CONCLUSION

The clinical importance of intestinal microbiota

L’importance clinique du microbiote intestinal

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Summary Clinicians have long learned of the pathogenic microorganisms that cause intestinal infections and that can be identified by culture methods from fecal, mucosa and blood samples. Nevertheless, much progress has been made due, in large part, to molecular biology which has enabled the discovery of pathogens that are not (yet) able to be cultured, but also to the protective role of some microorganisms within the microbiota. Ecological and clinical disturbances related to antibiotic therapy were decisive for demonstrating the beneficial effects of the endogenous microbiota and establishing the beneficial effects of biotherapeutic agents, known as probiotics. Most areas of gastroenterology (cryptogenic inflammatory bowel disease, irritable bowel syndrome, lymphomas, etc.), but also those in medicine overall (obesity for example), are affected by this new approach and are showing very significant progress. This article summarizes the historical steps of these discoveries and reviews what the “modern” clinician should know in this very dynamic area in regard to research and practical consequences.

Résumé Le clinicien a depuis longtemps appris à connaître des micro-organismes pathogènes responsables d’infections intestinales et qu’il identifie par méthodes de culture dans des prélèvements fécaux, muqueux ou sanguins. Cependant de nombreux progrès ont été réalisés, en bonne partie grâce à la biologie moléculaire, qui ont permis de découvrir des pathogènes non (encore) cultivables mais aussi le rôle protecteur de certains micro-organismes au sein du microbiote. Les perturbations écologiques et cliniques liées à l’antibiothérapie ont été décisives pour mettre en évidence des effets bénéfiques du microbiote endogène et établir les effets bénéfiques d’agents biothérapeutiques - probiotiques. Rares sont les domaines de la gastroentérologie (maladies inflammatoires cryptogénétiques de l’intestin, syndrome de l’intestin irritable, lymphomes.) mais aussi de la médecine dans son ensemble (obésité par exemple) qui échappent à cette nouvelle approche et progrès très significatifs. Cet article résume les étapes historiques de ces découvertes et fait le point sur ce qu’un clinicien « moderne » doit connaître dans ce domaine très dynamique en recherche et conséquences pratiques.

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“We all need a little help sometimes.” Jean de la Fontaine

There has been a marked increase in interest regarding the role of intestinal microbiota in physiology, as well as in various intestinal and extra-intestinal diseases. Progress made in molecular biology and bioinformatics has made it easier to describe. Knowledge of its own functions and its interactions with intestinal cells (enterocytes and immune cells) has grown, along with that of the important mechanisms of ecosystem biology. This has also resulted in an evolution of the concepts. This article summarizes for clinicians the progress made, current strategies of researchers in intestinal ecology and future directions.

History

Some microorganisms are pathogens and it is normal that clinicians are interested in studying them. Others however serve as protectors and should also be investigated so that they may be considered as treatment (left alone or even used deliberately as biotherapeutic-probiotic agents). Surprisingly, the former group were discovered first; the latter were discovered through observation of gastrointestinal disturbances induced by antibiotic treatments and have begun to be better understood.

History of bad microorganisms

The pathogenic role of some microorganisms was discovered very early in some cases following the initial discovery of microorganisms; for others however, this occurred much later. The sole presence of a microorganism in a subject is not enough to implicate it in a disease. Koch’s postulates, which were introduced in 1890 [1], had the goal of determining whether a given microorganism could be causative of a disease. This determination was made based on the presence of strict criteria:

- the microorganism must be present in every case of the disease;
- it must be isolated from the disease host and cultured;
- the specific disease must be reproduced when a pure culture of the microorganism is inoculated in a “susceptible” host;
- the microorganism must then be isolated from the experimental infected host.

These criteria have contributed enormously to the scientific approach and have been used to establish the role of many microorganisms in gastrointestinal infections [1]. Their limitations however have also been progressively understood. For example, there are cases in which the microorganism can not be cultured and others that have no animal model. Lastly, in some cases the infected individuals do not all develop the disease (susceptibility).

While microorganisms were visible on simple gastric biopsies, decades passed before the role of Helicobacter pylori was recognized in the pathogenesis of duodenal ulcers, gastritis and many gastric ulcers (and more recently lymphomatous and adenocarcinomatous tumoral complications of the stomach) [2]. The pathogenic role of Clostridium difficile also took a long time to establish [3,4], and major progress was made due to the significant increase of pseudomembranous colitis when lincomamines were first introduced [4]. Before C. difficile was considered and studies were done showing the role of its toxins in the genesis of symptoms and colonic lesions, clinicians attributed pseudomembranous colitis to Staphylococcus aureus (wrongly in the large majority of cases), since this microorganism was often isolated from the stools of the same diseased patients. Although pathogenic, staphylococcus was not the real culprit, but its frequent presence associated with C. difficile resulted in antibiotic-induced intestinal ecological disorders. The lesson for clinicians is that it is important to be aware of the wide ecological implications of antibiotic-induced disorders and not to focus only on the isolated results of some microorganisms. This should also be kept in mind when opportunistic pathogens are detected in the stools or mucosa of certain subjects with Crohn’s disease more often than in healthy subjects (although still very inconsistently) [5]. Although it is possible that in some subjects pathogens such as Mycobacterium avium paratuberculosis, adherent-invasive Escherichia coli (AiEC) or C. difficile participate specifically in inflammation, in the majority of cases this occurs through a mere increase in bacterial concentration (load) in the mucus layer [6].

Whipple’s disease presents another learning opportunity. The successive major steps for detecting the causal bacteria were: i) in 1907, the first clinical description of the disease; ii) in 1952, the discovery of the clinical efficacy of chloramphenicol; iii) in 1961, the demonstration of microorganisms in the macrophages of the lesions using electron microscopy; iv) in 1992, the identification of Tropheryma whippelii (by PCR); and v) in (only!) 2000, the culture of T. whippelii was finally possible [7].

The role of Campylobacter jejuni in alpha chain disease was discovered even more recently [8]. Microbial factors were suspected in view of the clinical efficacy of antibiotics in the first stages of the disease (before extensive lymphoma with adenopathies occurs); PCR enabled identification of the molecular signature of C. jejuni on anatomical fragments of the lesions. From this it could be seen that during this disease, microorganisms are the stimulus of lymphoproliferation. Helicobacter pylorus was also understood to be the stimulus of lymphoproliferation with gastric MALT lymphoma, and of the occurrence of gastric adenocarcinoma complicating atrophic gastritis with intestinal metaplasia. These disorders have taught clinicians one other thing: that the absence of microorganisms in late lesions of the disease should not dispel its major role in its pathogenesis in earlier stages.

Among the new data and concepts that the clinician should know these days in this field, it is certainly necessary to remember the following: i) the increasing proportion of bacteriologic techniques independent of culture, especially molecular methods detecting the presence of specific sequences of microbial groups or species [and which can be amplified by polymerase chain reaction (PCR)]; and ii) the possibility of inflammation, lymphoproliferation and even cancerogenesis stimulated by microorganisms. The dogma of “one bacteria for one disease” is no longer valid. One strain of bacteria can cause several disorders, and a single disease can probably result from different bacteria. This seems to be the case for cryptogenic inflammatory bowel diseases (IBD,
Crohn’s disease and ulcerative colitis) [6]. Some explanations suggesting the role of bacterial overgrowth in the intestine even concern some forms of irritable bowel syndrome [9], colon cancer [10] and obesity [11,12].

**History of good bacteria in the intestine**

The discovery of the physiological roles of “gut flora” (the term “microbiota” should now replace this former terminology) and of some beneficial microorganisms was aided and stimulated by the observation that antibiotic treatments often result in gastrointestinal disorders. The challenge then was to understand how the disturbances would generate various types of diarrhea, pain and an increased risk of developing irritable bowel syndrome [13]. This is how the barrier effect was demonstrated in the second half of the 20th century, thus helping in the fight against the pathogens [14,15], and the beneficial consequences of fermentation in order to avoid osmotic diarrhea after ingestion of undigested sugar [16]. Some investigators devoted their careers to determining whether the barrier effect was based on a limited number of endogenous microorganisms, or conversely (which seems more probable today) to a large group [14]. One of the most important clinical consequences of their work was the improved understanding of the protector role of some probiotic microorganisms for preventing or lessening the diarrhea related to the antibiotics [17]. It has now been established in this field that a single mechanism does not exist and that, to the contrary, many mechanisms can combine to bring about the protector role of some therapeutic yeasts or bacteria. For example, the effect of *Saccharomyces boulardii* in this area (proven by many randomized, controlled clinical trials and their meta-analyses) [18] involves the quantitative reduction of *C. difficile*, the inhibition effects of its toxins and more general antisecretory effects [19].

The discovery of a very reproducible bacterial overgrowth during cryptogenic inflammatory bowel diseases, which is different during Crohn’s disease and ulcerative colitis, also teaches us a great deal [5]. The reduction and restriction of biodiversity applies to firmicutes, and this undoubtedly favors the emergence of an increased number of usually non-dominant and opportunistic bacteria [5,20]. A decline in a common bacteria (*Faecalibacterium prausnitzii*) in the fecal microbiota and the adherent microbiota of the ileal mucosa is associated with Crohn’s disease [20]. A collaborative study showed that its level influenced the risk of postoperative recurrence of the disease, and that this bacteria exerts anti-inflammatory effects in vitro on isolated cells and in vivo on experimental colitis [20]. This result stimulates research into probiotics and supports the legitimacy of this therapeutic route.

Modulation of colonic fermentation is also an important research path. Some studies focus on the immediate possibility of reducing gaseous production of some subjects and the disorders that result from it. Others focus on modulating the risk of colon cancer, particularly by promoting distal colonic fermentation and the production of butyrate [10,21-23]. The consumption of hydrogen for producing other gases (methane for example) or for acetogenesis is also a major research path for the functional disorders [24]. A lot of research has also been generated as a result of the recent discovery of the possible role of ecological disturbances in the irritable bowel; the hope is to find a better way to treat the disease when it has begun and also to better prevent it in subjects with infectious intestinal episodes or those treated with antibiotics [9,13]. The description of the bacterial overgrowth during irritable bowel syndrome is increasingly detailed, as is the breakdown of the mechanisms of action of the probiotics [25-27]. Interventional studies using probiotics on potential models and (in all cases) on humans is therefore going to continue and probably even hopefully intensify with a possible preventative focus on mild disorders (discomfort) by functional foods, and another therapeutic focus on the use of probiotic microorganisms based on solid scientific proof [25-29]. Support for the process of resistance or resilience to the disturbances (for example, those that are antibiotic-induced) is an important and robust route, as seen for example by the recent studies of Anne Collignon with *S. boulardii* [28]. Although the use of probiotics has gone through a period of doubt at a time when a very large number were able to be marketed without proof of their efficacy [29], this is no longer the case, and as for any medication, all clinicians should now be capable of accessing source scientific data and identifying the efficient products.

**Geography and ecosystems**

A major fact to be understood is that ecological conditions differ in many parts of the gastrointestinal tube, constituting many niches or ecosystems. The gastric microbiota differ from that of the upper small intestine, which itself is different from the distal small intestine and the colon. There are also differences in the luminal microbiota of the right and left colons (including for example, the bacteria producing methane); the mucosa microbiota (which is also different) however is more stable (similar) from one intestinal or colonic site to another [30,31]. Reproducible and significant differences can even be found between the superficial and deep layers of the colonic mucosa [6]. Is one of these ecosystems more relevant than others (or even “the only” relevant one) for explaining certain diseases? No one knows. The relative stability of ecosystems is no longer often seen only in the large groups of microbiota which make it up, and molecular tools targeting portions of the microbial genome which are common to the members of these “groups” are widely used [32,33]. The compositional instability of a niche of microbiota is seen in various diseases, such as IBS [5]. The main activities of microbiota are largely redundant and are carried out by microorganisms that are dominant in number, although counterexamples exist, such as the production of methane by *Archae* [24,34].

**Lessons to be learned**

As the dominant bacteria of the microbiota can not be cultured to a large extent, doctors rarely learn their names (or even of their existence). An adaptation of the vocabulary (taxonomy) of the latter is therefore essential. A major division of colonic bacteria is that of the firmicutes, and this term should be known at a time when
concordant studies show a restriction of biodiversity of this division during inflammatory bowel diseases and an increase (compared to the Bacteroidetes) in obese subjects [5, 11, 12]. Should we be interested in what the microorganisms are (taxonomy)? Or what they can do (metagenome)? Or what they really do (metaproteome)? Here again are some interesting questions for researchers [32-36] and the need for clinicians to remain curious and open. Be that as it may, the very recent persuasive demonstration of a shared network of intestinal microorganisms common to human beings (“phylogenetic nucleus or core”) remains to be investigated and constitutes a solid foundation for making progress in intestinal ecology in the years to come [32, 35, 36].

As the microbiota is recognized as an entirely separate organ (and containing a diversity of genes that is at least 100 times greater than the other organs [36]), how can it be influenced in cases of bacterial overgrowth? It is clear that the research on substrates must continue, but also that the addition of new microorganisms with the goal of adding properties (vector microorganism) or changing the ecology (biotherapeutic agents). In any event, it is certain that just as those that were lucky enough to attend the seminar on intestinal microbiota which is summarized in this division during inflammatory bowel diseases and an increase (compared to the Bacteroidetes) in obese subjects [5, 11, 12]. Should we be interested in what the microorganisms are (taxonomy)? Or what they can do (metagenome)? Or what they really do (metaproteome)? Here again are some interesting questions for researchers [32-36] and the need for clinicians to remain curious and open. Be that as it may, the very recent persuasive demonstration of a shared network of intestinal microorganisms common to human beings (“phylogenetic nucleus or core”) remains to be investigated and constitutes a solid foundation for making progress in intestinal ecology in the years to come [32, 35, 36].

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Conflicts of interests

P. Marteau has collaborated in clinical studies and received consulting fees with the laboratories and companies of Biocodex, Danone, Merck Médication Familiale, Nestlé, and PiLeJe.

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