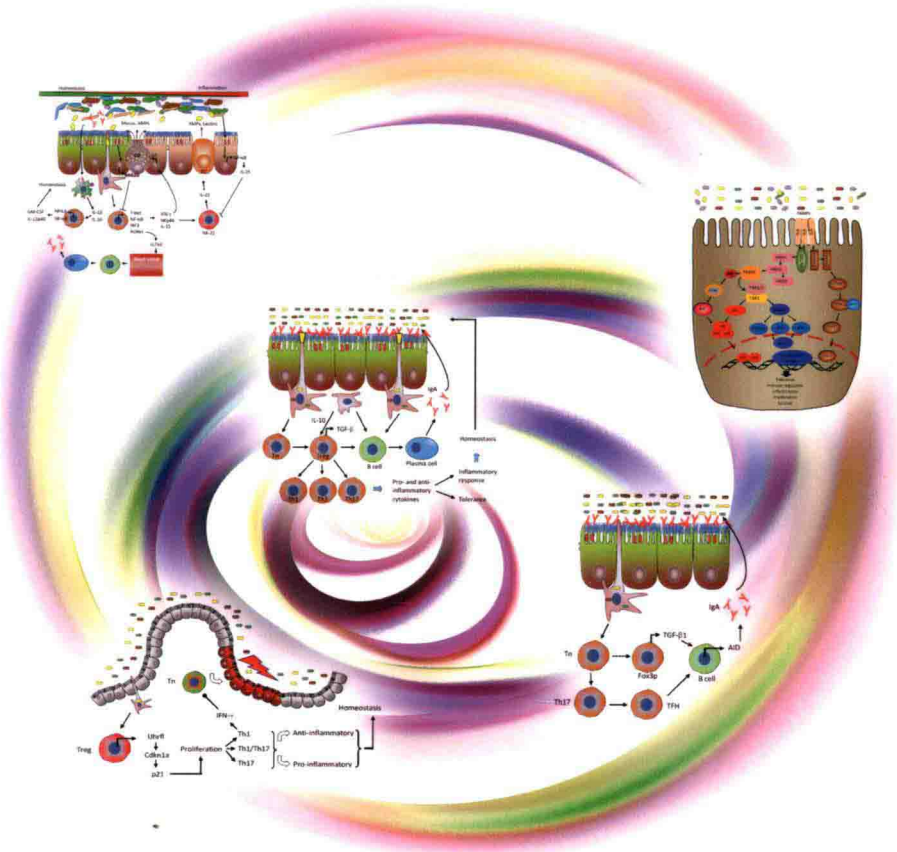
Joshua Joseph Malago

Contribution of Microbiota to the Innate and Acquired Gut Immunity during Health and Disease



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CONTRIBUTION OF MICROBIOTA TO INNATE AND ACQUIRED GUT IMMUNITY DURING HEALTH AND DISEASE

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PREFACE

The large number of microbials in the intestine that overrides the total human cells by ten folds alludes to significant contribution of the microbiota to human health. This is vivid in enteric and some systemic diseases emanating from disruption of the microbiota. The microbiota influences the development and functioning of both, innate and acquired immune systems for gut health. The effect of microbiota spills throughout the various components of the gut immune systems from "primitive" non specific pattern recognition receptors (PRR) to most specific adaptive T cell responses.

To induce immune responses, commensal microbes are recognized by PRRs, which in turn regulate mucosal innate immunity and inflammatory responses. PRRs detect microbe-associated molecular patterns (MAMPs or "infectious non-self") or endogenous "danger signals" derived from stressed, damaged or infected tissue to stimulate the intestinal innate immunity that initiates adaptive immune responses. MAMPs include peptidoglycans, lipoproteins, lipopolysaccharides, teichoic acids, CpG DNA motif, double strand RNA and flagellin. In a balanced microbiota profile, PRR signaling ensures immune homeostasis and protects the host against enteral pathogens. Chapter one of this book will discuss the influence of the microbiota to PRR signaling during health and disease for intestinal immunity.

Chapter two of the book focuses on a second level of innate immune system. This involves cells of the innate immune system that are responsible for driving non-specific innate immunity. They include natural killer cells, mast cells, eosinophils, basophils and the phagocytic cells including macrophages, neutrophils and dendritic cells. However, owing to the great commitment of macrophages and dendritic cells, a separate chapter for these two phagocytic cell types is allocated. Thus chapter two discusses the

influence of microbiota on innate cells engendering intestinal immunity under health and disease. It concludes the innate immune system of the intestine.

Macrophages and dendritic cells are professional antigen presenting cells. They sample antigens from the intestinal lumen, process, and present them to cells of the adaptive immune system. Despite of enormous types of enteral antigens ranging from harmful to beneficial, the antigen presenting cells are capable of efficiently discriminating them and driving respective responses to effector cells of the adaptive immune system. While dendritic cells are capable of priming T cell responses, macrophages do polarize the responses. As to how the microbiota influences the functioning of these cells, chapter three is devoted to discuss that phenomenon. The chapter links innate and adaptive intestinal immune systems since macrophages and dendritic cells lie in the interface between innate and adaptive immune systems.

The acquired or adaptive immunity of the gut is split in humoral and cellular components. The humoral immune system is mainly geared by gut-associated lymphoid tissue (GALT) whose components include effector (i.e., epithelial lymphocytes and lamina propria) and inductive (i.e., mesenteric lymph nodes, Peyer's patches, isolated lymphoid follicles, and cryptopatches) sites. It is interesting to note that microbiota influences GALT development and functioning during health and diseases. In germ free animals and those with disrupted microbita, GALT functioning is heavily compromised leading to diseases. Restoration of normal microbiotal profile to such individuals cures the disorders. Chapter four of this book will describe how the microbiota interacts with GALT and other components of the humoral immune system to maintain intestinal immunity under health and disease.

The last chapter, chapter 5, focuses on the second part of the adaptive immune system which is cellular immune system. This system is dominated by several CD4 and CD8 lymphocytes that drive the cellular adaptive immune system. The main components are $CD4^+$ cells which include T helper and regulatory T cells. Other T cells include cytotoxic T, memory, natural killer, and mucosa associated invariant T cells. While T helper cells drive most of the inflammatory responses, regulatory T cells downregulate these responses. As such, they are considered potential therapeutic agents of the future. Current knowledge indicates that the functioning of most, if not all, T cells is influenced by the microbiota. Chapter 5 is therefore devoted to discuss how the microbiota interacts with T cells during health and disease to foster intestinal immunity.

In the past few years we have encountered mounting evidence showing that the microbiota plays essential role in regulating and maintaining host's

intestinal immunity. This is done through various ways including; regulation of mucin gene expression by goblet cells, modification of glycosylation of mucus to interfere with bacterial adhesion, colonization and invasion, induction of secretion of antimicrobial peptides by intestinal Paneth cells, regulation of alterations of intestinal permeability caused by infection, stress, and inflammation, and influences on development of mucosal and systemic immunity. It is becoming well comprehended that microbiota is pivotal to the intestinal immunity through crosstalk with the epithelium, immune cells and the immune system in general. Disruption of microbiota balance often leads to disease. This book explores recent findings on how microbiota influences the intestinal immune responses, both innate and adaptive, to foster the intestinal mucosal immunity. The insight gained could contribute to designing approaches suitable for treating gastrointestinal diseases caused by disruption of the microbiota.

CONTENTS

Chapter 1

INFLUENCE OF MICROBIOTA TO PATTERN RECOGNITION RECEPTOR SIGNALING FOR INTESTINAL IMMUNITY

ABSTRACT

The contribution of intestinal epithelium to the innate immune system includes detecting luminal microbes, transducing signals, and activating inflammatory mediator release by epithelial and other cells of the immune system like the antigen presenting cells. Microbial antigens are detected by cells of the innate immune system through their pattern recognition receptors (PRRs). The PRRs recognize microbe-associated molecular patterns and generate signals that activate transcription pathways like nuclear factor kappa B and mitogen activated protein kinases. This activation leads to production of inflammatory and growth mediators that drive the immune system to elicit tolerance or immune response designated at maintaining immune homeostasis. Key to this signaling is the gut microbiota. Intestinal epithelial cell sensing of optimally balanced microbiota favors immune homeostasis whereas sensing under disrupted microbiota impairs immune function and predisposes to disease. Understanding the PRR-microbiota signaling would be useful in designing therapeutics for various immune-mediated disorders caused by imbalances of microbiota.

INTRODUCTION

Microorganisms and other noxious agents that successfully enter the gastrointestinal tract encounter intestinal physicochemical barrier as well as cells and mechanisms of innate immune system. In order for them to establish in the gut and cause disease, they must cross these elements. The exposure of intestinal epithelial cells (IECs) to this wide range of enteral agents triggers highly controlled responses to effect appropriate functions. Usually the responses involve elimination of the microorganisms or neutralization of toxins and other agents. To meet this function, IECs recognize antigens and respond by synthesizing a wide range of products including pro- and anti-inflammatory mediators, mucus, and antimicrobials (Lee et al., 2006; Malago 2014). They also transmit signals to underlying cells in the mucosa to drive the desired responses (DePaolo et al., 2012; Pickard et al., 2014).

Despite of enormous diversity in enteral antigens, the innate immune system is capable of discriminating potential pathogens from beneficial microbiota through a restricted number of preformed receptors named the pattern recognition receptors (PRR). They include membrane-bound toll-like receptors (TLR) and cytoplasmic nucleotide oligomerization domain (NOD)-like receptors (NLR). A series of TLR (including TLR1, 2, 3, 4, 5, 9) and NOD1/2 receptors are expressed by IECs to recognize motifs conserved by bacteria but not higher eukaryotes (Medzhitov 2007; Barton and Kagan 2009; Franchi et al., 2009; Langefeld et al., 2009; Wagner 2012; Kamdar et al., 2013; Kant et al., 2013). The expression may also follow recognition of damaged, injured or stressed cells that send out alarm signals that are recognized by the same receptors as those for pathogens (Matzinger 2002). Innate response is usually triggered when microbes are identified by the PRR which allows immediate recognition of bacteria and rapid response by cells of the innate immune system (Langefeld et al., 2009; Wagner 2012; Kamdar et al., 2013; Kant et al., 2013). Endocytic PRRs are involved in phagocytosis without relaying intracellular signals. They recognize receptors on phagocytes such as macrophages to clear apoptotic cells (Jeannin et al., 2008).

Signaling through PRR system generates transmembrane signals on cell surface that transduce the signals through kinase enzymes before activating putative transcription pathways like nuclear factor kappa B (NF-κB) and mitogen activated protein kinase (MAPK) (Figure 1) (Malago et al., 2002; Zhong et al., 2007) Activation of NF-κB drives transcription of a series of genes coding for inflammatory cytokines, enzymes and growth factors

(Malago et al., 2002). Important end results of NF-κB products to innate immune system are pro-inflammatory cytokines, especially chemoattractants like interleukin (IL)-8 and tumor necrosis factor (TNF)-α that attract inflammatory cells, particularly neutrophils, to the site of insult. The NF-κB activation also leads to generation of nitric oxide that imparts its antimicrobial activity. Part of this signaling activates cells underlying lamina propria (LP) including a population of innate lymphoid cells (ILCs) (Figure 2). The activated ILCs produce effector cytokines essential for protective and pathogenic roles during intestinal inflammation (Schenten and Medzhitov 2011). It is the LP dendritic cells (DCs) that determine whether anti- or pro-inflammatory response should be elicited in response to a particular antigen.

The influence of microbiota to the PRR signaling is evident. It activates and shapes the downstream responses of PRR signaling to maintain gut immunity (Bermudez-Brito et al., 2014; Yu et al., 2014). To meet this function, the microbiota drives PRR signaling to generate immune homeostasis in a steady state gut environment and induce immune responses to combat and eliminate noxious agents and restore immunity during perturbations. Disruption of the microbiota fails the signaling optimal performance and often leads to immune mediated disorders like inflammatory bowel disease and colorectal cancers (Song et al., 2014; Yu et al., 2014). Similarly, chemical colitis animal models show a decrease in protective bacteria but an increase in aggressive bacteria. They also show a decrease in the richness and diversity of both mucosal and luminal microbiota. These changes correlate with the severity of colitis as well as the expression of PRR (Xue et al., 2013). The expression of PRR in such animals is pivotal to the development of colitis. Indeed, the microbiota effect on PRR signaling is pertinent for gut immunity. It is therefore compelling to gain more insight into the microbiota-PRR interaction that might help designing medications for various forms of intestinal disorders emanating from disruption of this signaling. In this chapter, the contribution of microbiota to the PRR signaling and responses is discussed. Possible opportunities for employing the microbiota to alleviate gastrointestinal diseases are also highlighted.

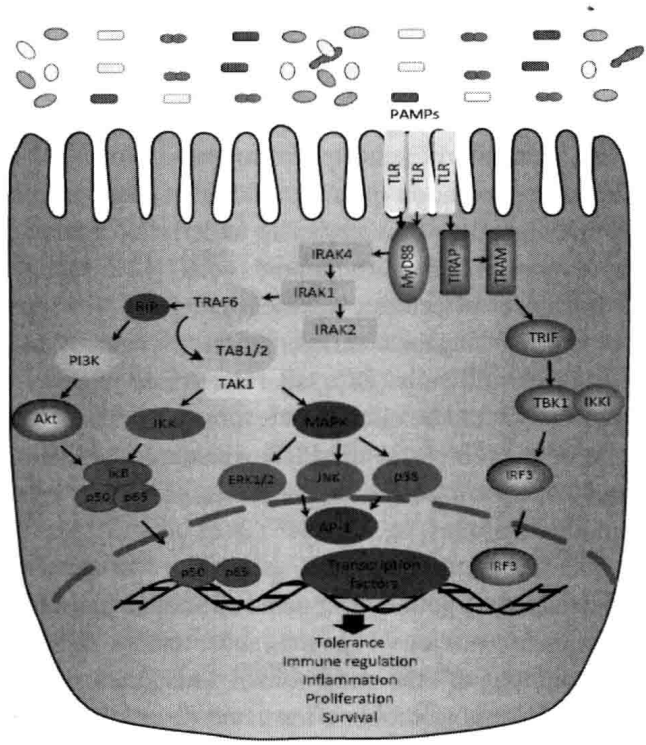

Figure 1. Microbiota-Toll like receptor signaling for intestinal immunity.

Microbiota signaling through TLR in a MyD88-dependent manner activates various intermediate factors that culminate in activation of p50-p65 dimer, ERK1/2, JNK, and p38. The MyD88-independent signaling leads to activation of IRF3. All the activated factors translocate intranuclearly and cause production of mediators for tolerance, immune regulation, inflammation, proliferation, and survival. All these effector products and functions are essential for maintaining intestinal immunity during health and disease. AP, activator protein; ERK, extracellular-regulated kinases; IκB, inhibitory kappa B; IKK, inhibitory kappa B kinase; IRAK, interleukin-1 receptor-associated kinase-like; IRF, interferon regulatory factor; IRF, interferon-regulatory factors; JNK, c-Jun N-terminal kinase activation; MAPK, mitogen activated protein kinase; MyD88, myeloid differentiation primary response gene (88); NF-κB, nuclear factor kappa B; PAMPS, pathogen-associated molecular patterns; RIP, routing information protocol; TAB, TAK1 binding protein; TAK, serine/threonine kinase; TBK, TANK-binding kinase; TIRAP, TIR-domain-containing adaptor protein; TLRs, Toll-like receptors; TRAF, TNF (tumor necrosis factor) receptor-associated factor; TRAM, TRIF related adaptor molecule; TRIF, TIR (Toll/IL-1 receptor) domain-containing adaptor protein inducing interferon-β.

PRRs FOR INTESTINAL INNATE IMMUNITY

PPR is a primitive part of the immune system that detects microbial antigens and transducer signals. To induce immune responses, commensal microbes are recognized by PRRs, which in turn regulate mucosal innate immunity and inflammatory responses. PRRs are proteins expressed by cells of innate immune system to detect microbe-associated molecular patterns (MAMPs or "infectious non-self") or endogenous "danger signals" derived from stressed, damaged or infected tissue. The detection leads to stimulation of intestinal innate immunity that initiates adaptive immune responses (Schlee 2013). MAMPs include peptidoglycans, lipoproteins, lipopolysaccharides, teichoic acids, CpG DNA motif, double strand RNA and flagellin. They mediate their signals through activation and maturation of antigen presenting cells (APCs) such as DCs (Szabo and Rajnavolgyi 2013).

TLR SIGNALING

Among PRRs, TLR are the first-discovered and the most well characterized member of PRRs. They are expressed by epithelial cells and APCs (including DCs and macrophages) and are responsible for initial recognition of specific pathogen-associated molecular patterns (PAMPs), discrimination between pathogens and harmless microbes and development of appropriate innate and acquired immune responses (Michelsen and Arditi 2007). They can be located at the cell surface, e.g., TLR1, 2, 4, 5, 6, 10 (Kawai and Akira 2006; Barton and Kagan 2009; Wagner 2012; Kamdar et al., 2013) or intracellularly, e.g., TLR3, 7, 8, 9 (Rakoff-Nahoum and Medzhitov 2008; Wagner 2012; Kamdar et al., 2013). Activation of TLR signaling through recognition of PAMPs leads to dimerization and induction of cell signaling through a series of adaptor molecules such as MyD88, TIR (Toll/IL-1 receptor) domain-containing adaptor protein inducing interferon (IFN)-β (TRIF), interferon regulatory factor (IRF), and TNF receptor-associated factor (TRAF) (Figure 1). This signaling activates NF-κB, activator protein (AP)-1, or IRFs (Kawai and Akira 2006; Rhee 2011). The transactivation of these factors leads to transcriptional activation of genes encoding for pro-inflammatory cytokines, chemokines and co-stimulatory molecules as well as type I interferons, which subsequently control the activation of antigen-

specific adaptive immune response (Bermudez-Brito et al., 2014; Song et al., 2014).

Influence of Microbiota on TLR Signaling during Health and Disease

Signaling through TLR co-ligands

The TLR-microbiota interaction modulates immune genes for gut immunity ranging from tolerance to combating pathogens. For instance, TLR2/6 co-ligands prime DCs to become tolerogenic and promote the polarization of IL-10-producing regulatory T cells (Tr1). Furthermore, activation of co-ligands TLR2/1 directs DCs to produce greater amounts of IL-12p40 and low levels of IL-10, thereby promoting the differentiation of Th1 or Th17 cells (DePaolo et al., 2008; DePaolo et al., 2012). The variations in these responses are due to differences in the down-stream effector pathways. Whereas TLR2/1 signals through p38-MAPK, TLR2/6 mediates its effect through c-Jun N-terminal kinase (JNK) activation (Figure 3) (DePaolo et al., 2008).

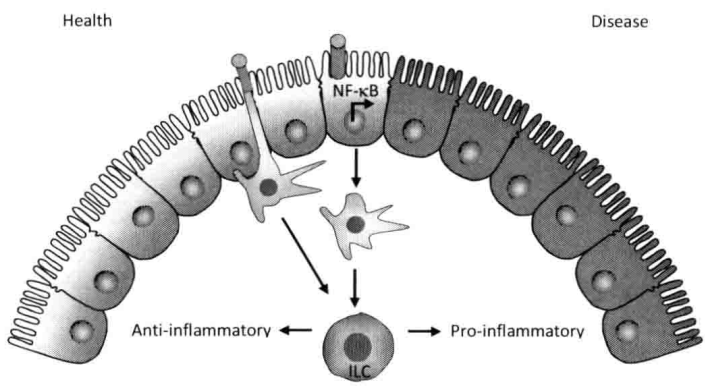

Figure 2. Microbiota-induced activation of innate lymphoid cells through Toll-like receptor signaling.

Commensal bacteria signal through Toll-like receptors found on epithelial and dendritic cells. This signaling activate transcription factors leading to stimulation of lamina propria innate lymphoid cells (ILC) that, depending upon the nature of antigen, induce immune homeostasis (via anti-inflammatory role) or inflammatory reaction during disease. NF-κB, nuclear factor kappa B.

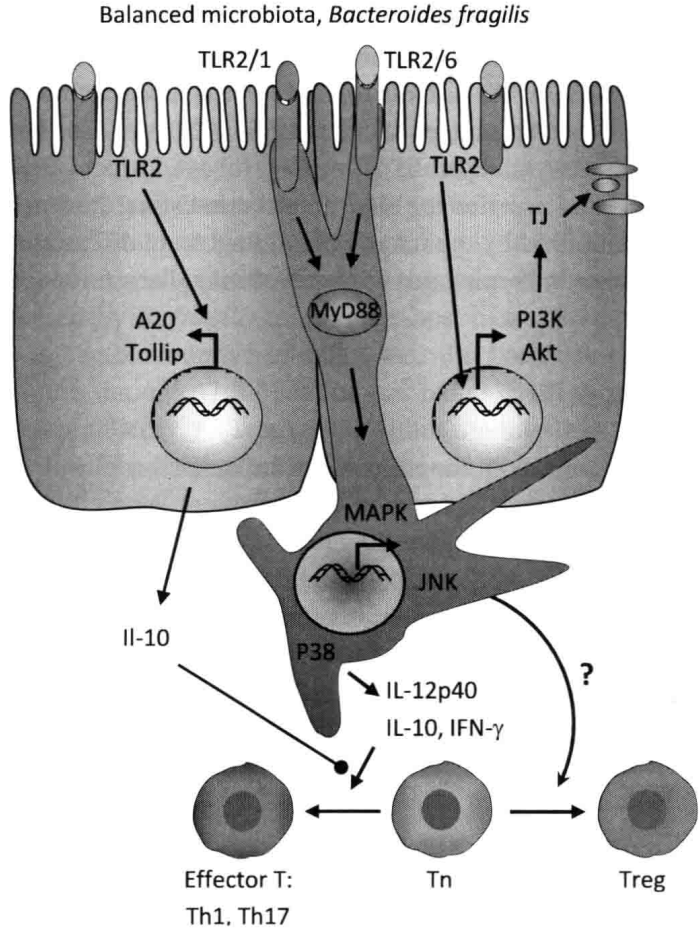

Figure 3. Microbiota-Toll-like receptor interaction in intestinal epithelial and dendritic cells. Balanced microbiota or specific commensal species like *Bacteroides fragilis* are capable of signalling through co-ligands TLR2/1 and TLR2/6 on dendritic cells. The signalling is MyD88-dependent and activates MAPK to drive T cell differentiation and protect the intestine. In addition, the microbiota and *Bacteroides fragilis* protect the intestine by signalling through intestinal epithelial TLR2. This signalling curbs generation of inflammatory effector T cells to prevent inflammation and induces production of tight junction proteins that strengthen the intestinal epithelial barrier integrity to inhibit microbial translocation into the submucosa to cause diseases. IFN, interferon; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; Th, T helper cell; TJ, tight junction proteins; TLR, Toll-like receptor; Tn, naïve T cell; Treg, regulatory T cell.

TLR signaling through ligands also benefits microbiota during disease by providing nutrition from within the body. This has recently been reported by Pickard and colleagues (Pickard et al., 2014) who observed that systemic exposure of TLR ligands induces secretion of IL-22 by DCs that activates IECs to express fucosyltransferase 2 leading to α(1,2)-fucosylation of the IECs. The fucosylated proteins are shed into the intestinal lumen to produce fucose that is metabolized by the microbiota during acute disease. In so doing, the microbiota assists in keeping gut immunity during disease-induced stress.

Signaling through TLR1

Literature is scarce about the microbiotal influence on TLR-1 signaling to drive intestinal immunity. Nonetheless, there is evidence that microbiota influences intestinal innate immune functioning through activation of TLR1. Some members of the microbiota, e.g., *Lactobacillus rhamnosus* or their cell-free culture supernatants increase the expression of TLR1 and suppress pro-inflammatory cytokine production by DCs challenged by pathogenic *Escherichia coli* (Bermudez-Brito et al., 2014). The TLR1-mediated anti-inflammatory property is important in curbing impending intestinal disease.

Signaling through TLR2

The microbiotal lipoproteins modulate TLR2 signaling to induce tolerance and prevent inflammation in healthy and diseased gut. This is mediated through increased expression of negative regulators, such as Tollip and A20, and activation of cell signaling pathways that induce production of anti-inflammatory IL-10 (Boone et al., 2004; Cario et al., 2007). The produced IL-10 inhibits macrophage and DC effector functions to limit inflammatory responses (Cario 2008). Alterations in TLR2-mediated NF-κB activation in APCs (such as caspase activation and recruitment domain (CARD15) or NOD-2 mutations), lead to mucosal inflammation through exaggerated IFN-γ, IL-12 and IL-23 production (Figure 3) (Watanabe et al., 2004). A recent study on mechanisms of microbiotal tolerance to the intestinal environment has indicated involvement of dephosphorylation and inactivation of microbiota (symbiont) MAMP lipid A by host alkaline phosphatases (APs). The APs work in synergy with a peptidoglycan recognition protein. The latter inactivates symbiont-exported peptidoglycan monomer to alter the symbiont