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MEDIATORS AND OUTCOMES OF BACTERIA-EPITHELIAL CELL INTERACTIONS

SIMON R. CARDING

School of Biochemistry and Microbiology, The University of Leeds, Leeds, England

SUMMARY

Intestinal mucosal surfaces are in continuous contact with heterogeneous populations of commensal microorganisms, which collectively make up the intestinal microbiota. Historically, the barrier function of intestinal epithelial cells (IEC) has been considered to be important in preventing or limiting the interaction of non-invasive bacteria from making contact with, and activating the mucosal immune system, and therefore maintaining immune (oral) tolerance to commensal bacteria. More recent investigations suggests that IEC may also play a role in sensing the external environment and communicating this information to the local immune system to affect appropriate responses. In this review the basis of IEC recognition of microorganisms and how disruption or breakdown in the interaction between IEC and the commensal microbiota are thought to underlie the development of chronic intestinal inflammation are discussed. In particular, the function of the cell surface pattern recognition receptors (PRR), Toll-like receptors, and the cytosolic nucleotide binding site plus leucine-rich repeat protein, NOD2, in IEC and the outcome of the triggering of these PRRs for IEC function are discussed.

INTRODUCTION

A single layer of epithelial cells is all that separates the host from the external environment. These cells are constantly interacting with a vast number of bacteria that are resident in the colon and distal small intestine and whose products such as endotoxin would in other sites of the body cause inflammation and tissue injury. Yet in the intestinal tract there is usually no evidence of any inflammation and instead commensal bacteria exist in a mutually beneficial or symbiotic relationship with the host. Although the nature of this relationship and the mechanisms by which it is regulated are poorly understood, its importance is evident from numerous studies demonstrating that dysregulation of this interaction is associated with chronic inflammation of the type seen in patients with inflammatory bowel disease (IBD). An important question therefore is how are pro-inflammatory responses to members of the commensal microbiota avoided while maintaining the capacity to orchestrate vigorous defence to any microorganisms that pose a threat to the host, and what role do IEC play in sensing and discriminating between innocuous versus dangerous bacteria in the gut?

INTESTINAL EPITHELIAL CELLS ARE MORE THAN A PHYSICAL BARRIER

The intestinal epithelium forms a highly impermeable barrier to most enteric antigens including microorganisms (*Didierlauerent*, 2002). Barrier function can broadly be described as being either physical, biochemical or immunological in nature all of which are to some extent dependent upon commensal bacteria for their establishment and/or efficient operation. These barriers must be breached by pathogenic microorganisms in order to invade and cross the epithelium.

The integrity of the physical intestinal barrier depends on specialised structures involved in cell-cell contacts known as tight junctions and adherens junction. The tight junction located at the apical region of epithelial lateral membranes provide a barrier that is selectively permeable to certain hydrophilic molecules, ions and nutrients, whereas the adherens junctions mediates strong cell-to-cell adhesions between adjacent IEC (*Cereijido* et al., 1998; *Liu* et al., 2000; *Mitic* and *Anderson*, 1998; *Moncrief* et al., 1995; *Obsio Jr*. et al., 1997; *Wu* et al., 1998). Tight junctional complexes are targets of pathogens such as *Bacteroides fragilis*, *Clostridia* sp. and enteropathogenic *Escherichia coli* (*Berkes* et al., 2002). Conversely, certain strains of probiotic lactobacilli have been shown to enhance epithelial barrier function (*Isolauri* et al., 1993; *Mao*, 1996) and to attenuate epithelial invasion by pathogenic bacteria (*Madsen* et al., 2003; *Resta-Lenert* and *Barret*, 2003). Compromised or leaky junctional complexes that facilitate translocation of commensal bacteria have been associated with the pathogenesis of IBD (*Gassler* et al., 2001). The major biochemical barrier of the intestinal epithelial layer is the mucus layer that overlies it that comprises mucin glycoproteins and members of the trefoil factor family. In addition, antimicrobial peptides (AMP) produced by Paneth cells in the crypts of the small intestine also contribute to the biochemical and anti-microbial defences of the epithelium. Induction of expression of the potent AMP, Angiogenin-4, by Paneth cells has been shown to be under the influence of commensal bacteria which together with the species specificity of Angiogenin-4 anti-microbial activity (*Hooper* et al., 2003) suggests that bacterial interactions with Paneth cells help shape the composition of the commensal microbiota. The major immunological defence mechanism of the intestinal epithelium is IgA, which is transported from the lamina propria and secreted into the lumen by IEC where it acts to prevent commensal bacteria breaching the mucosal barrier. IgA is not, however, exclusively produced in response to pathogenic microorganisms. In a seminal study *Schroff* and colleagues (1995) showed that upon colonisation of the gut commensal bacteria elicit adaptive an immune response which by virtue of coating bacteria in the intestinal lumen with IgA and preventing further translocation and stimulation of gut lymphoid tissue are naturally self-limiting. The generality of this phenomena is however uncertain since not all commensal bacteria are equally effective at inducing "protective" IgA (*Cebra*, 1999; *Moreau* et al., 1978) which may be related to differences in expression of pathogenicity factors such as molecules that mediate adhesion and invasion or that are endotoxic. How and where IgA production is initiated in the gut associated lymphoid tissues (GALT) has recently been established. Intestinal dendritic cells (DCs) have been shown to transport live commensal bacteria from the gut lumen to the intestinal mesenteric lymph nodes where IgA^+ lymphoblasts are activated enabling them to migrate to the lamina propria where they become

plasma cells (*Macpherson* and *Uhr*, 2004). IEC are thought to contribute to this process by producing cytokines in response to commensal bacteria that recruit DCs to the gut mucosa, acting therefore as a link between the innate and adaptive immune systems. How then is IEC recognition of enteric microorganisms mediated?

IEC RECOGNITION OF MICROORGANISMS

Host cells use distinct receptors (designated pattern-recognition receptors; PRR) for recognition of highly conserved structures of microorganisms, which have been called pathogen-associated molecular patterns (PAMPS) (*Medzhitov* and *Janeway*, 2002). However, considering that both pathogenic and non-pathogenic microbes can express the same molecular patterns these molecules are better described as microbe-associated molecular patterns (MAMPS) (*Didierlauerent*, 2002). Based upon their sub-cellular distribution, mammalian PRR can be divided into two types, the cell surface Toll-like receptor family (TLR) which recognise various PRRs of bacteria (lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PGN), flagellin, methylated DNA), viruses (single and double stranded RNA) and yeast (zymosan), and the cytoplasmic nucleotide-binding oligomerisation domain (NOD) family of proteins which recognise components of PGN (*Inohara* et al., 2002; *Medzhitov*, 2001). IEC expression of functional TLRs and NOD proteins is contentious and by no means clear.

TLR FUNCTION IN IEC

Most studies investigating PRR expression by IEC have relied on established intestinal epithelial cell lines which have provided evidence for both the presence and absence of TLRs (*Abreu* et al., 2002; *Bocker* et al., 2003; *Cario* and *Podolsky*, 2000; *Cario* et al., 2000; *Melmed* et al., 2003). Demonstrating that any TLRs expressed by IEC are functional is critical. Although some IEC lines have been shown to express TLR mRNA production of TLR protein and the co-factors required for recognition of certain MAMPs have been shown to be negligible rendering them functionally unresponsive (*Abreu* et al., 2001; *Funda* et al., 2001). The inability to detect TLR expression by IEC *in vivo* has led to the notion that IEC are normally unresponsive or ignorant of commensal bacteria (*Cario* and *Podolsky*, 2000). The cellular localisation of any TLR expression by IEC may also be significant in terms of discriminating between commensal and pathogenic microbes. Expression of TLR5, which recognises bacterial flagellin, in the intestinal epithelium has been shown to be restricted to the basolateral surface of IEC (*Gewirtz*, 2001) and may therefore be effectively hidden from all enteric microbes except those (pathogenic strains) able to subvert the barrier function of IEC. Not all studies of TLR5 expression by IEC however, agree with this finding (*Sierro*, 2001). The finding that expression of TLR4 and the co-receptors (MD-2 and CD14) required for recognition of LPS in crypt epithelial cells are primarily intracellular within the Golgi apparatus (*Hornef* et al., 2003), is thought to enable IEC to selectively respond to internalised MAMPs from invasive bacteria while remaining tolerant

of the excessive amounts of MAMPs and LPS present in the lumen. Conflicting evidence suggest that TLR4 trafficking occurs across the epithelium implying that IECs have the capability of sensing and sampling luminal bacterial antigens using receptors localised to one surface polarised IECs (*Cario* et al., 2002). The variability in TLR expression and responsiveness seen among IEC lines may at least in part be explained by observations in mouse models of spontaneous or induced intestinal inflammation that IEC responses to MAMPs vary according to their anatomic location along the intestine and on whether they are in the lower crypt or villous tip (*Ortega-Cava* et al., 2003; *Suzuki* et al., 2003).

There is also uncertainty regarding the consequences of TLR-MAMP interactions and the downstream signalling pathways utilised in IEC. The intracellular signalling pathways downstream of these PRR have principally been mapped using haematopoietic cell lines and ultimately result in nuclear factor-kappa B (NF-κB, p65) activation leading to the production of antimicrobial factors, cytokines and chemokines (*Aderem*, 2001; *Akira* et al., 2001; *Irie* et al., 2000). Two divergent pathways have been identified (*Akira*, 2003). In the first, stimulation of PRR by MAMP leads to the recruitment of the adaptor molecule, myeloid differentiation factor 88 (MyD88) that binds to the conserved Toll/Interleukin-1 receptor (TIR) domain (*Means* et al., 2000) enabling phosphorylation of serine kinase IL1-receptor-associated kinase (IRAK), which in turn leads to recruitment and activation of TNF-associated factor 6 (TRAF-6). The IRAK-TRAF6 complex then interacts with another complex containing TGFß-activated kinases leading to the activation of IKK and finally NF-κB activation. Alternative or MyD88-independent pathways activate caspase-1 (IL-1ß converting enzyme; ICE) that converts the inactive form of pro-inflammatory cytokines, IL-1β and IL-18, into the secreted and active form (*Akira* et al., 2001; *Seki* et al., 2001). Other MyD88-independent pathways involve the induction of IFN-inducible genes such as the CXC chemokine IP-10 (*Akira* et al., 2001; *Irie* et al., 2000; *Kawai* et al., 2001). Several inhibitory molecules have recently been identified that interfere with TLR signalling at various points in the signalling cascade. One of these, Toll-interacting protein (TOLLIP) which binds to MyD88 and suppresses IRAK phosphorylation (*Miyake*, 2004), has been shown to be expressed at high levels in IEC lines after exposure to LPS (*Otte* et al., 2004) resulting in hypo-responsiveness to not only TLR4 ligands but also TLR2 ligands. TLR homologues lacking signalling domains have also been identified in antigen presenting cells and dendritic cells (*Divanovic* et al., 2005). A Probiotic strain of *Bacteroides thetaiotaomicron* has been shown to induce expression of the peroxisome-proliferator-activated receptor-γ (PPAR-γ) in IEC lines, which is a negative regulator of NF-κB activation (*Kelly* et al., 2004) accounting perhaps for the bacterium's anti-inflammatory role.

To attempt to redress the conflicting data concerning TLR expression by IEC our own studies have focused on examining the functionality of TLRs expressed by primary IEC *in vivo* and *in vitro* using a novel IEC culture system capable of supporting populations of murine colonic epithelial cells (CEC) that maintain the properties and characteristics of their *in vivo* counterparts during prolonged culture (*Baumgart* et al., 1998; *Telega* et al., 2000). CEC from wild type adult mice analysed directly *ex vivo* express detectable levels of both TLR2 and TLR4 (*Singh* et al., 2005). In response to LPS, LTA, PGN or the

synthetic TLR2 ligand, Pam3Cys, CEC proliferated *in vitro* increasing in number by more than twofold compared to cells cultured in media alone (*Singh* et al., 2005). In addition, MAMPs and in particular LPS, up-regulated production of the cytokines IL6 and MCP-1 by primary CEC (*Singh* et al., 2005) both of which in addition to being involved in activation of the inflammatory response (*Conti* and *DiGioacchino*, 2001; *Hibi* et al., 1996; *Mukaida* et al., 1998; *Shephard*, 2002) also contribute to epithelial growth and homeostasis (*Grossmann* et al., 1989; *Naka* et al., 2002; *Yoshizaki* et al., 1990) and epithelial restitution (*Low* et al., 2001). These findings are consistent with the recently proposed role for TLR-mediated response in IEC homeostasis (*Rakoff-Nahoum* et al., 2004) with the balance between promoting inflammation and epithelial homeostasis perhaps being determined by the strength and duration of the MAMP stimulus.

IEC CAN DISTINGUISH DIFFERENT COMMENSAL BACTERIA

A CEC:bacteria co-culture system was used to compare the ability of different representative colonic commensal bacteria to modulate the production of different cytokines (n=15) by primary CEC. Exposure of primary CEC to *Bacteroides ovatus*, *E. coli* (SLF) or *Lactobacillus rhamnosus* all of which were human or rodent colonic isolates induced or up-regulated different patterns of cytokine production and secretion (*Lan* et al., 2005). *E. coli* selectively induced production of MIP-1α and MIP-1β and defensin 3 whereas *B. ovatus* and *L. rhamnosus* exclusively induced MCP-1 and MIP-2α expression, respectively. Other cytokines (TNFα, RANTES and MEC) were induced or up-regulated in response to some but not all three of the bacteria whereas others (IP-10, ENA78) were up-regulated in response to all bacteria. Evidence of bacterial interference and suppression of CEC cytokine production was also obtained from mixed bacterial:CEC co-cultures. Probiotic *L. rhamnosus* suppressed *E. coli*- and *B. ovatus*-induced production of pro-inflammatory cytokines, identifying CEC as a potential cellular target for probiotoic bacteria *in vivo*. Although the ability of probiotic bacteria to down modulate pro-inflammatory cytokine (TNFα and IL-6) production has been demonstrated previously (*Borruel* et al., 2002; *Schultz* et al., 2003), the identity of the cells effected by the bacteria and the cellular source(s) of the cytokines was not established in these studies. Although the mechanism(s) of action of probiotics remains unclear (*Ghosh* et al., 2004) our findings suggest a mechanism by which non-pathogenic or probitoic bacteria might suppress or limit the ability of other "pathogenic" bacteria to promote or sustain inflammatory responses. How *L. rhamnosus* interferes with the ability of *E. coli* or *B. ovatus* to induce cytokine production by CEC is not yet known but may include the production of AMPs (e.g. bacteriocins), expression of MAMPs of higher density and affinity by *L. rhamnosus*, and the induction of TLR antagonistic signalling pathways or molecules. The CEC:bacteria co-culture system we have developed should be of value in investigating these potential mechanisms in more detail.

IEC TLR RESPONSIVENESS CHANGES DURING THE DEVELOPMENT OF CHRONIC INFLAMMATION

Since the regulation of TLR expression in the intestinal epithelium may change during the development of chronic inflammation in patients with IBD (*Cario* and *Podolsky*, 2000), we examined the responsiveness of primary CEC in a murine model of ulcerative colitis during the development of chronic inflammation. Before or coincident with disease onset, CEC from Interleukin-2-deficient ($IL2^{-/-}$) mice demonstrated a switch in TLR responsiveness from being TLR4 dominant in healthy wild type mice to decreased TLR4 responsiveness and increased TLR2 responses resulting in exacerbated IL6 and MCP-1 secretion in $IL2^{-/-}$ mice (*Singh* et al., 2005). Changes in TLR responsiveness or underlying abnormalities in regulation of TLR-mediated responses appear therefore be associated with the development of chronic inflammation and presumably reflect differences or changes in the interaction of CEC with commensal bacteria in the healthy versus inflamed intestine.

NOVEL TLR SIGNALLING PATHWAYS IN IEC

Based upon the analysis of haematopoietic cells and immortalised epithelial cell lines the downstream signalling pathways from TLRs have been shown to ultimately result in MyD88-dependent NF-κB (p65) activation (*Akira*, 2003). In primary IEC, however, MyD88-independent pathways are primarily utilised for TLR signalling (*Singh* et al., 2005). Following stimulation of CEC with MAMPs several MAPK family members are activated in association with transient caspase-1 activation and predominance of the inhibitory, p50, subunit of NF-κB (*Singh* et al., 2005). This is consistent with the divergence of TLR-mediated intracellular signalling pathways (*Kawai* et al., 2001; *Muzio* et al., 1998) that may be selectively used by different cell types (haematopoietic versus epithelial). The differences in TLR-signalling pathways used by primary versus established IEC may be explained by the developmental control of TLR expression and function. Analysis of NF-κB activation in the colonic mucosa has shown that expression of the p65 subunit is restricted to rapidly dividing cells within the crypts and that the inhibitory p50 subunit predominates in mature enterocytes (*Inan* et al., 2000). MAMP responsiveness by IEC may therefore, be restricted to specific developmental or maturational stages, with less differentiated cells being less or hyporesponsive while fully differentiated cells can respond to MAMPs (*Cario* et al., 2002). This could be mediated by the action of specific negative regulators of TLR signalling such as PPAR-γ (*Kelly* et al., 2004), single immunoglobulin-IL-1 receptor related molecule (*Wald* et al., 2003) or of TLR cofactors (*Akashi* et al., 2000; *Shimazu* et al., 1999) or functionless TLR homologues (*Divanovic* et al., 2005) the expression of which would be developmentally regulated.

The importance of regulating TLR-mediated signalling is demonstrated by the sustained expression and lack of down-regulation of caspase-1 activity in primary CEC during the development of intestinal inflammation (*Singh* et al., 2005). The failure or breakdown in the regulation of caspase-1 activity in CEC may therefore play a role in the development or maintenance of intestinal inflammation. The observation that caspase-$1^{-/-}$ animals are resistant to

chemically-induced colitis (*Siegmund* et al., 2001b) and that anti-IL-18 antibodies ameliorate colitis in a number of murine models of IBD (*Kanai* et al., 2001; *Siegmund* et al., 2001a; *ten Hove* et al., 2001; *Wirtz* et al., 2002) are consistent with this interpretation. Of note, up-regulation of IL-18 expression has been demonstrated in the inflamed lesions of the intestine of IBD patients localised primarily to macrophages and epithelial cells, and caspase-1 has been identified as a potential therapeutic target for IBD (reviewed in *Siegmund*, 2002).

NOD2 FUNCTION IN IEC

Both NOD1/CARD4 and NOD2/CARD15 are members of the caterpillar gene family that encodes proteins with a variable but limited number of N-terminal domains, followed by a nucleotide-binding domain (NBD) and leucine-rich repeats (LRR) that are highly conserved in plants and vertebrates (*Ting* and *Davis*, 2005). NOD1 and NOD2 are capable of detecting bacterial peptides of PGN with NOD1 detecting muropeptide found mainly in Gram-negative bacteria, whereas NOD2 detects muramyl dipeptide, which is present on all bacteria containing PGN (*Inohara* and *Nunez*, 2003; *Inohara*, 2002). The interaction of these peptides with the LRR domain of NOD proteins leads to oligomerisation and recruitment of the adapter protein RICK (RIP-like CARD containing domain) which in haematopoietic cells results in NF-κB activation (*Kobayashi* et al., 2002; *Ogura* et al., 2001). NOD2 expression is restricted to the cytoplasm of monocytes, epithelial cells and Paneth cells in the small intestine (*Lala*, 2003). Although there is strong data linking NOD2 gene polymorphisms and mutations in the LRR domain of NOD2 with Crohn's disease that results in defective signalling in response to MAMPs (*Bonen*, 2003), the precise mechanisms of action of NOD2 is uncertain. Three different models of NOD2 function have been proposed based upon studies carried on transgenic mice with targeted mutations or deletion of the NOD2 gene (Figure 1). In the first model, based upon the analysis of spleen cells from mice deficient in exon 1 of the NOD2 gene preventing the NOD2 protein from being expressed, *Wantanabe* and colleagues (2004) propose that the interaction of NOD2 with MDP normally limits TLR2 signalling activated by PGN at the cell surface. This affects activation of the NF-κB subunit c-Rel, leading to an increase in the production of IL-12. Mutant NOD2 in Crohn's disease is unable to sense MDP and thus removes a constraint on the TLR2 pathway thereby invoking inflammation. In the second model based upon the outcome of infection of mice in which exon 3 had been deleted with the enteric pathogen *Listeria monocytogenes, Kobayashi* and co-workers (2005) propose that NOD2 plays an important role in the regulation of α-defensins made by Paneth cells enabling protection against pathogenic bacteria. Mutations in NOD2 cause a decrease and therefore loss of the α-defensins and loss of protection from invading pathogenic bacteria. Interestingly, in contrast to the Wantanabe study, analyses of bone marrow-derived macrophage (BMDM) responses in mice generated by Kobayashi failed to detect any increase in the production of pro-inflammatory cytokines, including IL-12, in response to various TLR ligands, such as PGN. The third model based upon the analysis of BMDM from mice expressing a mutated form of NOD2 protein (NOD^{2939iC}) homologous to the major mutation in human Crohn's disease resulting in a truncated NOD2 protein, led *Maeda* and col-

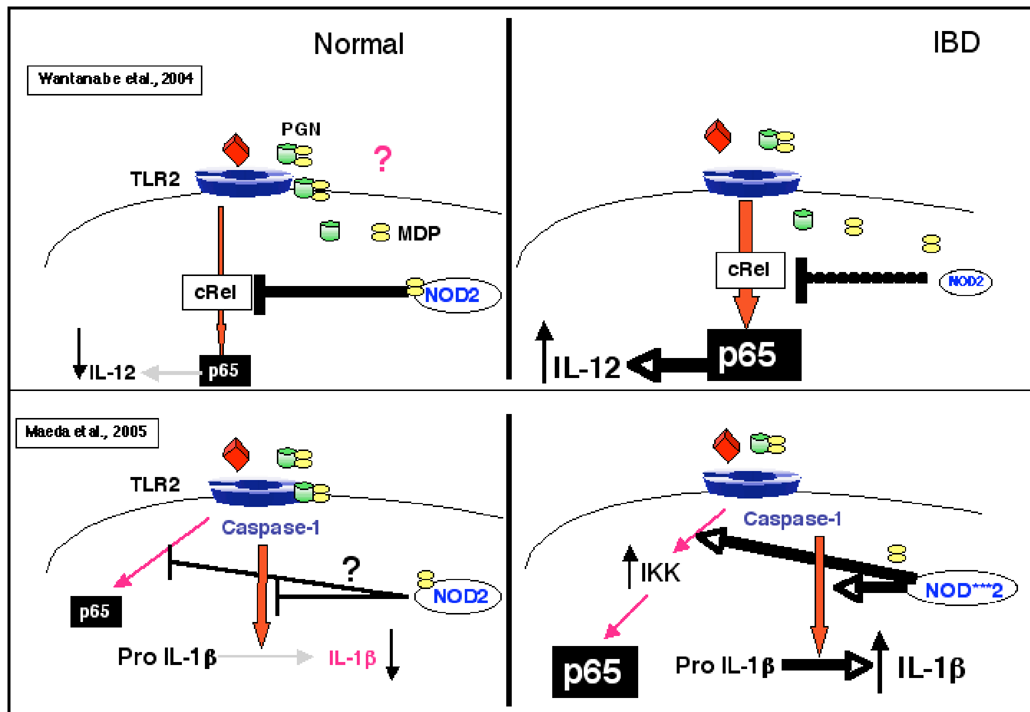

Figure 1: Models of NOD2 function based upon studies of haematopoietic cells. In the model proposed by *Wantanabe* and co-workers (2004) NOD2 activation by MDP reduces or interferes with TLR2-mediated IL-12 production by interfering or blocking activation of the c-Rel subunit of NF-κB. The mutated forms of NOD2 in Crohn's diseases patients are unable to provide this restraining effect and IL-12 production proceeds unabated leading to inflammation and disease. The model proposed by *Maeda* and colleagues (2005) is based on a gain-of-function of mutated forms of NOD2 that acquire the ability to activate IKK and caspase-1 which acts on the precursor form of IL-1β (and IL-18) enabling the secretion of biologically active cytokine leading to inflammation.

leagues (2005) to propose that the mutation in NOD2 results in a gain-of-function. Specifically, the N-terminal CARD domains of the mutated protein are now capable of activating caspase-1, which acts on the precursor form of IL-1β which is then secreted and can drive the inflammatory response in Crohn's disease patients.

Whilst all three models clearly identify a link between NOD2 activation and pro-inflammatory cytokine production there is no consensus as to how NOD2 influences cytokine production and whether NOD2 activation up- or down-regulates cytokine production. The reasons for these conflicting results are not clear but must be related to differences in the mutations introduced into the NOD2 gene (deletion of different exons or knock-in of a frame shift mutated gene) and the affect they now have on proteins and signalling complexes that NOD2 interacts with or is a part of. Importantly, none of these studies have examined the function of NOD2 in IEC, which is surprising since they are the first point of contact with enteric microbes, the outcome of which is of importance in determining the nature of the host response. Our own studies therefore, have focused on examining the function of NOD2 in primary CEC and have provided evidence for an anti-in-

flammatory role for this cytoplasmic PRR that may help explain the pathogenesis of chronic intestinal inflammation.

ROLE OF NOD2 IN PRIMARY CEC

The conditions under which NOD2 is expressed by primary CEC and the affects its activation has on their immune function were examined first. In a series of yet to be published studies NOD2 is expressed at low levels by CEC *in vivo* with expression localised to crypt regions and villous tips in normal healthy adult mice. This expression was shown to be dependent on the presence of commensal bacteria since it was not possible to detect any expression in the colonic mucosa of germ-free animals. Expression was dramatically and transiently up-regulated both *in vivo* after peroral infection with the bacterium, *L. monocytogenes* and the protozoan parasite, *Toxoplasma gondii* and *in vitro* upon exposure to pro-inflammatory cytokines (TNFα), MAMPS (PGN and MDP) and to varying degrees by different commensal bacteria.

Clues as to the function of NOD2 in CEC were obtained from analyses of cytokine production by CEC in response to MDP and other MAMPs. Activation of NOD2 in cultured primary CEC abrogated constitutive production of pro-inflammatory cytokines by CECs and counteracted the ability of TLR2 ligands to up-regulate cytokine production. The suppression of cytokine production by NOD2 activation was associated with expression of the p50 inhibitory subunit of NF-κB. Although these findings need to be corroborated by additional functional studies incorporating NOD2 deficient CEC, they suggest that NOD2 acts as an anti-inflammatory protein in CEC by regulating cytokine production. The transient nature of NOD2 expression by CEC in response to infection and exposure to MAMPs suggests that NOD2 gene transcription is normally tightly regulated and restricted to specific stages of the host response. Disruption of this regulation and sustained expression of NOD2 may therefore effect how it interacts with its associated downstream signalling molecules leading to qualitative or quantitative changes in NF-κB activation resulting in sustained or increased pro-inflammatory cytokine production.

It is interesting to note that elevated and sustained expression of NOD2 is associated with intestinal chronic inflammation (*Lala*, 2003; *Rosenstiel*, 2003) and increased levels of pro-inflammatory cytokine production, particularly IL6, by IECs in IBD patients (*Jones*, 1993; *Kusugami*, 1995; *Shirota*, 1990). Since the highest levels of NOD2 protein in the inflamed colon are localised to the crypts it may influence or be a consequence of the increased proliferation and IEC turnover that is required to repair and regenerate an intact epithelial barrier. It may also reflect disruption of NOD2 regulation, or a futile attempt to control and contain the inflammatory response. In an anti-inflammatory role, the high levels of pro-inflammatory cytokines and sustained NF-κB (p65) activation seen in Crohn's disease could be explained by the loss of inhibitory signals normally provided by NOD2 activation that restrict or prevent NF-κB activation in IEC. In view of the diversity of cellular functions associated with caterpillar proteins that range from influencing differentiation, proliferation and cell death (*Ting* and *Davis*, 2005) it is possible that there may be regional differences in NOD2 function in IEC in the colon; influencing or promoting epithelial anti-microbial defence (α-defensin production) and IEC differ-

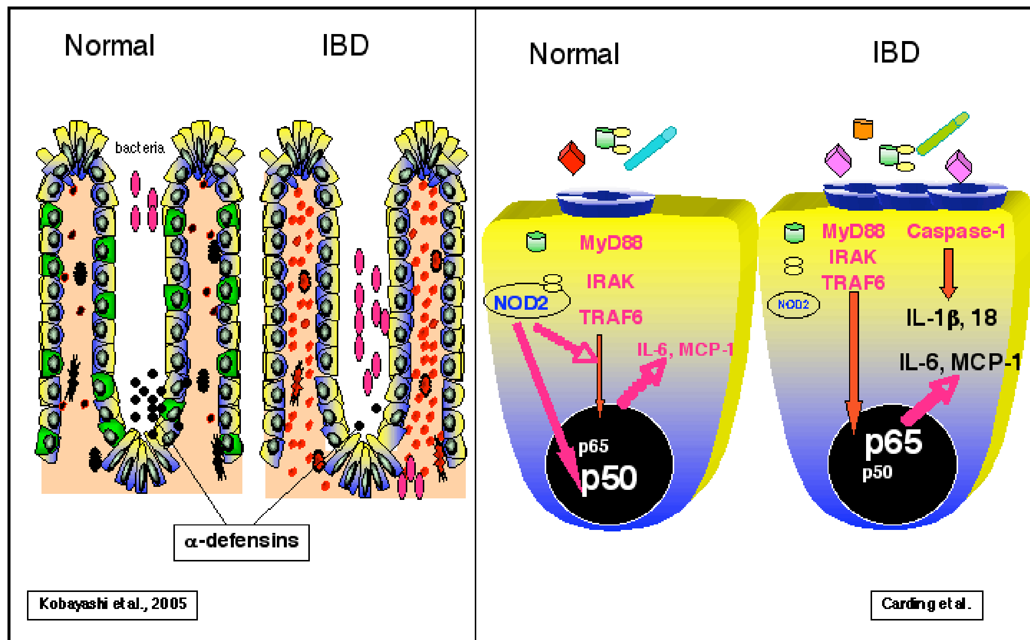

Figure 2: Models of NOD2 function in IEC. In the model proposed by *Kobayashi* et al. (2005) NOD2 regulates α-defensin production by Paneth cells in the crypts of the villi of the small intestine. Mutations in NOD2 lead to a decrease in defensin levels which compromises protection against invading bacteria. An alternative model based upon our own work (*Carding* et al., unpublished observations) is that NOD2 is an anti-inflammatory protein in CEC acting to suppress or restrict the production of NF-κB (p65)-dependent pro-inflammatory cytokines (e.g. IL6, MCP-1) induced by TLR-mediated recognition of commensal bacteria or enteric MAMPs. The loss-of-function of NOD2 in Crohn's disease effectively removes this control mechanism increasing the responsiveness of TLR-mediated signalling resulting in NF-κB (p65)- and caspase-1-dependent responses to commensal bacteria and inflammation and disease.

entiation and proliferation in the crypts, and apoptosis in IEC at the villus tips.

Although there are some similarities between our own findings and model of NOD2 function in primary IEC with those already described (Figures 1 and 2) they are not identical. The differences most likely reflect cell type (haematopoietic versus epithelial cells) or cell stage (immature versus mature) specific differences in NOD2 function that could for example, be mediated by differences in NF-κB subunit activation (p65 vs. p50) and types of cytokines produced (IL-12 or others). Considering that IEC are the first points of contact with enteric microbes the regulation of NOD2 function in IEC may be of central importance in determining the outcome of host-bacteria interactions and in mounting appropriate responses to commensal versus pathogenic bacteria. This does not exclude a (different?) role for NOD2 in other cell types once the epithelial barrier has been breached and they are exposed to MAMPs in the lamina propria or other tissues. Another important point that emerges from these studies is that it is unwise to extrapolate findings based upon one cell type to another unrelated cell type and in comparing responses of immortalised cells with that of primary cells.

CONCLUDING REMARKS

It is now clear that IEC express functional PRRs and that they are used to recognise and respond to microorganisms and microbial antigens in the gut lumen. This interaction or dialog between the two cell types is important in epithelial homeostasis, anti-microbial defences and influencing host innate and adaptive immune responses. This interaction is complex in that the response of IEC to microbial challenge is not stereotypical and they appear to possess the capacity to distinguish between different bacteria and to mount different (pro- or anti-inflammatory) responses. Disruption of this interaction and associated downstream signalling pathways may underlie the pathogenesis of chronic intestinal inflammation and IBD. Among the many questions that remain unanswered that relate to IEC-bacteria interactions, or conversations, are:

- What is the basis of the dialog between commensal bacteria and IEC? Face-to-face (cell-cell contact), long distance (soluble factors), or do they require a third party operator or interpreter such as intestinal DC that are often found in close association with IEC and can gain access to lumen contents via dendritic process that protrude between adjacent IEC?
- Which genes (lexicon) in each partner are required for this dialog?
- Are all bacteria and IECs equally equipped to converse? Are language skills developmentally acquired and are there different regional IEC dialects?
- How might this dialog be different in individuals prone to develop IBD, especially in the majority of IBD patients that have an intact and functional NOD2 gene? Deaf and/or dumb IEC perhaps? Are other known or unknown PRRs involved?
- Do probiotic bacteria converse with IEC in a different language to that of other enteric bacteria?
- Can this dialog be interrupted or corrected for prophylactic and therapeutic benefit?

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