23

Future Development of Infectious Microecology

Lanjuan Li *, **Yanfei Chen**
The State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University; Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, 310003, China
* E-mail: ljli@zju.edu.cn

More and more studies indicate interactions between infectious diseases and microbiota. Advances in molecular techniques have led to a greater appreciation of the diversity of human microbiota, the extent of interactions with the human host, and how that relates to inter-individual variation. Realization of the interaction between infectious agents and the microbiota will definitely deepen our understanding of infectious diseases.

23.1 Evolving View of Infectious Disease

Microbial ecology is the relationship of microorganisms with one another, and with their environment [1]. A microbial ecosystem is defined as a system that consists of all the microorganisms that live in a certain area or niche, which function together in the context of other biotic (plants and animals) and abiotic (temperature, chemical composition, and structure of the surroundings) factors of the niche [2]. The human body is home to many indigenous microorganisms, with distinct communities at different anatomical sites. Within the body of a healthy adult, microbial cells are estimated to outnumber human cells by a factor of one or two orders of magnitude. All the microbial cells constitute a small microecology, which is the simplest ecology on the earth. The composition of this microbial community is host specific, evolving throughout an individual's lifetime and

L. Li (ed.), *Infectious Microecology*

susceptible to both exogenous and endogenous modifications [3]. One of the main functions of this high density commensal microbiota that inhabits the intestine is to shield from infection. Colonization resistance is the mechanism whereby the host microflora protects itself against incursion by new and often harmful microorganisms [4]. An infectious disease is a clinically evident illness resulting from the presence of pathogenic microbial agents, including pathogenic viruses, pathogenic bacteria, fungi, protozoa, multicellular parasites, and aberrant proteins known as prions. Infectious disease results from the interplay between those few pathogens and the defenses of the hosts they infect. For a long time, much attention has been focused on identifying the bacteria which cause disease. It is of equal importance that bacteria associated with health also be researched, so that a microbiological view of infectious disease should be established.

23.2 Advances in Molecular Ecological Techniques

Due to long neglect, the complexity of the indigenous microbiota itself and the fact that many of its members resist cultivation, human microbiota is in fact new to science. Several developments have recently converged to renew interest in studying the normal human microbiota. Efforts to characterize microbial diversity increasingly rely on cultivation-independent, molecular techniques, since the vast majority of bacteria have yet to be cultivated. Advances in molecular techniques have led to a greater appreciation of the diversity of human microbiota, the extent of interactions with the human host, and how that relates to inter-individual variation.

Many of the tools developed for environmental microecology studies have recently been used on human samples, providing a more comprehensive view of our microbial symbionts. New technologies, such as metagenomics and metaproteomics, are shedding light on the wide diversity and function of the microbial consortium. Cell and animal experiments point to the intimate relationship between the immune system and the bacteria. Human microecology science is making rapid progress.

Genomics or metagenomics approaches have a tremendous capability to generate composition data and measure the metabolic potential encoded by the combined genomes of the gut microbiota. Because of its universal presence in cellular organisms, the presence of conserved regions, and its reliability, most molecular studies are based on the small subunit 16S rRNA gene for phylogenetic analysis. Several next-generation sequencing technologies, such as 454 pyrosequencing, have been introduced in recent years that dramatically outperform the traditional Sanger technology in terms of throughput and cost. These instruments, with the ability to produce millions of DNA sequence reads in a single run, are making great progress in human microecology research. For clinical diagnostic purposes, some simple and rapid methods for analysis of the composition of the gut microbiota such as the phylogeny chip and function chip have been developed [5].

Another post-genomics approach, metabonomics, has the capacity to measure the metabolic kinetics or flux of metabolites through an ecosystem at a particular point in time or over a course of time. Metabonomics thus derives data on the function of the gut microbiota *in situ* and how it responds to different environmental stimuli, *e.g.* substrates like prebiotics, antibiotics and other drugs and in response to disease.

Metagenomic sequencing has revealed information about the composition of genes in the gut microbiota. A major limitation of DNA-based approaches is that they predict potential functions, but it is not known whether the predicted genes are expressed at all or, if so, under what conditions and to what extent. In addition, it is not possible to determine whether the DNA is from cells that are active and viable, dormant or even dead. Verberkmoes *et al.* developed a novel high-throughput, non-targeted mass spectrometry (MS) approach, to determine the identities of thousands of microbial proteins in the faeces. This is the first step to developing an approach to obtain a first large-scale glimpse of the functional activities of the microbial community residing in the human gut [6].

Although in its infancy, the application of culture-independent tools has dramatically improved our ability to interrogate the vast diversity of unculturable microbial species. In the future, these three culture independent, high resolution approaches will be combined into a single "trans-genomic" approach which allows correlation of changes in metabolite profiles within human biofluids with microbiota composition metagenomic data. Such approaches are providing novel insight into the composition, function and evolution of our gut microbiota [7].

23.3 Normal Human Microbiota

As an ever-increasing body of evidence implicates the microbiota in defining states of health and disease, it is quite important to characterize the extent of normal microbiota [8]. With advanced technologies, we are able to learn more about the normal microbiota inside ourselves. Gut microbiota is the most complicated microbiota inside our body. Gut microbiota is an assortment of microorganisms inhabiting the length and width of the mammalian GI tract, which closely co-evolved with the human genome and diet. The distal GI tract houses up to 1,000 distinct bacterial species and an estimated excess of 1×10^{14} microorganisms. The composition of the microbiota in healthy people is not only unique, but also appears to be quite stable over time. There seems to be a vast "core" of approximately 300 phylotypes that are likely to exist in all individuals. The human oral microbiome is comprised of hundreds of microorganisms that mainly colonize on tooth surfaces as a biofilm. Dental plaque is a dynamic and extremely complex oral biofilm ecosystem. Oral bacterial researches have suggested that the oral cavity and intestinal tract harbor distinct sets of bacteria [9]. Two recent 16S rRNA gene tag pyrosequencing-based studies have suggested that there are approximately 250 – 300 species-level phylotypes in the mouth of any given individual, and that they segregate based on mucosal versus dental surfaces [10]. The

predominant taxa belongs to *Firmicutes* (genus *Streptococcus*, family *Veillonellaceae*, genus *Granulicatella*), *Proteobacteria* (genus *Neisseria*, *Haemophilus*), *Actinobacteria* (genus *Corynebacterium*, *Rothia*, *Actinomyces*), *Bacteroidetes* (genus *Prevotella*, *Capnocytophaga*, *Porphyromonas*) and *Fusobacteria* (genus *Fusobacterium*) [11]. The flora of the vagina and the urinary tract consist of a well-balanced system of about 50 bacterial strains. *Lacto bacilli* dominate the healthy flora of premenopausal women. The balance can be disturbed by the overgrowth of indigenous bacteria of the vagina like *Gardnerella*, *Bacteroides*, *Peptostreptococcus*, *Prevotella spp*. or *aerobic cocci*, or by the invasion of foreign microorganisms, such as *Escherichia coli*, *Enterococcus faecalis*, *Enterobacteriaceae*, *Staphylococci* or *Candida*.

Viral diversity and life cycles are poorly understood in the human gut and other body habitats. Reyes *et al.* sequenced the viromes (metagenomes) of virus-like particles isolated from faecal samples collected from healthy adult female monozygotic twins and their mothers at three time points over a one-year period and found that viromes are unique to individuals regardless of their degree of genetic relatedness [12].

23.4 Interactions between Infectious Diseases and Microbiota

For a long time, much attention has been focused on identifying the bacteria which cause infectious disease. Nowadays, more and more studies indicate interactions between infectious diseases and microbiota. Antibiotics, often used to cure infections, are causing more and more problems to the normal balance of microbiota. Not only bacterial infection but also virus infections are relevant to microbiota dysbiosis.

23.4.1 Disturbance of Normal Microbiota by Therapy

The human microbiota helps to protect the GI tract from enteric infections. A healthy microbiota is important in the host response to intestinal pathogens. Perturbations in the intestinal microbiota significantly impact the clinical incidence and severity of enteric infections. Disruption of the gut microbiome, termed dysbiosis, is frequently accompanied by overgrowth of pathogenic bacteria or fungi, in conjunction with significant loss of microbial diversity or key functional groups and an inflammatory response by the host, which contributes to disease development.

Mucositis, also referred to as mucosal barrier injury, is one of the most debilitating side effects of radiotherapy and chemotherapy treatment. Clinically, mucositis is associated with pain, bacteremia and malnutrition. Gut microbiota was found to participate in the development and severity of chemotherapy-induced mucositis. Recently, it has been shown that chemotherapy treatment is associated

with a decrease in the number of anaerobic bacteria and a decrease in microbial diversity [13]. The disappearance of commensal intestinal microbiota will minimize their protection of enterocytes against potential pathogens. Different initial microbial colonization may protect and predispose the pathophysiology of acute postradiotherapy diarrhea [14]. Stecher *et al.* have found that the presence of closely related species can increase the chance of invasion of newly incoming species into the gut ecosystem, and this principle might be of general validity for invasion of bacteria in preformed gut ecosystems [15]. However, research concerning the relationship between intestinal bacteria and chemotherapy-induced mucositis is still rare. Further research is needed to verify the mechanism of gut microbiota in chemotherapy-induced mucositis.

More and more evidence has shown that there is a new conceptual framework of the microbiota-gut-brain axis. Stressor exposure disrupts commensal microbial populations in the intestines, and leads to increased colonization by Citrobacter rodentium [16].

Antibiotics are the main, and often only, clinical intervention for prophylactic and active treatment of bacterial infections in humans. However, these drugs also shift the composition of commensal bacteria inside our bodies, especially those within the gut microbial community. More and more evidence show that antibiotic use can increase host susceptibility to pathogen infection. A number of opportunistic pathogens can cause disease during antibiotic therapy, including *Salmonella spp*., *Clostridium perfringens*, *Klebsiella oxytoca*, *S. aureus*, *Candida albicans*, and *C. difficile*. Of these, *C. difficile* is the most common cause of pathogen-associated antibiotic-associated diarrhea (15% – 25%), the most common cause of severe disease, and it causes nearly all cases of nosocomial pseudomembranous colitis [17]. Animal (hamster and mouse) and *in vitro* models show antagonism between conventional microbiota and *C. difficile* population growth [18].

Antibiotic-associated diarrhea (AAD) and Clostridium difficile infections (CDI) are associated with altered intestinal microflora and other symptoms that may possibly lead to death. The association between AAD or CDI and perturbations of the gut microbiota is well established but poorly understood.

Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase in colonization with potentially pathogenic bacteria in the gut [19]. This disturbance in the balance between anaerobic and aerobic bacteria will further increase the risk of gram-positive aerobic infections.

23.4.2 Microbiota and Viral Infection

Infectious diseases caused by a virus may also have relationships with the bacterial community of the host. Structural responses of gut microbiota were found on children with rotavirus infection [20].

Alteration in gut microbiota was also found in patients with HIV infection, and was supposed to be a key factor in HIV pathogenesis [21].

Indigenous microbiota was found to play a crucial role in the expansion and maintenance of viral-specific CD8 memory T cells in mice infected with murine cytomegalovirus [22].

23.4.3 Microbiota and Autoimmunity Disease

Dental caries, once thought to be a simple disease caused by *S. mutans*, is now unraveling as an extremely complex disease, which reflects the response of the tooth to a microbial challenge. Normally, low populations of acido-genic and aciduric bacterial species will increase following high-frequency carbohydrate exposure. The metabolism of carbohydrate by these microbiota results in the acidification of plaque (pH < 5), and acid-induced demineralization of the enamel and dentin occurs, eventually resulting in cavitation. Cariogenic plaques are comprised of numerous different microbial species, including *S. mutans* and other low-pH streptococci (*Streptococcus oralis*, *Streptococcus mitis*, *Streptococcus anginosus*), *Rothia*, *Actinomyces*, *Lactobacilli* and *Bifidobacterium spp*., and *Candida albicans* [23].

Studies of bacterial infections in developed countries suggest 75% of adults fully recover. However, around 25% have long lasting changes in bowel habits and a smaller number develop the irritable bowel syndrome (IBS). Post-infectious IBS (PI-IBS) usually follows bacterial infection with *Salmonella spp*., *C. jejuni* and *Shigella spp.* [24]. Why this inflammation persists in some, but not others still needs further research.

23.5 Therapy

Improved understanding of the normal gut microbiota has made the therapeutic manipulation of the gut ecosystem a valid and realistic future prospect.

It is well known that antibiotic use can cause short-term ecological disturbance in the microbiota, while another disturbing consequence of antibiotic treatment is long-term persistence of antibiotic resistance genes. Prudence in the administration of antibiotics could partially alleviate the emergence of antibiotic resistant pathogenic strains. Emphasis should be placed on alternative therapeutic options such as probiotics, immunotherapy, *etc.*

23.5.1 Probiotics or Prebiotics

In response to problems caused by antibiotic use, new biological treatment for infectious disease is needed. The use of probiotics, prebiotics and synbiotics is increasing in popularity for both the prevention and treatment of a variety of

diseases. Prebiotics are a group of non-digestible food ingredients including inulin, oligosaccharides, lactulose and resistant starch that are fermented by colonic commensal microbiota to potentially improve the health of the host by selectively stimulating the growth of certain gut bacteria. Lactic acid bacteria are thought to provide positive health effects for the host, and are usually referred to as probiotic bacteria. Bohle *et al.* found that the mucus adhesion promoting protein of Lactobacillus reuteri can be specially degraded to an antimicrobial peptide [25]. This finding gives some new perspectives on how probiotic bacteria may successfully contribute to a healthy microbiota. *Escherichia coli* O157:H7 is a food-borne pathogen causing hemorrhagic colitis and hemolytic-uremic syndrome, especially in children. The probiotic bacterium *Lactobacillus acidophilus* strain La-5 can secrete molecules, which is effective against enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 infection [26]. Meta-analysis of probiotics trials for the prevention of AAD showed an overall reduction in the risk of AAD when probiotics were co-administered with antibiotics [27].

Many probiotic strains have been tested for CDI. *Saccharomyces boulardii* is the best studied one among these strains. In a phase 3 trail, adult patients with CDI were randomized in a combination treatment of oral vancomycin and *S. boulardii* or vancomycin and placebo. Patients treated with vancomycin and the probiotic had significantly decreased recurrence rates compared with vancomycin with placebo [28].

Probiotics have shown effects on HIV-infected patients. In a randomized, placebo-controlled study of 24 subjects with HIV infection or AIDS, daily ingestion of probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, supplemented in yogurt, led to rapid resolution of diarrhea, flatulence and nausea, as well as a small increase in CD4 cell counts [29].

Oral or local application of yogurt in bacteria vaginosis or candida-vaginitis has shown promising results for decades. In a human study with 49 subjects, the local application of *L. rhamnosus* reduced the rate of urinary tract infections by 73% [30]. The strain showed tight adhesion to epithelial surfaces, hydrogen peroxide production, or the release of biosurfactants.

Probiotics also exert effects against viral infection such as rotavirus infection or viruses whose target organ is not the intestine. The mechanism is considered to be by immunostimulation and not by direct competition with the infectious agent.

In vitro models with complex and complete gut microbiota are required to accurately assess the potential and efficacy of probiotics on pathogen colonisation in the gut.

23.5.2 Other Bacteriologic Therapy

Most of the drugs used in clinics today were derived from living matter in the external environment. It's time to mine new drugs from microbial-derived signaling molecules in the inner environment of the gut. Bacteriocins are a family of anti-microbial peptides by which the producer organism can inhibit the growth of other organisms. The broad-spectrum bacteriocin, lacticin 3147, has been

shown to have resistance to *C. difficile* [31]. Some narrow-spectrum bacteriocins with relative specificity against specific organisms are being searched for now.

The gut microbiota is a source of immunomodulatory signals, and the microbiota composition has a profound impact on immunological differentiation. Some microbial-derived molecules with immunomodulatory potential have been well researched, which include bacterial nucleic acids or oligonucleotides containing hypomethylated CpG dinucloetides and cytoprotective or anti-inflammatory peptides [32]. The therapeutic potential of these molecules on infectious diseases is still under research. The exploration of the inner world of human microbiota for drug discovery or other bioactive development is in its infancy, but is very promising.

An alternative approach for shaping overall microbiota composition by probiotics or prebiotics aimed at reducing detrimental and potentially pathogenic bacteria is by means of specific bacteriophages. Bacteriophages are viruses that attach to their specific bacterial hosts and kill them by sequential internal replication and lysis. Bacteriophages administration is safe and can reduce the concentration of Listeria monocytogenes in the GI tract. It can also translocate to the spleen and liver in experimentally infected mice [33].

23.5.3 The Role of Microbiota in Drug Metabolism

Nowadays, it is clear that the complex microbial ecosystem in our intestines should be considered as a separate organ within the body, with a metabolic capacity which exceeds the liver, with a factor of 100. The intestinal microbiome is therefore closely involved in the first-pass metabolism of dietary compounds [34].

23.6 Summary and Prospects

This field is very much in its infancy, and considerable work still needs to be done to better understand the relationship between the microbiota and infectious diseases. Realization of the interaction between infectious agents and commensals in disease will require greater understanding of the normal microbiota, and the mechanisms of microbiota-host interactions. Technological advances in molecular analysis will definitely speed up the exploration.

There are still many concerns about the safety of probiotics use. Future work should define the possibly related molecular factors that promote probiotic functions, fitness, and facultative pathogenicity. Then we can give a definite answer as to when and how to use probiotic strains against infections.

Although many probiotic strains have been tested, the quality of evidence is still poor. Much of the mechanism remains unproven, *e.g.*, how probiotics work, which strains are effective, what can be expected to be achieved, and what dosage is required for effectiveness.

References

[1] Konopka A. What is microbial community ecology? ISME J, 2009, 3: 1223-1230.
[2] Raes J, Bork P. Molecular eco-systems biology: towards an understanding of community function. Nat Rev Microbiol, 2008, 6: 693-699.
[3] Xu J, Mahowald M A, Ley R E, *et al.* Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol, 2007, 5: e156.
[4] Gorbach S L, Barza M, Giuliano M, *et al.* Colonization resistance of the human intestinal microflora: Testing the hypothesis in normal volunteers. Eur J Clin Microbiol Infect Dis, 1988, 7: 98-102.
[5] Bjerketorp J, Ng Tze Chiang A, Hjort K, *et al.* Rapid lab-on-a-chip profiling of human gut bacteria. J Microbiol Methods, 2008, 72: 82-90.
[6] Verberkmoes N C, Russell A L, Shah M, *et al.* Shotgun metaproteomics of the human distal gut microbiota. ISME J, 2009, 3: 179-189.
[7] Tuohy K M, Gougoulias C, Shen Q, *et al.* Studying the human gut microbiota in the trans-omics era — focus on metagenomics and metabonomics. Curr Pharm Des, 2009, 15: 1415-1427.
[8] Fujimura K E, Slusher NA, Cabana M D, *et al.* Role of the gut microbiota in defining human health. Expert Rev Anti Infect Ther, 2010, 8:435-454.
[9] Bik E M, Long C D, Armitage G C, *et al.* Bacterial diversity in the oral cavity of 10 healthy individuals. ISME J, 2010, 4: 962-974.
[10] Keijser B J, Zaura E, Huse S M, *et al.* Pyrosequencing analysis of the oral microflora of healthy adults. J Dent Res, 2008, 87: 1016-1020.
[11] Zaura E, Keijser B J, Huse S M, *et al.* Defining the healthy 'core microbiome' of oral microbial communities. BMC Microbiol, 2009, 9: 259.
[12] Reyes A, Haynes M, Hanson N, *et al.* Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature, 2010, 466:334-338.
[13] van Vliet M J, Harmsen H J, de Bont E S, *et al.* The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS Pathog, 2010, 62: 1223-1236.
[14] Manichanh C, Varela E, Martinez C, *et al.* The gut microbiota predispose to the pathophysiology of acute postradiotherapy diarrhea. Am J Gastroenterol, 2008, 103:1754-1761.
[15] Stecher B, Chaffron S, Kappeli R, *et al.* Like will to like: Abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria. PLoS Pathog, 2010, 6: e1000711.
[16] Bailey M T, Dowd S E, Parry N M, *et al.* Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. Infect Immun, 2010, 78:1509-1519.
[17] Walk S T, Young V B. Emerging insights into antibiotic-associated diarrhea and clostridium difficile infection through the lens of microbial ecology. Interdiscip Perspect Infect Dis, 2008, 125081.
[18] Wilson K H. The microecology of Clostridium difficile. Clin Infect Dis, 1993, 16: S214-S218.
[19] van Vliet M J, Tissing W J, Dun C A, *et al.* Chemotherapy treatment in

pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. Clin Infect Dis, 2009, 49: 262-270.
[20] Zhang M, Zhang C, Du H, *et al.* Pattern extraction of structural responses of gut microbiota to rotavirus infection *via* multivariate statistical analysis of clone library data. FEMS Microbiol Ecol, 2009, 70: 21-29.
[21] Gori A, Tincati C, Rizzardini G, *et al.* Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J Clin Microbiol, 2008, 46:757-758.
[22] Tanaka K, Sawamura S, Satoh T, *et al.* Role of the indigenous microbiota in maintaining the virus-specific CD8 memory T cells in the lung of mice infected with murine cytomegalovirus. J Immunol, 2007, 178: 5209-5216.
[23] Filoche S, Wong L, Sissons C H. Oral biofilms: Emerging concepts in microbial ecology. J Dent Res, 2010, 89: 8-18.
[24] Spiller R, Garsed K. Infection, inflammation, and the irritable bowel syndrome. Dig Liver Dis, 2009, 41: 844-849.
[25] Bohle L A, Brede D A, Diep D B, *et al.* The mucus adhesion promoting protein (MapA) of Lactobacillus reuteri is specifically degraded to an antimicrobial peptide. Appl Environ Microbiol, 2010, 76:7306-7309.
[26] Medellin-Pena M J, Griffiths M W. Effect of molecules secreted by Lactobacillus acidophilus strain La-5 on Escherichia coli O157: H7 colonization. Appl Environ Microbiol, 2009, 75: 1165-1172.
[27] Doron S I, Hibberd P L, Gorbach S L. Probiotics for prevention of antibiotic-associated diarrhea. J Clin Gastroenterol, 2008, 42: S58-S63.
[28] Surawicz C M, McFarland L V, Greenberg R N, *et al.* The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. Clin Infect Dis, 2000, 31: 1012-1017.
[29] Anukam K C, Osazuwa E O, Osadolor H B, *et al.* Yogurt containing probiotic *Lactobacillus* rhamnosus GR-1 and *L. reuteri* RC-14 helps resolve moderate diarrhea and increases CD4 count in HIV/AIDS patients. J Clin Gastroenterol, 2008, 42: 239-243.
[30] Reid G, Bruce A W. Low vaginal pH and urinary-tract infection. Lancet, 1995, 346: 1704.
[31] Rea M C, Clayton E, O'Connor PM, *et al.* Antimicrobial activity of lacticin 3,147 against clinical Clostridium difficile strains. J Med Microbiol, 2007, 56: 940-946.
[32] Shanahan F. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Host-microbe interactions in the gut: Target for drug therapy, opportunity for drug discovery. Clin Exp Immunol, 2010, 160: 92-97.
[33] Mai V, Ukhanova M, Visone L, *et al.* Bacteriophage administration reduces the concentration of listeria monocytogenes in the gastrointestinal tract and its translocation to spleen and liver in experimentally infected mice. Int J Microbiol, 2010, 624234.
[34] Possemiers S, Bolca S, Verstraete W, *et al.* The intestinal microbiome: A separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. Fitoterapia, 2010, 82: 53-66.