The role of the intestinal microbiota in pneumonia and sepsis

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1.
General introduction

The intestinal microbiota

Humans carry with them trillions of bacteria, viruses, fungi and archaea that are collectively called the human microbiota. The number of bacterial cells in our body is as large as the number of human cells; the collective genome of the microbiota (the microbiome) is even larger than the human genome [1, 2]. We have co-evolved with these microorganisms for thousands of years, most probably to a mutual benefit.

Most of our resident bacteria are located in the gut, where they fulfill essential functions such as the breakdown of nutrients [1, 3]. However, since most of these bacteria cannot be cultured, we know very little about them; until very recently the intestinal microbiota and its interactions with the human body were largely unexplored terrain. With the development of sequencing techniques, using the bacterial gene for ribosomal subunit 16S as a unique phylogenetic classifier, a new research field has opened up. Currently, the role of the intestinal microbiota in a whole range of diseases and conditions is being investigated.

One of the most prominent breakthroughs has been the finding of the tremendous therapeutic effect of fecal transplantation for severe recurrent *Clostridium difficile* infection – far better than classical antibiotic therapy [4]. Using fecal transplantation as therapy for *C. difficile* infection has a strong theoretical basis, as it is thought that the disease is caused by eradication of the normal microbiota by antibiotics, leaving an open niche for pathogens. The same could hold true for inflammatory bowel diseases (IBD), where an incorrect immune response to resident gut bacteria could play an important role in pathogenesis of intestinal inflammation [5]. Several randomized trials using fecal transplantation for IBD are currently being performed; results from the first studies are not yet conclusive [5, 6]. Furthermore, obesity and metabolic diseases are likely to be influenced by the intestinal microbiota to some extent. Associative studies have shown that the increase in antibiotic usage – especially early in life - and the prevalence of obesity go hand in hand [7, 8]. The agricultural sector has been familiar with this phenomenon for over 70 years, feeding livestock antibiotics to increase its weight [8]. Lastly, the worldwide increase in allergic diseases has long been suspected to somehow be related to our decreased interaction with microbes, a theory also known as the hygiene hypothesis. New evidence is pointing to a positive correlation between the use of antibiotics in childhood and the incidence of allergic diseases [7]. Whether the intestinal microbiota also affects the innate immune system during systemic bacterial infections such as pneumonia-derived sepsis is as yet unknown.

Pneumonia and sepsis

Pneumonia is the third most common cause of death in the world [9]. Generally, pneumonia is subdivided into community-acquired and hospital-acquired pneumonia, as these are caused by different pathogens. The most common cause of community-acquired pneumonia is *Streptococcus pneumoniae*, also known as pneumococcus [10]. When

pathogenic bacteria such as *S. pneumoniae* enter the airways, several mechanisms of host defense are initiated. The innate immune system, which includes cells such as neutrophils and macrophages, is very important in the early phases of infection.

When host defenses fail and bacteria are able to spread throughout the body, sepsis may develop. This condition is characterized by a set of symptoms, including hyper- or hypothermia, tachycardia, tachypnea and leukocytosis or leukopenia [11]. The pathogenesis of sepsis is poorly understood; formerly it was thought to be mainly caused by an uncontrolled proinflammatory immune response or "cytokine storm" [12]. Most clinical trials in the past were aimed at dampening this exaggerated immune response, but these trials have largely failed [13]. Current theories state that both a hyper- and hyporesponsive phase are important in the course of sepsis [14]. The hyporesponsive state of the immune system ("immunoparalysis") leads to secondary infections that are an important cause of death in the Intensive Care Unit (ICU) [12]. New insights have thus inspired researchers to investigate possibilities to boost the immune system during sepsis.

Melioidosis

Melioidosis, caused by *Burkholderia pseudomallei*, is an endemic infectious disease in Southeast Asia and Australia [15]. The majority of admitted patients presents with pneumonia derived sepsis, but there is a wide range in clinical manifestations, including skin- and urinary tract infections. The disease has a high mortality, varying from 10% in Australia to 40% in Thailand [16]. No vaccine is currently available. *B. pseudomallei* is registered as a Tier 1 select agent due to its intrinsic resistance to standard antimicrobial agents, potential for easy dissemination and lack of a vaccine [17]. Melioidosis is considered to be a good model for Gram-negative sepsis [18].

Aim of this thesis

It has been suggested that the intestinal microbiota provides signals that stimulate or "prime" the systemic innate immune system [19]. These signals could be either bacterial components, such as peptidoglycan or lipopolysaccharide (LPS) from the cell wall, or bacterial products, such as short chain fatty acids (SCFA). This hypothesis has some parallels with the concept of trained immunity, which states that the innate immune system does have some kind of memory [20]. After infection or vaccination, innate immune cells were reported to display increased responsiveness upon secondary stimulation with microbial pathogens, increased production of inflammatory mediators and enhanced capacity to eliminate infection [21]. Several papers have now shown that decreased exposure to gut bacteria (i.e. mice that are born under germ-free conditions or that are treated with antibiotics) is associated with less effective host defenses during systemic bacterial infections [19, 22, 23].

Evidence of a so-called “gut-lung axis” is slowly accumulating [24-26]. Low intestinal microbial diversity and recurrent use of antibiotics in early infancy have been associated with the incidence of asthma [7, 25]. In mouse models, microbiota modulation through either antibiotic treatment or the amount of fiber in diet was shown to modulate allergic airway inflammation [27, 28]. Likewise, epidemiological studies provided evidence that a diet high in fiber is associated with better lung function and reduced risk of COPD [26]. A similar link seems to exist between gut microbiota and innate host defenses during bacterial pneumonia. In both Gram-negative and Gram-positive mouse pneumonia models using *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*, a protective effect from healthy, diverse intestinal microbiota was observed [29-31]. Defective immune responses in mice with a disturbed microbiota consisted mostly of decreased neutrophil numbers and -functions, but also alveolar macrophage functions were reported to be altered. If intestinal microbiota disruption by antibiotics indeed lowers innate immune defenses, this would be very important for all patients receiving antibiotics. This may be of special relevance for critically ill patients, as they receive a lot of antibiotics and are known to experience critical defects in their immune system.

The overall aim of this thesis was therefore to investigate the effect of the gut microbiota on innate immune responses during pneumonia and sepsis, using a translational approach. Our main hypothesis was that the gut microbiota plays a protective role in the host defense against systemic bacterial infections; i.e., that microbiota disruption by antibiotics would negatively affect the innate immune system during pneumonia and sepsis.

The first part of this thesis consists of human studies. In healthy subjects, we investigated the effect of antibiotic microbiota disruption on *ex vivo* innate immune responses (chapter 3). Next, we aimed to further explore our findings from this study *in vivo*, again in healthy subjects. We used the human endotoxemia model to compare innate immune responses in control- and antibiotic treated subjects (chapter 4). Very little is known yet about the precise effects of broad-spectrum antibiotics on the intestinal microbiota. In chapter 5, we investigated the fecal microbiota composition of ICU patients, comparing different groups of septic and non-septic critically ill patients. Chapter 6 describes the long-term effects on the intestinal microbiota of the broad spectrum antibiotics that were given to the healthy subjects from chapter 3 and 4.

In the second part of this thesis we investigated the effect of antibiotic induced microbiota disruption on host responses during pneumonia and sepsis in murine models. Mouse studies provide an opportunity to eliminate as many confounders as possible and are therefore suitable for gaining mechanistic insights. We tested our hypothesis during Gram positive *S. pneumoniae* pneumonia (chapter 7), sterile LPS-induced lung inflammation (chapter 8) and Gram negative *B. pseudomallei* pneumonia (melioidosis) (chapter 9). Chapter 10 describes the role of the intestinal microbiota in the phenotype of Toll like receptor (TLR)-5 deficient mice during melioidosis. Lastly, chapter 11 is the odd one out: here, we tested a DNA vaccine against *B. pseudomallei* flagellin as a candidate for protection against aerosolized *B. pseudomallei*.

References

1. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010;464:59-65.
2. Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell* 2016;164:337-340.
3. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207-214.
4. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N Engl J Med* 2013;368:407-415.
5. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis. *Gastroenterology* 2015;149:110-118 e114.
6. Moayyedi P, Surette MG, Kim PT, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. *Gastroenterology* 2015;149:102-109 e106.
7. Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun* 2016;7:10410.
8. Cox LM, Blaser MJ. Antibiotics in early life and obesity. *Nat Rev Endocrinol* 2015;11:182-190.
9. WHO. http://www.who.int/mediacentre/factsheets/fs310/en/. In.
10. van der Poll T, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* 2009;374:1543-1556.
11. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;101:1644-1655.
12. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. *Lancet Infect Dis* 2013;13:260-268.
13. Opal SM, Dellinger RP, Vincent JL, et al. The next generation of sepsis clinical trial designs: what is next after the demise of recombinant human activated protein C?*. *Crit Care Med* 2014;42:1714-1721.
14. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* 2013;13:862-874.
15. Wiersinga WJ, Currie BJ, Peacock SJ. Melioidosis. *N Engl J Med* 2012;367:1035-1044.
16. Currie BJ. Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. *Semin Respir Crit Care Med* 2015;36:111-125.
17. CDC. http://www.selectagents.gov/SelectAgentsandToxinsList.html. In.
18. Simpson AJ. Melioidosis: a clinical model for gram-negative sepsis. *J Med Microbiol* 2001;50:657-658.
19. Clarke TB, Davis KM, Lysenko ES, et al. Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat Med* 2010;16:228-231.
20. Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. *Cell Host Microbe* 2011;9:355-361.
21. Netea MG, Joosten LA, Latz E, et al. Trained immunity: A program of innate immune memory in health and disease. *Science* 2016;352:aaf1098.
22. Deshmukh HS, Liu Y, Menkiti OR, et al. The microbiota regulates neutrophil homeostasis and host resistance to Escherichia coli K1 sepsis in neonatal mice. *Nat Med* 2014;20:524-530.
23. Khosravi A, Yanez A, Price JG, et al. Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host Microbe* 2014;15:374-381.
24. Budden KF, Gellatly SL, Wood DL, et al. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol* 2017;15:55-63.

25. Marsland BJ, Trompette A, Gollwitzer ES. The Gut-Lung Axis in Respiratory Disease. *Ann Am Thorac Soc* 2015;12 Suppl 2:S150-156.
26. Young RP, Hopkins RJ, Marsland B. The Gut-Liver-Lung Axis. Modulation of the Innate Immune Response and Its Possible Role in Chronic Obstructive Pulmonary Disease. *Am J Respir Cell Mol Biol* 2016;54:161-169.
27. Trompette A, Gollwitzer ES, Yadava K, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 2014;20:159-166.
28. Kim YG, Udayanga KG, Totsuka N, et al. Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE(2). *Cell Host Microbe* 2014;15:95-102.
29. Bernard H, Desseyn JL, Bartke N, et al. Dietary pectin-derived acidic oligosaccharides improve the pulmonary bacterial clearance of Pseudomonas aeruginosa lung infection in mice by modulating intestinal microbiota and immunity. *J Infect Dis* 2015;211:156-165.
30. Clarke TB. Early innate immunity to bacterial infection in the lung is regulated systemically by the commensal microbiota via nod-like receptor ligands. *Infect Immun* 2014;82:4596-4606.
31. Gauguet S, D'Ortona S, Ahnger-Pier K, et al. Intestinal Microbiota of Mice Influences Resistance to Staphylococcus aureus Pneumonia. *Infect Immun* 2015;83:4003-4014.