The Microbiome of the Lung

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The interplay between environmental and genetic factors is believed to play a role in the induction of human disease, with infectious agents such as bacteria, viruses and parasites comprising a large subsection of environmental triggers. These infectious agents are not limited to those in the outside environment which are introduced into the body, but include as well those that are inherent in our body sites, such as the oral cavity, lung, and gastrointestinal tract. Alterations in these microbial populations, and indeed the introduction of new organisms, may induce or contribute to the development of disease. In order to understand which of those organisms are related to pathology, it is essential to know which species define the "normal" flora. This is defined by the microbiome, which is specific to a particular region or organ such as the gut, oral cavity, respiratory tree, or inguinal crease to name a few. The microbiome defines the flora of a particular region in a particular state in time and is (generally) reflective of normal flora.

Recently, the use of metagenomic screens of bacterial populations in the gastrointestinal tract has provided an idea of what "normal" might be in that region [1]. However, it must be noted that the normal profile in one individual may be abnormal for another, and these differences may also extend to particular races, genetic profiles, gender, or geographic regions. The composition of this flora may also change over time, as studies of the gut flora of neonates have shown that the percentage of prevalent bacteria changes from one generation to the next. These microbial compositions are important for defining or understanding disease in a particular organ, in addition to systemic disease or disease in a distant organ. For example, defining the microbiome of the urinary bladder and vagina may help us understand the role of urinary tract infections in autoimmune diseases. It is therefore likely that investigation of multiple body sites would provide data on local and systemic disease.

Whether or not components of the microbiome contribute to the development of disease is unclear. It may be the case that such organisms contribute to the development of disease directly, or that a dysbiosis of the normal flora is responsible for such processes. This highlights several limitations of the microbiome, in that it is limited to a particular body site and does not reflect the changes in microbial populations over time. Recently, we introduced the concept of the infectome, which related to all disease-causing organisms throughout life and is not limited to one body site [2]. Although the infectome is intended to take the microbiome a step further by providing a micropathological profile (or profiles) of a particular disease, the microbiome is essential to define the normal flora of a particular region, which can eventually be collated to define the microbiome of the entire body.

In this issue of *IMAJ*, Berger and Wunderink [3] tackle a very important topic: the current evidence for a true resident lung microbiota. Early classical culture-based studies led to the long-held belief that the human lung is sterile in normal conditions. At present, however, there is heated debate as to whether the microbiota found in the lower respiratory tract/lung is an epiphenomenon of aspirated upper respiratory tract communities, or whether those microbiota represent a permanent population able to grow in healthy as well as diseased individuals.

The controversy stems from the apparent inability – due to ethical reasons – to perform lung biopsies in healthy individuals. As the authors point out, meticulous examination of sputum presents severe limitations; as such, fluid is apparently an admixture contaminated with lower respiratory tract and upper respiratory tract organisms. Moreover, bronchoalveolar lung fluid collected by bronchoscopy is most likely contaminated by upper respiratory tract organisms as a result of a 'carry-over' phenomenon on the bronchoscope. Such difficulties are also seen in other endoscopic assessments such as those of the gastrointestinal system.

Elegant maneuvers, based mainly on the use of two bronchoscopes passing and sampling at three different levels, have been used to overcome these technical problems. Data stemming from these studies have led to the conclusion that the lower respiratory tract has a bacterial community very similar to that of the upper respiratory tract, with a biomass 2–4 logs lower in the former than in the latter community.

Examination of whole-lung samples under sterile conditions has been used to overcome these difficulties, leading to the notion that there is a small but noteworthy bacterial community within human lung tissue.

Recent culture-independent high throughput microbiological assessments, such as those based on massively parallel

pyrosequencing of bacterial 16S amplicons, have identified important changes in the lung microbiome in various lung diseases. Similar studies indicated that the lungs are also not sterile in individuals without profound lung diseases. To overcome the limitation imposed by the possibility that bacteria in the bronchoalveolar lavage samples could mirror upper airway contamination of the bronchoscopes used during the procedure, some of these studies have sampled multiple tissue sites from lung explants removed from patients undergoing transplantation for chronic obstructive lung disease. Because of the increased cost, small sample size is one of the most frequent limitations in this type of study.

There is no doubt, however, that such research designs will always be open to criticism and that researchers in the field will arguably raise their concerns as to whether the communities identified are true bacterial populations of the human lung, or communities identified due to contamination over sample acquisition.

A wealth of data being collected from ongoing studies including the National Heart, Lung and Blood Institute Lung Human Microbiome Project will help efforts to address these questions. Also, these types of errors are better addressed at the experimental level, i.e., by work performed using healthy animals or animals with lung diseases. Again, such work may provide a wealth of material, but will always leave unanswered the question as to whether findings seen in experimental models of human diseases induced in mice or other animals have immediate relevance to the 'real' thing noted in a human lung. Irrespective of that, animal studies have also provided convincing evidence that the normal human lung is not sterile.

What has become apparent from the paper by Berger and Wunderink [3] in this issue of the Journal is that research on the lung microbiome holds much promise. Most of us are totally unaware of techniques such as those used to study the bacterial composition of the healthy lung, as well as the dynamic diversity and relationship between the upper and lower airway microbiome in both healthy individuals and those with specific lung diseases [4]. For sure, assays that rely on extracting phylogenetic information from the bacterial *16S rRNA* gene through Sanger sequencing of clone libraries, microarray hybridization, T-RFLP analysis or pyrosequencing, will help us define the lung microbiome in health and disease.

Although none of these cutting-edge culture-independent techniques have reached a momentum such that they can be used in a routine clinical laboratory, lessons can be learnt and applied in studying the relationships between the gastrointestinal tract and systemic, autoimmune and other diseases. These findings lead us to believe that their incorporation in the study of the lung microbiota will radically change our thinking on host-microbiome interactions taking place within the lung.

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Capsule

The kinase mTOR modulates the antibody response to provide cross-protective immunity to lethal infection with influenza virus

Highly pathogenic avian influenza viruses pose a continuing global threat. Current vaccines will not protect against newly evolved pandemic viruses. The creation of 'universal' vaccines has been unsuccessful because the immunological mechanisms that promote heterosubtypic immunity are incompletely defined. Keating et al. found that rapamycin, an immunosuppressive drug that inhibits the kinase mTOR, promoted cross-strain protection against lethal infection with influenza virus of various subtypes when administered during immunization with influenza virus subtype H3N2. Rapamycin reduced the formation of germinal centers and inhibited class switching in B cells, which yielded a unique repertoire of antibodies that mediated heterosubtypic protection.These data established a requirement for the mTORC1 complex in B cell class switching and demonstrated that rapamycin skewed the antibody response away from high affinity variant epitopes and targeted more conserved elements of hemagglutinin. These findings have implications for the design of a vaccine against influenza virus.

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Eitan Israeli

"I am, indeed, a king, because I know how to rule myself"

Pietro Aretino (1492-1556), Italian author, playwright, poet and satirist who wielded immense influence on contemporary art and politics

"If the rich could hire someone else to die for them, the poor would make a wonderful living"

Anonymous