Розділ 1. Екологічна безпека

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MICROBIAL ENVIRONMENT, IMMUNITY AND HUMAN HEALTH

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Sophisticated analytical tools for elucidating genome structure and life of bacteria yielded a new paradigm on our relation to the microbial environment. Formerly, bacteria were seen as jeopardising invaders, and studies on bacterial pathogenesis were the basis for great medical progress in the restraint of infections. Now it becomes clear that Homo sapiens is not a single-species organism, but it is to understand as a complex ecosystem assembled of a 10:1 mixture of both environmental microbial and human cells.

The gargantuan diversity of prokaryotic life includes genetic multiplicity, metabolic endowment, colonised habitats, and response to physical and chemical stresses. Advances in microbiology, molecular biology, biochemistry and eukaryotic cell biology have enabled striking discoveries on how eukaryotic cells cooperate with colonising bacteria. Bacterial life in multi-species associations and biofilms limits competition, reduces temporal variance of productivity, enhances performance, and favours positive interactions among the allied members. Quorum sensing, hypermutable loci in the genome, horizontal transfer of genes distributed within the population and the enduring crosstalk with the epithelia and the innate immune system of the host warrant stability within the microbial community and maintenance of the mutually beneficial association with the host.

Factors accompanying our current life such as over- and malnutrition, physical inactivity, excessive use of tobacco, alcohol, drugs and medicine,

UV-irradiation, incorporation/inhalation of pesticides, toxic gases and heavy metals and growing-up in a sanitised world disturb the immune balance and the host-microbiota partnership. Phobia of germs, compulsive hygienic custom pattern, and the excessive use of antibiotic agents are detaining the immune system of the microbial information input upon which it is dependent.

This conglomerate of factors and habits conforms to a huge increase of chronic inflammatory diseases, e.g. atherosclerosis, inflammatory bowel diseases, diabetes, asthma, rheumatism, neurodermatitis, psoriasis, adipositas, and cancer. It may be necessary to invent measures of replacing the microbial input. Food additives with immune modulating (e.g. beta-glucans), prebiotic (e.g. oligofructosides) or probiotic (e.g. Bifidus, Lactobacillus) features are anticipated to improve a tilted host-microbiota partnership, immunity and constitutional performance.

Складні аналітичні підходи до вивчення структури і життя бактерій породили нове відношення що до мікробного оточуючого середовища. Раніше розглядали бактерії як такі, що створюють небезпечні умови для інших організмів, і дослідження патогенезу бактеріальних захворювань були основою для великого прогресу в медицині щодо стримування інфекцій. Зараз стає зрозумілим, що Людина розумна не окремий організм, це складна екосистема, яка складається у відношенні 10:1 з різних мікроорганізмів та клітин людини.

Різні середовища існування колонізували величезне генетичне і метаболічне різноманіття прокаріотів. Досягнення у мікробіології, молекулярній біології, біохімії і цитології еукаріотів дозволили з'ясувати, як клітини еукаріотів взаємодіють з бактеріями, які колонізують організми. В асоціаціях різноманітних бактерій обмежена конкуренція, скорочена часова різниця у відтворенні, виникають позитивні взаємодії їх членів і стимулюється робота. Відчуття «кворуму», наявність гіпермінливих місць у геномі, горизонтальний перенос генів між бактеріями в межах асоціації та стійкий взаємозв'язок з епітеліями та природною імунною системою хазяїна гарантують стабільність мікробного угрупування і взаємно корисні взаємодії.

Фактори, які впливають на наше життя — переїдання, недоїдання, обмежена фізична активність, надмірне вживання тютюну, алкоголю, наркотиків і ліків, ультрафіолетового опромінення, паління, дія комплексу пестицидів, отруйних газів і важких металів, виховання дітей у санованому світі — порушують імунний баланс і взаємодію організму хазяїна з бактеріями. Боязнь мікробів, суворі нав'язливі гігієнічні вимоги і надмірне застосування антибіотиків затримує розвиток імунної системи, тому що багато мікроорганізмів не потрапляють в організм людини.

Цей набір факторів і звичок відповідає величезному збільшенню хронічних захворювань, які мають важки наслідки, наприклад, атеросклерозу, хвороб шлунково-кишкового тракту, діабету, астми, ревматизму, нейродермітів, псоріазу, раку. Можливо треба запропонувати нові засоби заміни впливу мікроорганізмів , наприклад, використовувати харчові домішки, які містять імуномодулятори (наприклад, бетаглікани); пребіотикі (наприклад, олігофруктози) або пробіотикі (наприклад, Бифідобактерії, Лактобактерії). Є сподівання, що їх застосування покращить взаємодію мікроорганізмів і людини, її імунітет і сталу роботу її організму.

Сложные аналитические инструменты, объясняющие структуру и жизнь бактерий, привели к новому отношению к микробной окружающей среде. Прежде бактерии рассматривали как такие, которые создают опасные условия для других организмов, а исследования бактериального патогенеза были основанием для большого медицинского прогресса в сдерживании инфекций. Теперь становится ясно, что Человек разумный не отдельный организм, а сложная экосистема, состоящая в отношении 10:1 из разных микроорганизмов и человеческих клеток.

Разные среды обитания колонизировали гигантское генетическое и метаболическое разнообразие прокариот. Достижения в микробиологии, молекулярной биологии, биохимии и цитологии еукариот позволили показать как клетки еукариот сотрудничают с колонизирующими организм бактериями. В ассоциациях разнообразных бактерий ограничена конкуренция, сокращена временная разница воспроизводства, возникает положительное взаимодействие их членов и стимулируется работа. Ощущение «кворума», гиперизменчивые места в геноме, горизонтальный перенос генов между бактериями в пределах ассоциации и устойчивая связь с эпителиями и врожденной иммунной системой хозяина гарантирует стабильность микробного сообщества и взаимно полезного взаимодействия.

Факторы, сопровождающие нашу текущую жизнь, такие как сверхили недоедание, физическая бездеятельность, чрезмерное использование табака, алкоголя, наркотиков и лекарств, ультрафиолетовое облучение, действие комплекса пестицидов, ядовитых газов и тяжелых металлов и воспитание детей в санированном мире нарушают иммунный баланс и взаимодействие организма хозяина с бактериями. Боязнь микробов, строгие навязчивые гигиенические требования и чрезмерное использование антибиотиков задерживает развитие иммунной системы, т.к. многие микроорганизмы не попадают в организм человека.

Этот набор факторов и привычек порождает огромное увеличение хронических болезней, которые имеют тяжелые последствия,

например, атеросклероза, болезней кишечника, диабета, астмы, ревматизма, нейродермитов, псориаза, рака. Может быть необходимо предложить новые методы замены влияния микроорганизмов, например, пищевыми добавками, содержащими иммуномодуляторы (например, бета-гликаны), пребиотики (например, олигофруктозы) или пробиотики (например, Бифидобактерии или Лактобактерии). Как ожидают, их применение улучшит взаимодействие микроорганизмов и человека, его иммунитет и стабильную работу его организма.

1. Microbial diversity and human activity

Fifteen hundred scientists under the auspices of the United Nations Environment Programme (UNEP) completed in 1995 the Global Biodiversity Assessment (Heywood 1995). This appraisal alludes to the biological diversity at all levels — genes, species, and ecosystems — and is still extremely uncertain. Of its working figure of 13 million species, only approximately 1.7 million have been scientifically described and given Linnaean nomenclature. These living organisms interact among them and with the non-living environment and comprise the global ecosystems. The biological multiplicity is pivotally evident for human society and sustainable development. A reckoned 40 per cent of the global economy rests upon biological products and processes. Faced with the rate of species extinctions, which is hundreds or thousands of times the natural background level, the public begins to raise attention on the outcome of human activities on biodiversity. However, the appreciation on how activity of mankind, human health and biodiversity are intimately connected among one another will only increase in coming years.

Having a glance in a biology textbook of the 1980s one may realise that the living matter is subdivided in five kingdoms: Bacteria, Protoctista (nucleated unicellular organisms), Fungi, Plantae and Animalia. About 5000 species classified by means of traditional bacteriological techniques were allocated to non-eukaryotic organisms, and thus should contribute only a minor portion to the overall species variety (Skerman et al. 1989). Just for comparison: more than half a million insect species have been classified.

Beginning in the 1960s it became feasible to define prokaryotes on the basis of shared molecular characteristics when Zuckerkandl and Pauling (1965) expounded the role of nucleic acid as the master

candidate of a molecular phylogeny. Obviously, microbial morphology, motility, metabolism and serological types are too simply or inexplicable to serve as the basis for a phylogenetically compelling taxonomy. Sophisticated techniques such as reverse transcription (RT)-PVR, DNA micro arrays, in vivo expression technology (IVET), fluorescence in situ hybridisation (FISH), restriction fragment length polymorphism, subtractive hybridisation, signature-tagged mutagenesis, in situ isotope tracking, and the sequencing revolution replaced classical phenotypic characteristics by molecular criteria. This progress culminated in two scientific acmes at the end of the last century: i) the creation of a new natural system of organisms dividing life on our planet in three domains (Woese et al. 1990), and ii) the whole-genome random sequencing and assembly of a free-living microorganism, the r-proteobacterium Haemophilus influenzae Rd. (Fleischmann et al. 1995). Comparison of the unique sequences of nucleobases in the chromosomes has all of a sudden shown that the three primary domains are: Bacteria, Archaea, and Eukarya. These domains evolved in parallel along three phylogenetic lineages.

The small unit of ribosomal ribonucleic acid (16S rRNA from prokaryotic cells and 18S rRNA from eukaryotic cells) proved to be an elegant taxonomic tool in regard of the following: first, it is present in all organisms, and secondly, it contains highly conserved as well as variable nucleotide sequences. Divergence between species or strains may be evident when comparisons are made of the variable regions, and comparing highly conserved regions may elucidate divergence at the higher taxonomic ranks (domain, kingdom). Nowadays, techniques for high-throughput cultivation of more than 10,000 bacterial and fungal isolates per environmental sample are available (Zengler et al. 2002). That provides access to the immense reservoir of untapped microbial diversity by cultivation. Determination the species composition of a microbial community needs to be followed by establishing the functions of the community and of each of its members (metabolic fingerprinting by community level physiological profiling CLPP). After all, one has to have in mind that there is an ongoing debate on methods for defining species, mechanisms that lead to speciation and whether microbial species even exist. Achtman and Wagner (2008) propose that a method-free species concept based on cohesive evolutionary forces should direct decisions on the existence of species and procedures to define them.

From a commonplace of view we recognise only the multicellular creatures as the denizens of our earth. All are Eukarya, but many single-cellular belong to this domain, too. Bacteria and Archaea are exclusively composed of prokaryotic cells and are thus microbial. The major advances in molecular and cell biology and the application of these methodologies on microbiology produced cross-fertilisation of these disciplines and had impacts, which was unimaginable only a generation ago (Table 1). The gargantuan diversity of prokaryotic life includes genetic multiplicity, metabolic endowment, colonised habitats, and response to physical and chemical stresses. Microorganisms are the major life forms on earth and reside yet in extreme environments like very rocks, hot springs and submarine grounds. The amount of carbon that is piled up in prokaryotes matches that in the plant kingdom. In case of nitrogen and phosphorus the ratio is even ten in favour of the microbes. Since only less than 1% of species of the microbial world was cultivated and studied under laboratory conditions, we know nothing of them other than what can be inferred from their genomic sequence.

The majority of microorganisms lives in microbial dynamic assemblages. The stability and functions of these consortia are ruled by dependencies and antagonisms among the members. Life in multi-species associations and biofilms limits competition and favours positive interactions among the allied bacteria. A fraction of the microbial universe coexists with animals in mutually beneficial association. Such microbial communities have to assert themselves with the vicissitudes of their host (Handelsman et al. 2005). Microbial symbioses with animals warrant benefits to both host and bacteria and promote their macro-evolutionary persistency. Such symbioses have played an essential role in revival of the biosphere after periodic mass annihilation of major groups of organisms and whole ecosystems (Hickman 2005). Less than 0.0005 percent of the total domain Bacteria can cause disease. The relation in fungi is not quiet different: approximately 300 of the greater than 100,000 classified fungal species have pathogenic relationships with animals and plants (Steenbergen and Casadevall 2006). Advances in microbiology, molecular biology and eukaryotic cell biology have enabled striking discoveries on how eukaryotic cells cooperate with colonising bacteria and interact with pathogens during the infectious process. The partnering-up of these related fields progressed in a novel scientific discipline that has been termed cellular microbiology (Cossart et al. 1996).

Table 1 — **Impact of new analytical tools on cellular microbiology**

Topics	Impact	References
Diversity of prokaryotic life	Genetic diversity highest in the microbial world, prokaryotes encompass up to ten million species, wide range of energy substrates and the availableness of miscellaneous catabolic and anabolic pathways, occupying multiple habitats, multitude of responses to physical and chemical stresses	Stahl and Tiedje 2002 Torsvik et al. 2002
Ancestry	Eukarya, Bacteria and Archaea have split from a common ancestor 2—3.5 billion years ago	Woese et al. 1990 Doolittle et al. 1996
Biomass	$4—6\times10^{30}$ prokaryotic cells on earth containing $350—550\times10^{9}$ tons of carbon, $85—130\times10^{9}$ t nitrogen, $9—14\times10^{9}$ t phosphorus, cellular production rate for all prokaryotes on earth is estimated at 1.7×10^{30} cells/yr, it is highest in the open ocean	Whitman et al. 1998
Cultivability	90 to 99.9 % do not grow and replicate in cultures under laboratory conditions	Henderson et al. 1999 (p.19)
Mode of life	Multi-species associations, colonies, biofilms, quorum sensing systems as signal amplifiers within a bacteria and as signal disseminators between bacteria	Palmer et al. 2007 Fuqua et al. 1994 Dunny and Winans 1999
Symbiosis	1000—3000 species (phylotypes) mutually coexist with man, specific gene-gene interactions within the microbiome (genomic plasticity)	Eckburg et al. 2005 Dethlefsen et al. 2007 Ehrlich et al. 2008
Pathogenicity	About 50 bacteria cause human diseases	Rappuoli 2006

The current debate on the use and misuse of antibiotics perpetuates a long history of semantic thoughts on microbes as friend or foe of man. In the late seventieth century, Antonie van Leeuwenhoek, who ranks first to had inspected the indiscernible minuscule life, already expressed his astonishment on the ample colonisation of our body and its environment by «animalcule» what means «little animals»: «...there are more animals

living in the scum on the teeth in a manrs mouth, than there are men in the whole kingdom». Bacteriology started two centuries later with the pioneering work of Robert Koch, Louis Pasteur, Sergei Winogradsky and other scientific heroes. Koch and Pasteur developed pure culture techniques to identify causative agents of many diseases that affect humans and animals. Contrariwise, Sergei Winogradsky — born in Kiev 1856 — invented a simple columnar device for culturing a large diversity of bacterial life in natural environments. Among the bacteria within such a single column there grow members of all four basic life strategies: phototrophs, chemotrophs, autotrophs, and heterotrophs. The Winogradsky column evolved, thus, as classic tool for studying the relationships between different types of microorganisms in mixed communities. In addition, it is an elegant illustration of how microorganisms occupy highly specific niches according to their environmental tolerances and their requirements of energy and carbon.

In the aftermath microbes were dichotomic seen from the viewpoint of practical purposes (Gutzeit 1918, Duddington 1961). The «good» ones help in fermentations in the dairy industry, the manufacture of vinegar, beer, coffee, cocoa, food supplements, and feed for farm animals as well as in biomining (bioleaching) and sewage treatment. The «bad» ones are looked upon as pests or can be at least a nuisance in many everyday situations. According to the widely held opinion, germs should be eliminated or constrained in their activities. Following such options many a scientist lumbered in a meander. Ilja Metchnikoff, for example, assumed «autointoxication» of the human body by the intestinal microflora and practised surgical ablation of the colon. Later, however, by contemplating about longevity in Bulgarians he suggested that that may be linked to the helpful activity of lactic acid bacteria ingested with sour milk.

The elimination of bacterial pathogens appeared to become a reality when antimicrobial agents were introduced into clinical practice in the 1940s and 1950s. That was one of the most outstanding public health achievements of the 20th century. In marvelling the unimaginable aid to legions of infected patients the Western societies lost its fear of bacterial infection. In 1969 the then United States Surgeon General presumed to prophesy in his address to Congress «We can close the book on infectious diseases». One outcome of this misjudgement was the drop in research into the mechanisms by which bacteria cause communicable diseases. The other was the assumption on the selective

toxicity of a particular antibiotic to a certain bacterial pathogen: due to fundamental differences to eukaryotic cells the antibiotic therapy might not threat the host.

Nonetheless, already in the late 1940s strains of Staphylococcus aureus developed resistance against penicillin. The number increased steadily and at present MRSA (methicillin-resistant Staphylococcus aureus) is a menace of hospital settings. Resistance has developed meanwhile against all available classes of antibiotics (Virk and Steckelberg 2000, Smith and Coast 2002). Rowe-Magnus and Mazel (2006) published a survey on the evolution, biochemical mechanisms of antibiotic resistance and their genetic determinants. Species of the genus Bacteroides have the highest resistance rates of all anaerobic pathogens, the multiple mechanisms of which are discussed by Wexler (2007). In addition, there are alert marks just heralding man-made tumbling into a new predicament: Resistant Staphylococcus aureus changed its epidemiology and advanced from being a pathogen primarily related with nosocomial infection to one that has begun to recurrently infect individuals outside the hospitals (Chambers 2001). Obviously the «golden age of antibiotics» turns up jaded after merely two generations.

By traditional view, the transmission of pathogenic bacteria has been assumed to take place through ingestion of infected stuff or liquids and intimate contact with contaminated surfaces. Transmission over short distances is also possible when the pathogen is enclosed in large droplets, as in sputum. This route should be rendered more precisely on the basis of reports on airborne transmission of communicable diseases (Roy and Milton 2004). Gandara et al. (2006) gave account on the common appearance of cultivable strains of Staphylococcus aureus resistant against penicillin, ampicillin and cefaclor in aerosols sampled within residential homes. The infection of children without known risk factors with this pathogen has to be regarded as an exceptionally precarious observation (Gorak et al. 1999). Experiments on mouse and human cell lines demonstrate the ability of indoor air bacteria and fungal spores in boosting the production of proinflammatory mediators (Huttunen et al. 2003). The trends in the epidemiology of antibiotic-resistant pathogens give persuasive justification for more profound scientific exploration of both the indoor sources of, and morbid effects correlated with, indoor residential exposure to airborne pathogens.

Findings emphasise that current concentrated animal feeding operations (CAFOs) can result among others in the emanation of antibiotic-resistant bacteria into the general environment (Platz et al. 1995). Multiresistant strains were detected in the air plume up to 150 m downwind of a swine confined animal feeding operation (Gibbs et al. 2006). Naпve subjects living in close proximity to such facilities and employees who work inside suffered from respiratory inflammatory symptoms (Heederick et al. 2007). The paucity of data in regard of environmental and community adverse health effects of modern production of pigs and poultry resulted in a growing controversy in the public (Cole et al. 2000, Rieger 2001). Intensified cooperation among animal producers, feed professionals and health officials as well as forced research in cross-linked human and veterinary health surveillance systems are indispensable to successfully address these issues. Special attention should be given to high-risk groups such as children and asthmatics. A flicker of hope comes from Denmark, where the prevalence of macrolide-resistant species of Campylobacter dropped subsequently to the banning of all growth promoters from feeding in livestock.

2. Man and microbes are companions

Microbes make up a considerable component of the human body. The external surface — like the skin and its adhering structures — and the internal surfaces such as the mucosal layers of the gastrointestinal, respiratory and genito-urinary system and the cavities they enclose, harbour microbial communities of different complexity. The term microbiota, or indigenous microflora, or commensal microflora, respectively, encompasses all of the bacteria, archaea, fungi, proctists, and viruses. The bacterial component at any body site was chiefly studied, but little is known about the other types. The microbiota in the adult has a mass of about 1—2 kg, which corresponds to that of liver and outruns most other human tissues. Although this represents only about 2% of the whole body mass, in terms of cell numbers the total microbial population exceeds our total number of somatic and germ cells by at least one order of magnitude (Xu and Gordon 2003). The number of genes in this «microbiome» may overtake the human genome about 100 times. In conclusion, human sapiens may not be regarded as a single-species organism, but as a complex ecosystem

compiled of a 10:1 mйlange of both microbial and human cells (McVean et al. 2005, Eckburg et al. 2005, Ley et al. 2006). The microbiome and hosts genome collectively constitute the hologenome that includes all genes from the host and from all symbionts. It is flexible and varies with age, health, diet, and environmental factors. The 6.5 billion humans living on earth represent a microbial reservoir of 10^{23}—10^{24} prokaryotic cells. Thus, human intestine and that of other mammals share a considerable portion of the total microbial mass on our planet that is estimated to about $4—6\times10^{30}$ prokaryotic cells. The human bowel harbours 10^{11}—10^{12} prokaryotic cells per ml, the highest observed density for any microbial habitat (Whitman et al. 1998).

During foetal life the human being like all other mammals dwells in a sterile environment. Birth is the encounter stage in microbial colonisation of the newborn that discharges into a life-long partnership. First microbes stem from the mother, members of clinical staff, equipment and the close environment. Later, germs may attain from more distant sites of environment and from food. One may wonder in all innocence what causes the whittling down of a spectrum of more than fifty phyla of Bacteria and 13 phyla of Archaea staying alive on earth to just four phyla of Bacteria — the Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria — that overwhelmingly dominate microbial communities of man. In the colon Bacteroidetes and Firmicutes contribute more than 95% of the microbiota, only one member of Archaea (Methanobrevibacter smithii) is present? Chlamydiae, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Fusobacteria, Verrucomicrobia, and Spirochaetes establish the minor balance. High levels of strain variation complete this little dissimilarity in the deep phylogenetic lineages. That type of an extreme fan-like phylogenetic architecture of the gut microbiota might have arisen in evolution from the diversification of a distinct limited primary community into strains (Ley et al. 2006). A more detailed view of the differences between genomes of human individuals and the microbiota may foreshadow a deeper comprehension on evolutionary and biological forces that have made us (Dethlefsen et al. (2007).

Apparently there are stringent requirements for membership in the microbial community. Whether or not a bacterium affiliates the local biota depends on the nutritional and physicochemical conditions and the host defence on site. It obeys two fundamental rules formulated by Liebig and Shelford. The nutrient present in the lowest concentration

in comparison with the needs of the organism determines the yield of this organism according to Liebigrs law of minimum. Usually the host and accompanying microbes are the sources of nutrients, intestinal microbes utilise in addition ingested foodstuffs. Shelfordrs law states that each of the physicochemical constraints operating on site have to remain within the tolerance range to make available the survival of a species within an ecosystem. The effectiveness of these basic laws is amended by beneficial and antagonistic interactions among the species present, colonisation history, host characteristics (genotype, age, gender, life-style, diseases). The dual hegemony of Firmicutes and Bacteroidetes apparently originates from their particular and complementary metabolic functions within the microbial community.

The «living-together» is variable with respect to species and topological distribution of microbes involved and the mode of cooperation with the host. The biochemical features of binding and identification of molecules determine the rules of pacing, extent, and pattern of microbial diversity. Different methodologies employed, variation between individuals, changes in microbial nomenclature and taxonomy, and so on hamper the compilation of published data on the distribution of particular fractions of microbes. Nonetheless, an approach is made to survey the diversity of indigenous microbiota in various sites of adult humans (Table 2). Most of the data on the microbiota still have been derived from culture-based investigations. However, cultivation methods under-represent the extent of bacterial diversity. For instance, in samples from human microbiota about 10—50% of bacteria can cultivated, from environments (soil, lakes, seawater, sediments) less than 1%.

In spite of evident variations in tissue structural and functional properties of our outer and inner surfaces and environmental determinants, each microbial community is generally dominated by a limited number of species. Only five phyla contribute more than 90% and establish the core set of regional microbiotas. That was shown for the oral cavity, the distal oesophagus, the stomach, and the colon, where Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes and Fusobacteria are dominating. It holds to be true for skin, too, though Fusobacteria are missing there. Infrequent taxa compose the remainder of the population and may vary between times of sampling and among individuals.

The density of sudoriferous (sweat-producing) and sebaceous (sebum-producing) glands at the particular area principally influence the

Table 2 — **Indigenous microbiota at various sites of human body**

Body site	Number	Diversity	Bacteria
1	**2**	**3**	**4**
Skin, hairfollicle	10^3 -10^6 per cm^2	Stable scaffold of small number of genera	Actinobacteria, Firmicutes, Proteobacteria, Corynebacterium, Staphylococcus, Streptococcus, Propionibacterium, Micrococcus, Kocuria, Malassezia, Brevibacterium, Dermabacter, Acinetobacter, Methylobacterium
Conjunctiva of eye	10^2 - $5x10^4$	Usually not more than 2 species	CNS (coagulase negative staphylococci), Propionibacteria, Corynebacterium, Streptococcus, Lactobacillus, Sarcina, Bacillus
Oral cavity	10^8 per ml saliva 10^7-10^9 per cm^2 tongue	High	Firmicutes, Bacteriodetes, Streptococcus, Actinomyces, Neisseria, Haemophilus, Eubacterium, Lactobacillus, Fusobacterium, Abotriophia, Gemella, Veillonella, Prevotella, Bifidobacterium, Staphylococcus, Propionibactrium, Porphyromonas Plaques: Streptococcus mutans, Streptococcus viridans, Actinomyces, Veillonella, Fusobacterium, Prevotella, Eubacterium, spirochaetes Porphyromonas gingivalis, Bacteroides forsythus, and Treponema denticola
Gastro-intestinal tract	10^{14} 10^{11} – 10^{12} cells per ml	Upper part: low Lower part: high	Oesophagus: Staphylococci, lactobacilli, Corynebacterium, Prevotella, Veillonella. Stomach: Hel. pylori, acid-tolerant streptococci, staphylococci and lactobacilli. Ileum: Streptococci, enterococci and coliforms in the lumen; on the mucosa Bacteroides, Clostridium,

Body site	Number	Diversity	Bacteria
1	2	3	4
			Bifidobacterium. Cecum: Enterobacteriacea, lactobacilli, Bacteroidetes, Clostridium. Colon: Bacteroides, Eubacterium, Bifidobacterium, Clostridium; (by culture-independent methods: Firmicutes, Bacteroidetes, Clostridia, Bifidobacterium, Atopobium)
Respiratory tract		Upper part: high Lower part: no resident microflora	Streptococcus, Neisseria, Haemophilus, CNS, Corynebacterium, Propionibacterium, Prevotella, Veillonella, Mollicutes. Microbes detectable in the lung, which are aspirated with microbe-containing secretions from the upper respiratory tract
Female reproductive system	10^9	High <4—8 per individual	Lactobacilli, CNS, Gram-negative anaerobic bacilli (GNAB), Gram-positive anaerobic cocci (GPAC), Corynebacterium, Bacteroides, Prevotella, Gardnerella vaginalis, Atopobium vaginae

Compiled from Paster et al. (2001), Wang et al. (2003), Pei et al. (2004), Eckburg et al. (2005), de Backer et al. (2007), deFranco et al. (2007), Dethlefsen et al. (2007), Gao et al. (2007), Wilson (2008).

composition of cutaneous microbiota. Corynebacterium, Propionibacterium, and Staphylococci inhabit at any site, propionibacteria dominate sebum-rich sites (forehead, scalp), corynebacteria moist areas (axillae, perineum), and staphylococci are the predominating species in dry regions such as arms and legs. Microbial communities inhabit nose and the pharynx, where they adhere to a substratum in order to prevent

expulsion by the respiratory flow. This region is a carriage site of a number of very important human pathogens (Strep. pyogenes, Strep. pneumoniae, H. influenzae, Staph. aureus, N. meningitides). The airways in the lungs are sterile to a large extent.

The gastrointestinal tract expanding from the oral cavity until colon and rectum is highly diverse from anatomical, functional and microbial points of view. With a surface of 200—300 m^2 it is the largest body surface in contact with the environment. About 700 bacterial phylotypes have been characterised in the oral cavity with considerable variations between various sites. Besides of the densely colonised tongue the teeth are the predominant area in this location. Bacteria on tooth surfaces live in biofilms that are known as plaques. Adhering to these surfaces is of pivotal significance for any potential microbial coloniser, since in this cavity biting, chewing, tongue movements, and salivary flow generate the highest mechanical forces in the body and the danger of displacement. «Pioneer colonisers» such as Actinomyces equipped with adhesins start colonisation of tooth's surface followed by biofilm-promoting Streptomyces. «Secondary colonisers», unable either to adhere, or survive on the bare tooth surface, join expansion to the plaque by autogenic growth.

The largest assembly of microorganisms undoubtedly inhabit the intestine. Most are refractory to cultivation. Physicochemical and nutrient gradients exist along the gut and in the transversal direction of the lumen. This and the complex microstructure of epithelia and the mucous layer on their exterior as well as the high rate of cell renewal make available many niches for microbial existence. So extreme species diversity (up to 1,000 species), inter-subject variability and differences between anatomical and microanatomical locations might be not astonishing (Eckburg et al. 2005). Nevertheless, a few generally consistent traits appear evident (Table 3).

The part of the body that is beneath the skin and the mucosal epithelia is devoid of microorganisms. Therefore, one function of epithelia is to prevent contact of the microbes with the rest of the body. This is realised in various modes (Fig. 1): Several junctions (tight junctions, zonula adherence, desmosomes) link epithelial cells with each other and with the underlying connective tissue matrix to establish a physical barrier. A coat of keratinised dry and dead horny cells, which are not accessible for microbes, further protects the skin. Special

Table 3 — **Characteristic traits of the intestinal microbiota**

Trait	Property and function of gut microbiota	Reference
Biodiversity	Restricted by stringent requirements for membership in the microbial community: - enzymes to utilise available nutrients - cell-wall accessories to contact the proper habitat and to conciliate the supervising immune system, - evade bacteriophages, - fast growing to prevent washout, - mutability to be able to adaptation - stress resistance in order to leap to other hosts overcoming an awkward or toxic environment	Ley et al. 2006
Share of anaerobes	> 99.9% of the cultivable population in adults are obligate anaerobes	Moore and Holdeman 1974
Bifidus spp.	Dominating in the preweaning microbiota, in particular Bifidobacterium longum with high activities of ß-galactosidases to utilise lactose of mothers milk	Schell et al. 2002
Regional population density	Increases from the duodenum containing 10^3 cfu/ml (that is 10^3 organisms per ml luminal contents) to the colon with 10^{11} cfu per ml contents. Thus, it increases by about 8 orders of magnitude along the gut	Savage 1977
Involvement in host metabolism	Microflora operates as a postnatally acquired, multifunctional autochthonous organ, metabolic diversity by far outperforms that of human tissues, complements the metabolism of host, e.g. cracking of indigestible polysaccharides of plant or microbial origin, degradation of dietary oxalates, biotransformation of conjugated bile acids, synthesis of vitamins and amino acids	Salmond et al. 1995

Trait	Property and function of gut microbiota	Reference
Immunity	Coaching the immune system tolerance against a wide mix of environmental and food antigens, decreases number and extent of allergic responses	Braun-Fahrländer et al. 2002
Intestinal epithelia	Maintenance of intestinal epithelial homeostasis, protecting epithelial cell layers and stimulating the development of intestine´s elaborate submucosal network of interconnected capillaries	Rakoff-Nahoum et al. 2004, Stappenbeck et al. 2002
Fat storage in host	Decreases lipid accumulation in adipose tissue, increases lipid mobilisation, prevents adiposity	Ley et al. 2005, Bäckhed et al. 2007

glycoproteins (mucins) secreted by the epithelium prevent mucosal surfaces from microbial sticking. One part, the membranous mucins are fixed to the cells and form the «glycokalyx». Secreted mucins form large linked polymers that are closely associated with the glycokalyx. The epithelium secretes microbicidal peptides, and local B cells secrete IgA. M-cells conduct transport of intact macromolecules from the lumen to the opposite side by an endocytose mechanism. The most remote cells on the top of the villus are regularly being expelled and are thrown out together with mucus and attached microbes.

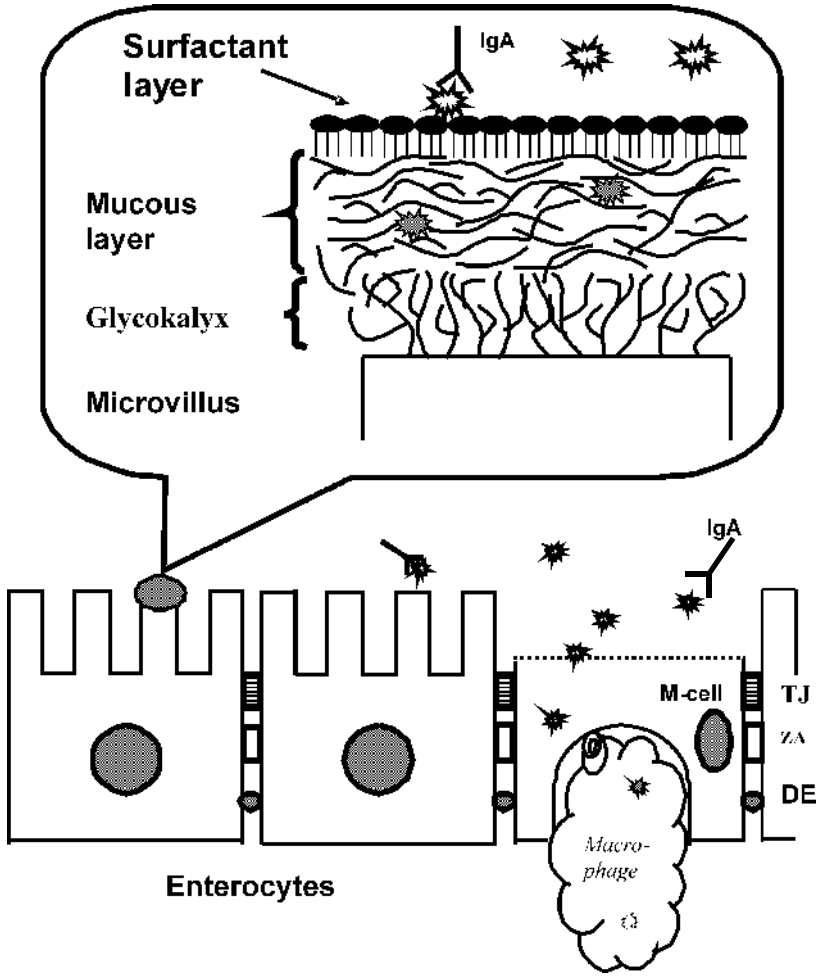

Figure 1. Physicochemical and immune barriers against microbial invaders. TJ = Tight junction; ZA = Zonula adherence; DE = Desmosom.

= Immunoglobulin A attacking pathogen microbes

3. Stabilisation of the microbiota

The microbiota may maintain sustainable stability by two mechanisms: firstly, by interactions among the members of the microbial population, and secondly by interaction with the host.

The general types of interactions between microbial species are shown in the Table 4. These might be positive or negative for the members involved that means beneficial for survival or extinction, respectively. It should be noted that there is some confusion with respect to the term «symbiosis». Now and then it is used to characterise an exclusively beneficial cooperation such as synergism or mutualism. But, strictly speaking, it bears on any long-term relationship between two populations.

Several mechanisms that may be involved in gut stability are suggested in the Table 5. From the viewpoint of system biology species richness insures ecosystems against functional failure since various species give a better warranty that some will maintain functioning even if others fail. An understanding of long-term effects of biodiversity on ecosystem processes may be given by the insurance hypothesis (Yachi and Lereau, 1999). These authors developed a general stochastic dynamic model to assess the effects of species richness on the productivity of the ecosystem, based on individual species' productivity responses to environmental fluctuations. The model shows two major insurance effects: i) a buffering effect, i.e., a reduction in the temporal variance of productivity, and ii) a performance-enhancing effect, i.e., an increase in the temporal mean of productivity.

This and other models were designed to predict the «pure» effects of species richness by randomising and averaging across perturbing environmental factors. Later on Norberg et al. (2001) developed a more realistic, yet general, model linking diversity and ecosystems functioning by modelling the means and variances of phenotypes within a group of species. The presented framework suggests that phenotypic variance within functional groups is linearly related to their ability to respond to environmental changes. Most intriguing is the ratio between short-term productivity with low variance and fixed optimal phenotypes, and long-term productivity in changing environments with higher phenotypic variance. The adaptive model had a higher productivity than the model with a fixed phenotype. The contribution of Norberg et al. is a

Table 4 — **Type of interactions between two microbial populations A and B**

	A	**B**	**A**	**B**
Commensalism	+	0	Supplies vitamins and amino acids, degrades polymers to monomers, solubilisation of insoluble compounds, neutralisation of toxic substances	Utilises essential substances that it is not able to synthesise, utilises monomers, utilises solubilised compounds, protects against toxic substances
Synergism	+	+	Syntrophism (Cross-feeding, food chains, food webs)	
			Bacteroides produces sulphate, Ent. faecalis converts arginine to ornithine and utilises putrescine	Desulfovibrio utilises essential sulphate, E. coli converts ornithine to putrescine that can be further utilised by this species
Mutualism	+	+	A form of synergism in which the relationship between the two organisms is obligatory	
Competition	-	-	Competitve exclusion E. coli and Clostridium difficile compete on certain sugars methanogens and sulphate-reducing bacteria compete on H_2	
Amensalism	+	-	Produces toxic substances: bactericins, hydrogen peroxide, acidic end products	Cessation of growth and killing in an incompatible environment (Candida unable to grow on skin and vagina owing to low pH)
Parasitism	+	-	Protoctists Entamoeba gingivalis in the oral cavity, Dientamoeba fragilis in the intestine	Bacteria are the major food for protoctists

Table 5 — **Mechanisms of maintaining stability in the intestinal microbiota**

Factor	Benefit for population stability
Environment	Host provides optimal and stable conditions for life of microbiota, e.g. supply of nutrients, maintaining temperature and pH, keeping away perturbing foreign microorganisms
Species richness	Phenotypic variance insures microbial ecosystems against functional failure in accord to the insurance hypothesis: - buffering effect to respond to environmental perturbations - performance-enhancing effect
Community formation	More or less tight aggregations of microbial colonies, and formation of biofilms produce locally optimal conditions and protection: - „supergenome" in the population provides momentary adaptation to changes in the environment - coordinated population behaviour by quorum sensing
Separation	Life within the mucous layer along the epithelium and in spatial niches offers protection against competitors, mucous glycoproteins are utilised as food

persuasive affirmation of linking diversification, abundance and evolution as bolstered by Darwin in 1859.

There is now expanding belief that microbes of a single species or a number of different species aggregate to communities of different complexity in the colon and other sites. That encompasses individual cells attached to a surface, loosely aggregated microcolonies as well as biofilms. Four decades ago Guggenheim and Schroeder (1967) observed aggregation and clumping of Streptococcus mutans a few seconds after addition of sucrose into the incubation medium. Formation of high-molecular dextran was surmised to be responsible for this effect, which was soon confirmed experimentally by Gibbons and Fitzgerald (1969). Dextran binds receptor molecules on the bacterial surface and links cells to a network. These findings were conjectured as of clinical relevance for cariogenic plaque formation and may be seen as the first report of elucidation of specific macromolecular interactions to the phenomenon what we now know as biofilm. Probert and Gibson (2002) allude to experimental research on microbial biofilms already in 1936. A biofilm is apprehended as a community of surface-adherent

microorganisms enmeshed within a matrix of extracellular polymeric substances. The matrix components are usually polysaccharides. Its formation is a pivotal strategy of bacterial survival in an inhospitable environment. That makes the affiliates in a way functionally similar to a multicellular organism. The matrix of the biofilm is produced by the cells constituting the biofilm, its structural integrity depends essentially on an intact bacterial metabolism (Il"ina et al. 2004, Branda et al. 2005).

Planktonic bacteria when going to group together in a biofilm modify specific patterns of protein and gene expression, metabolic profile and specific physiology. They approach a new state that is termed «biofilm phenotype» (Whiteley et al. 2001, Sauer et al. 2002).

Biofilm communities tend to be more resistant to antimicrobial stressors, involving those exerted by host-defence, than bacteria of the similar species living planktonic. That is apparently the manifestation of several factors including vitiated penetration of the antibiotic, metabolic activity and phenotypic variability of biofilm cells.

Microbes growing in a colony may faster become antibiotic-resistant due to a greater opportunity of horizontal gene transfer. Such inducible competence and transformation mechanisms gave the basis of the «distributed genome hypothesis» or «supergenome hypothesis» (Ehrlich et al. 2005). It implies the following conceptions (Ehrlich et al. 2008): a huge phenotypic and genotypic diversity within a microbial species; an almost universally polyclonal microbial colonisation; and microbiomes in different hosts are considerably different. A microbial species is thus classified by a core genome that each member of a species possesses. It contains in addition distributed genes, which encompass genes that are not shared by all strains of a species. The species E. coli, for instance, possesses about 4000 obligate/core genes. In addition, there are about 2000 facultative genes, of which about 200-1500 genes are found in each individual strain. The assemblage of facultative genes codifies additional functions, which are not mandatory for survival. Rare metabolic routes, resistance against toxins, pathogenic or symbiosis features or speciation for colonisation of certain niches are such functions. Thus, the facultative genes are distributed in a gene pool. Optimised procedures of horizontal gene transfer afford immediate distribution among all members of the species. New strains can come up through inducible autocompetence and autotransformation systems. Contingency genes (hypermutable loci in the genome) are distributed

within the population and give the property of changes in phenotype at a frequency up to 1:100-1000 bacteria per generation. Typically, activities of genes are affected that interact with the environment. These mutations are random in time and cause heterogeneity within a population of replicating bacteria. That is an important mechanism of momentary adaptation to changes in the environment.

Coordinated population behaviour to gain maximal benefit in a competitive environment implies intercellular communication. Bacteria send out and perceive «hormone-like» signal molecules that increase in concentration as a function of cell density («autoinducer»). The whole population initiates a concerted action as soon as the population density and the corresponding signal concentration approach a certain threshold. Few signal transmitting molecules have been identified: post-translationally modified peptides (Hardman et al. 1998), diketopiperazines furanosyl borate diester (Daniels at al. 2004), inorganic polyphosphates (Brown and Kornberg 2004), acetyl phosphate (Wolfe 2005), N-acylhomoserine lactones (Swift et al. 2001, Dong and Zang 2005). Target genes can synchronously be expressed among a local community and biological activities can be coordinated (Miller and Bassler 2001). This community genetic regulatory phenomenon was coined «quorum sensing». Evidence is provided that quorum-sensing signals influence cellular processes like biofilm association, responses to physicochemical stresses, organelle biogenesis, cell cycle regulation, phase variation in stationary-phase planktonic cultures, virulence factor expression, symbiosis, production of antibiotics, motility, swarming, sporulation, and conjugation.

Bacteria embedded in a biofilm show considerable variability in metabolic and reproductive activity (Fux et al. 2005). The formation of the polymer matrix may increase or decrease in dependence on biofilm evolution (Nadell et al. 2008). Biofilms may contain bacteria of different species. A curious observation on the «social life» within a biofilm was reported recently (Diggle et al. 2007). These researchers showed exploitative behaviour of some individuals. Such bacteria avoid the expenditure of producing signal molecules and of partaking in cooperative activities, and thus proliferate faster than the kin relatives.

Salmond et al. (1995) viewed the action of bacterial biofilms as an example of multi-cellularity in prokaryotic populations. The previous communications on the fascinating and diverse social life of bacteria will focus increasing attention into this field.

During the last decades a variety of factors that contribute obviously to the increased rate of infections by bacteria forming biofilms, e.g. excessive use of antibiotics, disturbances between intestinal flora and hosts immune system, failures in nutrition, damage of epithelia by intracorporeal medical devices with inappropriate coatings and improper application, failure of diagnostic procedures to recognise bacteria growing in a biofilm (Matsumoto and Takahashi 1994). Biofilms in water and its impact in human disease transmission were recently conferred by Huq et al. (2008). Kaufmann et al. (2008) considered the use of quorum-sensing blockers for therapeutic purposes. Studies with Pseudomonas aeruginosa show the role of quorum-sensing and neuraminidase-dependent biofilm formation for respiratory infection. Therefore, perturbance of quorum sensing by macrolides and inhibition of that enzyme may be a novel potential therapeutic perspective to prevent pneumonia (Otto 2004, Tateda et al. 2004, Raffa et al. 2005, Rice et al. 2005, Soong 2006).

4. Hosts immunity and microbiota

Generally, each microbe when entering a human tissue may initiate ruinous outcomes for the host. Physical, chemical and immune barriers created by the intestinal epithelium and the adjacent lymphatic system protect the host against such unwelcome intrusion of enteric microorganisms. Four cell lineages, including enterocytes (form a thin layer of epithelial cells that separates the gut lumen from the mucosal immune system; support specific transport functions for nutrients and give information about the external milieu), hormone-producing entero-endocrine cells, goblet cells (mucin production), and Paneth cells (production of antimicrobial peptides and proteins), compose the epithelial barrier. Intercellular junctional protein complexes such as tight junctions, adherens junctions, and desmosomes maintain the integrity of the epithelium. In addition, M cells are specialised epithelial cells belonging to the follicle-associated epithelium that wraps Peyer's patches. This cell type is involved in the communication between intraluminal microbiota and lymphatic tissue by sampling luminal antigens directly (see Fig. 1). Peyer's patches, mesenteric lymph nodes, diffusely scattered lymphocytes, cryptopatches and great numbers of IgA producing plasma cells in the intestinal lamina propria and epithelium constitute the

GALT (gut-associated lymphoid tissue). It expands along the intestinal mucosa. One should remember that almost all components of the immune system are abundant at the end of a full-term pregnancy, but effective protection of the intestinal mucosa necessitates the microbial incitement of initial bacterial settling.

It is customarily assumed that the immune system exclusively provides defence to the host against a multiplicity of bacterial threats from the environment. However, host immune system and the microbiota of its most adjacent environment establish a symbiotic, highly redundant network. It works as a two-way communication system and brings together the biodiversity of bacteria and the potentially multiple immune response of the host. In this network the host fulfils its keen rationale to keep under control the composition of the microbiota. Species-specific response to suppress or eliminate a particular microbial strain may be disabled, however, owing to the enormous strain diversity. Instead, mechanisms evolved to handle whole matching sets of bacteria with mutual structural and/or functional features. This mission has to be done for the most part by the innate immune system. It provides the first frontier of immune defence against infectious agents and on which the acquired immune system depends for activation and for the majority of its effector functions.

To live together with numerous bacteria in our gut without any harmful effects is an outstanding feat by the local epithelium and the joining lymphoid tissue. The gut microflora, a great variety of antigens brought along with food, and potential pathogen germs continuously challenge the gastrointestinal defence. Intruded microbials were held off by a plethora of sensing and effector functions to initiate proper immune responses. Intact food antigens have to be excluded, an arrangement has to be made with commensals, and pathogens have to be abolished. That is an extremely intricate issue. Obviously a fine borderline secedes the homeostatic balance upheld in the presence of the commensal microbiota and the devastating response to invading bacterial pathogens. For this balance to maintain, a fine-tuned communication between microbiota, epithelium and the underlying lymphoid cells is mandatory. This enduring dialogue is coined microbial-epithelial crosstalk. It ensures tolerance to the normal bacterial flora and prevents inflammatory derailments and their detrimental clinical outcomes (Sansonetti 2004).

Microbes present multifaceted surface structures that phagocytes should identify for immediate killing and triggering the expression of cytokines and chemokines that orchestrate host antimicrobial defence. The affiliated epithelium and the immune cells must first of all recognise present microbes, distinguish them from host cells, and discriminate pathogens from members of the indigenous microflora. Surface structures of microbes that both animals and insects are missing are the basis for this recognition. Janeway suggested this concept in 1989: «... the immune system has evolved specifically to recognize and respond to infectious microorganisms, and that this involves recognition not only of specific antigenic determinants, but also of certain characteristics or patterns common on infectious agents but absent from the host.» These structures were firstly coined PAMPs, for «pathogen-associated molecular patterns». Since such structures are also present in most non-pathogenic species, the term «microbe-associated molecular patterns» MAMPs should be preferred. The recognising molecules on host cells are termed pattern recognition receptors (PRR). In the Table 6 are surveyed PRRs, the corresponding ligands, and the microbes to be recognised by this system as gleaned in the last one decade.

PRR are protein molecules located in outer and inner cell membranes and in the cytoplasm of immune cells and cells of other tissues. A collection of soluble pattern recognising molecules, mainly but not exclusively produced by the liver, circulates in body fluids and contributes to opsonification and complement activation. Recognition is dependent on receptor-ligand interaction between pattern recognising protein molecules on host cells and MAMPs of the microorganism (Medzhitov and Janeway Jr. 1997). Toll-like receptors (TLR), the Я-glucan-receptor Dectin 1 and the mannan receptor on the outer cell membrane and the soluble intracellular proteins NOD1 and NOD2 appertain to cell-associated PRR. They are germ-line encoded and predominantly expressed on macrophages/monocytes, neutrophils, fibroblasts, B cells, dendritic cells, mucosa epithelium cells, endothelial cells, and regulatory T cells (Treg). Thirteen TLRs have been found in mammals as yet.

Although the recognition mechanism at the molecular level is well studied, the mechanisms of discrimination and confining potentially harmful bacteria and allowing indigenous microbiota to remain are far from being elucidated. That is even more amazing, since the cells make use of mutual molecular signalling routes. PRRs bound to membranes

such as TLRs act as signal-transducer. Binding of the particular MAMP on the extracellular portion of the corresponding PRR that bears a motif of leucine-rich repeats initiates dimerisation and/or complexation with other receptor molecules on the inside of the cell membrane. A set of adaptor proteins is recruited to amplify the down-stream signalling. The reaction cascade discharges into activation of transcription factors (NF-κB, Activator protein1), which enter the cell nucleus and lead to a distinct array of gene expression products (Fig. 2). These gene products (cytokines, interleukins) arrange innate immune responses and direct development of antigen-specific acquired immunity (Medzhitov and Janeway 1997, Hallman et al. 2001, Akira and Takeda (2004), Oda and Kitano (2006), deFRanco et al. 2007, Krishnan et al. 2007).

In this framework MyD88 acts as the primary adaptor for microbial signalling and NF-κB is accepted as the central mediator of the immune response. Specificity of signalling is approached by collaboration among TLRs and of TLRs with other receptors, differential utilisation of adaptor proteins, collaboration among multi-transcription factor binding sites in the promoter region of a certain gene, interaction with TLR-independent signalling routes, and so on. Negative and positive feedback loops preclude rampant inflammation that gives rise to ample tissue damage and fatal clinical outcomes. In addition to an appropriate response to a pathogen TLRs play a role in immune cell differentiation and maturation. Since TLRs are able to recognise endogenous molecules of the host (e.g. heat shock proteins, fibronectin, RNA, ATP) a function of these PRR may be anticipated in restraint of immune disorders.

Abbreviations used in the Table 6. Components of antimicrobial defence

Abbreviations

S = skin
E = eye (tear)
O = oral cavity
G = stomach
R = respiratory tract
F = female genital tract
U = urinary tract
LPS = lipopolysaccharide
SLPI = Secretory leukocyte proteinase inhibitor
$SPLA_2$ = Secretory phospholipase A_2

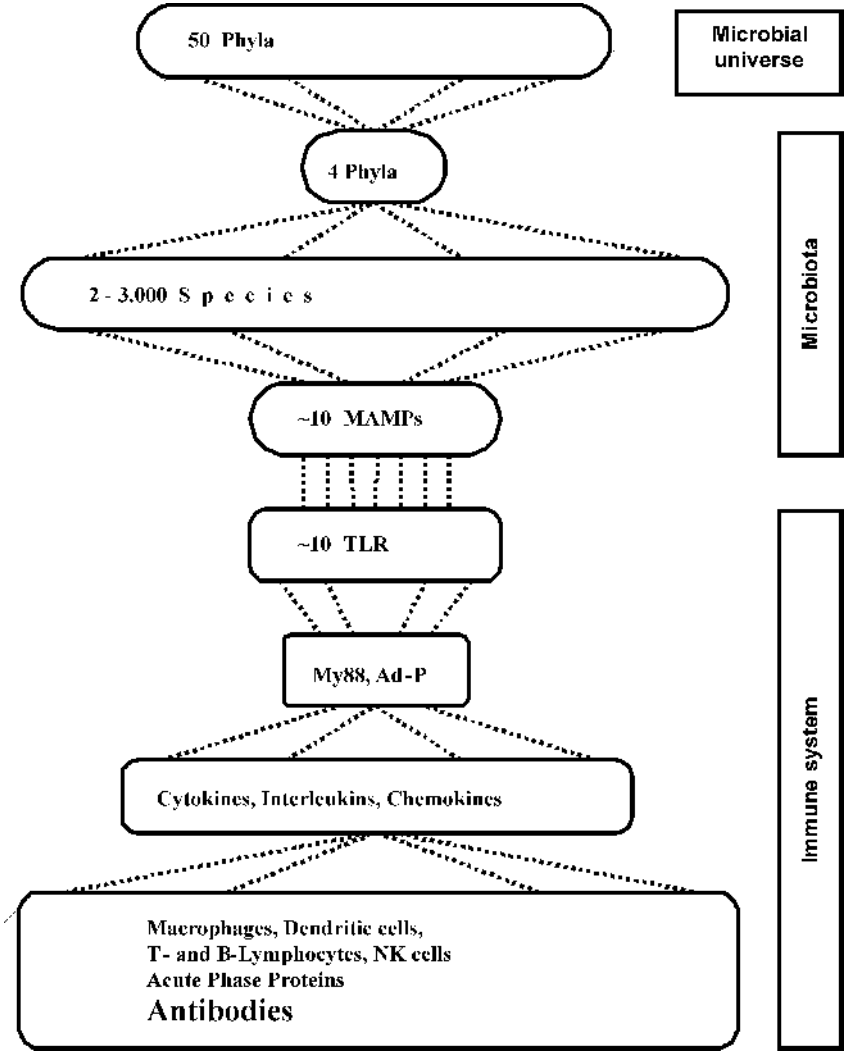

Figure 2. Bow-tie architecture of microbiota-human symbios.

Table 6 — **Non-cellular components of antimicrobial defence**

Component	S	E	O	G	I	R	F	U	Target of antimicrobial and protective activities
Peptide, Protein									
Anionic peptide						+			E. coli, Staph. aureus, Ps. aerug., K. pneumoniae
BPI			+		+	+			Gram-negative species, neutralises LPS
Cathelicin	+		+		+	+	+		Synergic activity with lysozyme against many bacteria, neutralises LPS, attracts neutrophils, monocytes and T-lymphocytes (CD4)
Coeruloplasmin		+							Binding of copper
Collectins						+			Surfactant proteins; Klebsiella pneumoniae
Complement			+						Microbial lysis and opsonisation,
α-Defensin-5					+		+		Cand. albicans, E. coli, Staph. aureus, Lis. monocytogenes, Bac. cereus
ß-Defensin-1	+	+	+	+	+	+	+	+	Gram-pos./neg. species, Cand. albicans
ß-Defensin-4				+		+	+		E. coli, Staph. aureus, Ps. aeruginosa, K. pneumoniae, attracts monocytes
Calprotectin			+				+		E. coli, Staph. aureus, Klebsiella, Cand. alb.
Dermcidin	+								E. coli, Staph. aureus, Cand. alb., Ent. faecalis
Elafin	+					+	+		Staph. aureus, Ps. aeruginosa, neutralises LPS, inhibits neutrophil elastase
Fibronectin		+							Facilitates phagocytosis
Glandulin						+			Gram-neg. species
Hemocidins							+		E. coli, Salmonella, Ent. faealis
Hepcidin		+						+	Candida albicans, E. coli, Staph. epidermidis
Histatins			+						Yeasts, suppression of cytokine induction and plaque formation, attracts monocytes

Component	S	E	O	G	I	R	F	U	Target of antimicrobial and protective activities
IgA, IgM					+				Prevent microbial adhesion, acivate complement,
Lactoferrin		+	+		+	+	+		Fe-binding, activates NK cells, Strep. mutans, E. coli, Vibrio cholerae, Bac. subtilis
Lactoperoxidase		+	+		+	+			Redox catalyst, Staph. aureus, streptococci, H. influenzae, E. coli, Prevotella, Pseudomonas
Lysozyme	+	+	+		+	+	+		Produced by staphylococci, agglutinates Gram-pos. bacteria
Mucin-7		+							N-terminal cleaved peptides antimicrobial active
Psoriasin	+								E. coli, chemokines
Reg IIIy					+				List. monocytogenes and innocua, Ent. faecalis
Sialin		+							Binds microbes
SLPI		+	+	+	+	+	+		E.coli, Ps. aerugin., Staph. aureus, inhibits LPS
$SPLA_2$	+				+	+			Gram-positive species
Statherin			+			+			Pseudomonas aeruginosa
Transferrin			+						Fe-binding
Other									
Acid pH	+			+			+		Acids produced by host (G) or Bacteria (S,F)
Bile acids					+				Predominantly Gram-positive species
CO_2	+								Produced by bacteria, inhibits dermatophytes
Shedding	+		+	+	+		+	+	Shed cells carry microbes
Flow		+	+				+	+	Microbes displaced from surfaces

Compiled from Beisswenger and Bals 2005, Лійvin-Le Moal and Servin (2006), McAuley et al. 2007, Wilson 2008.

This complex immune system and the coexistence with the microbiota pose numerous questions, such as: Why there is no permanent inflammation on the mucosal/epithelial surfaces («commensal paradox»)? There are counteracting mechanisms that bolster or hinder microbial diversity: Why in vertebrates the microbial diversity is greater than in invertebrates, although vertebrates possess a stronger immune system, which may damp diversity? What consequences have bacterial life and sympatry in micro-niches, resource competition, promoted gene flow through random mating, and competitive exclusion on species diversity? How robustness, fragility, resource limitation and performance are involved in the symbiotic association of microbiota and hosts immunity? What are the consequences of systemic or local disturbances? Did the vertebrate immune system evolve under selection pressure to maintain a diverse and stable microbiota? Systems biological methodology gains in importance to disentangle the possible logic behind the network and is beginning to answer such questions (Csete and Doyle 2004, Seymour 2005, Kitano and Oda 2006, Kitano 2007).

In Seymour's model the host possesses a number of distinct niches, which harbour generalist and specialist species with varying rates of growth and reproduction. A low rate of mutation between closely related species and some migration along routes linking niches are permitted. The host acts like a predator in an ecological community, but one with adaptable prey preferences, and uses its immune system to retard the growth rate of the microbiota. The diversity between niches increases with the strength of immunity. In the absence of systematic disturbance a reasonable strong immune response yields highly diverse communities dominated by several distinct species of specialists. Stochastic disturbances such as transitory compromises of immune response constrained diversity of microbiota. Disturbances having a preference to niches yield competitive dominance by the generalist members. Seymour supposes that the high diversity with the relatively even abundance of most species could be related with the commensal paradox: inflammation demands «above threshold» signals to trigger the local cytokine network.

A number of investigators attempted to unveil the design principle and the structural topology of biological networks in relation to their function by use of mathematical models and computer simulation. For it, complex biological networks were deconstructed into conceptually

simple entities (modules) that are made up of many species of interacting molecules (Hartwell et al. 1999, Papin et al. 2004). These networks have topologies that are principally different to those in simple randomly linked networks. The choice of bow-tie as a structural building unit proved to be a concise and smart option for constructing metabolic and signal transduction networks (Zhao et al. 2006, Kitano 2007).

One of the fundamental characteristics of biological systems is robustness. It is a «property that allows a system to maintain its function against internal and external perturbations» (Kitano and Oda 2006, Oda and Kitano 2006). Kitano and co-workers propose that the intracellular signal transduction pathways and the intercellular signal processes of both the innate and acquired immune system consist of a modularised tandem bow-tie pattern (Fig. 2). The MyD88-adapter-protein complex and NF-κB act as bottlenecks in this topology. This main bow-tie network has extensive collateral pathways with multiple positive/negative system controls and crosstalk regulations that may modulate downstream performance. Such an architecture is characterised by a great «fan-in» of signals recognised by PRR, and a great «fan-out» of cytokines, chemokines, and interleukins. In the consequence, the response of the signalling network to a specific set of stimuli depends on the activation levels and temporal dynamics of molecules in this theoretical hyperspace.

That modularised bow-tie design leads to inherent trade-offs among robustness, fragility, resource limitation, and performance. A modular signalling network that is nested by many relatively independent and robust bow-tie units, will give more advantages in generating coordinated response to various stimuli from environment. Both symbiotic partners — host's immune system and a diverse microbiota — coevolved in a symbiotic relationship to optimise robustness against pathogen challenges and nutrient perturbations. This concept is in harmony with «conclusions from a number of studies that these structures result from universal and fundamental organising principles for efficiency and robustness, rather than frozen accidents of evolution» (Zhao et al. 2006). There is a further point of benefit for the host: the enduring homeostasis of microbiota might be exceptionally important against autoimmune disorders and other immune derailments which are predicated on the intrinsic tendency of the immune system toward hyperactivity.

5. How to improve a tilted host-microbiota partnership?

Factors accompanying our current life such as over- and malnutrition, physical inactivity, excessive use of tobacco, alcohol, drugs and medicine, UV-irradiation, incorporation/inhalation of pesticides, toxic gases and heavy metals and growing-up in a sanitised world disturb the immune balance and the host-microbiota partnership with the consequence of a huge increase of chronic inflammatory diseases, e.g. atherosclerosis, inflammatory bowel diseases, diabetes, asthma, rheumatism, neurodermatitis, psoriasis, adipositas, and cancer. The anticipation to improve the host-microbiota partnership, immunity and constitutional performance by food additives is based on four aspects: i) using the recent advance in understanding of the dynamic interplay between host immune system and microbiota, ii) recognising the established history of using health-promoting extracts of fungi, algae and herbs in traditional oriental medicine and in food supplementation, iii) assuring usefulness and safety of food ingredients for disease prevention and treatment, and iv) the bioactive molecules to come into question can be given to the diet, without the prerequisite to cope with phase-I/II/III clinical trials. Orally administered agent's come into direct contact with the largest immune tissue GALT and its microenvironment in the intestine, which may have an explicit effect on the microbiota and the ensuing final immune response.

There may be three options to accomplish such an entitlement:

i. Immunomodulating option: Uptake of immune modulating ingredients to prevent inflammatory lapse or trace back a derailed immune system into balance.

ii. Prebiotic option: Uptake of non-digestible substrates to feed health-promoting members of indigenous microbiota, e.g. Bifidus spp.

iii. Probiotic option: Uptake of health-promoting bacteria such as Lactobacillus spp. and Bifidus spp.

Compounds that can interact with the immune system to upregulate or downregulate the host response are termed immunomodulator, or synonymously immunostimulant, immunopotentiator, immune enhancer, or biological response modifier. The concept of immunomodulation is to induce the same response as that taking place during the initial phase in a common infection without producing sustained dangerous inflammation or harsh side-effects. A particular immune sub-function

has to be increased or suppressed in such a way as to enhance or complement a desired response. Such a proceeding is promising since i) it strengthens hosts own defence mechanisms, and ii) it excludes the use of species-specific antibiotics.

The inflammatory reaction mostly attains a fine balance that eliminates pathogen microbes and repairs damaged tissues. Strictly regional low-grade inflammations may occur now and then and should be understood as a sub-clinical everyday occurrence. However, the fine-tuned response can go awry and a persistent inflammation arises, often lacking a recognisable infection. Cytokines, interleukins and chemokines are local messenger molecules that mediate information between cells. These mediators have a great impact on cell division, differentiation, growth and immunity. It makes sense to envisage the cytokines as constituting a network of inherent agonist and antagonist functions, e.g. pro-inflammatory and anti-inflammatory reactivity, or growth-promotion and suppression (Balkwill 2000). At an inflammatory site pro- as well as anti-inflammatory mediators are consecutively up-regulated followed by down-regulation. In states of chronic inflammation the equilibrium is skewed towards the inflammatory arm. The outbalanced cytokine network induces inflammatory cell recruitment of the tissue(s) concerned, vitiation of cell functions, and tissue-matrix injury.

Many immunomodulating compounds are polysaccharides or polysaccharide-protein complexes, such as the zwitterionic polysaccharides, mannans, protein bound polysaccharides PSK and PSP, and hyaluronan. One of the best studied is the group of beta-glucans. These polysaccharides are widely distributed in the biological matter in, for example, yeast, fungi (excluding zygomycetes), bacteria, and lower and higher plants. They occur as a primary constituent in the cell wall of fungi and bacteria. (1→3)-beta-glycosidic bonds link the glucose units in the backbone. Side chains are formed as (1→4)-linkages or as (1→6)-linkages. (1→3),(1→6)-beta-D-Glucans are non-toxic, non-immunogenic, and non-allergenic. beta-Glucans react with PRRs (e.g. Dectin1) on monocyte/macrophages, natural killer cells, neutrophils, dendritic cells, vascular endothelial cells, keratinocytes and fibroblasts. The macrophage has been identified as the master cell in the first line of defence against most infections as well as in mediating the response of the innate and acquired immunity. This cell type can be activated as the result of a common infection process or by products of fungi or bacteria.

Binding of beta-glucan to macrophages initiates complexation of the beta-glucan receptor with TLR2 and TLR6 and activation of signal transduction pathways involving NF-κB-like transcription factors (Luhm et al. 2006). It alerts the innate immune system and primes it to respond immediately and properly to infections. The anti-inflammatory efficacy has been shown in-vitro and in-vivo (Gerber et al. 2005, Lull et al. 2005, Dillon et al. 2006, Jung et al. 2007). As an example, the Figure 3 shows the results of a study on thoroughbred race-horses performed in springtime. In that period regular hair coat replacement, environmental changes, intensification of training loads, transportation, heavy physical exertion and competitive races, and frequent contacts to other animals bring about an enduring metabolic, immune and psychological stress. The animals respond with elevation of acute phase proteins in blood (fibrinogen, haptoglobin, SAA), which are accepted markers of inflammation. These changes are ameliorated by the immune modulating activity of yeast beta-glucan.

In 1995 Gibson and Roberfroid inaugurated the prebiotic concept as part of the functional food strategy. A prebiotic should fulfil the following criteria: inertness against hydrolytic cleavage and absorption in the upper part of the gut, acting as selective substrate for one or few health-promoting colonic bacterial species, and constraining pathogens via keeping up growth and/or activity of health-promoting species. Gibson et al. (2004) suggested a definition of prebiotics taking into account nutritional, biochemical, physiological and microbial areas that may benefit from a selective targeting of particular micro-organisms: «A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health». At present the only compounds with prebiotic functions are carbohydrates. The genera commonly targeted are Bifidobacterium and Lactobacillus, the growth and activity of which should be increased.

Human breast milk may be seen as the prebiotic per se. The bifidus factor — a glycoprotein — is the specific entity responsible for the dominance of bifidus bacteria in the colon of newborns fed breast milk. A range of oligosaccharides has been proven in experimental studies to exert prebiotic activity, e.g. oligosaccharides of fructose, galactose, mannose, xylose, and (1→6)-linked glucose, lactulose, isomaltooligosaccharide, palatinose, and tagatose. However, in food manufacturing

around the globe inulin, fructooligosaccharides, and galacto-oligosaccharides are the dominating types. There are attempts to develop a «second generation» of prebiotics with enhanced activity and desirable attributes such as fine control of microflora modulation, specific inhibition of pathogen adhesion, varying viscosity, lack of side-effects, and persistence to distal regions of the colon (Rastall and Gibson 2002).

The term «probiotic» was coined by Lilly and Stillwell (1965) in contrast to antibiotic to describe substances secreted by one microorganism, which stimulates the growth of another. Following Havenaar and Huis Inrt Veld (1992) and Schrezenmeir and de Vrese M (2001) a probiotic is defined as «A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host». This definition is applicable independent of the probiotic site of action and the route of administration. A synbiotic — in the strongest sense of the term — is a blend of a prebiotic and a probiotic «... in which

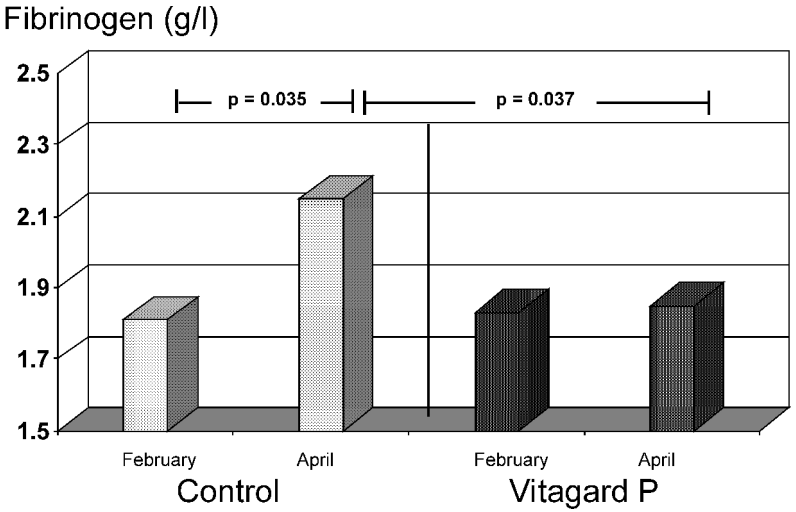

Figure 3: Yeast (1?3),(1?6)-beta-D-glucan (Vitagard P) attenuates the springtime stress-associated increase of the acute phase protein fibrinogen in blood serum of race-horses.

the prebiotic compound selectively favours the probiotic compound» (Schrezenmeir and de Vrese M, 2001).

The beneficial effects of probiotics and prebiotics on human health are critically surveyed by Hanske and Blaut (2006). The probiotics aimed for human use must fulfil a number of criteria: preferentially from human origin, genetically stable, non-pathogenic and safe, insensitive to gastric acidity and bile salt, and to remain viable during technological processing. The comprehension of the mechanisms underlying the health promoting claim(s) should be verified by a scientifically established methodology to improve the credibility of the prebiotic and probiotic conception. Utilisation of advanced molecular methods for monitoring variations in the particular microbiota are compulsory.

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