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MILESTONE 13

Mechanisms of colonization resistance

In 1917, as war was tearing its way across Europe, a fascinating scientific observation was being made. The German physician Alfred Nissle had been looking for novel therapies to tackle enteric infections, which in this pre-antibiotic era represented an enormous burden on troops. He noted that one soldier in particular, who had participated in a military campaign in the Balkans, proved stubbornly resistant to dysentery when many of his comrades had been laid low by the disease. Speculating that a component of this soldier's intestinal microbiota might be responsible for this resistance, Nissle acquired stool samples and was able to isolate a strain of bacteria that came to be known as *Escherichia coli* Nissle 1917. Laboratory testing, as well as some self-experimentation on the part of Nissle, showed that this novel strain of *E. coli* was indeed able to antagonise pathogenic bacteria and it soon entered clinical practice. Although his identity is lost to history, the soldier's donation of his unique *E. coli* strain is still used to this day as the active component of the probiotic Mutaflor.

> "The host's microbiota can manifest colonization resistance through a number of potential mechanisms"

In many ways, the findings of Nissle were built on earlier concepts articulated by the 'father' of cellular immunology Élie Metchnikoff, who in a monograph in 1910 had lauded the consumption of soured milk (rich in bacteria) as a means to stave-off infectious disease and enhance human longevity. Indeed, peasants from the Balkans and Caucasus had long-been famous not only for their centenarians but also for millennia-old traditions of yoghurt-making. However, while it seemed that certain strains of bacteria could have beneficial properties on their host, perhaps in part through their direct antagonism of enteric pathogens, the mechanistic basis of these remarkable effects were almost wholly unknown. Arguably, the first in-roads into this question were made in the mid-1920s, in Belgium, by an often-overlooked early pioneer of microbiology — André Gratia. Along with his colleagues, most notably Sarah Dath, Gratia observed antagonism between co-cultures of different strains of *E. coli*. This effect was attributed to a secreted factor, which came to be known as 'colicin'. This protein now represents the first described member of an unrelated family of narrow-spectrum, bacterially-produced antibiotics known as 'bacteriocins'.

Another important step in understanding the role played by the host's microbiota in resistance to enteropathogenic bacteria was made in the 1950s, by Marjorie Bohnhoff and colleagues at the University of Chicago, and subsequently in the early 1970s by Dirk van Waaij and colleagues in the Netherlands. Secondary infections are a common occurrence following a course of antibiotics, suggesting that some perturbation of the microbiota might be responsible. In order to model this phenomenon, these studies found that mice that had their microbiota heavily-depleted by antibiotics were drastically more susceptible to oral challenge with even mildly pathogenic strains of *Salmonella* or *E. coli*. A 1971 paper by van Waaij and colleagues was especially important for its coining of the term 'colonization resistance' and placing it into a quantitative framework.

The host's microbiota can manifest colonization resistance through a number of potential mechanisms, for example, 'passively' by out-competing bacteria for space and trophic resources, or more actively by the generation of bacteriocidal factors. Three key papers in 2007 illuminated different aspects of colonization resistance. The probiotic strain *Lactobacillus salivarius* UCC118 is known to produce the bacteriocin Apb118. Conor Gahan and colleagues observed that this probiotic strain could protect mice against infection with *Listeria monocytogenes* and this effect was wholly dependent on the production of Apb118. However, *L. salivarius* UCC118 also conferred protection against a strain of *Salmonella* resistant to Apb118, suggesting that colonization resistance by this probiotic is more multi-faceted than

simply the production of a bacteriocin. A second pair of unrelated papers set out to understand how enteropathogens could overcome colonization resistance. In separate mouse studies and using different enteropathogens (*Citrobacter rodentium* or *Salmonella*), it was shown that intestinal inflammation altered the composition of the host's microbiota and made them susceptible to colonization by the invading bacteria. In both cases the bacteria needed to be able to elicit gut inflammation in order to establish themselves — in other words, this appeared to be a case of the enteropathogen co-opting the host's immune response to its advantage.

Colonization resistance has proved to be a useful model for understanding the dynamics of microbial communities in the gut and other barrier surfaces, such as the skin, however in one sense it is strikingly similar to the much earlier ecological concept of 'allelopathy'. Initially outlined in the 1930s to describe interactions between certain plant species, allelopathy was later broadened to describe the suppression of any competitor organism by another through the generation of biologically active factors.

As we teeter towards the dangers a post-antibiotic era, further insights from the study of colonization resistance could offer the hope of novel antimicrobial therapies.

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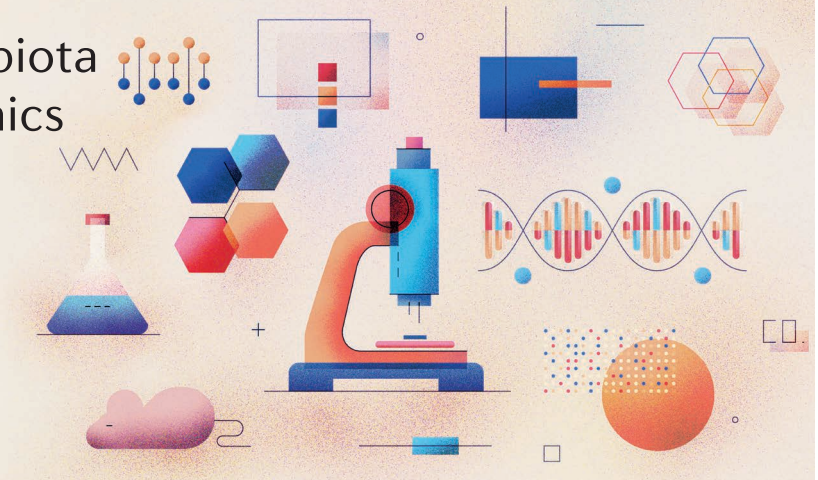
MILESTONE 14

Functional human microbiota analyses in vivo using 'omics technologies



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Eline Klaassens and colleagues applied a metaproteomics approach to uncultured faecal microbiota, providing the first insights beyond taxonomic identification. This was followed by numerous studies using 'omics methods, such as metabolomics and metatranscriptomics, as well as the development of multi-omics pipelines; methods that are still uncovering the functions of the microbiota today.

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