Molecular Analysis of Microbiota-Host Cross-Talk in the Intestine

Andrew S. Neish*

Department of Pathology, Emory University School of Medicine, Atlanta, GA 30322, U.S.A.

Received for Publication, September 18, 2009

The resident microbiota of the mammalian intestine influences diverse homeostatic functions of the gut, including regulation of cellular growth, restitution after injury, maintenance of barrier function, and modulation of immune responses. However, it is unknown how commensal prokaryotic organisms mechanistically influence eukaryotic signaling networks. We have shown that epithelia contacted by enteric commensal bacteria in vitro and in vivo rapidly generate reactive oxygen species (ROS), and distinct microbial taxa have markedly different potencies in stimulating this response. This physiologically generated ROS is known to participate in a variety of cellular signal pathways via the rapid and transient oxidative inactivation of a number of regulatory enzymes. We show that these oxidant sensitive enzymes include key control points in the proinflammatory NF-κB pathway and in the regulation of cytoskeletal dynamics. Accordingly, we show various commensal bacterial have the ability to suppress inflammatory signaling and stimulate cell motility both in cell culture and in animal models. These events are consistent with known effects of the microbiota and selected probiotics. Collectively, our studies outline a molecular mechanism that may account for aspects of microbial–host cross-talk in the intestine in normal physiology and during therapeutic intervention with probiotics.

Key words: signaling; inflammation; microbiota; probiotic; reactive oxygen

EUKARYOTIC/PROKARYOTIC INTERACTIONS IN THE GASTROINTESTINAL TRACT

Commensal host-microbe interactions have coevolved over millennia in many animals, with the human luminal ecosystem representing a highly medically relevant example (40). The vast majority of these microbes represent about 500 genera of bacteria, broadly grouped into two taxonomic divisions, the Bacteroidetes and Firmicutes. An accurate accounting of the microbiota is not practical by conventional microbiological techniques; however recent high-throughput sequencing and molecular taxonomic methodologies have greatly increased our understanding of the population composition, dynamics, and ecology of the gut microflora [reviewed in (4, 13, 15, 18, 66)]. The gut is sterile in utero and is colonized immediately after birth, rapidly developing into a diverse and stable community, though marked variations in microbial composition between individuals is typical (14). Total numbers vary from $10^{11}$ cells/gram luminal content in the ascending colon, $10^{7-8}$ in the distal ileum, and $10^{2-3}$ in proximal ileum and jejunum. Most members of the microbiota are autochthonous, meaning indigenous and stable, though allochthonous, or transient members are known (certainly most enteric pathogens fall into this category).

This complex microbial ecosystem is separated from the host interior by only a single layer of epithelial cells (or epithelial derived, e.g. mucus layer). Impressively, epithelia and the complete mucosa perform vital fluid and nutrient absorptive functions, and must do so in presence of the microbiota and their products. Epithelial cells, by definition, act as interfaces between the host and the environment, and are equipped with apical surface specializations (microvilli, mucus production, vectorial ion secretion, intercellular junctions) to permit physiological function while contacting the microbiota. However, studies with germ-free mice have revealed that the microbiota is not functionally insulated from the mucosa, but in contrast, gut bacteria can fundamentally influence epithelial metabolism, proliferation and survival, and barrier function (18, 19, 21, 35, 59). For example, the small intestinal villi of the germ-free gut are elongated, while crypts are atrophic, show a slower turnover of the epithelial cells (50) and defective angiogenesis (60). Such mice monocolonized with a single gut symbiont species (Bacteroides thetaiotaomicron) exhibit robust host transcriptional

*Corresponding author. Mailing address: 105-F Whitehead Building, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, 615 Michaels St, Atlanta, GA 30322, U.S.A. Phone: +404-727-8545. Fax: +404-727-8538. E-mail: aneish@emory.edu

This review was presented at the 13th Annual Meeting for Intestinal Microbiology, Shirogane, Tokyo, Japan, 2009
Intestinal bacteria thrive in a stable, nutrient rich environment but also serve beneficial functions to the host including energy salvage of otherwise indigestible complex carbohydrates, vitamin and micronutrient syntheses, stimulation of immune development and competitive exclusion of pathogenic microorganisms (18, 36). Thus there is a dynamic interaction between the microbiota and the host, where the epithelia forms the major interface, resulting in for the most part a mutually beneficial relationship. However, in other cases, the normal flora of the intestine may be sufficient to provoke intestinal inflammation, such as that seen in IBD [which includes Ulcerative colitis (UC) and Crohn’s disease (CD)] (56). Additionally, there is much current interest in quantitative and/or qualitative abnormalities of the flora that may be associated with other systemic immune, allergic, metabolic and infectious disorders (44, 65). There is also increasing interest in potential therapeutic benefits of supplementing the normal flora with exogenous viable bacteria. This approach, termed probiotics, has been reported to dampen inflammation, improve barrier function and improve reparative responses in vitro and has shown promise as therapy in several inflammatory and developmental disorders of the intestinal tract (20, 46). Thus, there is increasing and compelling evidence that the gut flora beneficially affects intestinal -and systemic- homeostasis and thus health. However, little is known of how the host perceives non-pathogenic bacteria, or how the microbiota mechanistically influences gut biology.

PATTERN RECOGNITION RECEPTORS AND EPITHELIAL PERCEPTION OF BACTERIA

All eukaryotic cells have the ability to respond to and manage threats from bacterial pathogens —and by extrapolation, respond to and manage commensals. Transmembrane and intracytoplasmic receptors, such as the now well studied Toll-like receptors and related nod proteins, are designated “pattern recognition receptors” or PRRs. PRRs recognize and bind to conserved structural motifs present on the surface of a wide range of microbes, which are termed MAMPs, or “microbe associated molecular patterns”. For example, TLR4 recognizes lipopolysaccharide and TLR2 binds specific peptidoglycans -both components of bacterial cell walls (55). TLR5 detects the bacterial protein flagellin (69). The now well known association of Crohn’s disease with mutant forms of Nod2 clearly underscores the importance of PRR monitoring in intestinal health (56).

PRRs are expressed in most cells; however, given the vast microflora, the dominant interaction of bacteria with host cells occurs in the intestine, especially the epithelia. PRRs and their downstream signaling pathways, such as the MAPK and NF-κB systems, have an ancient lineage, exhibiting impressive structural and functional homology even at the level of invertebrates and plants. These systems represent entwined cytoplasmic information relays, which when activated employ rapid post translational events (covalent protein modifications and regulated protein degradation) to transduce PRR binding into well defined inflammatory and apoptotic tissue responses that evolved to eliminate pathogenic threats (1, 40, 54). However, while PRR mediated signaling clearly has a central and dominant role in initiating cellular inflammation during infection, it is now also apparent that basal tonic TLR (and possibly other PRR) mediated signaling in response to the normal flora and their products is necessary for mucosal health. Murine models with defective PRR signaling are hypersensitive to a variety of intestinal insults and stressors, and supplementation of TLR ligands such as CpG DNA and flagellin can have cytoprotective effects (8, 51). Regenerative responses to colonic injury are markedly attenuated in germ-free animals, indicating a discernable role of the flora in stimulation of epithelial proliferation and response to injury, and restitution is reduced in MyD88 (a signaling intermediate required by multiple TLRs) null mice, reinforcing the notion that PRR mediated signaling is necessary for trophic/restorative effects (50). These and related observations with mice null in epithelial NF-κB pathway components (6, 9, 43, 68) support the hypothesis that a constitutive degree of PRR signaling is necessary for normal gut homeostasis, presumably because of the tonic upregulation of cytoprotective genes (gene products with anti-apoptotic, chaperone/stress response, and antioxidant effects) (68) and underscores the importance of gut-prokaryotic interaction as a beneficial and necessary relationship.

FORMYLATED PEPTIDE RECEPTORS

An understudied form of PRRs are the formylated peptide receptors (FPR). While not typically considered PRRs in the same class as leucine rich repeat bearing TLRs or Nods, the FPR are clearly, by definition, PRRs that recognize and respond to bacterial products. Classically, the FPRs are seven membrane pass, G-protein linked surface receptors expressed on neutrophils and macrophages, where they perceive bacterial cell wall products and stimulate phagocyte function (38). The best characterized ligands are formylated peptides, which are
characterized as high affinity with an ED50 for fMLP in humans by the originally characterized FPR and the phagocytes to bacterial ligands. Subsequent signaling trifurcates to PI3K MAPK signaling pathways, calcium release, and GTPase activation which eventuate in 1) changes in actin dynamics and initiation of chemotaxis, 2) transcriptional upregulation of inflammatory effectors and cytokines, and 3) the activation of NADPH dependant oxidase enzymes and ROS generation (respiratory burst). Thus, the FPRs are a key PRR that controls the biological response of professional phagocytes to bacterial ligands.

The formylated peptide receptors are represented in humans by the originally characterized FPR and the closely related FPRL1 and FPRL2. FPR has been characterized as high affinity with an ED50 for fMLP in the nanomolar range, while the low affinity FPRL1/FPRL2 responds to the same agonist at micromolar ranges (32). Importantly, immunohistochemical staining has shown the formylated peptide receptors are expressed on the apical surface of the intestinal epithelia, prompting interest that this and related epithelial receptors may mediate physiological responses in the gut (3). Furthermore, we have shown that in epithelial cells, the MAPK ERK is activated by formylated peptides in a FPR dependant manner. The potential role of these receptors in transducing signals from the microbiota is a topic of current investigation.

**PHYSIOLOGICAL GENERATION OF REACTIVE OXYGEN SPECIES**

The rapid generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H2O2), hydroxyl radicals and a variety of their degradation products are a result of excitation or incomplete reduction of molecular oxygen. ROS are short lived reactive molecules and at high levels are considered potently microbicidal, necessary for the killing of engulfed organisms. ROS production in response to FPR stimulation is a cardinal feature of the cellular response of phagocytes to both pathogenic and symbiotic bacteria. Phagocytes generate ROS via a very well studied enzymatic apparatus. The neutrophil NADPH oxidase, Nox2 (formerly gp120phox), is a constitutively inactive multi-subunit complex comprised of a membrane bound dimer of p22phox and gp91phox (31). The in vivo role of this enzyme in host defense is vividly illustrated by the fact that the genetic absence of Nox2 function results in chronic granulomatous disease (CGD), a condition where phagocytes fail to induce ROS and patients are predisposed to recurrent pyogenic infections. Invertebrate phagocytes stimulated by formylated peptides generate ROS (MAMPs) in the same manner as mammalian neutrophils, and plants also utilize induced ROS in response to bacterial pathogens and symbionts, continuing the theme of conversion of basic machinery of microbial perception and effector pathways (29, 31, 47, 57, 61). Drosophila requires commensal microbe-induced hydrogen peroxide (H2O2) to maintain gut epithelial homeostasis (16, 17). However, in the case of the fly, the ROS generation occurs in the epithelia, and is necessary for control of the luminal flora. This latter observation suggests a conserved role for epithelial ROS (as opposed to strictly phagocyte) generation in gut homeostasis and microbial control. Additionally, it is now apparent that the ROS generating enzymes activated by FPRs in neutrophils (Nox2) have functional paralogous enzymatic complexes in non-phagocytic cells (31). Indeed, a family of NADPH oxidase enzymes, the Nox’s and Duox’s is seen in many non-phagocytic tissues, with two, Nox1 and Duox2, strongly expressed in the intestinal epithelia (the inducible ROS observed in Drosophila intestine is produced by the fly ortholog of Duox). In general, the non-phagocytic NADPH oxidases exhibit similar, but not identical organization to the phagocyte enzyme.

Recently, we have shown that several species of normal human gut bacteria can induce rapid, “deliberate” generation of ROS within epithelial cells (30). Furthermore, these cells immediately show increased oxidation of soluble redox sinks, such as glutathione and thioredoxin, and exhibit an increase in redox stimulated transcriptional activation, both reflecting a cellular reaction to increased ROS. Interestingly, different strains of commensal bacteria can elicit marked differences in ROS levels in contacted cells. We have found that the Lactobacilli are especially potent in ROS production in cultured cells and in vivo, though all bacterial tested have some ability to alter the redox environment of the cell. This is not surprising given that phagocytes can induce a respiratory burst regardless of whether they encounter nominal pathogens or stray commensals. As mentioned, Nox enzymes play a central role in ROS generation in phagocytes; whether the Nox’s or Duox’s are involved in the generation of ROS in mammalian epithelia or if this ROS also has microbiostatic functions is not known.

High ROS stimulating bacteria, such as Lactobacilli, may possess specific membrane components or even secreted factors that activate cellular ROS production.
For instance Yan reported soluble factors of *Lactobacilli* that mediated beneficial effects in *in vivo* inflammatory models (67). Alternatively, high ROS stimulating bacteria may simply possess enhanced adhesion or ability to penetrate mucin layers and gain more proximal access to cellular receptors such as TLRs and FPRs. As the FPRs are expressed on apical surfaces and are known to directly stimuli ROS production in phagocytes, these are interesting candidates for this function. Alternative possibilities include endogenous production of ROS from prokaryotic enzymes, though experiments showing ROS stimulation with non-viable and denatured bacterial components make this notion less likely. Additional sources of cellular ROS generation could include 5-lipoxygenase, xanthine oxidase and mitochondrial respiratory chain enzymes. Clearly, bacteria, unlike individual peptides and cytokines, are multifaceted biological stimuli and clearly would be expected to elicit a complex range of cellular receptors and influence diverse processes.

**ROS MEDIATED SIGNALING**

ROS also have functions beyond microbial killing. Controlled generation of ROS by activation of receptors for various hormones, cytokines and growth factors mediate critical roles in the modulation of signal transduction pathways seen in all multi-cellular life, plants and animals alike (29, 45, 47, 61, 62). The specificity of biological responses to altered levels of ROS can be modulated by the specific molecular species of ROS, the intensity/duration of the signal, the subcellular sites of production and the developmental stage of the cell (45, 62). ROS are short lived molecules and can have a very small functional radius of action, which contributes to the selectivity of action. Indeed certain receptors physically interact with a ROS generating Nox enzyme, presumably to limit ROS mediated influences to the immediate vicinity of effector proteins (28).

A major mechanism by which ROS are thought to exert their effects on signal transduction pathways is by their ability to reversibly oxidize cysteine residues in specific target proteins (5). Only a subset of proteins can be modified by this reaction as oxidation of cysteine requires this amino acid to be present in the thiolate anion form (Cys-S−), whereas most cysteines (pK_a ~ 8.5) are protonated (Cys-SH) at physiological pH. Only some cysteine residues exist as a thiolate anion at neutral pH as result of lowering of their pKa value by vicinal charged amino acids (52). Specific examples of such oxidant sensitive proteins include protein tyrosine phosphatases (PTPs), the lipid phosphatase (PTEN), MAP kinase phosphatases (MAPK-P or DUSPs), and low-molecular-weight protein tyrosine phosphatases (LMW-PTPs) (10, 23, 64). More recently examples of ROS mediated inactivation of enzymes have come from studies by Bossis and Melchoir (7) and from our own laboratory (30) with the sumoylation and the neddylation enzymes, respectively. Sumoylation and neddylation are the conjugation of ubiquitin-like proteins, Sumo or Nedd8, to target lysine residues of substrate proteins. The latter, Nedd8 plays a role the control of the key inflammatory transcription factor, NF-κB, discussed next.

**MICROBIAL EFFECTS ON INFLAMMATORY SIGNALING**

While it is obvious that the host must defend against threats posed by bacterial pathogens, the benefits conferred by the microbiota require that immune and inflammatory systems not eliminate them entirely. The epithelia can suppress TLR signaling or reduce TLR expression to moderate immunoinflammatory signaling (1, 55). Additionally, individual members of the microbiota are able to actively modulate signaling intensity (22, 26, 41). A variety of reports have described commensals -many employed as probiotics- are able to suppress eukaryotic inflammatory signaling pathways such as NF-κB and block inflammatory effector functions (34, 37, 48, 67). Several mechanisms have been described. The gut symbiont *Bacteroides thetaiotaomicron* has been elegantly shown to inhibit NF-κB pathways by regulating cytoplasmic to nuclear translocation of the p65 NF-κB subunit (25). Several laboratories have demonstrated that intestinal bacteria are able to influence inflammatory pathways, and very likely other cellular regulatory processes, by manipulating the ubiquitin system (22, 42, 49, 63). Ubiquitination is a covalent modification increasingly recognized to play a regulatory role in a wide spectrum of biochemical events, generally by targeting modified proteins for controlled degradation via the proteasome organelle. An example of a signaling component regulated by ubiquitination is the inhibitory component of the NF-κB pathway, IκB (24), and there are numerous examples of pathogens that utilize preformed effector proteins to influence IκB ubiquitination and thus innate immunity (2, 27, 53). Members of the microbiota interacting with epithelial cells *in vitro* are capable of blocking IκB ubiquitination and thus NF-κB activation by interference with the function of the IκB ubiquitination ligase, SCF/TrCP(Skp1, Cdc53/Cullin, F box receptor) (12, 33, 42). This enzymatic complex is activated by a second
covalent modification, neddylation, on the regulatory subunit of the complex, cullin-1. Neddylation is the covalent modification of the SCF ubiquitin ligases by the ubiquitin-like protein Nedd8. The event is emerging as a central regulatory event in cellular processes that are controlled by protein degradation, including NF-κB and β-catenin. Neddylation occurs by an enzymatic series analogous to the ubiquitination reaction, specifically catalyzed by a Nedd8 ligase called Ubc12. We have shown that contact of commensal bacteria with epithelia in vitro and in vivo resulted in the rapid and reversible loss of the Nedd8 modification, accounting for the loss of overall SCF ubiquitin ligase function and consequent blockade of NF-κB activation (12). Prompted by observations that other enzymes involved in modification of regulatory proteins by ubiquitin-like enzymes (the SUMOylation process) were controlled by transient oxidative inactivation, we investigated if the neddylation reaction was influenced by oxidative signaling. We demonstrated that both endogenous ROS (H₂O₂) and ROS generation by bacterial contact was able to transiently inactive the Nedd8 ligase, Ubc12 (30). These results demonstrated that commensal bacteria directly modulate a critical control point of the ubiquitin-proteasome system and is the first example of a eukaryotic signaling pathway influenced via bacterially stimulated ROS, and furthermore provides a detailed molecular mechanism for bacterial suppression of host inflammatory pathway. Nevertheless, it is highly unlikely that ROS mediated signaling is limited to a single pathway or class of enzyme, suggesting other signaling pathways could plausible be affected by microbially generated/stimulated ROS.

**MICROBIAL EFFECTS ON EPITHELIAL CELL FUNCTION, GROWTH AND SURVIVAL**

As mentioned, germ-free mice show defective epithelial proliferation and wound healing, indicating commensal enteric bacteria are able to stimulate epithelial cell migration post-injury and during

Fig. 1. Diagram of the NF-κB pathway. NF-κB is activated by sequential modifications of IκB; phosphorylation (by IKK), ubiquitination (by the SCF complex) and degradation (by the proteasome). Free NF-κB dimer can then translocate to the nucleus and activate transcription. The SCF ubiquitin ligase (cullin subunit) must be modified by the ubiquitin-like protein Nedd8 for activity, and the neddylation reaction is mediated by the oxidant sensitive ligase Ubc12. Intracellular ROS from bacterial contact transiently inactivates Ubc12 and thus blocks activity of downstream functions, including IκB ubiquitination/degradation and NF-κB mediated signaling.
development. How commensal bacteria affect this process is unclear. Epithelial cell migration depends on coordinated changes in actin cytoskeleton involving spatial and temporal changes in adhesion of the protruding membrane edge to the cell extracellular matrix at specialized signaling nidus points called focal adhesions (FA). FA assembly is regulated by focal adhesion kinase, a 125 kDa protein that is maintained in an inactive dephosphorylated form by the constitutive action of redox sensitive tyrosine phosphatases, LMW-PTPase and SHP-2 (39). Past reports have shown that endogenous physiological stimuli, such as growth factors and integrin engagement with the epithelial basement membrane induced local ROS production via activation of Nox1, resulting in rapid oxidative inactivation of these PTPase’s, and consequent phosphorylation of FAK and initiation of cellular motility (11). Accordingly, we have shown that interaction(s) of wounded intestinal epithelia with natural commensal bacterial strains is associated with rapid accumulation of ROS, especially at the leading edge of the migrating monolayer. Elicitation of ROS results in reversible oxidation of target low pKa cysteines in LMW-PTP and SHP-2, and thereby a consequent increase in phosphorylation of focal adhesion kinase (FAK). Concomitantly, commensal bacteria mediate an increase in number of FA at the migrating edge of the monolayer, and increased cell adhesion and velocity of epithelial migration. Functionally, commensal bacteria...
mediate enhanced wound closure in an in vitro model of injury and enhanced resolution of dextran sodium sulfate-induced mucosal damage in a mouse model. Thus ROS production associated with commensal-epithelial contact can stimulate epithelial motility and likely contribute to wound restitution. This data suggests another means for how the microbiota mediates homeostatic effects on the gut lining and a mechanism for certain beneficial effects of probiotics.

PROSPECTS

We have shown that epithelia exhibit increased ROS generation in response to commensal bacteria, in a manner similar to the events induced in phagocytic cells, suggesting a deep functional conservation. Indeed, recent data in invertebrates suggest that ROS generation for signaling and microbiocidal functions in the gut epithelia may represent the ancestral form of response to bacteria (16). We have shown ROS generated in epithelial in response to bacteria serves a signaling function (as in many non epithelial cells), and likely there are numerous ROS sensitive enzymes that could be influenced by changes in cellular redox status. As mentioned, reversible oxidative inactivation of a wide range of regulatory enzymes is an increasingly recognized mechanism of signal transduction (10, 62). For example, the DUSPs are redox sensitive PTPases that serve as negative regulators of various MAPKinases. Plausibly, the myriad functions of the MAPKinase pathways may be regulated by microbial induced redox events. Current proteomic approaches that exploit reactive cysteines to label individual peptides may be employed as a high throughput system to screen for oxidant sensitive regulatory proteins (58). Alternatively (but not contradictory), an epithelial antimicrobial function (as in phagocytes and the Drosophila gut) of bacterial elicited ROS, especially in limited locations such as the intestinal crypt are also likely, and are questions to be resolved.

The source of ROS is an intriguing topic. Clearly the Nox enzymes, especially Nox1 and Duox2 are prime candidates given their pattern of tissue expression, but other sources such as mitochondria respiration chain enzymes, lipoxigenases and others could contribute to redox control in the cell. FPRs are attractive candidates for receptor stimulated ROS production, given that many of the same mechanisms that mediate FPR signaling in professional phagocytes are conserved in epithelial cells. Additionally, it is also unclear whether certain commensals could generate ROS by their own enzymatic machinery and influence eukaryotic signaling by exogenous ROS (conversely, some bacteria could achieve this result by producing anti-oxidants).

ROS mediated signaling may occur during rapid quantitative changes in microbial populations or qualitative changes in the composition in the gut, during development, or with probiotic therapy. The observation that different taxa of bacteria exhibit markedly different potencies in the ability to elicit/provide ROS supports the idea that qualitative changes in community composition can affect host biology. This notion may be relevant to the development and optimization of probiotics, and may explain a parameter that defines a healthy vs “dysbiotic” microbiota. Long term biochemical accommodation to tonic bacterial presence, as in the colon, may affect different aspects of redox biology.

In conclusion, cellular ROS by microbe-epithelial contact is a conserved processes with many known, expected and plausible consequences, making this mechanism attractive as a general and non-species selective means by which a complex floral community could influence a wide range of host signaling and homeostatic processes (33). It is hoped that a fuller understanding of this mechanism may advance our understanding of the natural microbiota and exploitation of probiotic organisms.

REFERENCES

(1) Abreu MT, Fukata M, Arditi M. 2005. TLR signaling in the gut in health and disease. J Immunol 174: 4453–4460.
(2) Angot A, Vergunst A, Genin S, Peeters N. 2007. Exploitation of eukaryotic ubiquitin signaling pathways by effectors translocated by bacterial type III and type IV secretion systems. PLoS Pathog 3: e3.
(3) Babbin BA, Jsaatis AJ, Ivanov AI, Kelly D, Laukoetter M, Nava P, Parkos CA, Nusrat A. 2007. Formyl peptide receptor-1 activation enhances intestinal epithelial cell restitution through phosphatidylinositol 3-kinase-dependent activation of Rac1 and Cdc42. J Immunol 179: 8112–8121.
(4) Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. Science 307: 1915–1920.
(5) Barford D. 2004. The role of cysteine residues as redox-sensitive regulatory switches. Curr Opin Struct Biol 14: 679–686.
(6) Ben-Neriah Y, Schmidt-Supprian M. 2007. Epithelial NF-kappaB maintains host gut microflora homeostasis. Nat Immunol 8: 479–481.
(7) Bossis G, Melchior F. 2006. Regulation of SUMOylation by reversible oxidation of SUMO conjugating enzymes. Mol Cell 21: 349–357.
(8) Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL,
Osterman AL, DiDonato JA, Feinstein E, Gudkov AV. 2008. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science 320: 226–230.

(9) Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF, Karin M. 2003. The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. Nat Med 9: 575–581.

(10) Chiarugi P, Buricchi F. 2007. Protein tyrosine phosphorylation andversible oxidation: two cross-talking posttranslation modifications. Antioxid Redox Signal 9: 1–24.

(11) Chiarugi P, Pani G, Giannoni E, Taddei L, Colavitti R, Raugei G, Symons M, Borrello S, Galeotti T, Ramponi G. 2003. Reactive oxygen species as essential mediators of cell adhesion: the oxidative inhibition of a FAK tyrosine phosphatase is required for cell adhesion. J Cell Biol 161: 933–944.

(12) Collier-Hyams LS, Sloane V, Batten BC, Neish AS. 2005. Cutting Edge: Bacterial modulation of epithelial signaling via changes in neddylation of cullin-1. J Immunol 175: 4194–4198.

(13) Dethlefsen L, McFall-Ngai M, Relman DA. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature 449: 811–818.

(14) Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. 2005. Diversity of the human intestinal microbial flora. Science 308: 1635–1638.

(15) Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JJ, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. Science 312: 1355–1359.

(16) Ha E-M, Oh C-T, Bae YS, Lee W-J. 2005. A direct role for dual oxidase in drosophila gut immunity. Science 310: 847–850.

(17) Ha E-M, Oh C-T, Ryu J-H, Bae Y-S, Kang S-W, Jang I-h, Brey PT, Lee W-J. 2005. An antioxidant system required for host protection against gut infection in drosophila. Developmental Cell 8: 125–132.

(18) Hooper LV, Gordon JJ. 2001. Commensal Host-Bacterial Relationships in the Gut. Science 292: 1115–1118.

(19) Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JJ. 2001. Molecular analysis of commensal host-microbial relationships in the intestine. Science 291: 881–884.

(20) Hord NG. 2008. Eukaryotic-microbiota crosstalk: potential mechanisms for health benefits of prebiotics and probiotics. Annu Rev Nutr 28: 1–17.

(21) Ismail AS, Hooper LV. 2005. Epithelial cells and their neighbors. IV. Bacterial contributions to intestinal epithelial barrier integrity. Am J Physiol Gastrointest Liver Physiol 289: G779–G784.

(22) Iyer C, Kosters A, Sethi G, Kumnumakkara AB, Aggarwal BB, Versalovic J. 2008. Probiotic Lactobacillus reuteri promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-kB and MAPK signalling. Cellular Microbiology 10:1442–1452.

(23) Kamata H, Honda S, Maeda S, Chang L, Hira H, Karin M. 2005. Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120: 649–661.

(24) Karin M, Ben-Neriah Y. 2000. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol 18: 621–663.

(25) Kelly D, Campbell JJ, King TP, Grant G, Jansson EA, Coutts AG, Pettersson S, Conway S. 2004. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. Nat Immunol 5: 104–112.

(26) Kelly D, Conway S, Aminov R. 2005. Commensal gut bacteria: mechanisms of immune modulation. Trends Immunol 26: 326–333.

(27) Kim DW, Lenzen G, Page AL, Legrain P, Sansonetti PJ, Parsot C. 2005. The Shigella flexneri effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. Proc Natl Acad Sci U SA 102: 14046–14051.

(28) Kim Y-S, Morgan MJ, Choksi S, Liu Z. 2007. TNF- induced activation of the Nox I NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 26: 675–687.

(29) Kotchoni SO, Gachomo EW. 2006. The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. J Biosci 31: 389–404.

(30) Kumar A, Wu H, Collier-Hyams LS, Hansen JM, Li T, Yamouh K, Pan ZQ, Jones DP, Neish AS. 2007. Commensal bacteria modulate cullin-dependent signaling via generation of reactive oxygen species. Embo J 26: 4457–4466.

(31) Lambeth JD. 2004. NOX enzymes and the biology of reactive oxygen. Nat Rev Immunol 4: 181–189.

(32) Le Y, Murphy PM, Wang JM. 2002. Formyl-peptide receptors revisited. Trends Immunol 23: 541–548.

(33) Lee WJ. 2008. Bacterial-modulated signaling pathways in gut homeostasis. Sci Signal 1: pe24.

(34) Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. 1999. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 116: 1107–1114.

(35) Madsen K, Cornish A, Soper P, McKainney C, Jijon H, Yachimiec C, Doyle J, Jewell L, De Simone C. 2001. Probiotic bacteria enhance murine and human
intestinal epithelial barrier function. Gastroenterology 121: 580–591.

(36) Marchesi J, Shanahan F. 2007. The normal intestinal microbiota. Curr Opin Infect Dis 20: 508–513.

(37) Menard S, Candah C, Bambou JC, Terpend K, Cerf-Bensussan N, Heyman M. 2004. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut 53: 821–828.

(38) Migeotte I, Communi D, Parmentier M. 2006. Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. Cytokine Growth Factor Rev 17: 501–519.

(39) Mitra S, Hanson D, Schlaepfer D. 2005. Focal adhesion kinase: In command and control of cell motility. Nat Rev Mol Cell Biol 6: 56–68.

(40) Neish AS. 2003. Microbes in gastrointestinal health and disease. Gastroenterology 136: 65–80.

(41) Neish AS. 2009. Microbes in gastrointestinal health and disease. Curr Opin Infect Dis 20: 508–513.

(42) Neish AS. 2004. Does the microbiota regulate immune responses outside the gut? Trends Microbiol 12: 562–568.

(43) Ogier-Denis E, Mkaddem SB, Vandewalle A. 2008. NOX enzymes and toll-like receptor signaling. Semin Immunopathol 30: 291–300.

(44) Park J, Floch MH. 2007. Prebiotics, probiotics, and dietary fiber in gastrointestinal disease. Gastroenterol Clin North Am 36: 47–63.

(45) Pauly N, Puciarriello C, Mandon K, Innocenti G, Jamet A, Baudouin E, Herouart D, Frendo P, Puppo A. 2006. Reactive oxygen and nitrogen species and glutathione: key players in the legume-Rhizobium symbiosis. J Exp Bot 57: 1769–1776.

(46) Petrof EO, Kojima K, Ropeleski MJ, Musch MW, Tao Y, De Simone C, Chang EB. 2004. Probiotics inhibit nuclear factor-κappaB and induce heat shock proteins in colonic epithelial cells through proteasome inhibition. Gastroenterology 127: 1474–1487.

(47) Pull SL, Doherty JM, Mills JC, Gordon JI, Stappenbeck TS. 2005. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. Proc Natl Acad Sci USA 102: 99–104.

(48) Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 118: 229–241.

(49) Rhee SG, Kang SW, Jeong W, Chang TS, Yang KS, Woo HA. 2005. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. Curr Opin Cell Biol 17: 183–189.

(50) Wills-Karp M, Santeliz J, Karp CL. 2001. The
germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol 1: 69–75.

(66) Xu J, Mahowald MA, Ley RE, Lozupone CA, Hamady M, Martens EC, Henrissat B, Coutinho PM, Minx P, Latreille P, Cordum H, Van Brunt A, Kim K, Fulton RS, Fulton LA, Clifton SW, Wilson RK, Knight RD, Gordon JL. 2007. Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol 5: e156.

(67) Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. 2007. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 132: 562–575.

(68) Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, Du Y, Yost EA, Gruber AD, May MJ, Greten FR, Eckmann L, Karin M, Artis D. 2007. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. Nature 446: 552–556.

(69) Zeng H, Carlson AQ, Guo Y, Yu Y, Collier-Hyams LS, Madara JL, Gewirtz AT, Neish AS. 2003. Flagellin is the major proinflammatory determinant of enteropathogenic Salmonella. J Immunol 171: 3668–3674.