A new vision of immunity: homeostasis of the superorganism

G Eberl^{1,2}

The immune system is commonly perceived as an army of organs, tissues, cells, and molecules that protect from disease by eliminating pathogens. However, as in human society, a clear definition of good and evil might be sometimes difficult to achieve. Not only do we live in contact with a multitude of microbes, but we also live with billions of symbionts that span all the shades from mutualists to potential killers. Together, we compose a superorganism that is capable of optimal living. In that context, the immune system is not a killer, but rather a force that shapes homeostasis within the superorganism.

INTRODUCTION

The capacity to discriminate between good and evil is crucial for survival. Our senses inform us on the nature of the environment and generate negative feelings of fear and disgust, or positive feelings of comfort and love. Accordingly, decisions are taken to follow a secure path. Societies have developed vast cultures to define good and evil to guarantee the survival of the system, be it a religion, a nation, or a political party. An extreme version of such a culture is Manichaeism, created during the third century AD in Babylon by the prophet Mani, and successfully spread within decades to all corners of the known world. Manichaeism describes the cosmological struggle between the good spiritual world of light and the evil material world of darkness, and prescribes how human society should guide this struggle to its resolution by separating good from evil. As esoteric as Manichaeism theology might sound, its dualistic principle seems nevertheless to pervade our usual perception and description of the world, and thereby to shape microbiology and immunology alike.

At the heart of the dualistic view in microbiology and immunology is the “germ theory of disease” or “pathogenic theory of medicine”, stating that microbes are the cause of a range of diseases. The first mention of such a theory goes back to 36 BC when Marcus Terentius Varro, a Roman scholar, wrote that “certain minute creatures, which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose, and there cause serious diseases”.¹ Following up in 1020, the Persian polymath Avicenna stated in *The Canon of Medicine* that bodily secretions are contaminated by “foul foreign earthly bodies” before a person becomes infected.² The

formal demonstration of this theory by Robert Koch and Louis Pasteur in the late 19th century marks the birth of microbiology, hygiene, and vaccine development.³ However, in the early 20th century, Ilya Metchnikoff proposed that bacteria are also agents of good. He suggested that lactic acid-producing bacteria prolong life by inhibiting the growth of putrefactive (proteolytic) bacteria, such as common gut microbiota members of the *Clostridia* class, which produce toxic substances and might provoke intestinal “auto-intoxication”.⁴ The term “probiotic”, defined as “a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance,” was coined in 1953 by Werner Kollath to contrast with antibiotics.⁵ The use of probiotics in a number of ailments, including inflammatory bowel disease (IBD) and cancer, has shown some level of protective effects by strains of the *Lactobacillus*, *Bifidobacteria*, *Bacteroides*, *Escherichia*, and *Faecalibacterium* genera.⁶

The discovery of innate immune receptors or pattern-recognition receptors (PRRs) in the late 1990s marks a fundamental turn in immunology.^{7,8} PRRs recognize microbe-associated molecular patterns (MAMPs), thereby allowing the immune system to detect microbes and act accordingly. They are believed to universally elicit rejection and lead to antimicrobial immunity. Reflecting this view, MAMPs are in general termed PAMPs for pathogen-associated molecular patterns.⁹ Thereby, immunology has assigned to the immune system a dualistic perception of the world, aiming it at the detection and destruction of microbes. The inevitable corollary of this view posits that activation of the immune system leads or at least aims at destruction of the

¹Department of Immunology, Institut Pasteur, Lymphoid Tissue Development Unit, Paris, France. ²CNRS URA1961, Paris, France. Correspondence: G Eberl (gerard.eberl@pasteur.fr).

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activator, and thus defines the activator as a pathogen. However, immune responses come in different blends, best characterized by generation of proinflammatory interferon- γ -producing Th1 cells and interleukin-17 (IL-17)-producing Th17 cells; IgE- and allergy-promoting Th2 cells; and anti-inflammatory IL-10-producing or Foxp3⁺ regulatory T cells.¹⁰ Therefore, the current view holds that pathogens elicit proinflammatory responses, whereas innocuous microbes, such as probiotics, elicit anti-inflammatory responses if detected at all.¹¹ Considerable efforts are made to understand the distinction between pathogens and innocuous microbes, which point to differences in the structure and expression of PAMPs, virulence factors, and invasive properties.⁹

Here, I challenge this dualistic view of microbes and of the immune system. I propose instead that microbes navigate between shades of good and evil, a position that is determined during interaction with the host, and a position that can change with host, tissue, and time. Facing the microbes, the immune system does not react to combat evil, but merely shapes the microbial environment to allow the organism to live with the microbes. It is not a fight between good and evil, it is rather an equilibrium between microbes and host that generates a superorganism.¹²

THE SUPERORGANISM

The role of the symbiotic microbiota

The human intestine hosts an astronomical 10^{14} bacteria, roughly 100 times the number of cells in our body, and close to 1000 distinct species,¹³ not taking in account archaea, fungi, and viruses. This microbiota is usually termed commensal, even though there is a considerable degree of mutualism with the host (**Box 1**). The microbes benefit from a selective environment that is regularly flooded with nutrients, and the host benefits from microbial activity that complements its digestive pathways, degrades xenobiotics, regulates epithelial homeostasis,¹⁴ and provides a barrier against potential pathogens.^{15–18} Microbial communities reside on all body surfaces, including the entire length of the digestive tract, the vagina, and the skin. Altogether, the partnership of the host with its microbiota can be described as a new functional entity termed a *superorganism* (**Figure 1**). This superorganism encodes $\sim 2 \times 10^4$ host genes and an estimated $\sim 10^6$ microbial genes,^{12,13} and in addition to the mammalian metabolic pathways, operates a plethora of microbial metabolic pathways collectively termed the metabolome.¹⁹

Box 1 Definitions

The recent literature is relatively confusing regarding its usage of the terms “symbiosis” and “commensals”. Symbiosis is generally understood as a relationship between two organisms from which both organisms benefit. In that definition, symbiosis is synonymous with mutualism.⁹² Furthermore, qualified as commensals are microbes living within a host. However, a number of such commensals are actually mutualists, helping for example in digestion and others are opportunistic pathogens, such as *Staphylococcus aureus* on the skin. Thus, the usage of the term “commensal” in that context may not be adequate. In this review, I use the original definitions of these terms to categorize the different types of microbes in their relationship with the host.

Symbiosis

Originated from the Greek words *syn* and *biosis*, meaning “with” and “living”. The original meaning of symbiosis is the “living together of unlike organisms”, first

coined in 1879 by the mycologist Heinrich Anton de Bary.⁹² The common usage of the term “commensal” should therefore be replaced by “symbiont” to designate the microbiota living within a host. The symbiotic relationships can be formally categorized as mutualistic, commensal, or parasitic, even though parasites are rarely considered symbionts.

Mutualism

Originated from the Latin word *mutuus*, meaning lent, borrowed, or mutual. A relationship between two organisms where both organisms benefit. For example, bacteria that expand in the intestinal niche and provide metabolic pathways complementing the digestive functions of the host.

Commensalism

Originated from the Latin word *cum mensa*, meaning “sharing a table”. A relationship between two organisms where one organism benefits but the other is unaffected. Microbes that expand in the intestine obviously benefit from the intestinal niche. However, it is more difficult to ascertain that microbes have no effect on the host, so as to be described as commensals.

Parasitism

Originated from the Greek words *para* and *sitos*, meaning “beside” and “food”, or one who eats at another’s table. A relationship between two organisms where one organism benefits at the expense of the other. Defines the behavior of a pathogen.

Superorganism

Originated from the Latin word *supra*, meaning “above”, and the Greek word *organon*, meaning “organ, instrument, tool”. In biology, an organism is a living system capable of autonomous metabolism and reproduction. A superorganism is a living system of a superior degree of complexity, consisting of many organisms. It may be defined more generally as a “collection of agents that can act in concert to produce phenomena governed by the collective”. Examples of superorganisms include ants and termite societies.

The effect of the microbiota extends beyond complementation of the host. In germfree mice, the intestinal immune system is underdeveloped.⁶ Lymphoid tissues such as Peyer’s patches (PPs), mesenteric lymph nodes (LNs), and the splenic white pulp remain small, and isolated lymphoid follicles (ILFs), present in hundreds in the lamina propria of normal mice, fail to develop.²⁰ Furthermore, intestinal lymphocyte populations are markedly reduced, including intra-epithelial T cells, lamina propria T cells, and IgA-producing B cells, and pro-inflammatory Th17 cells^{21–23} and innate IL-22-producing Natural Killer (NK)-like cells fail to be recruited or generated.^{24,25} Expression of anti-bacterial peptides by Paneth cells is also reduced.²⁶ Thus, the intestinal microbiota, mostly studied at the bacterial level, has an important role in the development and maturation of the immune system. Evidence accumulates that the collection of viruses, or virome, that stably infects the host, also has a major role in shaping the immune system, as discussed recently by Virgin *et al.*²⁷

Evolution of the superorganism

Organisms are selected by their fitness within a particular environment. Traditionally, an organism is defined by its genome, and inter-individual competition sorts out the fittest genome. However, selection might also operate at higher levels of organization, such as the group where the social abilities of individuals may provide a collective advantage to the group and hence to each individual of the group.²⁸ At a lower level, the mutualistic partnership between a host and its microbiota provides digestive, protective, and immune advantages to the superorganism. Selection can operate both at the individual host and microbial levels to evolve the best partnership, and the best partnership can be selected to evolve the fittest superorganism. Reflecting this selection process, only few bacterial phyla successfully colonize the human intestine, and the majority of bacterial species are

Figure 1 The superorganism. In the context discussed here, the superorganism is the composite of a host with its symbiotic microbiota. These two worlds have evolved to live together and establish an equilibrium that optimizes the fitness of the superorganism, and thereby the fitness of both the host and the members of the microbiota. The microbial metabolome¹⁹ complements the mammalian metabolome in a number of functions best described in the intestine,¹⁶ whereas the mammalian metabolome forms a niche that allows survival of selected microbes.

members of either the *Firmicutes* or the *Bacteroidetes* phyla.²⁹ On the host's side, a restricted number (~50 known) of PRRs define the universe of MAMPs that are sensed by the innate immune system,^{30–32} a limited reactivity that is nevertheless expected to provide a broad vision of the microbial world to the host. Beyond innate immunity, a virtually unlimited number of receptors are generated by lymphocytes to fill any potential gaps, a diversity suggested to allow vertebrate organisms to host a more complex microbiota.³³ Furthermore, the immune system must have co-evolved with the microbiota to adjust its reactivity and maintain homeostasis of the superorganism. In conclusion, the dualistic view that separates the host from its microbiota is of course valid in terms of separation of the genomes, but appears to be overruled at the functional level in the superorganism (**Box 2**).

Box 2 Predictions

The value of a concept or a model is measured by its predictive power. Therefore, what is the predictive power of the *superorganism* concept? What does the continuum model add to the classical dualistic models of immunity? Some leads are proposed here.

The superorganism

The host and its symbiotic microbiota constitute a superorganism with superior efficiency in digestion, defense, and detoxification, to list a few items, as compared with the bare host (**Figure 1**). Many systems are actually affected in germfree mice,⁹³ and therefore, the concept of superorganism is largely validated. Importantly the intestinal microbiota not only affects the intestine but also more distant organs, such as the pancreas,⁸⁷ and the hypothalamic–pituitary–adrenal axis during stress response in mice.⁹⁴ The mechanism leading to such effects is still a matter of speculation, but a recent paper shows that microbiota-derived peptidoglycans are present in the serum and bone marrow where they enhance neutrophil function.⁹⁵ Thus, it is predicted, and testable, that homeostasis of the superorganism not only requires an extensive local crosstalk between symbionts and the immune system, but also a more general systemic effect of microbiota based on invaders or circulating MAMPs.

The continuum model

The continuum model questions the dualistic view of the immune system based on self/non-self discrimination or physiologic versus pathological inflammation (**Figure 2**). Instead, it proposes that triggers of immunity, microbes for that matter, and the immune responses they elicit are best described by a continuum of properties. The type of microbes and immune responses are defined by their position on

this continuum, and the properties of individual microbes can vary between the two extremes depending on their interaction with the host. Thus it is predicted, and already documented,^{17,27,47} that microbes perceived as pathogens may become mutualists and that microbes perceived as mutualists may become pathogens, depending on the context. Another consequence of the continuum model is that proinflammatory immunity, which is viewed as elicited during a pathological situation, is also required to maintain steady state. Similarly, regulatory immunity is required to maintain steady state but is also involved during microbial infection to establish long-term resistance.⁹⁶ It is predicted that blocking proinflammatory immunity during steady state may lead to loss of homeostasis and, conversely, that blocking regulatory immunity during pathology may lead to loss of homeostasis during steady state.

MICROBES AND IMMUNITY

The nature of microbes

Given the proximity with so many microbes, it is expected that we are in constant danger of invasion, and that lowering our guard should be dangerous. As a matter of fact, immunosuppressed individuals are prone to opportunistic infections by ubiquitous and otherwise symbiotic microbes.^{27,34} It seems, therefore, safe to state that the immune system protects us from microbe invasion. However, it is an inaccurate leap forward to state that the immune system protects us from pathogens. How could it define a pathogen? Does a microbe express markers of pathogens before attack? This fundamental question is at the root of the current vision to develop immunotherapies and vaccines.^{9,35} The discovery of PRRs suggested indeed that expression of PAMPs defines a pathogen, but this view is problematic. Toll-like receptor-4 (TLR-4), which recognizes lipopolysaccharides (LPS), a component of the outer membrane of Gram-negative bacteria, best illustrates this problem. Given its wide expression, LPS can hardly be defined as a PAMP. Nevertheless, it has been shown that LPS expressed by prominent symbionts, such as *Bacteroides*, is modified and poorly recognized by TLR-4.^{11,36} However, potential pathogens such as *Helicobacter pylori*³⁷ and *Porphyromonas gingivalis*³⁸ also express variant forms of LPS that evade efficient recognition by TLR-4. Therefore, the term MAMPs seems to better describe the nature

of bacterial moieties recognized by PRRs,^{9,39} and cannot be the basis of pathogen recognition.

Recently, it was suggested that pathogenic microbes are recognized by “patterns of pathogenesis,”⁹ or POPs. Rather than by its structure, a pathogen would be defined by its characteristic behavior. A first POP is growth, as pathogens are able to grow in their host upon invasion. A second POP is cytosolic invasion, as many pathogens deliver active proteins, or virulence factors, into the cell host through syringe-like secretion systems or pores, which interfere with activation of the immune system.⁴⁰ A third proposed POP is disruption of the normal function of the host cell cytoskeleton, as bacterial pathogens and viruses have been shown to exploit the actin network to move within and between cells. During such pathogenic behaviors, MAMPs access forbidden compartments of host tissues and cells. In such a context, MAMPs would be recognized by PRRs as genuine PAMPs and elicit an appropriate inflammatory immune response. In accordance with this view, many PRRs are segregated to react only within a POP context.⁴¹ In the intestine, TLR-5 is expressed mainly on the basolateral side of the intestinal epithelial cells⁴² and on dendritic cells in the intestinal lamina propria.⁴³ Thus TLR-5 is not contacted by its flagellin ligand that is ubiquitous in the intestinal lumen, but by bacteria that cross the epithelial barrier and invade the forbidden compartment of the lamina propria. Some PRRs are only expressed within the cytosol, such as NOD-1 and NOD-2, as well as a number of components of the inflammasome and nucleic acid-recognizing molecules, and can only react to MAMPs that penetrate the cell.⁹ The definition of PAMPs in that context is nevertheless complicated by the existence of membrane transporters encoded by the host, as in the case of NOD-2,⁴⁴ or endosome portals for both NOD-1 and NOD-2⁴⁵ that can translocate MAMPs into the cytosol.

The “patterns of pathogenesis” POP hypothesis was proposed with the aim to understand how the immune system recognizes pathogens, and thus was framed within a dualistic view of microbes and immunity.⁹ It can be taken a step further to suggest that a POP does not define a pathogen, but merely a pathogenic behavior that can be adopted by any microbe. The distinction may seem subtle, but allows a particular microbe to be considered a mutualist or a pathogen, depending on time and context. In that view, a pathogenic microbe is not a pathogen *per se*. Its pathogenicity is determined by its interaction with the host, and can change with host, location in the host, and the immune state of the host. The interaction between a microbe and its host determines the position of the microbe on the scale of mutualists to pathogens, and its position on the scale can change continuously. Many examples exist of bacteria and viruses²⁷ that can change sides, such as the symbiotic intestinal bacteria that become a source of pathogens during colitis,⁴⁶ and pathogenic herpesviruses that cause an acute infection of the host before turning into a helpful ally against bacterial infection during latency.⁴⁷ These and other examples will be discussed in detail below.

The nature of immunity

Microbes, be it bacteria, fungi, or viruses, grow and move. However, the niches offered by the host to the microbes in the

gastrointestinal tract, the skin, and elsewhere considerably restrict the type of microbes able to colonize.²⁹ For example, most bacteria colonizing the human intestine are members of only two phyla, the *Firmicutes* and the *Bacteroidetes*. In addition, containment of the microbiota is enforced by multiple epithelial cell layers, such as in the skin, or thin layers of epithelial cells in the digestive tract and lungs, which produce antibacterial peptides and are protected by mucus produced by Goblet cells. In addition, B cells in mucosal surfaces produce large amounts of IgA that are transported across the epithelial barrier and released into the lumen. There, IgA contribute to the containment of microbes beyond and within the mucus, and to opsonization of microbes for sampling or destruction by phagocytes.¹⁵

However, the quality of the containment can change, due for example to environmental variations, exposure to chemicals, and injury. Breaches might be generated and microbes might penetrate the forbidden compartments of host tissues and cells. When this happens, the immune system is triggered through PRRs, such as by TLR-5 on the basolateral side of epithelial cells and on phagocytes in the intestinal lamina propria, which activate cascades of proinflammatory pathways.^{42,43} The consequence is recruitment and activation of polymorphonuclear cells, macrophages, and lymphocytes; elimination of the intrusive microbes; and eventual wound healing. Ideally, containment and homeostasis is re-established. The rules of engagement of proinflammatory immunity are best described by the “danger model”, which posits that modification of self, through invasive microbes and injury of tissues and cells, is the ultimate trigger.^{48,49} Thus, another dualistic view of immunity has emerged: one arm of immunity is involved in every day’s containment of well-behaving symbiotic microbes, whereas another arm of immunity is involved in the elimination of invasive bad-behaving pathogenic microbes that breach containment (**Figure 2**).^{11,15,40}

It might be inferred from this view that homeostasis of the superorganism commands the immune system to fight breaches of containment *at all cost*. However, such a conclusion is a dubious leap forward on the role of proinflammatory immunity and the nature of homeostasis in the superorganism. Even though proinflammatory immunity may follow dualistic rules of engagement to fight the pathogenic behavior of microbes, it cannot avoid breaches of containment. Such breaches seem to occur constantly and are really part of homeostasis. Direct evidence of such breaches is difficult to obtain, but the elevated proinflammatory state of the intestinal immune system is well documented. In specific pathogen-free mice, the proportion of proinflammatory Th17 cells producing IL-17, which induces granulopoiesis and recruitment of neutrophils,^{50,51} is significantly higher in the intestine and skin than in any other tissues.^{52,53} This state is induced by the intestinal microbiota^{22,23} and is regulated by IL-10 to avoid overdrive of intestinal proinflammatory immunity; IL-10-deficient mice suffer from severe colitis.⁵⁴ The notion of “physiologic inflammation” has been suggested a hundred years ago by Metchnikoff in the context of homeostatic removal of dead cells,⁵⁵ and was more recently discussed by Sansonetti and Di

Figure 2 The dualistic and the continuum models of the microbial and immunological worlds. The ancestral view of immunology states that the immune system is educated not to react to self and to react to non-self. In that view, self is good and induces tolerance during lymphocyte maturation and selection, or anergy of self-reacting mature lymphocytes. Non-self, including microbes, as well as allotypic and xenotypic tissues, are evil and elicit an immune response aimed at destroying non-self. All can be boiled down to self/non-self discrimination.^{97,98} The modern view of immunology states that the immune system reacts primarily to danger signals.^{48,49} The immune system is still educated to be tolerant to self, but the notion of self is modified. Good includes normal self and mutualistic microbes, whereas evil includes altered self such as dead cells releasing danger signals and pathogenic microbes that alter the antigenic landscape of normal self. In that context, the normal self induces a physiological level of inflammation that contributes to homeostasis through for example containment of the intestinal microbiota, whereas injury and pathogens induce pathological inflammation that leads to “full-blown” inflammation.⁴⁰ The continuum model states that the perceived duality of mutualistic and pathogenic microbes, normal and altered self, and regulatory or inflammatory immunity, represents extremes of a continuous reality. Microbes can express different levels of mutualistic or pathogenic properties and these levels can vary during interaction with the host. Similarly, the state of self and of immune responses can navigate between well-described extremes, and the most likely states are combination of these extremes.

Santo⁴⁰ in reference to immunity induced for containment of symbionts, distinct from proinflammatory immunity to pathogens. However, physiologic inflammation, which might be defined as immunity required for maintaining homeostasis in the superorganism, appears also to include proinflammatory immunity (**Box 2**).

HOMEOSTASIS IN THE SUPERORGANISM

Activation of the immune system during homeostasis

Homeostasis in the superorganism is defined by the optimal cohabitation of the host with its microbiota. It is a dynamic equilibrium between host and microbes, where growth and movement of the microbes are constantly kept in check by mechanical, chemical, and immunological containment by the host. The dynamic nature of this equilibrium results from variations in the composition of the microbiota, caused by changes in environmental factors such as chemistry, food, and incoming microbes, and variations in the state of the host through mutations, injury, and other forms of stress. The host must have developed a flexible system that adapts to these variations and maintains homeostasis of the superorganism. The immune system perfectly matches this function: it contributes to containment and equilibrium through for example expression of IL-13 that induces production of mucus by Goblet cells,⁵⁶ expression of IL-22 that induces the production of antibacterial peptides by epithelial cells,⁵⁷ and generation of IgA-producing B cells.¹⁵ Furthermore, it can be activated to a higher state of inflammation by breaches in containment that provoke deviations from the equilibrium. Thus, the immune system emerges as a crucial force to maintain homeostasis (**Figure 3**).

In the superorganism, the immune system is never at rest. It is like a spring: the more microbes colonize the host niches or behave like pathogens, the stronger they pull the spring of immunity, and the stronger the spring of immunity pushes the microbes back. In germfree animals, the immune spring is close to rest, but in animals grown in a normal microbial world, the immune spring is always under tension, the tension required to maintain homeostasis. This tension also contributes to defining the niche for symbionts, and thus to select incoming microbes and preserve homeostasis. This principle is nicely illustrated by the following experiment: when mice, and humans, were treated with a mix of antibiotics to eradicate most of their intestinal microbiota, they became highly sensitive to infection by an antibiotic-resistant strain of *Enterococcus*.¹⁷ Because of the lack of symbionts, intestinal epithelial cells expressed low levels of antibacterial peptides that normally provide selection against the *Enterococcus* strain. However, resistance was re-established by concomitant treatment of mice with LPS, which binds TLR-4 and re-induces the expression of antibacterial peptides. Thus, the symbiotic microbiota pulls the string of immunity and induces the production of antibacterial peptides that contribute to defining an intestinal niche permissive for symbionts, but mostly toxic for potential pathogens such as antibiotic-resistant *Enterococcus*.

Development of the immune system for homeostasis

In the absence of microbiota, the immune system does not fully develop. The set of lymphoid tissues is incomplete and immature, and lymphocytes populations in the intestine are reduced in numbers or fail to develop.⁶ Particular members of the intestinal microbiota, such as segmented filamentous bacteria, are potent activators of intestinal T-helper cells, including Th17 cells^{22,23} and in germfree mice, Th17 cells and NKp46⁺ cells that produce

Figure 3 The continuum of microbial states and immune responses: a dynamic equilibrium. Microbes, including members of the symbiotic microbiota, are not inherently mutualistic or pathogenic, but navigate between shades of mutualism and parasitism. Facing the microbes, the immune system is not designed to discriminate between mutualist or pathogens, but merely to react to signals, including MAMPs and antigens. The nature of the immune response is not purely regulatory or inflammatory, but more generally adjusts to the nature of the trigger it faces, like a spring that is pulled by the intensity of the microbial challenge. Furthermore, the immune system has the capacity to evolve when challenged, through generation of different types of lymphocyte subsets such as Th1, Th2, Th17, Treg, Th22 cells,¹⁰ and follicular T helper cells,⁹⁹ and generation of memory lymphocytes and lymphoid tissues.⁶ This adds another level of adaptability to the immune system and provides it with the necessary flexibility to maintain homeostasis of the superorganism. MAMP, microbe-associated molecular pattern.

IL-17 and/or IL-22 are markedly reduced.^{21–25} These cytokines have an important role in the containment of microbiota, as IL-17 induces the recruitment of neutrophils⁵⁰ and both cytokines induce the production of antibacterial peptides by epithelial and Paneth cells, such as β -defensins, RegIII γ , and S100 family members.^{57–59}

The microbiota-induced development of lymphoid tissues is particularly instructive. Secondary lymphoid tissues, such as LNs and PPs, are programmed to develop in the sterile environment of the fetus.⁶⁰ Lymphoid tissue inducer cells are recruited to LN and PP anlagen where they activate specialized stromal cells to produce chemokines and cytokines for subsequent recruitment of B and T cells.⁶¹ After birth, bacterial colonization induces the enlargement of mesenteric LNs and PPs, but does not alter their basic organization, nor are microbes required to maintain this organization. By contrast, ILFs, present in the adult intestinal lamina propria as hundreds of small B-cell follicles, are not programmed during ontogeny, but induced by colonizing microbiota.⁶² Peptidoglycans shed by dividing bacteria are recognized by NOD-1 in epithelial cells, which then upregulate the expression of β -defensin-3 and of the chemokine CCL20. Both are ligands of CCR6 expressed by lymphoid tissue inducer cells clustered in cryptopatches of the lamina propria. Lymphoid tissue inducer cells in cryptopatches then initiate local lymphoid tissue

development and recruitment of CCR6⁺ B cells.²⁰ Importantly, ILFs generate IgA-producing B cells that contribute to the containment of the intestinal microbiota, thus establishing a negative feedback loop on their own development.⁶³ Therefore, ILFs seem to be an optimal system to maintain intestinal homeostasis, as the more bacteria are present in the intestine, the more ILFs are generated that produce IgA, but the more IgA is produced, the less bacteria will be capable of inducing the formation of ILFs. It remains to be established whether a “breach in containment” is required to activate NOD-1 and formation of ILFs, or whether a mechanism of active sampling of luminal content by epithelial cells and transfer to cytosolic NOD-1 is sufficient to activate this pathway.⁴⁵

Unexpected mutualists for a changing homeostasis

The interaction between a microbe and its host determines the position of the microbe on the scale of mutualists to pathogens, and its position on the scale can change continuously. It may be added that not only the host but also the environment decides the position of the microbe on the scale. Environmental factors may include climate, chemicals, microbes (such as phages), or anything that conditions the fitness of the superorganism. In a changing context, new combinations of microbes and host may yield a competitive advantage to the superorganism, and old foes might become new mutualists. Conversely, old friends may turn bad.

A striking example has been reported in mice infected with a γ -herpesvirus.⁴⁷ Early after infection, γ HV68 undergoes lytic replication in a number of cell types, before establishing life-long latency in memory B cells, macrophages, and dendritic cells. Such a latent infection confers mice resistance to infection with *Listeria monocytogenes* and *Yersinia pestis* through a state of increased and persistent immune activation. It was thus proposed that viral latency can define a mutualistic relationship between the host and the virus. In this case, the superorganism includes a former pathogen that eventually has been brought under control: its status within the superorganism changed from an invasive pathogen to a contained mutualist. Homeostasis is modified as the proinflammatory state of the immune system is increased, providing an advantage to the superorganism in the face of *Listeria* infection.

Another example is immunological memory. Being exposed constantly to microbes that breach containment, the host develops a collection of memory lymphocytes. The host becomes thereby more reactive to such microbes and alters the niche available to the microbial community. As in the previous example, this evolution of the superorganism is expected to make homeostasis more resistant to pathogenic invasion and thus more robust. Two non-mutually exclusive mechanisms generate and maintain immunological memory: differentiation of long-lived memory lymphocytes during a primary immune response, and latent infection by the microbe or long-lasting antigen depots to uphold specific T- and B-cell activation.^{64–67} In another case of herpesvirus infection, cytomegalovirus establishes latency after systemic infection in both mouse and man.⁶⁸ In infected individuals, accumulation of cytomegalovirus-specific CD8⁺

T cells can reach the exceptional proportion of 10% of total CD8⁺ T cells, in a phenomenon known as memory inflation. It was shown that this high proportion of cytomegalovirus-specific CD8⁺ T cells was maintained by long-lived memory cells as well as by continuous generation of short-lived and functional T cells by the latent infection. Such latent infections are thus expected to protect the superorganism from secondary infections, and may operate upon childhood infections and vaccination with other microbes. In this case, homeostasis of the superorganism is modified as immunological memory supported in part by the latent cytomegalovirus infection increases resistance to new infections by itself or related viruses. The old foe remains a foe, but its “domestication” can make it a member of the superorganism to fight its wild relatives.

It has recently been discussed that the host is in a situation of metastable equilibrium with such old foes or pathogens, as a decrease in immunosurveillance, or progressive erosion of this equilibrium, may turn latent microbes into a life-threatening pathogen.²⁷ This view can be applied to the whole symbiotic microbiota, and is the basis of ruptures of homeostasis discussed in the next chapter.

RUPTURE OF HOMEOSTASIS

A rupture in homeostasis can be caused by the environment, invading microbes, symbiotic microbes, or the host, and can have severe consequences if the superorganism cannot re-establish equilibrium between the microbiota and the host. Here, we will examine ruptures of homeostasis caused by alterations in the immune system, as well as two examples involving subtle alterations in the host metabolic and tissue regulation. Examples of progressive or abrupt rupture of homeostasis involving the host virome,²⁷ or strong pathogens such as *Shigella* or Influenza A virus, are well described^{11,69} and will not be discussed here.

Weak immunity

Too weak an immune system exposes the superorganism to a conversion of mutualists and commensals to pathogens. The immune forces that normally contain symbionts and establish equilibrium are at risk to be overwhelmed, and microbes that are not normally behaving as pathogens may invade forbidden areas and become pathogens. Immunocompromised people, such as patients suffering from AIDS, treated in intensive care units, receiving chemotherapy, or organ transplants, are exposed to life-threatening opportunistic infections by microbes that are otherwise harmless or ubiquitous symbionts. The latter include the yeast *Candida albicans*,⁷⁰ a member of the human microbiota of the skin, intestine, and vagina, and *Pseudomonas aeruginosa* and *Staphylococcus aureus*,⁷¹ both present in the skin microbiota.⁷² Recently, it has been reported that lipoteichoic acid derived from *Staphylococcus* strains inhibits inflammatory cytokine release from keratinocytes through a TLR-2-dependent mechanism,⁷³ suggesting a possible mutualistic role of *Staphylococci* in skin homeostasis. Additional benefits these symbionts may provide to the host include exclusion of related microbes from the niche.

A number of examples have been reported on the loss of containment of the intestinal microbiota in mice deficient for components of the innate or the adaptive immunity. When specific pathogen-free mice are fed orally with the symbiont *Enterobacter cloacae*, dendritic cells collect bacteria in the PPs sub-epithelial dome before migrating to the draining mesenteric LN where live bacteria can be recovered.⁷⁴ In the absence of immunoglobulins in J_H-deficient mice, containment of the symbionts is less efficient and significantly more bacteria are recovered from the mesenteric LNs. A similar loss of containment is found in mice deficient for the Toll-like receptor-signaling molecules Myd88 and Trif, or deficient in the phagocyte oxidative burst.³⁴ When specific pathogen-free mutant mice are fed with the symbiont *Escherichia coli* K-12, live bacteria are recovered from the spleen, and serum IgG are produced against members of the symbiotic microbiota, indicating bacterial leakage into the bloodstream. Similar findings were reported earlier in mice deficient for Myd88 or TLR-4.⁷⁵ The consequence of decreased containment of the intestinal microbiota is increased morbidity in Myd88- and J_H- double-deficient mice,³⁴ and increased susceptibility of TLR-4- or Myd88-deficient mice to IBD induced by sodium dextran sulfate.⁷⁵

Strong immunity

Too strong an immune system destabilizes the microbiota and causes dysbiosis, or alteration in the symbiotic microbial community. In the case of *Drosophila* flies, immune hyperactivity against intestinal symbionts can be fatal.⁷⁶ Wild-type flies harbor an intestinal community dominated by five bacterial species, including *Gluconobacter* strain EW707 and *Acetobacter* strain EW911. Mutant flies that show uncontrolled, bacteria-induced nuclear factor- κ B activation, due to knockdown of the regulatory Caudal transcription factor, suffer excessive apoptosis of epithelial cells and progressed to death. It was shown that the mutant flies harbor an altered intestinal bacterial community, where EW911 becomes a minor population and EW707 expands. EW707 alone is pathogenic to germfree flies, but EW911 protect the flies from the damaging effect of EW707. Thus, even though EW707 is a “regular” member of the symbiotic microbiota, its population size has to be kept in check by the presence of other symbionts to maintain homeostasis in wild-type flies. A beneficial role of EW707 for the host during homeostasis remains to be established.

Furthermore, given the nearly unlimited source of antigens generated by the intestinal microbiota, an aggressive immune system can progress into overdrive, generate acute and chronic pathological inflammation, and cause severe damage to the tissue. Such as scenario unfolds during Crohn’s disease and mutations that map to the *CARD15* locus significantly increase disease susceptibility in patients.⁷⁷ *CARD15* codes for the innate receptor NOD-2, which recognizes muramyl dipeptides shed mostly by Gram-positive bacteria.⁷⁸ Contradictory data have been reported on the effect of such mutations on the activity of NOD-2. It was found that mutations either increase NOD-2 activity and induce elevated nuclear factor- κ B activity

and IL-1 β secretion,⁷⁹ or abolish NOD-2 activity and decrease containment through antibacterial peptides.⁸⁰ Decreased or lack of NOD-2 activity, reflecting the mutations found in Crohn's patients, leads to increased stimulation in response to TLR-2 stimulation and consequent hyperactivity toward the microbiota.⁸¹

Dysbiosis

Dysbiosis can be caused by dysregulated immunity and have life-threatening consequences as we just discussed. But dysbiosis can also be induced by subtle shifts in immunity or by non-immunological factors and elicit dramatic consequences.

Propionibacterium acnes is a major constituent of the microbiota on human skin and feeds on fatty acids produced by sebaceous glands.⁷² However, disruption of homeostasis can lead to skin disease caused by *P. acnes* such as acne. Acne unfolds as hair follicles undergo hyperkeratinization, which blocks sebum egress and causes its accumulation within the follicle.⁸² Under such conditions, *P. acnes* expands and damages the follicle and local containment through increased production of bacterial enzymes. The immune system reacts to occasional breaches in containment and invasion of *P. acnes* and generates local inflammation and folliculitis. The situation worsens when other skin symbionts, such as *Staphylococcus aureus*, penetrate the breaches and cause more severe infections.

Another, much publicized, example of dysbiosis is shown to lead to obesity. The intestinal microbiota of obese ob/ob mice and patients was sequenced and compared with that of lean mice and patients.^{83,84} The microbiome and its inferred metabolome, the collection of metabolic pathways encoded by the microbiota, show that the microbiota of obese individuals is more efficient than that of control individuals in harvesting energy from nutrients, in particular through enhanced catabolism of dietary polysaccharides.⁸⁴ As a result, the consumable energy remaining in feces is lower in obese individuals. Remarkably, transfer of the cecal microbiota of ob/ob mice to wild-type controls induces a significant increase in body fat as compared with control transfers, showing the dominant effect of the dysbiosis present in ob/ob mice. These data also suggest that obesity can be inherited from the mother not only through genes and behavior, but also through symbiotic bacteria. It was further shown that ob/ob mice and obese patients have an increased proportion of *Firmicutes* versus *Bacteroidetes* symbionts, the two dominant phyla in the mouse and human intestine.^{83,85} However, the primary cause of dysbiosis in ob/ob mice and obese patients remains a fundamental and fascinating problem.⁸⁶

A striking effect of dysbiosis was also reported in the susceptibility of NOD mice to type-1 diabetes.⁸⁷ A series of experiments was designed to assess whether progression to disease was dependent on proinflammatory signals delivered through innate receptors such as TLRs. Apparently confirming this hypothesis, Myd88-deficient NOD mice show marked resistance to development of the disease. T cells reactive to diabetes-associated peptides are found in the spleen and in the mesenteric LN of such mice, yet are markedly reduced in the pancreatic LNs. These data indicated that Myd88-deficient NOD mice do not suffer

from systemic immunosuppression but rather from localized immunosuppression in the pancreatic LNs that drain both the pancreas and the intestine. Surprisingly, germfree Myd88-deficient NOD mice develop diabetes to the same extent as germfree or normally colonized Myd88-sufficient NOD mice, indicating a role for the intestinal microbiota in protection from disease progression in the absence of Myd88. A key experiment showed that Myd88-deficient mice develop an altered intestinal microbiota that somehow suppresses the generation of diabetogenic T cells in the pancreatic LN: when NOD newborn mice were raised with Myd88-deficient mothers, disease progression was mitigated. Myd88-deficient mice harbor a decreased ratio of *Firmicutes* versus *Bacteroidetes* symbionts as compared with Myd88-sufficient mice, as well as increased proportions of *Lactobacillaceae*. A cause-to-consequence relationship has not been established yet in this model, but a probiotic mix containing four species of *Lactobacillaceae* has been previously shown to protect NOD mice from diabetes and induce a concomitant increase in IL-10 production.⁸⁸

Restoration of homeostasis for prevention of disease and therapy

Prevention and therapy of a rupture in homeostasis, leading to disease, might be directed against the primary cause in the host or the microbiota, such as immune dysregulation or potential pathogens. It might also aim at restoring homeostasis through engineering of the symbiotic microbiota.

In cases of immunodeficiencies, the key is to maintain equilibrium between host and microbiota through restoration of normal immunity or a decrease in the microbial load through chemotherapy. In cases of pathological immune hyperactivity, unfolding for example during Crohn's disease, the most widely used therapy is immunosuppression through administration of anti-tumor necrosis factor- α antibodies. Decreasing the bacterial load through antibiotic therapy would in principle also be a valid approach during acute inflammatory disease, but in the long run exposes the host to invasion by antibiotic-resistant microbes that take advantage of empty niches, and deprives the host of microbial metabolic pathways involved in protection, digestion, and catabolism of xenobiotics.

The study by Pamer and co-workers proposes a new strategy to combine antibiotic treatment with replacement of microbiota.¹⁷ In their study, susceptibility of antibiotic-treated mice to an antibiotic-resistant *Enterococcus* could be mitigated through concomitant administration of LPS. The LPS induced TLR-4-mediated production of antibacterial peptides targeting the pathogenic bacteria, an effect normally induced by the symbiotic microbiota. Thus, in patients treated with antibody cocktails, some degree of homeostasis could be restored by complementing the dwarfed microbiota with selected microbiota-derived compounds. Therefore, deciphering the activity of microbial components on the immune system and beyond the immune system might lead to an extension of such replacement strategies. As microbial metabolic pathways would still be missing, such strategies would have to be complemented with specific diets.

IBD, including Crohn's disease and ulcerative colitis, is associated with dysbiosis.^{89,90} It has therefore been proposed that dysbiosis is a potential target for the treatment of IBD. Potential treatments are based on the use of antibiotics targeting specific types of bacteria associated with IBD, or on the use of pre- and probiotics that would favor the restoration of a normal microbiota. Nevertheless, a cause-to-consequence relationship between dysbiotic microbiota and IBD remains to be clearly established. An alternative strategy is based on the use of bacterial strains that exert an immunomodulatory effect on the immune system. The *Firmicutes Faecalibacterium prausnitzii* is significantly reduced in patients suffering from Crohn's disease.⁹¹ *In vitro*, *F. prausnitzii* induces lower IL-12 and interferon- γ and higher IL-10 production by mononuclear cells, and *in vivo*, modulated TNBS (2,4,6-trinitro benzene sulfonic acid)-induced colitis. Similar anti-inflammatory effects and protection from IBD have been shown for different species of *Lactobacilli*, *Bifidobacteria*, *Bacteroides*, and *E. coli*.⁶ Thus, homeostasis might be restored through modifications of the microbial community through administration of probiotics or prebiotics that favor the emergence of such probiotics. This modified microbiota would exert an anti-inflammatory effect on the hyperactive immune system found in IBD patients and establish a new and potentially viable homeostasis. An important issue however is the stability of such modified homeostasis with time and its robustness in pathological settings.

We have discussed how stable dysbiosis, transmitted by the mother to her offsprings or maintained by mutations in the immune system, promotes obesity⁸⁴ or prevents the progression of type-1 diabetes,⁸⁷ respectively. These examples show how altered but stable associations between microbiota and host influence the metabolic and inflammatory state of the superorganism. It is therefore expected that a fine understanding of the crosstalk and equilibrium between microbiota and host, and eventually of the rules that govern the superorganism, should lead to preventive and therapeutic strategies in numbers of ailments that are characterized by disrupted homeostasis.

CONCLUSIONS

The superorganism is discussed here as the association of the mammalian host with its symbiotic microbiota. This concept offers a novel perspective of self and microbiota, where self is embedded in the microbial world and depends on it for full development and optimal survival. The superorganism requires forces, such as the immune system, to maintain homeostasis. During pathogenesis, the equilibrium between the microbiota and the immune system can become dangerously unstable and glide toward collapse and death of the superorganism. In that context, it appears important to decipher the cellular and molecular crosstalk between the symbiotic microbiota, the host, and its immune system. The molecular messengers of this crosstalk may lead to a new generation of preventive and therapeutic avenues: it will be possible to diagnose and understand ruptures of homeostasis, elaborate strategies to prevent progression to disease, and design therapies for return to homeostasis.

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DISCLOSURE

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