CTMI (2006) 308:1–18

Role of the Innate Immune System and Host–Commensal Mutualism

S. Rakoff-Nahoum · R. Medzhitov (✉)

Howard Hughes Medical Institute and Section of Immunobiology, Yale University School of Medicine, New Haven, CT 06510, USA
ruslan.medzhitov@yale.edu

Abstract Host organisms live in intimate contact with indigenous microflora. The interactions between the host and commensal microbiota are highly complex and heterogeneous. A growing body of evidence indicates that commensal symbionts provide many benefits to the host physiology, particularly in the gastrointestinal system. The molecular mechanisms of the mutualistic interactions between the host and commensals are largely unknown but can be due either to bioactivity of the commensals or to the reaction of the host immune system to the commensal-derived products. Recent advances in our understanding of the innate immune system allow re-evaluation of some of the older findings regarding the mechanisms of benefits conferred by

microflora. Here we review the examples of the benefits of host–commensal interactions that are due to recognition of commensal microbial products by the host innate immune system.

1 Introduction

All metazoans are engaged in complex interactions with microorganisms. The nature of these interactions can range from highly antagonistic to mutually beneficial and is determined by evolutionary adaptations of the host and the microbial species. Depending on the outcome of the interaction, the microorganisms are referred to as pathogens, if the interaction results in a loss of fitness of the host, or commensals, if the interaction is either beneficial or neutral for the host. It is generally thought that the host immune system protects from infection by pathogens but somehow avoids responding to commensals, as such responses can be detrimental under certain conditions. This view presupposes that there is something fundamentally different in the way the immune system deals with commensals versus pathogens. The problem with this view, however, is that the distinctions between commensal and pathogenic microbes are often arbitrary, as the ability to cause the disease is not always dependent on characteristics intrinsic to the microorganisms. Further complicating the matter is the fact that the receptors used to sense infection by the innate immune system do not discriminate between pathogens and commensals. This raises several important questions regarding the nature of the interactions between commensal microflora and the host innate immune system and the mechanisms that regulate these interactions. Indeed, the failure of mechanisms that regulate intestinal immune responses can lead to a variety of immunopathologies, including the development of inflammatory bowel disease (IBD). Nevertheless, several aspects of metazoan physiology, most notably of the gastrointestinal system, have clearly coevolved with, and are dependent upon, mutualistic interactions with indigenous microflora.

The aim of this review is discuss the benefits of the indigenous microflora on host biology, especially in the intestine. Emphasis is placed on what is known about the mechanisms by which these benefits occur. In particular we highlight evidence that suggests that recognition of microbes by Toll-like receptors (TLRs) plays a role in mediating host–microbe mutualism.

The life histories of plants and animals are replete with mutualistic relationships with the microbial world. Most complex metazoans are colonized with a consortium of microorganisms comprising an indigenous microflora.

In some cases, the host dependence on symbionts can be truly remarkable. In the plant kingdom, infection of legumes, such as soybeans, by the gram-negative *Rhizobium* leads to root nodulation allowing for the fixation of N_2 by the bacteria, thus providing the host plant with selective advantage in soils with nitrogen deficiency (Fisher and Long 1992). The benefits of microbial colonization of insects leads to increased nutritional sources (Dillon and Dillon 2004), protection from infection with enteropathogens (Dillon and Dillon 2004), and increased life span (Brummel et al. 2004). In the symbiosis between *Vibrio fisheri* and the Hawaiian sepiolid squid, *Euprymna scolopes*, colonization leads to postembryonic development of the squid light organ (Nyholm and McFall-Ngai 2004). The commensal microflora also plays a role in the lives of other aquatic animals, as demonstrated in studies of axenically derived zebra fish (Rawls et al. 2004) and of deep sea vent worms (Jeanthon 2000).

Host–microbe interactions play a profound role in the biology of mammals. Mammals are colonized with a diverse and abundant indigenous microflora. It is estimated that 500–1,000 different species of 10^{14} microorganisms may colonize mammals such as rodents and humans, although the number of species is most likely an underestimate (Sonnenburg et al. 2004). Commensal bacteria colonize many organs of the mammal, including the gingiva, oropharynx, skin, and genitourinary and respiratory tracts. However, the greatest density, magnitude, and diversity occur in the gastrointestinal tract, particularly at the large intestine. Since the original observation that microbial colonization plays a role in the physiological development of rodents, 40 years ago (Schaedler et al. 1965), numerous benefits of the indigenous microflora to mammalian biology have been revealed. These benefits range from those affecting metabolism, development, or various organ systems to tolerance to mucosal antigens and resistance to infection (Berg 1996).

There have been three ways in which the role of the commensal microflora in mammalian biology has been assessed. The most common is the comparison of conventionally raised animals with those that are germ-free, reared in positive-pressure isolators after being delivered by sterile Cesarean section. In gnotobiotic studies, germ-free animals are colonized with a known species or group of species of microorganisms. A third, and very different, approach to study the affect of the commensals on host biology is to deplete conventionally raised mice of their microflora with antibiotics. Although this approach has the advantage of being able to assess the effect of the microflora on animals with normal development, a caveat is the inability to determine the effectiveness of the depletion of commensals because of the inability to culture or identify the majority of microfloral species.

2
Different Types of Host–Microbial Mutualism

Despite the enormous benefits of microorganisms in host biology, relatively little is known about the molecular details of host–commensal interactions. Until recently, it was thought that the majority of benefits provided by the microflora were conferred because of the "bioactivity" of commensals. Two well-known examples of this type of benefits are the roles of indigenous symbionts in energy and nutrient utilization and in resistance to infection by pathogens. In the former, bacterial enzymes, such as those that process polysaccharides normally indigestible by host enzymatic machinery, allow for emergent carbon sources. In the latter, the indigenous flora helps to prevent infection by pathogens by production of antimicrobial compounds or competing for niches necessary for infection.

In addition to these contributions to host biology, recent studies have revealed that commensals and the host are engaged in a much more intimate and complex interactions. Both in vitro and in vivo studies using human intestinal epithelial cell lines have revealed a role of nonpathogenic species of bacteria in the regulation of host cell signaling. An unidentified factor produced by nonvirulent *Salmonella* strains was shown to inhibit IκB degradation and NF-κB activation by blocking IκB polyubiquitination (Neish et al. 2000). Recently, the nonpathogenic $PhoP^c$ *Salmonella* mutant was demonstrated to increase nuclear localization of β-catenin by inhibiting its ubiquitination and proteosomal degradation (Sun et al. 2004, 2005). Kelly et al. have shown that the intestinal symbiont *Bacteroides thetaiotamicron* may regulate the NF-κB pathway by mediating nuclear export of RelA via a PPARγ-dependent mechanism (Kelly et al. 2004).

Elegant studies by Hooper et al. have characterized a system of bidirectional communication between *B. thetaiotamicron* and intestinal epithelial cells (Hooper et al. 1999). In this relationship, the bacteria upregulate the expression of α1.2-fucosyltransferase in the host cell, which leads to the appearance of fucosylated glycans on the ileal epithelium. The bacteria utilize the fucose residue as an energy source, and in addition fucose levels feed into a regulatory operon in which high fucose levels repress the signal to the epithelium stimulating fucosylated glycan synthesis.

Thus nonpathogenic bacteria and intestinal symbionts have the capacity to interact with and modulate NF-κB and β-catenin signaling and metabolic pathways in host cells. NF-κB plays a critical role the inflammatory response, and β-catenin is an essential regulator of intestinal epithelial proliferation and differentiation. Although the physiological role of commensal-mediated reg-

ulation of this pathway is not known, it likely contributes to the maintenance of intestinal tissue homeostasis.

Recent studies in mice have revealed that recognition of the indigenous microflora by TLRs, best known for their role in host defense from infection, may be responsible for mediating some of the benefits of colonization by the indigenous microflora (Araki et al. 2005; Cario et al. 2004; Fukata et al. 2005; Pull et al. 2005; Rakoff-Nahoum et al. 2004). TLRs recognize conserved molecular products present on microorganisms. Ten to fifteen different TLRs have been identified in mammals. A number of TLR ligands have been identified so far, including TLR2 ligands lipotechoic acid (LTA) and bacterial lipoprotein (BLP), TLR4 ligand LPS, TLR3 ligand double-stranded RNA, TLR5 ligand flagellin and TLR9 ligand hypomethylated CpG DNA. TLRs signal through at least four adaptor molecules, MyD88, TRIF, TIRAP, and TRAM. Downstream signaling leads to the activation of NF-κB, IRFs, and MAP kinase pathways (Takeda et al. 2003).

3
Host–Microbe Mutualism in Mammals: Benefits and Mechanisms

3.1
Metabolism and Energy Utilization

The metabolic activity of the indigenous gastrointestinal flora has been described as equaling that of the liver (Berg 1996). Among many other abilities, commensals produce short-chain fatty acids (SCFA), synthesize vitamin B12, and deconjugate bile acids. Compared to conventionally raised animals, germ-free animals show defects in vitamin synthesis (including, biotin, folate, and vitamins B and K), bile acid transformation, carbohydrate and fatty acid digestion, synthesis of SCFA, xenobiotic transformation, and other signs of metabolic deficiencies (Berg 1996; Midvedt 1999; Savage 1986). Many of these benefits are indeed mediated by the novel enzymatic bioactivity of the indigenous flora. However, recent studies by Backhed et al. reveal that some of the effects of intestinal symbionts on host metabolism may be due to regulation of host metabolic enzymes (Backhed et al. 2004). Conventionalization of germ-free mice led to adipogenesis and increased insulin resistance, revealing a role of the microflora in positively regulating fat storage. Gene expression analysis revealed that the microbiota suppressed the expression of fasting-induced adipocyte factor (Fiaf) by intestinal epithelium, which was partly responsible for this phenotype. It is not known how colonization leads to the modulation of the expression of this factor by the intestinal epithelium.

3.2 Organ Development

Many organs, including the heart, liver, spleen, and adrenal glands, are undersized in germ-free rodents. Blood volume is decreased in these animals, perhaps leading to the decreased cardiac output and peripheral blood flow observed (Berg 1996; Savage 1977). Germ-free animals compared to those either conventionally raised or colonized with known species of bacteria show many differences in intestinal anatomy and morphology. Germ-free animals have decreased intestinal mass and surface area, thinner villi, and decreased rates of peristalsis. Intestines show compromised development of the vasculature (Stappenbeck et al. 2002) and abnormalities in glycosylation patterns, mucin production, and proliferation and differentiation of epithelial cells (Banasaz et al. 2002; Gordon et al. 1997). It is not currently known how the commensal microflora mediates these developmental changes.

3.3 Development of the Mucosal Immune System

The commensal microflora has a profound effect on the gut-associated lymphoid tissue (GALT). The GALT can be subdivided into three main sections, lymphoid follicles such as Peyer's patches, lamina propria (LP), and intraepithelial (IE) compartments, all of which require the commensal microflora for aspects of normal development (Jiang et al. 2004; Macpherson et al. 2001).

Compared to conventionalized or gnotobiotic animals, those raised under germ-free conditions have smaller, underdeveloped Peyer's patches, which lack germinal centers. Perhaps one of the most striking effects of the commensal microflora on the GALT is the expansion of IgA-secreting plasma cells in the lamina propria and the increased secretion of intestinal IgA that may contribute to the natural antibody pool (Jiang et al. 2004; Macpherson et al. 2001). In rabbits, commensal microflora induce the somatic diversification of the primary antibody repertoire (Lanning and Knight 1998). The number of total $CD4^+$ T cells and the ontogeny of mucosa-associated invariant T cells (MAIT) present in the lamina propria are commensal dependent (Treiner et al. 2003). Germ-free mice show a reduction in $CD8\alpha^+$ $\alpha\beta TCR^+$ T cell numbers in the IEL (Umesaki et al. 1993) compartment, but no difference in $CD8\alpha^+$ $\gamma\delta TCR^+$ T cells (Bandeira et al. 1990).

Given their known role in the immune system, it would seem likely that TLR signals induced by the commensal microflora may be important in the development of the mucosa-associated lymphoid tissue of the intestine. However, in initial analyses of adult animals, mice deficient in MyD88, TLR2, or TLR4 do not show gross abnormalities in the development of Peyer's patches,

differences in the morphology of small or large intestinal villi, or phenotype of intraepithelial lymphocytes with regard to αβ vs. γδ TCR and CD4 vs. CD8α surface expression (Iiyama et al. 2003).

However, on further analysis, significant differences were noted in the size of Peyer's patches at 2 weeks of age between TLR4$^{-/-}$, but not TLR2$^{-/-}$, and WT mice (Iiyama et al. 2003), suggesting that TLR4-dependent signaling by ligands present during this early postnatal stage may be important for Peyer's patch development but may be compensated for later by other mechanisms.

A more focused analysis of small intestinal intraepithelial lymphocytes revealed decreased numbers of CD8αα TCRα^{+} IEL in various strains of mice deficient in TLR4 signaling (Kaneko et al. 2004). Investigation into the mechanism causing this reduction of IEL subset revealed diminished IL-15 expression in isolated intestinal epithelium from TLR4-deficient mice. This suggests that the mechanism of commensal-dependent development of certain subsets of intraepithelial lymphocytes, whether of thymic (Eberl and Littman 2004) or extrathymic (Lefrancois and Olson 1994; Rocha et al. 1994; Saito et al. 1998; Umesaki et al. 1993) origin, may actually be due to constitutive signaling of TLR4 in the intestine induced by the commensal microflora.

Thus in the mouse, TLR-mediated signals are important in various aspects of the development of intestinal lymphoid tissue. It is unknown what role commensal-TLR interactions play in GALT development in humans, given the differential programs of GALT organogenesis between these two species (McCracken and Lorenz 2001).

3.4 Regulation of Host Immune Responses

3.4.1 Positive Regulation

The commensal microflora plays an important role in the induction and regulation of immune responses, both systemically and locally. Germ-free animals show defective induction of peripheral delayed-type hypersensitivity to sheep red blood cells (MacDonald and Carter 1979) and antibody responses to haptenated antigens (Bos and Ploplis 1994; Ohwaki et al. 1977)). In vitro studies of splenic and peritoneal macrophages from germ-free mice showed decreased proliferation, MHC class II expression, and production of IL-1, IL-6, and IL-12 (Nicaise et al. 1998, 1999). Peritoneal macrophages from germ-free rats showed defects in chemotaxis (Jungi and McGregor 1978), whereas those from bronchoalveolar lavage were found to have decreased lysosomal enzyme activity (Starling and Balish 1981). Germ-free mice show decreased lytic activity of natural killer cells (Bartizal et al. 1984). It is not known how

the indigenous microflora mediates these functions; however, a role for TLRs is likely given that such TLR signaling is known to activate many of these events.

3.4.1.1 IgA Immune Responses

Association of germ-free animals with known species of commensal bacteria leads to germinal center reactions in Peyer's patches in which B cells become committed to the production of IgA. Studies of the antigen specificity of the IgA revealed that the commensal microflora may be inducing IgA specific to commensal antigens (specific IgA) and also nonspecific IgA, termed "natural IgA" (Bos et al. 2001; Macpherson and Uhr 2004). Analysis of the relative proportions of commensal-specific and natural IgA of the monoassociated germ-free mice demonstrated that the overwhelming majority of IgA was not specific to the commensals. For example, colonization of germ-free mice with SFB results in the production of IgA of which only 1% is antigen specific to the commensal (Talham et al. 1999).

These findings suggest polyclonal activation of the natural B cell pool. TLR ligands, including LPS, are well known to be polyclonal activators of B cells (Andersson et al. 1972; Armerding and Katz 1974). It is thus very likely that ligation of TLRs either directly on B cells or on accessory cells (such as DC) in the intestine, may be responsible for the polyclonal expansion of B cells and production of intestinal IgA on conventionalization of germ-free animals (Fagarasan and Honjo 2003; Jiang et al. 2004). Although the functions of the natural IgA induced nonspecifically by commensals remain unknown and controversial, it is possible that these antibodies function similarly to natural serum IgM [although these are not commensal dependent (Haury et al. 1997), acting as an early defense system, before the induction of an antigen-specific immune response (Baumgarth et al. 1999; Ochsenbein et al. 1999)]. Thus this may be another example in which a commensal dependent benefit to host biology may be mediated by TLR recognition of the indigenous microflora.

3.4.2 Negative Regulation

Commensal microbiota may also be involved in negative regulation of certain types of immune responses. Germ-free animals have been shown to have defects in inducing systemic tolerance to orally administered foreign antigens (Wannemuehler et al. 1982) and in the regulation of atopic response in the intestine (Sudo et al. 1997; Tanaka and Ishikawa 2004).

3.4.2.1 Oral Tolerance

A role of TLRs in inducing systemic tolerance to oral antigens comes from two lines of evidence. Unlike conventionally raised animals, germ-free BALB/c mice given sheep RBC via gastric gavage were able to induce potent systemic immune responses as determined by detection of high frequencies of antigen-specific antibody (IgG, IgM, and IgA)-producing splenocytes on intraperitoneal immunization with antigen. When these germ-free mice were given a single oral dose of LPS, they became hyporesponsive to systemic challenge (Michalek et al. 1982; Wannemuehler et al. 1982). The role of LPS in the induction of oral tolerance was further suggested by similar studies in which tolerance could not be induced in C3H/HeJ (LPS insensitive) compared with C3H/HeN (LPS-sensitive) mice (Kiyono et al. 1982; Mowat et al. 1986). Both the lack of oral tolerance in germ-free mice and the role of LPS in mediating this tolerance were challenged by studies in germ-free and conventionalized C3H/HeJ mice (Moreau and Corthier 1988) using OVA as fed antigen. However, these studies used a high dose of OVA, which is known to induce T cell deletion rather than induction of regulatory T cells (Chen et al. 1995) and may have been the mode of action in experiments using SRBC.

3.4.2.2 Regulation of Atopic Allergic Responses

The "hygiene hypothesis" suggests that the absence of microbial infection may lead to the development of IgE-mediated atopic allergy by Th2-polarized immune responses (Strachan 1989; Umetsu et al. 2002). It is hypothesized that this may occur through the lack of microbial induced Th1 responses and associated with increased Th2 responses, or by a defective generation of immunoregulatory mediators such as regulatory T cells or interleukin-10 (Macpherson and Harris 2004). Evidence that the indigenous microflora may play a role in regulating IgE responses comes from two lines of evidence: studies in germ-free mice showing increased IgE response to antigen (Sudo et al. 1997; Tanaka and Ishikawa 2004) and observations showing the relationship between the composition of the intestinal microflora and the incidence of atopic allergy in animal models and humans (Bjorksten et al. 2001; Kalliomaki and Isolauri 2003; Kirjavainen et al. 2002).

A role for commensal-TLR interaction in the regulation of intestinal atopy was first suggested by studies in which C3H/HeJ mice, compared to BALB/c, showed severe anaphylaxis, antigen-specific IgE, and plasma histamine levels on oral administration of both cow's milk and peanut allergen (PNA) (Morafo et al. 2003). Bashir et al. demonstrated that commensal-TLR signaling was

responsible for inhibiting this IgE hyperresponsiveness (Bashir et al. 2004). This was evidenced by increased levels of serum IgE in TLR4-deficient mice compared to WT on oral administration of PNA. Antibiotic depletion of commensals in WT mice phenocopied the high serum levels of IgE seen in TLR4$^{-/-}$ mice and was reversed on feeding the mice with CpG oligonucleotides. Thus it appeared that both TLR4 and TLR9 ligands on commensal microflora were capable of inhibiting the IgE induced by oral allergen. In the intestine, immunostimulatory DNA from probiotic bacteria may act on TLR9 and mediate anti-inflammatory effects in an animal model of colitis (Rachmilewitz et al. 2004). A recent report, however, questions the specificity of immunoregulatory DNA for TLR9, showing that germ-free mice fed nucleic acids devoid of CpG motifs were able to skew intestinal immune responses to a Th1 bias (Sudo et al. 2004).

3.5 Resistance to Infection with Pathogens

Studies in both germ-free animals and those depleted of commensals by antibiotics have revealed a role of the indigenous microflora in providing colonization resistance, which helps to prevent infection of the host by pathogenic microorganisms. In humans, a well-known phenomenon is the diarrhea caused by *Clostridium difficile* overgrowth in the intestine following the use of broad-spectrum antibiotics (Stoddart and Wilcox 2002). In mice, oral administration of antibiotics leads to a decrease in the number of pathogenic organisms, such as *Vibrio* and *Shigella*, required for effective colonization (Freter 1955). Infection studies in germ-free mice have demonstrated the beneficial role of the commensal microflora in protecting the host from intestinal pathogens (Filho-Lima et al. 2000; Hudault et al. 2001)

Passive mechanisms may be particularly operative in colonization resistance to infection by intestinal pathogens. Proposed passive mechanisms of such protection include (a) niche competition, both for nutrients and epithelial attachment sites, (b) production of antimicrobial compounds, such as colicins and microcins, and (c) production of metabolites that may be unfavorable to the growth of intestinal pathogens.

However, recent studies have suggested that the intestinal microflora may stimulate host cells in the intestine to produce antimicrobial factors, and this may be one of the ways in which the flora mediates colonization resistance. Paneth cells located at the base of small intestinal crypts, below the stem cell zone (Brittan and Wright 2002; Marshman et al. 2002), may play an important role in maintaining a sterile environment so as to protect the stem cell niche. These cells contain apically oriented intracellular granules containing

numerous factors with known antimicrobial effects such as phospholipase2, lysozyme, α-defensin, and angiogenin 4 (Ouellette 1999). Comparisons of genes induced in the ileum on conventionalization or monospecific association of *B. thetaiotamicron* with germ-free mice identified upregulation of a family of antimicrobial proteins known as angiogenins (Hooper et al. 2001, 2003). In particular, angiogenin 4 (which was specifically expressed in Paneth cells on colonization of germ-free mice) was shown to have potent microbicidal activity against the bacterial intestinal pathogens, *Enterococcus faecalis* and *Listeria monocytogenes*. Interestingly this protein had very limited ability to kill *B. theatiotamicron* itself.

How does the intestinal flora or a symbiont such as *B. thetaiotamicron* induce the production or secretion of antimicrobial factors? Studies performed on isolated intestinal crypts have suggested a role of TLR ligation on Paneth cells in inducing degranulation. Contact with bacteria, both live and dead, has been shown to induce degranulation of Paneth cells (Ayabe et al. 2000). A role for TLRs and NOD proteins in inducing Paneth cell degranulation was suggested by studies showing that degranulation can be induced in isolated small intestinal crypts by various TLR and NOD ligands, such as LPS, LTA, lipid A, and muramyl dipeptide (MDP) (Ayabe et al. 2000). The degranulation results in a release of lysozyme, angiogenin 4 (Hooper et al. 2003) and α-defensins (Ayabe et al. 2000). In addition, intraperitoneal injection of CpG oligonucleotides was shown to result in Paneth cell degranulation and increased expression of cryptdin-1 in isolated intestinal crypts. Consistent with these findings, TLR9 was found to be expressed in Paneth cells of both murine and human small intestine, located mainly at intracellular granules (Rumio et al. 2004).

3.6 Maintenance of Tissue Homeostasis and Repair

The indigenous microflora may be important in maintaining barrier function, epithelial integrity, and wound healing in many organs. Germ-free mice have been shown to be extremely susceptible to nonspecific intestinal epithelial injury (Kitajima et al. 2001; Pull et al. 2005). Ex vivo studies of rat bowel loops revealed that some commensal bacteria (such as *Lactobacillus brevis*), but not others (*Bacteriodes fragilis*, *Escherichia coli*), may maintain epithelial integrity (Garcia-Lafuente et al. 2001). The commensal microflora may also play a role in the maintenance of liver homeostasis after injury (Cornell et al. 1990). In various models, germ-free animals show defective healing after intestinal resection (Okada et al. 1999), skin incision (Okada 1994), tooth extraction (Rovin et al. 1966), and tongue injury (Rovin et al. 1965).

The ability of the indigenous flora to produce (SCFA, which are known to affect various aspects of intestinal epithelial cell biology, perhaps by acting as an inhibitor of histone deacetylase, may be one way in which microbial symbionts confer this benefit to the host (Blottiere et al. 2003; Sanderson 2004).

A new role for beneficial commensal-TLR interactions in protecting the large intestine from injury has recently been suggested (Araki et al. 2005; Cario et al. 2004; Fukata et al. 2005; Pull et al. 2005; Rakoff-Nahoum et al. 2004). Animals deficient in MyD88, TLR2, and TLR4 showed severe mortality, weight loss, and intestinal bleeding and epithelial cell injury (Rakoff-Nahoum et al. 2004). WT mice were rendered susceptible to the epithelial injurious agent DSS when depleted of all culturable commensals by treatment with broad-spectrum antibiotics. The susceptibility of commensal-depleted animals was completely reversed by oral pretreatment with either LPS or LTA (Rakoff-Nahoum et al. 2004). Additional studies in MyD88$^{-/-}$ mice revealed an inability to regenerate colonic epithelial cells after whole body irradiation (Rakoff-Nahoum et al. 2004) and DSS administration (Pull et al. 2005). Together, these studies demonstrated that activation of TLRs by commensal-derived products is essential for protecting the intestinal epithelium from injury and for the induction of tissue repair responses. In vitro studies have recently demonstrated that TLR2 signaling in intestinal epithelium may aid in maintaining epithelial barrier function by increasing tight junction formation (Cario et al. 2004).

A LPS-unresponsive mouse strain (C3H/HeJ) was found to have a defect in hepatocyte proliferation after partial resection compared with LPS-responsive C3H/HeN mice (Cornell et al. 1990). A critical role of TLRs in repair of the liver after injury was further demonstrated in studies using MyD88$^{-/-}$ mice (Seki et al. 2005). As the portal vein contains commensal-derived TLR ligands, such as LPS, this protective effect is likely mediated by commensal product-TLR interactions in the liver.

A series of experiments performed in rats suggested that microflora of the skin may aid in skin wound healing. In experiments originally performed to study the effects of heating scalpels on wounds, the authors revealed a surprising role for *Staphylococcus aureus* in accelerating the healing of epidermal-dermal incisions (Levenson et al. 1983). Further investigation into this phenomenon revealed that this benefit was mediated by *S. aureus* cell wall and specifically by its peptidoglycan (Kilcullen et al. 1998). Thus although in many circumstances, such as in deep tissue wounds, the microflora of the skin may cause infection, pattern recognition of the commensal bacteria of the skin may be important in skin tissue regeneration and repair.

4 Innate Immune Recognition of Commensals in Symbiosis in Other Organisms

Recent investigations in squid and zebra fish indicate that signaling via TLRs (or other pattern recognition receptors) may be involved in mediating host–microbe mutualism in animals other than mammals. Studies in the symbiosis between the bacteria *Vibrio fisheri* and the Hawaiian sepiolid squid, *Euprymna scolopes* have revealed that LPS, peptidoglycan, and tracheal cytotoxin may be responsible for various aspects of light organ development (Foster et al. 2000; Koropatnick et al. 2004; Nyholm et al. 2002). The microbial flora of the zebra fish digestive tract was found to induce a widespread host gene expression program (Rawls et al. 2004). Fifty-nine of the 212 genes upregulated on microbial colonization of zebra fish were shared with those induced on introduction of commensals to mice, revealing an evolutionarily conserved response to the intestinal microflora. These include genes involved in many biological processes such as epithelial proliferation, nutrient metabolism, and innate immunity. It is not known whether TLRs are responsible for the recognition of symbionts or symbiont-derived products in either squid or zebra fish. Homologs of TLR and TLR signaling intermediates have recently been identified in zebra fish (Jault et al. 2004; Meijer et al. 2004). One report has shown that LPS may decrease glutathione-*S*-transferase activity in these fish, decreasing the detoxification of cyanobacteria-derived toxins (Best et al. 2002); however, the role that this activity and LPS-zebra fish interactions may have on other aspects of zebra fish biology is unknown.

5 Conclusions and Perspectives

Complex metazoans coexist with different types of microorganisms ranging from overt pathogens to beneficial symbionts. This co-existence requires that the host (a) be able to mount protective immune responses to potential pathogens, (b) allow for the mutualist microbes to confer benefit to host biology, (c) prevent constitutive immune responses to the indigenous microbes that would cause immune-mediated pathology, and (d) prevent opportunistic infections caused by endogenous pathogens (components of the indigenous microflora).

A predominant theory has been that anatomic compartmentalization fulfills these four conditions. According to this hypothesis, symbionts are confined to living outside of the host by a physical barrier. This barrier simultaneously prevents both recognition by the host immune system and consequent immunopathology and also opportunistic infection by the indigenous flora.

On infection with a pathogen, virulence factors enable the pathogen to traverse this barrier. It is thought that this desequestration allows for recognition of the microbe by the host immune system and the induction of host defense responses.

However, as discussed in this review, many of the benefits conferred by the indigenous microflora do not simply occur by passive means and indeed could not occur if there was strict compartmentalization between the host and their symbionts. In many instances, activation of host cell signaling is required. Furthermore, we have highlighted evidence to suggest that many of the benefits conferred by symbiotic microorganisms occur through recognition of commensals by the TLRs. It is this same family of receptors that are known to be responsible for orchestrating host defense responses to microbial pathogens. Thus it appears that TLRs do not distinguish between pathogenic and nonpathogenic microbes on the basis of their ligand specificity.

A future challenge will be to determine how the four conditions of host–microbe interactions outlined above may be fulfilled given that recognition of microbes by TLR is involved both in protection from microbial pathogens and in mediating the benefits of colonization with symbiotic bacteria. It appears that active regulatory mechanisms, in addition to physical barriers such as anti-inflammatory cytokines, may be instrumental in setting a threshold of the immune response of host organisms to their indigenous flora. How these factors modulate TLR signaling and allow for the induction of both TLR-mediated host defense to pathogens and homeostatic interactions with the flora, while simultaneously preventing commensal-induced immunopathology, is unknown.

TLRs are thought to have evolved to protect the host from microbial pathogens. However, as illustrated by many examples discussed in this review, TLR-mediated recognition of commensals is also highly beneficial to the host. Therefore, both TLR-mediated recognition of pathogens and TLR-mediated recognition of commensals have their own benefits that would provide selective advantage to the host. This implies that either one of the two functions of TLRs could have evolved on its own right. Thus the question arises as to which of the two functions has been the primary driving force in the evolution of the TLR family and which was the secondary adaptation. As discussed at the beginning of this text, the distinctions between commensals and pathogens are not absolute and static, but rather relative and dynamic. It is likely, therefore, that TLRs have evolved to detect microorganisms and to induce responses ranging from inflammatory and antimicrobial to tissue protective and reparative. Which type of response would dominate in any particular situation is likely determined by multiple variables, including microbial strategy of host colonization.

References

Andersson J, Sjoberg O, Moller G (1972). *Eur J Immunol* **2,** 349–53.

Araki A, Kanai T, Ishikura T, Makita S, Uraushihara K, Iiyama R, Totsuka T, Takeda K, Akira S, Watanabe M (2005). *J Gastroenterol* **40,** 16–23.

Armerding D, Katz DH (1974). *J Exp Med* **139,** 24–43.

Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME, Ouellette AJ (2000). *Nat Immunol* **1,** 113–8.

Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI (2004). *Proc Natl Acad Sci USA* **101,** 15718–23.

Banasaz M, Norin E, Holma R, Midtvedt T (2002). *Appl Environ Microbiol* **68,** 3031–4.

Bandeira A, Mota-Santos T, Itohara S, Degermann S, Heusser C, Tonegawa S, Coutinho A (1990). *J Exp Med* **172,** 239–44.

Bartizal KF, Salkowski C, Pleasants JR, Balish E (1984). *J Leukoc Biol* **36,** 739–50.

Bashir ME, Louie S, Shi HN, Nagler-Anderson C (2004). *J Immunol* **172,** 6978–87.

Baumgarth N, Herman OC, Jager GC, Brown L, Herzenberg LA (1999). *Proc Natl Acad Sci USA* **96,** 2250–5.

Berg RD (1996). *Trends Microbiol* **4,** 430–5.

Best JH, Pflugmacher S, Wiegand C, Eddy FB, Metcalf JS, Codd GA (2002). *Aquat Toxicol* **60,** 223–31.

Bjorksten B, Sepp E, Julge K, Voor T, Mikelsaar M (2001). *J Allergy Clin Immunol* **108,** 516–20.

Blottiere HM, Buecher B, Galmiche JP, Cherbut C (2003). *Proc Nutr Soc* **62,** 101–6.

Bos NA, Jiang HQ, Cebra JJ (2001). *Gut* **48,** 762–4.

Bos NA, Ploplis VA (1994). *Eur J Immunol* **24,** 59–65.

Brittan M, Wright NA (2002). *J Pathol* **197,** 492–509.

Brummel T, Ching A, Seroude L, Simon AF, Benzer S (2004). *Proc Natl Acad Sci USA* **101,** 12974–9.

Cario E, Gerken G, Podolsky DK (2004). *Gastroenterology* **127,** 224–38.

Chen Y, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL (1995). *Nature* **376,** 177–80.

Cornell RP, Liljequist BL, Bartizal KF (1990). *Hepatology* **11,** 916–22.

Dillon RJ, Dillon VM (2004). *Annu Rev Entomol* **49,** 71–92.

Eberl G, Littman DR (2004). *Science* **305,** 248–51.

Fagarasan S, Honjo T (2003). *Nat Rev Immunol* **3,** 63–72.

Filho-Lima JV, Vieira EC, Nicoli JR (2000). *J Appl Microbiol* **88,** 365–70.

Fisher RF, Long SR (1992). *Nature* **357,** 655–60.

Foster JS, Apicella MA, McFall-Ngai MJ (2000). *Dev Biol* **226,** 242–54.

Freter R (1955). *J Infect Dis* **97,** 57–65.

Fukata M, Michelsen KS, Eri R, Thomas LS, Hu B, Lukasek K, Nast CC, Lechago J, Xu R, Naiki Y, Soliman A, Arditi M, Abreu MT (2005). *Am J Physiol Gastrointest Liver Physiol* **288,** G1055–65.

Garcia-Lafuente A, Antolin M, Guarner F, Crespo E, Malagelada JR (2001). *Gut* **48,** 503–7.

Gordon JI, Hooper LV, McNevin MS, Wong M, Bry L (1997). *Am J Physiol Gastrointest Liver Physiol* **273,** G565–70.

Haury M, Sundblad A, Grandien A, Barreau C, Coutinho A, Nobrega A (1997). *Eur J Immunol* **27,** 1557–63.
Hooper LV, Stappenbeck TS, Hong CV, Gordon JI (2003). *Nat Immunol* **4,** 269–73.
Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001). *Science* **291,** 881–4.
Hooper LV, Xu J, Falk PG, Midtvedt T, Gordon JI (1999). *Proc Natl Acad Sci USA* **96,** 9833–8.
Hudault S, Guignot J, Servin AL (2001). *Gut* **49,** 47–55.
Iiyama R, Kanai T, Uraushihara K, Ishikura T, Makita S, Totsuka T, Yamazaki M, Nakamura T, Miyata T, Yoshida H, Takeuchi O, Hoshino K, Takeda K, Ishikawa H, Akira S, Watanabe M (2003). *Scand J Immunol* **58,** 620–7.
Jault C, Pichon L, Chluba J (2004). *Mol Immunol* **40,** 759–71.
Jeanthon C (2000). *Antonie Van Leeuwenhoek* **77,** 117–33.
Jiang HQ, Thurnheer MC, Zuercher AW, Boiko NV, Bos NA, Cebra JJ (2004). *Vaccine* **22,** 805–11.
Jungi TW, McGregor DD (1978). *Infect Immun* **19,** 553–61.
Kalliomaki M, Isolauri E (2003). *Curr Opin Allergy Clin Immunol* **3,** 15–20.
Kaneko M, Mizunuma T, Takimoto H, Kumazawa Y (2004). *Biol Pharm Bull* **27,** 883–9.
Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S (2004). *Nat Immunol* **5,** 104–12.
Kilcullen JK, Ly QP, Chang TH, Levenson SM, Steinberg JJ (1998). *Wound Repair Regen* **6,** 149–56.
Kirjavainen PV, Arvola T, Salminen SJ, Isolauri E (2002). *Gut* **51,** 51–5.
Kitajima S, Morimoto M, Sagara E, Shimizu C, Ikeda Y (2001). *Exp Anim* **50,** 387–95.
Kiyono H, McGhee JR, Wannemuehler MJ, Michalek SM (1982). *J Exp Med* **155,** 605–10.
Koropatnick TA, Engle JT, Apicella MA, Stabb EV, Goldman WE, McFall-Ngai MJ (2004). *Science* **306,** 1186–8.
Lanning DK, Knight KL (1998). *Curr Top Microbiol Immunol* **229,** 45–57.
Lefrancois L, Olson S (1994). *J Immunol* **153,** 987–95.
Levenson SM, Kan-Gruber D, Gruber C, Molnar J, Seifter E (1983). *Arch Surg* **118,** 310–20.
MacDonald TT, Carter PB (1979). *J Immunol* **122,** 2624–9.
Macpherson AJ, Harris NL (2004). *Nat Rev Immunol* **4,** 478–85.
Macpherson AJ, Hunziker L, McCoy K, Lamarre A (2001). *Microbes Infect* **3,** 1021–35.
Macpherson AJ, Uhr T (2004). *Science* **303,** 1662–5.
Marshman E, Booth C, Potten CS (2002). *Bioessays* **24,** 91–8.
McCracken VJ, Lorenz RG (2001). *Cell Microbiol* **3,** 1–11.
Meijer AH, Gabby Krens SF, Medina Rodriguez IA, He S, Bitter W, Ewa Snaar-Jagalska B, Spaink HP (2004). *Mol Immunol* **40,** 773–83.
Michalek SM, Kiyono H, Wannemuehler MJ, Mosteller LM, McGhee JR (1982). *J Immunol* **128,** 1992–8.
Midvedt T (1999). Microbial Functional Activities. *In* "Probiotics, Other Nutritional Factors, Intestinal Microflora" (L. a. Y. Hanson RH, ed.), pp. 79–96. Lippincott-Raven, Philadelphia.
Morafo V, Srivastava K, Huang CK, Kleiner G, Lee SY, Sampson HA, Li AM (2003). *J Allergy Clin Immunol* **111,** 1122–8.
Moreau MC, Corthier G (1988). *Infect Immun* **56,** 2766–8.

Mowat AM, Thomas MJ, MacKenzie S, Parrott DM (1986). *Immunology* **58,** 677–83.
Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL (2000). *Science* **289,** 1560–3.
Nicaise P, Gleizes A, Sandre C, Forestier F, Kergot R, Quero AM, Labarre C (1998). *Scand J Immunol* **48,** 585–91.
Nicaise P, Gleizes A, Sandre C, Kergot R, Lebrec H, Forestier F, Labarre C (1999). *Eur Cytokine Netw* **10,** 365–72.
Nyholm SV, Deplancke B, Gaskins HR, Apicella MA, McFall-Ngai MJ (2002). *Appl Environ Microbiol* **68,** 5113–22.
Nyholm SV, McFall-Ngai MJ (2004). *Nat Rev Microbiol* **2,** 632–42.
Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F, Hengartner H, Zinkernagel RM (1999). *Science* **286,** 2156–9.
Ohwaki M, Yasutake N, Yasui H, Ogura R (1977). *Immunology* **32,** 43–8.
Okada M (1994). *Surg Today* **24,** 347–55.
Okada M, Bothin C, Kanazawa K, Midtvedt T (1999). *Br J Surg* **86,** 961–5.
Ouellette AJ (1999). *Am J Physiol Gastrointest Liver Physiol* **277,** G257–61.
Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS (2005). *Proc Natl Acad Sci USA* **102,** 99–104.
Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E (2004). *Gastroenterology* **126,** 520–8.
Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R (2004). *Cell* **118,** 229–41.
Rawls JF, Samuel BS, Gordon JI (2004). *Proc Natl Acad Sci USA* **101,** 4596–601.
Rocha B, Vassalli P, Guy-Grand D (1994). *J Exp Med* **180,** 681–6.
Rovin S, Costich ER, Fleming JE, Gordon HA (1965). *Arch Pathol* **79,** 641–3.
Rovin S, Costich ER, Fleming JE, Gordon HA (1966). *J Oral Surg* **24,** 239–46.
Rumio C, Besusso D, Palazzo M, Selleri S, Sfondrini L, Dubini F, Menard S, Balsari A (2004). *Am J Pathol* **165,** 373–81.
Saito H, Kanamori Y, Takemori T, Nariuchi H, Kubota E, Takahashi-Iwanaga H, Iwanaga T, Ishikawa H (1998). *Science* **280,** 275–8.
Sanderson IR (2004). *J Nutr* **134,** 2450S-2454S.
Savage DC (1977). *In* "Microbial Ecology of the Gut" (R. T. J. a. B. Clark T, ed.), pp. 277–310. Academic Press.
Savage DC (1986). *Annu Rev Nutr* **6,** 155–78.
Schaedler RW, Dubs R, Costello R (1965). *J Exp Med* **122,** 77–82.
Seki E, Tsutsui H, Iimuro Y, Naka T, Son G, Akira S, Kishimoto T, Nakanishi K, Fujimoto J (2005). *Hepatology* **41,** 443–50.
Sonnenburg JL, Angenent LT, Gordon JI (2004). *Nat Immunol* **5,** 569–73.
Stappenbeck TS, Hooper LV, Gordon JI (2002). *Proc Natl Acad Sci USA* **99,** 15451–5.
Starling JR, Balish E (1981). *J Reticuloendothel Soc* **30,** 497–505.
Stoddart B, Wilcox MH (2002). *Curr Opin Infect Dis* **15,** 513–8.
Strachan DP (1989). *BMJ* **299,** 1259–60.
Sudo N, Aiba Y, Oyama N, Yu XN, Matsunaga M, Koga Y, Kubo C (2004). *Int Arch Allergy Immunol* **135,** 132–5.
Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y (1997). *J Immunol* **159,** 1739–45.

Sun J, Hobert ME, Duan Y, Rao AS, He TC, Chang EB, Madara JL (2005). *Am J Physiol Gastrointest Liver Physiol*., in press.
Sun J, Hobert ME, Rao AS, Neish AS, Madara JL (2004). *Am J Physiol Gastrointest Liver Physiol* **287,** G220–7.
Takeda K, Kaisho T, Akira S (2003). *Annu Rev Immunol* **21,** 335–376.
Talham GL, Jiang HQ, Bos NA, Cebra JJ (1999). *Infect Immun* **67,** 1992–2000.
Tanaka K, Ishikawa H (2004). *Histol Histopathol* **19,** 907–14.
Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O (2003). *Nature* **422,** 164–9.
Umesaki Y, Setoyama H, Matsumoto S, Okada Y (1993). *Immunology* **79,** 32–7.
Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH (2002). *Nat Immunol* **3,** 715–20.
Wannemuehler MJ, Kiyono H, Babb JL, Michalek SM, McGhee JR (1982). *J Immunol* **129,** 959–65.