitors, b) restored or stimulated immune lung cell populations, c) restored outcomes of sublethal *P. aeruginosa* lung infection and d) increased survival of lethal *P. aeruginosa* lung infection. Flt3-Ligand promotes the generation of a primarily myeloid cell containing colonies. Flt3-Ligand is essential is to the generation of DC. Furthermore, Flt3-Ligand transgenic mice show a significant expansion of Flt3-positive cells and progenitors (myeloid cells, DCs, MPP, CMP, Granulocyte-Macrophage Progenitors, CLP, and EPLM progenitors) [15]. Administration of human Flt3-Ligand into mice increases DC-marker positive cells in the bone marrow, liver, Peyer's patches, thymus, peritoneum, and lung [16]. In a murine model of influenza A virus infection, Beshara *et al.* showed that Flt3-L overexpression reduced the dissemination of *S. pneumoniae* instilled into the lungs by enhancing bone-marrow cDC progenitors and restoring lung cDCs [17]. These results suggest that its protective effect is not limited to *P. aeruginosa*.

Conclusion

Contrary to emerging controversial postulates [18], the gut microbiota, independently from the lung microbiota, might be crucial to developing an appropriate lung immune response against *P. aeruginosa*.

Treating antibiotic-induced dysbiosis through FMT or treating the associated lung immune depression through Flt3-Ligand restore *P. aeruginosa* lung infection outcomes in mice, suggesting that both may be therapeutic options to modulate the immune consequences of antibiotic-induced gut dysbiosis. Interestingly, recombinant human Flt3-L (CDX-301, Celldex Therapeutics, Hampton, NJ) is currently undergoing over 30 clinical trials to treat various malignancies.

Furthermore, given the FMT recent safety issues [19], it is of particular interest that deleterious effects of prior antibiotics on lung immunity may be modulated beyond the gut microbiome.

Declarations

Ethics approval and consent to participate:

The national ethics committee for animal study approved all experiments (APAFIS number 7166).

Consent for publication:

Not applicable

Availability of data and material :

Not applicable

Competing interests:

The authors declare that they have no competing interests

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Author contributions:

MB, TG, CF, MF, DB and RLG contributed to data acquisition, analysis and interpretation, RD, KF, CF, BG, PG and EK contributed to data interpretation and critically revised the manuscript. RD, BG, PG, and EK conceived and designed the study. All authors substantially contributed to editing, revising, and finalizing the manuscript. All authors approved the final manuscript.

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References

1. Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat. Rev. Microbiol. 2011;9:233–43.
2. Dang AT, Marsland BJ. Microbes, metabolites, and the gut-lung axis. Mucosal Immunol. 2019;352:539.
3. Robak OH, Heimesaat MM, Kruglov AA, Prepens S, Ninnemann J, Gutbier B, et al. Antibiotic treatment-induced secondary IgA deficiency enhances susceptibility to Pseudomonas aeruginosa pneumonia. J. Clin. Invest. 2018;128:3535–45.
4. Brown RL, Sequeira RP, Clarke TB. The microbiota protects against respiratory infection via GM-CSF signaling. Nat Commun. 2017;8:1512.
5. Boutoille D, Marechal X, Pichenot M, Chemani C, Guery B, Faure K. FITC-albumin as a marker for assessment of endothelial permeability in mice: comparison with 125I-albumin. Exp. Lung Res. 2009;35:263–71.
6. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 2019;37:852–7.

7. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods. 2016;13:581–3.
8. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018;6:226.
9. Tsapogas P, Mooney CJ, Brown G, Rolink A. The Cytokine Flt3-Ligand in Normal and Malignant Hematopoiesis. Int J Mol Sci. 2017;18:1115.
10. Fagundes CT, Amaral FA, Vieira AT, Soares AC, Pinho V, Nicoli JR, et al. Transient TLR activation restores inflammatory response and ability to control pulmonary bacterial infection in germfree mice. J. Immunol. 2012;188:1411–20.
11. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa e Melo F, Roelofs JJTH, de Boer JD, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. Gut. 2016;65:575–83.
12. Russell SL, Gold MJ, Willing BP, Thorson L, McNagny KM, Finlay BB. Perinatal antibiotic treatment affects murine microbiota, immune responses and allergic asthma. Gut Microbes. 2013;4:158–64.
13. Josefsdottir KS, Baldridge MT, Kadmon CS, King KY. Antibiotics impair murine hematopoiesis by depleting the intestinal microbiota. Blood. 2017;129:729–39.
14. Thackray LB, Handley SA, Gorman MJ, Poddar S, Bagadia P, Briseño CG, et al. Oral Antibiotic Treatment of Mice Exacerbates the Disease Severity of Multiple Flavivirus Infections. Cell Rep. 2018;22:3440–6.
15. Tsapogas P, Swee LK, Nusser A, Nuber N, Kreuzaler M, Capoferri G, et al. In vivo evidence for an instructive role of fms-like tyrosine kinase-3 (FLT3) ligand in hematopoietic development. Haematologica. 2014;99:638–46.
16. Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. J. Exp. Med. 1996;184:1953–62.
17. Beshara R, Sencio V, Soulard D, Barthélémy A, Fontaine J, Pinteau T, et al. Alteration of Flt3-Ligand-dependent de novo generation of conventional dendritic cells during influenza infection contributes to respiratory bacterial superinfection. Sant AJ, editor. PLoS Pathog. 2018;14:e1007360.
18. Roquilly A, Torres A, Villadangos JA, Netea MG, Dickson R, Becher B, et al. Pathophysiological role of respiratory dysbiosis in hospital-acquired pneumonia. Lancet Respir Med. 2019;7:710–20.
19. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant. N. Engl. J. Med. 2019;381:2043–50.

Figures

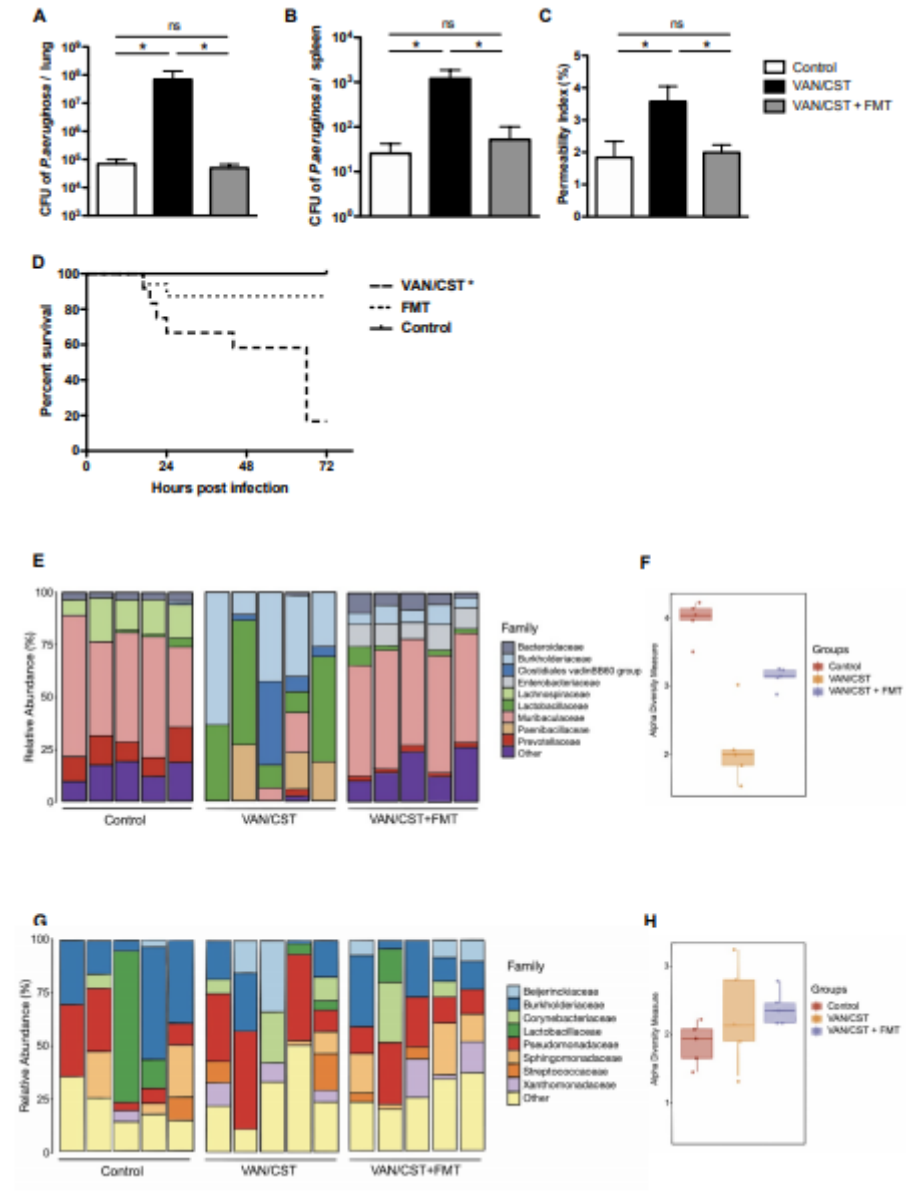

Figure 1

Gut microbiota and lung infection by P. aeruginosa. (A) (B) Lung and spleen bacterial loads and (C) alveolar-capillary barrier permeability measured 24-hours after intranasal sub-lethal P. aeruginosa (PAO1), in mice previously treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not. (D) 72-hour survival of mice challenged with sublethal P. aeruginosa (PAO1) treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not. All experiments, 5 mice per group, except for mortality, 10 mice per group in duplicate; results are shown as mean ± SD; *: $p<0.05$. (E) Bacterial family as percentages of sequenced 16S rDNA in the stool of uninfected mice treated or not by oral vancomycin (VAN) and colistin (CST) or fecal microbiota transplant (FMT); (F) Alpha diversity (Shannon index) of distinguishable taxa in sequenced stool samples; (G) Bacterial family as percentages of sequenced 16S rDNA in the stool of uninfected mice treated or not by oral vancomycin (VAN) and colistin

(CST) or fecal microbiota transplant (FMT); (H) Alpha diversity (Shannon index) of distinguishable taxa in sequenced stool samples. All microbiota experiments, 5 mice per group.

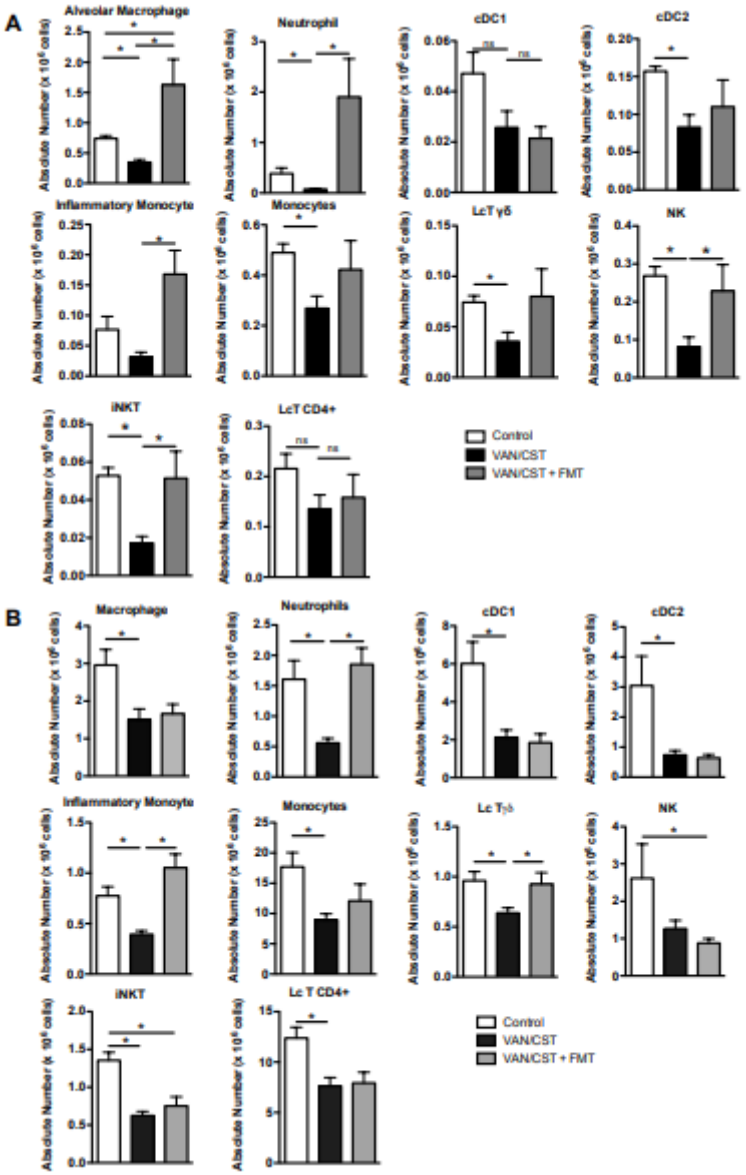

Figure 2

Effects of oral non-absorbable antibiotics on lung and spleen immune cell populations. (A) Flow cytometry of immune cells from lung homogenates in mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not. (B) Flow cytometry of immune cells from spleen homogenates in mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not. All experiments, group size 5 mice per group, results are shown as mean ± SD; *: $p<0.05$.

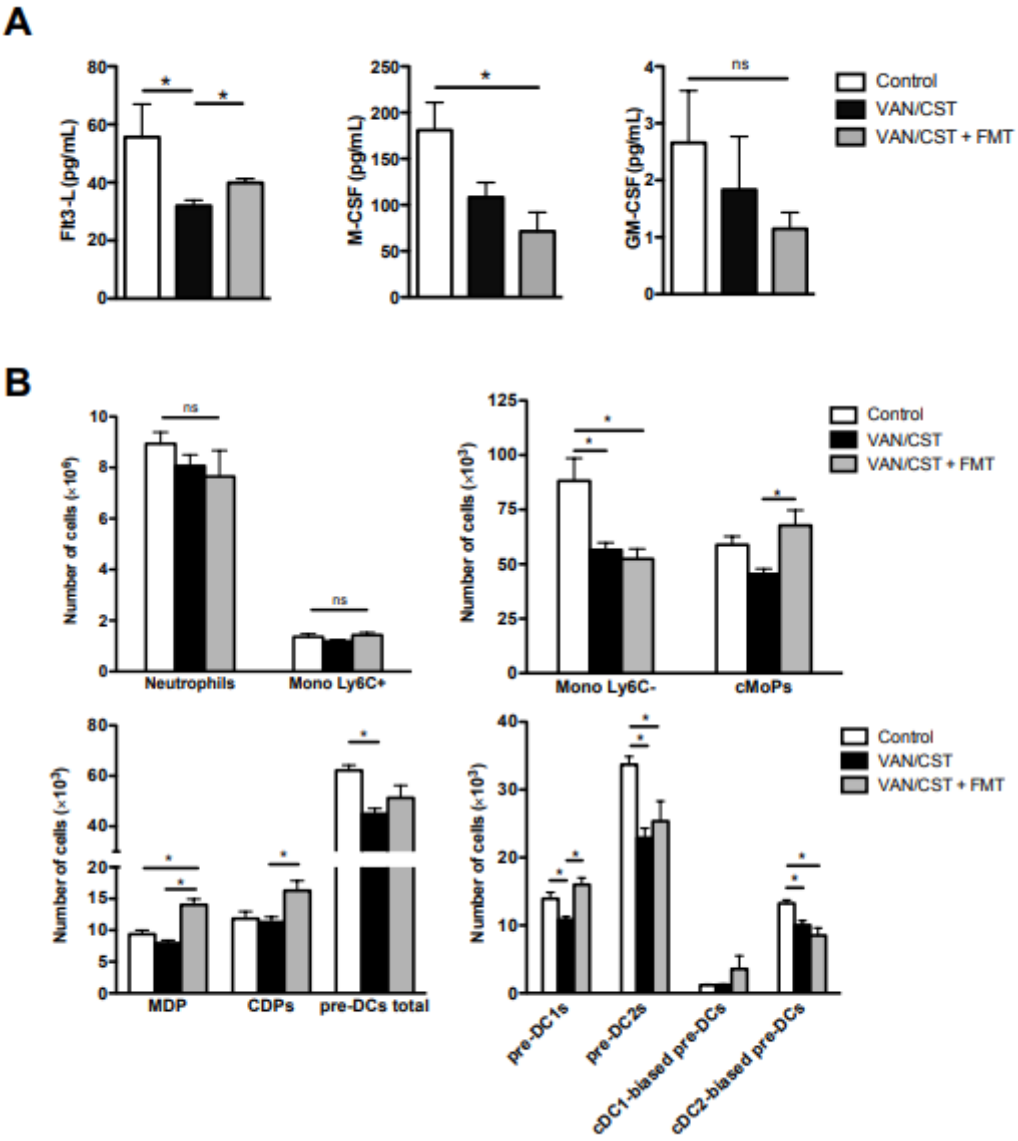

Figure 3

Effects of oral non-absorbable antibiotics on hematopoietic factors and bone marrow monocyte/dendritic cell progenitors. (A) ELISA of hematopoietic factors in serum of mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not, (B) Flow cytometry of monocyte/DC progenitors in the bone marrow in mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by fecal microbiota transplant (VAN/CST/FMT) or not. For all experiments, group size 5 mice per group; results are shown as mean ± SD; *: $p<0.05$.

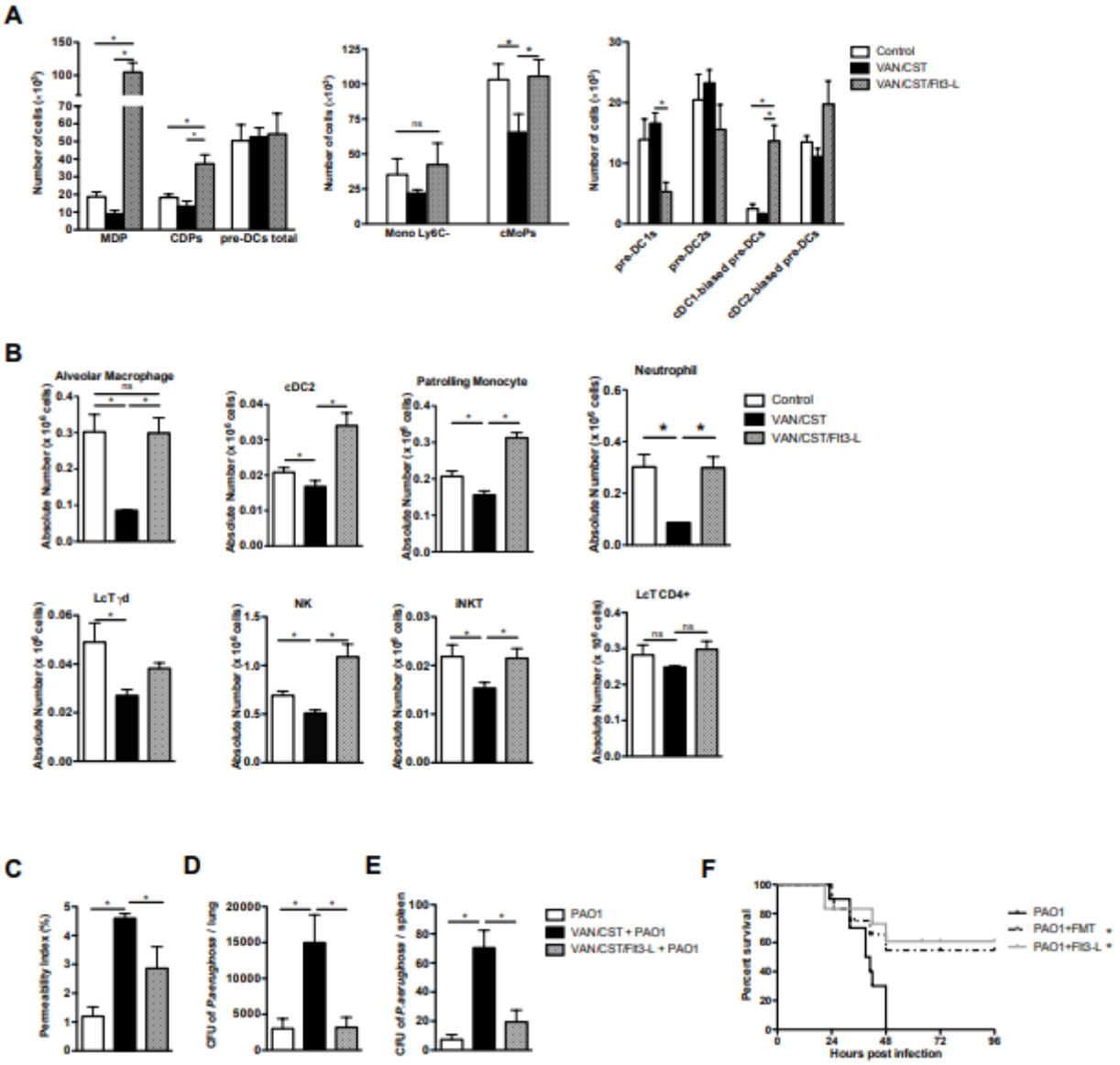

Figure 4

Effects of Flt3-Ligand on lung immune cell populations and on outcomes of lung infection. (A) Flow cytometry of monocyte/DC progenitors in the bone marrow of mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by systemic Flt3-Ligand administration (VAN/CST/Flt3-L) or not, (B) Flow cytometry of immune cells from lungs of mice with 7-days oral vancomycin/colistin (VAN/CST) followed by systemic Flt3-Ligand administration (VAN/CST/Flt3-L) or not. (C-D) Lung and spleen bacterial counts and (E) alveolar-capillary barrier permeability, measured in mice treated or not (controls) with 7-days oral vancomycin/colistin (VAN/CST) followed by systemic Flt3-Ligand administration (VAN/CST/ Flt3-L) or not. For all experiments, group size 5 mice per group; results are shown as mean ± SD; *: $p<0.05$; (E) Survival of mice after lethal P. aeruginosa pneumonia treated or not by FMT or Flt3-L, group size 10 mice per group; *: $p<0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplemental4.pdf
- Supplemental3.pdf
- Supplemental2.pdf
- Supplemental1.pdf