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Infection, inflammation, and chronic diseases: consequences of a modern lifestyle

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Infectious diseases, including tuberculosis, malaria, hepatitis, pneumonia, dysentery, and helminth infestations, still constitute a profound threat in developing countries. Curiously, their decline in high-income societies is paralleled by an unprecedented emergence of allergic disorders, notably asthma and atopy, and chronic inflammatory and autoimmune diseases, such as Crohn's disease, type 1 diabetes, and multiple sclerosis. Several changes in lifestyle are associated with this transition, including diminished exposure to soil and animals, nutritional bias, obesity and increased exposure to pollutants and antibiotics, which all impact the intestinal microbiota. Understanding the mechanistic links behind the epidemiological observations, the complexity of a changing microbiome, and the immunoregulatory consequences of microbial encounter in barrier organs was the subject of the 99th Dahlem Conference.

Epidemiology: an astounding association

In the last century, high income regions have seen a sharp decline in hepatitis A, childhood diarrhea, and parasitic diseases. In the 1960s, a substantial increase in asthma, type 1 diabetes (notably in children under 5 years of age) and multiple sclerosis (MS) occurred, and accompanied rising incidences of hay fever and atopy in the same Westernized societies [1]. Recent studies on border populations in Karelia (Russia) and Finland, as well as in migrant populations from developing to industrialized countries confirm that household income and gross domestic product positively correlate with the incidence of allergic and autoimmune disorders, and negatively correlate with exposure to certain infectious agents [2,3]. Growing up in farms (in proximity to livestock and with exposure to non-pasteurized milk) protects against asthma, allergic rhinitis, and inflammatory bowel disease (IBD), but not against type 1 diabetes or rheumatoid arthritis [4].

Individuals having multiple, particularly older, siblings or having stayed in daycare are more likely to be protected against asthma and atopy. The latter finding led to a new theoretical framework for the development of allergies that was coined the "hygiene hypothesis" in 1989 [5]. This term, unfortunately, focused scientific and media attention on the rituals of current household hygiene, rather than on the multitude of major lifestyle changes that more likely impacted on increased incidences of chronic inflammatory and autoimmune diseases (CID) in high-income countries. In its more current version, changes in environmental exposure to infectious agents, allergens, feces, pollutants, antibiotics etc., in concert with specific variations in disease genes affecting receptors that recognize environmental stresses, or regulating barrier integrity, are thought to lead to a breakdown of the normally coordinated, homeostatic inflammatory response. This, in turn, precipitates chronic disorders (Figure 1). At the 99th Dahlem Conference (Berlin, June 9–12, 2009), 37 experts from the fields of molecular genetics, evolutionary biology, population epidemiology, structural and cell biology, immunology, nutritional sciences, experimental animal research, and clinical medicine, engaged in an interdisciplinary examination of the role of microbes (symbionts, commensals and pathogens) as elicitors, perpetuators, modulators and terminators of inflammatory responses (Box 1).

Lifestyle changes and microbial environment: towards a mechanistic hypothesis

Major improvements in sanitation and clean water supply, concomitant with a reduction in gastrointestinal infections in newborns, preceded the steep rise in allergic diseases. Viruses, helminths and bacteria have all been associated with protection from CID. In particular, gastrointestinal infections have a major protective impact. For example, Turkish immigrants in Germany, who continually travel to Turkey, have a higher rate of salmonella infections, but fewer allergies, while Turkish families who closely emulate a German lifestyle do not [6]. In addition, *Helicobacter pylori* infections are inversely associated with asthma in urban populations [7]. However, it remains unclear whether the occurrence of certain pathogens serves as a marker of poorer hygiene (and, therefore, potentially trigger multiple changes in the environment or the gut-associated microbiome, not only decreased fecal contamination), or whether a causal association indeed exists between

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[3] see Box 1.

1471-4906/$ – see front matter doi:10.1016/j.it.2010.02.003

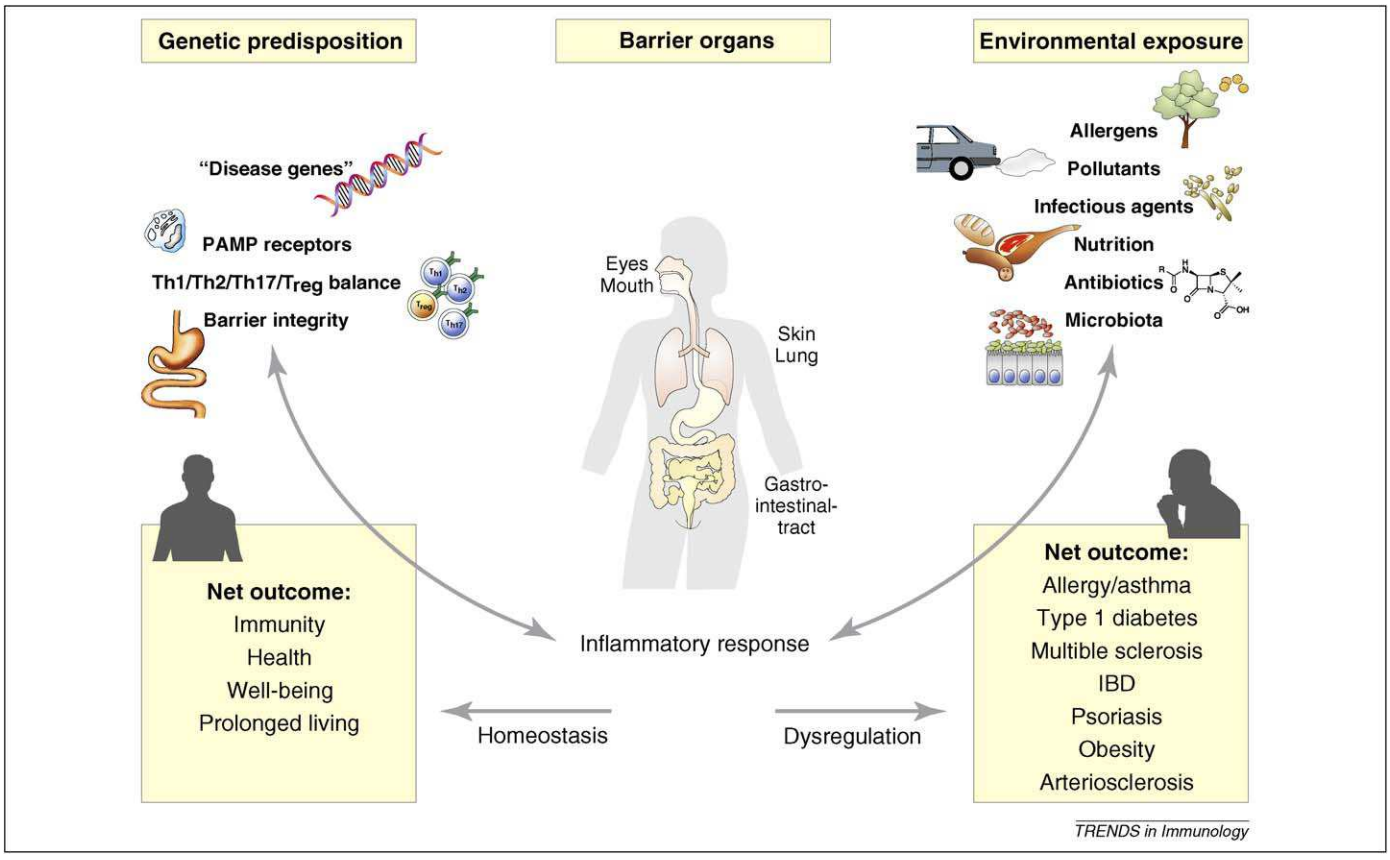

Figure 1. Gene-environment interactions leading to inflammatory responses in barrier organs. Barrier organs (lung, skin, gut) are exposed to environmental stresses. Depending on the genetic predisposition (for example, in pattern recognition receptors for microbial molecular patterns (PAMP receptors) or pathways of immune regulation (Th1, Th2, Th17, Treg cells), stress signals are integrated at the site of assault to maintain homeostasis (beneficial results, left-hand box). If dysregulation occurs, inflammation is not terminated appropriately, and detrimental outcomes ensue (right-hand box). Restoration of homeostasis may, in some cases, be achieved by manipulating the microbiome.

declining infection rates and increases in allergic disease (see Box 2).

In patients suffering from MS, helminth infections can safeguard against relapse. This is a result of the development of myelin-specific regulatory T cells that secrete immunomodulatory cytokines, and is not just a bystander effect of helminth infestation [8]. Similarly, in mice infected experimentally with helminths, the induction of specific regulatory T cells (Treg or Tr1 cells) protect against excessive inflammation [9]. It has been concluded that chronic exposure to helminths, similar to that occurring in early human hunter-gatherer populations, conditioned the immune system to tolerate the unavoidable, and thus, in evolutionary terms, "turned the inevitable into a necessity" [10]. In modern times, with the eradication of helminths in high-income countries, this stimulus is lost, and inflammatory responses to infectious agents can evade control by immunoregulatory circuits. An alternative view is that a change in immune conditioning may have also uncovered the pathogenic potential of yet undiscovered persistent infectious agents capable of causing allergies, CID, as well as cancer [11]. With regard to the latter, an enterotoxin secreting strain of the human commensal, *Bacterioides fragilis* not only chronically colonized mice, but also triggered colitis and strongly induced colonic tumors in a susceptible mouse strain. [12]. Accordingly, insufficient or deviant immune activation for antimicrobial defense, rather than excessive reaction, may in some cases be the ultimate cause of CID.

A likely consequence of a change in lifestyle is the development of dysbiosis, or disturbed variability of the gut microbiome. Mammals maintain a lifelong association with innumerable commensal microorganisms that inhabit almost every environmentally exposed surface of the body, including the gastrointestinal tract, the oral cavity, the nasopharynx, the respiratory tree, the vagina and the skin. Astoundingly, the gastrointestinal tract harbors

Box 1. 99th Dahlem Conference

The Dahlem Workshop model is based on background papers (provocative mini-reviews and opinion statements) prepared in advance and discussed in the atmosphere of a think-tank workshop by a panel of experts. Participants of the 99th Dahlem Conference on ''Infection, Inflammation and Chronic Inflammatory Disorders'' were (conference organizers, workshop conveners and session rapporteurs are underlined): Frederick M. Ausubel, Jean-Francois Bach, Yasmine Belkaid, Bruce Beutler, John Bienenstock, Anita van den Biggelaar, Fredrik Bäckhed, Thomas Bosch, Lucienne Chatenoud, Robert Coffmann, Stephen M. Collins, Max D. Cooper, Stefan Ehlers, Paul Ewald, Alan Ezekowitz, Matthias von Herrath, Jules Hoffman, Patrick Holt, Jean-Luc Imler, Christopher Karp, Dennis L. Kaspar, Stefan H. E. Kaufmann, Rick Maizels, Paolo Matricardi, Samuel Miller, Stephen D. Miller, Lorenzo Moretta, Erika von Mutius, Liam O'Mahoney, Thomas Platts-Mills, Eyal Raz, Graham Rook, Paul Schulze-Lefert, Fergus Shanahan, Alan Sher, Ulrich Steinhoff and Dale T. Umetsu. Background papers are published separately in a special issue of *Clinical and Experimental Immunology, 160 (1), 1–135 (April 2010)*.

Box 2. From epidemiology to molecular pathogenesis – an example

Epidemiological studies have shown that infection with the hepatitis A virus (HAV) is associated with a reduction in the risk of developing atopic diseases [6]. A potential role for HAV as a safeguard against atopy has been strengthened by genetic studies demonstrating that polymorphisms in the gene encoding the receptor for HAV, *TIM1/HAVCR1*, are associated with protection against atopy. Furthermore, recent studies revealed that TIM-1 is a receptor for phosphatidylserine, and thus can mediate phagocytosis of apoptotic cells. Since clearance of apoptotic cells is critical for the development of immune tolerance, HAV and TIM-1 may regulate immunity and tolerance by promoting engulfment of apoptotic cells. Further studies to elucidate the function of TIM-1 and its interactions with HAV will provide insights into how infection regulates chronic inflammatory disease [53].

$\sim 10^{14}$ microorganisms of ~1,000 different species, with a total mass of 1000 grams. Many of these microorganisms remain unknown. Collectively, the microbiota can be regarded as an additional 'forgotten organ' with the capacity to execute various physiologic functions, and, as a result, may significantly influence host biology [13,14].

Recent advances in culture-independent molecular-based methods have begun to reveal the full extent of the complex composition of the different microbiota, only a minor fraction of which can be cultivated on standard microbiological media. The colonic microbiota is the most densely populated microbial ecosystem known, and is dominated by bacteria belonging to the phyla (classes) Firmicutes, Bacteroidetes, Actinobacteria and Verrucomicrobia. Altered gut (fecal) microbiota has been associated with several metabolic and inflammatory diseases in humans, although this does not by itself establish causality [15,16]. More specifically, IBD and obesity are characterized by reduced microbial diversity implying the necessity of a 'complete' microbiome (the collective microbial genomes) for a healthy microbiota and human host. Recent data suggest further that humans maintain a "core microbiome" in the absence of a strictly defined microbiota. This means that, as long as relevant genes are present, it is less important which species carry them [17]. Thus, it may become feasible to identify marker genes or indicator species within the microbiota, such as the beneficial *Faecalibacterium prausnitzii*. These markers could then be exploited as indicators of a healthy diversity, and for differentiation from a disease-promoting dysbiosis [18].

The microbiota produces a broad spectrum of metabolites that can interact with the host. These include fermentation products (e.g., short chain fatty acids), neuroactive molecules (e.g. gamma-amino butyric acid, nitric oxide, hydrogen sulphide, carbon monoxide, ATP, basic amines) and immunostimulatory bacterial constituents (e.g. lipopolysaccharides, capsular polysaccharides, and DNA). The impact of these molecules on immune and neural systems, as well as on specific cell types, such as epithelial and enteroendocrine cells has been largely underestimated. This cross-talk at the interface of the host receptors and the microbe-derived ligands may not only contribute to immunologic disorders but also to the pathogenesis of neuropsychiatric disorders [19,20].

Immunoregulatory consequences of microbial encounter

The importance of interkingdom signalling is most evident from functional and morphological comparisons of colonized and germ-free mice. For example, germ-free mice born and raised under sterile conditions have a rudimentary immune system, abnormal gastrointestinal function (such as altered intestinal permeability, motility, and mucin secretion), and exhibit reduced vascular, nutritional, and endocrine functions. They are more prone to infection than conventionally colonized animals, and show increased locomotor activity, behavioural changes and exaggerated hypothalamic-pituitary-adrenal (HPA) responses to stress. These alterations reverse upon bacterial colonization, the timing of which may be critical in some instances, such as in the HPA stress response [21].

Germ-free animals, born and raised under sterile conditions, display many defects in the development of intestinal tissues. Certainly in the gastrointestinal tract, and probably at other colonized sites, immune responsiveness is enhanced by microbial colonization [22]. Since several different bacterial species (e.g. *Lactobacillus sp., Bacterioides sp.*, segmented filamentous bacteria) can reconstitute the immune system of germ-free animals, there must be considerable redundancy in the microbial triggers involved. Reconstituting the bacterial flora, however, is particularly successful with only a restricted number of host-specific bacterial species at a relatively early post-natal stage [23]. The presence of trillions of bacteria places a tremendous antigenic and immunostimulatory burden on the intestinal immune system. Hence, it must be able to respond selectively to or tolerate bacterial products of the microbiota. Germ-free animals have a particular deficit in immunoregulation, and in the induction of tolerance to antigens ingested orally [24]. Breakdown of tolerance leads to severe tissue damage, such as that found in IBD. The failure to adequately prime and differentiate regulatory T cells is one likely basis for various chronic diseases (possibly including depressive and neurodegenerative disorders) [20].

Immune homeostasis, however, should not be interpreted as the absence of a response to the microbial world, but as the outcome of appropriate immune activation, immune counter-regulation, and immune-driven trophic and repair processes. Following microbial colonization, the system does not completely reset to baseline conditions [25]. In addition, there is clear tissue- (and even niche-) specificity, both in the character of the immune-microbial interface and of immunoregulation. While the gut microbiota has been relatively well characterized, the exact composition of the lung and skin commensals, particularly concerning the non-culturable microorganisms, is only beginning to be explored. Importantly, early life (including prenatal) experience with the microbial world plays a critical role in controlling functional maturation of the developing immune system, and influences its subsequent trajectory [26].

Complex and simple model systems to assess the implications of the lifestyle hypothesis

Despite a great diversity in counter-regulatory pathways, mouse models have suggested common cellular and

molecular mechanisms underlying counter-regulation of diverse classes of organisms, e.g., bacteria, fungi and helminths, commensals, symbionts and pathogens, as well as anatomical sites. In the afferent arm, these include Toll-like receptors (TLRs) and other pattern-recognition receptors (PRRs). In the effector arm they comprise cytokines, notably interleukin (IL)-10 and transforming growth factor-beta (TGF-β), and regulatory lymphocytes, e.g. Treg and Tr1 cells, and dendritic cell subpopulations [25,27,28].

In terms of understanding the immunological effects of the microbiome in experimental mouse systems, the critical micro-anatomical sites of interactions remain to be identified. Also, we need to understand whether the presence of unique species or the existence of dynamic diversity serves as a driving force. It has become clear that current immunological research is likely to be heavily confounded by distinct microbial environments in various mouse facilities [29]. Stable, reproducible models exploiting mice colonized with a defined flora are necessary. On the other hand, studies in germ-free mice are likely compromised by the fact that microbes function in qualitatively different ways in monocultures versus communities, and by the potential lack of colonization with human pathogens or commensals.

One critical issue is the selection of specific microbiota by distinct hosts. Evidence from basal metazoans such as hydra indicate that a given species can stably maintain a unique microbiota for decades. In a mutant strain of *Hydra magnipapillata*, removing gland cells and neurons from the epithelium caused significant changes in hydra's microbial community, whereas the absence of interstitial stem cells in nematocytes had no effect on the microbiota [30]. A likely mechanism is differential production of defensin-like molecules [31]. This implies strong host-dependent selective pressure on maintenance of a unique microbiota for commensalism. Specific selection of distinct microbes such as *Rhizobium* spp. for nitrogen fixation in legume root nodules, or of *Vibrio fischeri* in bobtail squid, illustrate benefits for host physiology conferred by single microbial species [32,33]. The dialog between the drosophila host and its gut microflora involves antimicrobial peptides. Inhibition of the expression of Caudal, a homeobox protein repressing NF-κB-dependent antimicrobial peptide production in the gut, alters composition of the microflora [34]. Increased production of antimicrobial peptides in the gut of Caudal-silenced flies leads to decreased numbers of dominant members of the normal gut flora. Remarkably, this decrease is accompanied by a profound increase of a *Gluconobacter* strain that is otherwise a minor constituent of the gut microflora, leading to epithelial damage. Drosophila and hydra thus serve as promising model systems for in-depth analyses of the contribution of antimicrobial peptides to the interkingdom dialog between host and its endogenous microbial flora.

Human studies that are likely to be informative in terms of driving future mechanistic investigations include: comparison of the microbiome and early immune development in populations undergoing rapid socioeconomic transition; the relationship between the microbiome and early immune development in infants born via Caesarian versus vaginal delivery in Westernized populations; comparison of the airway microbiome and early immune development.

Are there endogenous ligands for innate receptor activation?

Many seemingly "sterile inflammatory diseases" exhibit the same type of inflammation (e.g., in rheumatoid arthritis, ankylosing spondylitis, sarcoidosis) as that elicited by microbes, yet no microbes have been assigned a unique etiologic importance in these diseases. Endogenous ligands may drive innate responses in these pathologies, although the pathways activated, and the proximal ligands, have yet to be identified unequivocally, and their interactions characterized structurally [35].

In one canonical autoimmune disease, systemic lupus erythematosus (SLE), TLR9 and TLR7 activation are possibly pathogenically important, maintaining an auto-amplification loop that drives the expansion of B-cell clones with specificity for nucleic acids [36]. Inherited autoinflammatory diseases (Blau's syndrome, neonatal onset multisystem inflammatory disease, cold-induced autoinflammatory syndrome and familial Mediterranean fever) provide instructive examples of harmful consequences of chronic dysfunction of the Nod-like receptor (NLR) pathways [37].

In many inflammatory diseases, particularly those involving the intestinal tract, PRR activation probably drives pathology, but—at least partly—depends upon the microbial flora. Some mutations that produce classical inflammatory or autoimmune diseases (e.g., mutations of *Ptpn6*, that encodes the anti-inflammatory SHP1 phosphatase) do so only in the presence of microbes, and the inflammatory phenotype is not manifested in germ-free animals [38]. In other instances, the microbial flora exacerbates disease initiated by, for example, experimental *Toxoplasma gondii* infection in mice [39]; this may reflect a switch from "toning effects" of microbiota on immunoregulation to "disease-enhancing" stimuli when the anatomic barrier becomes defective or undergoes repair.

Frequently, the discrimination between commensals and pathogens is largely context-driven: a commensal that finds itself in the wrong anatomical space will elicit a strong innate response. Moreover, a microbe may become a pathogen only by virtue of its ability to damage a host cell (e.g., through its possession of a type III secretion system), and trigger a host response in this way. *Candida albicans* is an illustrative example of this dynamic scenario: this commensal can drive pathology, such as IBD, only when a particular assembly of pathogen-associated molecular patterns interacts with a defined, and sometimes disturbed, repertoire of PRR [40].

The future of anti-inflammation research: exploiting the microbiome for diagnosis and therapy

Despite increasing insights into colonic microbiota, the field of metagenomic research has faced several technical shortcomings: (1) samples are obtained and stored differently in various studies; (2) different lysis protocols are being used; (3) different regions of the 16S gene are analyzed; (4) different analysis platforms are used (qPCR,

DGGE, cloning and full-length sequencing, 454 pyrosequencing). These differences make comparisons between studies next to impossible. Thus, harmonization or even standardization should become a priority of future research. Transcriptomics (including mRNA and microRNA) of the host and microbiome together with proteomics and metabolomics should be pursued as complementary strategies [41].

Although we have begun to accumulate knowledge on gut microbiota, most, if not all, studies focus on stool samples reflecting the communities of the distal colon but not the more proximal ones. Furthermore, different regions of the intestinal microbiota harbor unique communities (e.g., luminal vs. mucus-associated). Thus, a systematic investigation of the gut microbiota is needed along the length of the gut (longitudinal) as well as its radial extension (lumen vs. mucus). In addition, kinetic studies are key to determining whether the gut microbiota remains stable over time in a given individual. Finally, studies of the microbiota of other epithelial surfaces should be performed. Much may be learned about the evolution of the microbiota by comparing the microbiota of hunter-gatherers of remote areas of the globe and individuals with a Westernized lifestyle.

Given that changes in microbial exposure are partly responsible for increased CID incidences in Westernized societies, the opportunity exists to reduce the risk of disease by manipulating the microbiome (Figure 1). Experimentally, colonization of the small intestine of mice with a single commensal microbe, segmented filamentous bacterium (SFB), was sufficient to induce the appearance of CD4(+) T helper cells that produce IL-17 and IL-22 (Th17 cells) in the lamina propria. Colonization with SFB was correlated with increased expression of genes associated with inflammation and antimicrobial defense and resulted in enhanced resistance to the intestinal pathogen *Citrobacter rodentium* [42]. In humans, strategies that mimic microbial exposure in an earlier era, such as that of the hunter-gatherer lifestyle, could be particularly appropriate, provided that potentially harmful side-effects can be excluded. It would be important to identify markers that indicate microflora conducive to health versus microflora facilitating disease, since intervention strategies require such surrogate markers to predict risk of disease. The somewhat "romantic" view, however, which holds that restoring the microbiota of our distant ancestors would be purely beneficial, needs to be viewed from an evolutionary medicine standpoint: each individual species of the microbiome is fighting for its niche in a struggle for survival and is part of a highly organized community that is not necessarily aimed at the wellbeing of a respective human host [11]. Thus, the benefits of microbiome manipulation likely come at a price.

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO). The most frequently used bacterial strains include *Escherichia coli* strain Nissle, *Lactobacillus rhamnosus* GG, *Bifidobacterium breve*, and a mixture of eight different probiotic bacteria called VSL#3 [44]. Probiotics are used with the intention to (i) block pathogenic bacterial effects by producing bactericidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium; (ii) enhance innate immunity, modulate pathogen-induced inflammation via PRR-regulated signaling pathways, and fine-tune T cell regulation; (iii) stabilize intestinal epithelial homeostasis by promoting intestinal epithelial cell survival and enhancing barrier function [43]. Robust evidence for a positive impact of probiotics on human health, however, is rare [44]. The best evidence thus far is in the context of prevention or therapy of acute enteric infections, such as rotavirus diarrhea, travellers' diarrhea or antibiotic-associated diarrhea [45]. In addition, a beneficial effect of some, but not all, probiotics has been documented in irritable bowel syndrome [46]. Strategies to modify immunoallergic disorders in human adults have been disappointing, and in stark contrast to results in animal models, in which probiotics have generally been administered early in life and prior to disease onset. Thus, timing and dose of administration appear critical. In two studies, however, *Lactobacillus rhamnosus* GG was effective in preventing early atopic disease in infants and young children at high risk [47,48].

In some instances the molecular mechanism of probiotic action has been conclusively demonstrated. Polysaccharide A of *Bacterioides fragilis* was found to be protective against experimental colitis in mice by inducing interleukin-10-producing CD4+ T cells [49]. In one instance, a single antimicrobial factor (bacteriocin) was shown to account fully for the protective effect of a particular strain of *Lactobacillus salivarius* against experimental listeriosis of mice, whereas the same microorganism protected against salmonellosis, but by a different mechanism [50]. Thus, data from one organism or one indication cannot be extrapolated to other settings, and much of the confusion concerning the efficacy of probiotics results from the lack of detailed molecular characterization of the (often proprietory) bacterial strains and components used.

To mine the microbiota for factors that influence the development and efficacy of the immune system, to restrict the growth of undesired microorganisms such as *Clostridium difficile*, or to affect nutrient availability (retinoic acid, resorbable fat quantity, etc.), it will be critical to breathe new life into classical bacterial physiology and establish culture procedures for species that have not been grown to date. The wealth of bioactive molecules to be harnessed from these versatile organisms for prevention and therapy is virtually untapped, and thus provides a rich source [51]. Given that helminths have well-documented effects on Treg cell induction in many model systems, formal trials are already being conducted in inflammatory bowel disease, allergies, and multiple sclerosis using ova of the pig whipworm or hookworm [52].

In summary, the Dahlem Conference emphasized that open-minded communication between diverse disciplines uncovers not only gaps in our knowledge, but also novel strategies to bridge them: after all, elucidation of inter-kingdom crosstalk requires full use of our interdisciplinary capacities.

Acknowledgements

SE is supported in part by the Cluster of Excellence "Inflammation at Interfaces" and funding by the DFG and BMBF. SHEK receives financial

support from the EC, BMBF, BMGF and others. The 99th Dahlem Conference received a generous grant from the Volkswagen Foundation. The authors would like to thank M. Brückner for organizing all technical aspects of the conference and M. L. Grossman for help in preparing this manuscript.

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