Histol Histopathol (2011) 26: 1453-1464

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Potential role of chitinases and chitin-binding proteins in host-microbial interactions during the development of intestinal inflammation

Hoa T. Tran[1], Nicolas Barnich[2,3] and Emiko Mizoguchi[1,4]

[1]Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, USA, [2]Universite Clermont 1, Pathogenic Bacterienne Instinale, JE2526, Unite Sous Contrat Institut National de la Recheche Agnomique 2018, Clermont-Ferrand, France, [3]Institut Universitaire de Technologie en Genie Biologique, Aubiere, France and [4]Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital, Harvard Medical School, Boston, USA

Summary. The small and large intestines contain an abundance of luminal antigens derived from food products and enteric microorganisms. The function of intestinal epithelial cells is tightly regulated by several factors produced by enteric bacteria and the epithelial cells themselves. Epithelial cells actively participate in regulating the homeostasis of intestine, and failure of this function leads to abnormal and host-microbial interactions resulting in the development of intestinal inflammation. Major determinants of host susceptibility against luminal commensal bacteria include genes regulating mucosal immune responses, intestinal barrier function and microbial defense. Of note, it has been postulated that commensal bacterial adhesion and invasion on/into host cells may be strongly involved in the pathogenesis of inflammatory bowel disease (IBD). During the intestinal inflammation, the composition of the commensal flora is altered, with increased population of aggressive and detrimental bacteria and decreased populations of protective bacteria. In fact, some pathogenic bacteria, including Adherent-Invasive *Escherichia coli*, *Listeria monocytogenes* and *Vibrio cholerae* are likely to initiate their adhesion to the host cells by expressing accessory molecules such as chitinases and/or chitin-binding proteins on themselves. In addition, several inducible molecules (e.g., chitinase 3-like 1, CEACAM6) are also induced on the host cells (e.g. epithelial cells, lamina proprial macrophages) under inflammatory conditions, and are actively participated in the host-microbial interactions. In this review, we will summarize and discuss the potential roles of these important molecules during the development of acute and chronic inflammatory conditions.

Key words: Chitinase, Inflammatory bowel disease, Bacteria, Colonic epithelial cells, CEACAM

Offprint requests to: Emiko Mizoguchi, MD, PhD, Assistant Professor of Medicine, Harvard Medical School, Massachusetts General Hospital, GRJ 825D, 55 Fruit Street, Boston, MA 02114, USA. e-mail: emizoguchi@partners.org

Introduction

A large number of microorganisms exist in the intestine of a human, which include approximately 10-100 trillion bacteria belonging to 15,000~36,000 species. (Frank et al., 2007; Xu et al., 2007). An interaction between enteric microorganisms and host cells, including both immune and non-immune cells, plays a crucial role in maintaining the homeostasis of large as well as small intestines. Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic inflammatory disorders that affect individuals throughout life.

Abbreviations. AIEC, adherent-invasive *Escherichia coli*; AMCase, acidic mammalian chitinase; CBPs, chitin-binding proteins; CD, Crohn's disease; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CECs, colonic epithelial cells; CF, cystic fibrosis; CHI3L1, chitinase 3-like 1 (YKL-40); EPEC, enteropathogenic *E. coli*; Fn3, fibronectin type III domain; GlcNAc, N-acetyl-D-glucosamine; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; M cells, microfold cells; Tir, translocated intimin receptor; UC, ulcerative colitis; WT, wild-type

Inappropriate host-microbial interactions initiate the disregulation and disruption of intestinal immune balance, which result in intestinal disorders (Mizoguchi et al., 2003; Sadlack et al., 1993; Rath et al., 1996). In the intestinal mucosa of IBD patients, in particular CD patients, a tremendous number of microorganisms generate a thick aggregate, called biofilm formation, which is composed of extracellular DNA, proteins and polysaccharides in various configurations (Swidsinski et al., 2002). Of note, pathogenic bacteria called Adherent-Invasive *E. coli* (AIEC) was found to have a relatively increased rate (30-50%) compared to other bacteria in the inflamed mucosa (Baumgart et al., 2007; Martinez-Medina et al., 2009a). Although AIEC has been detected with high frequency in the ileum biopsy of CD patients, it has also been found in approximately 6% of healthy individuals, suggesting that AIEC is an opportunistic pathogen, which is associated with in the development of CD in genetically predisposed hosts (Darfeuille-Michaud et al., 1998; Martinez-Medina et al., 2009a). In those hosts, it has been predicted that an abnormal expression of specific receptor(s) which are expressed on host intestinal epithelial cells (IECs) under inflammatory conditions might interact with a bacterial lectin-like component (Barnich et al., 2007).

IECs, together with specialized lymphocytes and other mononuclear cells, play an important role in host-microbial interaction. An alteration of IECs may be involved in the initiation and/or perpetuation of disease, IECs not only perform a barrier function, but also actively participate in the induction of innate as well as acquired immune responses. Our group previously identified that a gene encoding chitinase 3-like 1 (CHI3L1, also known as YKL-40) was significantly upregulated during the recovery phase of acute colitis as well as chronic colitis in animal models of colitis. Furthermore, the expression of CHI3L1 was significantly upregulated in the active phase, but not inactive phase, of CECs and lamina proprial macrophages in UC and CD patients compared to the healthy individuals (Mizoguchi, 2006). Of note, the induction and overexpression of CHI3L1 on CECs significantly enhances the bacterial adhesion and invasion on/into CECs. Based on this result, it was predicted that the overexpression of CHI3L1 could be one of the leading causes of the enhancement of selected bacterial adhesions to the mucosal surface in IBD patients (Mizoguchi, 2006).

It has been reported that the increased rate of bacterial invasiveness causes serious damage in the host cells during the development of IBD (Nell et al., 2010). The invasiveness of bacteria is regulated by many factors including: 1) the ability of bacterial colonization on/into the host cells, 2) the secretion of extracellular proteins to facilitate invasion, and 3) the ability to combat host defense systems. The virulence factors of pathogenic bacteria seem to play a central role in host-microbial interactions, which include pyelnephritis-associated pili, fimbriae, Dr-binding adhesions, type 1 fimbria, arobactin, toxins, and arobactin (Johnson, 1991; Martinez-Medina et al., 2009b).

In general, invasive bacteria produce bacterial chitinases to allow them to invade into and to survive in host cells (Chaudhuri et al., 2010). For example, *Listeria monocytogenesis*, a major food-borne pathogen, produces two chitinases and a chitin-binding protein (CBP), which have been implicated in the enhancement of bacterial infectious rate in human intestinal cells (Chaudhuri et al, 2010). *L. monocytogenes* actively produce a CBP, which exhibits an affinity toward chitin and chito-oligosaccharide. The biological role of those bacterial chitinases and CBPs has been well characterized: *L. monocytogenes* can utilize chitin as a source of energy and nutrition by digesting it with chitinases. As a result, the bacteria can live on or invade into the chitin-containing host promptly and efficiently. Therefore, it has been predicted that both chitnases and CBP play a critical role in degrading chitin and/or adhering to the chitinous surfaces of host cells (Vaaje-Kolstad et al., 2005a; Bhattacharya et al., 2007). We will further discuss the biological function of chitinases and CBP of *L. monocytogenes*.

Interestingly, gene encoding chitinases and CBPs were also identified in microorganisms that were unable to use chitin as a sole source of carbon, suggesting that they might have biological/physiological functions other than binding to chitinous material or breaking down chitin. A growing number of reports indicate that bacterial chitinases and CBPs play a role as virulence factors. In this review article, we will summarize and discuss some potential roles of bacterial chitinases and CBPs in the pathogenesis of inflammation. We will also discuss selected molecules which may regulate the interactions between IECs and pathogenic as well as potentially pathogenic bacteria under inflammatory conditions, including IBD.

Definition/classification of chitin, chitinases and CBPs

Chitin, a linear ß-1,4-linked polymer of N-acetyl-D-glucosamine (GlcNAc), is the second most abundant polysaccharide in nature after to cellulose. It is found in the microfibrial sheaths of helminthes and the exoskeletons of insects and crustaceans (e.g. crab, shrimp). The major role of chitin is to protect the organism from harsh conditions and host anti-parasite/pathogen immune responses. So far, no endogeneous chitin has been identified in mammals. Chitin plays an important role as a nutrient source for microorganisms that could utilize colloidal chitin as a source of carbon and nitrogen. Many organisms contain chitinases and CBPs genes, which encode proteins that can break and bind to chitin, respectively. Both chitinases and CBPs have diverse functions, depending on the species.

Chitinases (EC 3.2.1.14) are glycosyl hydrolases that catalyze the hydrolysis of the 1,4-beta-linkages in chitin.

Chitnases are found in a wide range of species from all kingdom of life (Kasprzewska, 2003; Arakane and Muthukrishnan, 2010; Lee et al., 2010), including those that are known not to synthesize chitin, such as bacteria, viruses, higher plants as well as mammals. Based on the site on chitin where the chitinolytic enzymes act, chitinases are divided into exo-chitinases and endo-chitinases (Dahiya et al., 2006; Henrissat and Davies, 1997). Endo-chitinases cleavage chitin randomly at internal sites, generating soluble oligomers such as $(GlcNAc)_{2-4}$. Exo-chitinases act on the non-reducing end of chitin, which are digested into chitobiosidases (or chitin-1,4-ß-chitobiosidases) and chitobiases (or ß-(1,4)-N-acetyl-Glucosaminidases). Endo-chitionases break down chitin progressively from the non-reducing end to produce $(GlcNAc)_2$ with no monomer GlcNAc, while exo-chitinases are enable to split further the products of endo-chitinases and chitobiosidases in order to generate GlcNAc monomers (Dahiya et al., 2006, Henrissat and Davies, 1997). Both chitinases are required for the efficient breakdown of chitin into the metabolizable monomer GlcNAc, which can generate energy, CO_2, and NH_3, required for other organisms.

Based on amino acid sequence similarity of the catalytic domain, chitinases are classified into family-18 and -19 glycosyl hydrolases in the Carbohydrate-Active enZymes CAZy databases (http://www.cazy.org) (Henrissat and Davies, 1997; Fukamizo, 2000). The chitinase 18 family is found in a wide variety of organisms, including bacteria, archaea, fungi, some plants, insects, animals as well as viruses. In contrast, chitinase 19 family is only present in some plants and bacteria (e.g. *Streptomyces* species). Chitinase families 18 and 19 share no significant similarity in terms of amino acid sequences and 3-D structure and are believed to have evolved from different ancestors (Suzuki et al., 1999). The Chitinase 18 family can be further divided into 3 subfamilies: A, B and C based on the similarity in the amino acid sequence of their catalytic domain (Watanabe et al., 1993). While subfamily A chitinase has a small insertion of the α+ß fold region between the 7th and 8th (α/ß)8 barrel of the catalytic domain, subfamily B and C chitinases do not have this insertion (Watanabe et al., 1993). *Serratia marcescens* chitinases are a good example of bacterial chitnases with different subfamilies. The chitinase classification of other bacterial species is most frequently referred to as *S. marcescens*.

Chitinases may contain only the catalytic domain or associated with other domains (Fig. 1). 2 domains that are commonly found in chitinases are fibronectin type III domain (Fn3) (Watanabe et al., 1994) and chitin-binding domain type III (ChtBD3) (Hashimoto et al., 2000). Other domains in chitinases include polycystic kidney disease (PKD) domain (Orikoshi et al., 2005), cadherin-like domains (Morimoto et al., 1997), and cystein-rich domain (CRD) (Suarez et al., 2001; Iseli et al., 1993). Bacterial Chitin-binding Domain and PKD have been found to bind directly to chitin, and therefore promote

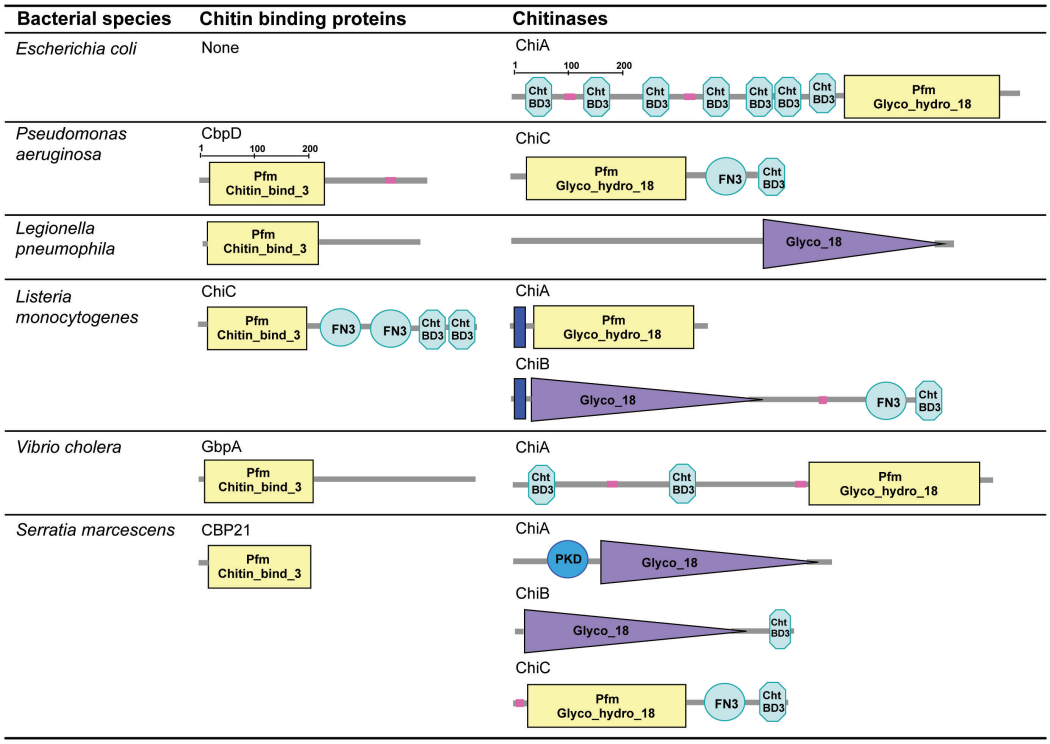

Fig. 1. Domain architecture of bacterial chitin binding proteins and chitinases discussed in this review. Abbreviations used in this table are as follows: ChtBD3 or Pfam Chitin_bind_3, Chitin-binding domain type 3; FN3, Fibronectin type 3 domain; Glyco_hydro_18 or Glyco_18, Glycosyl hydrolase family 18; PKD, polycystic kidney dise

interaction between the substrate and enzyme and increase the enzyamatic activitie of chitinases (Hashimoto et al., 2000; Orikoshi et al., 2005). Although cadherin-like domain and Fn3 are unable to bind to chitin directly, their presence also enhances the enzymatic activities of chitinases (Watanabe et al., 1994; Morimoto et al., 1997).

Chitin-binding domain has a binding ability to chitin, and plays a pivotal role in enhancing the chitinase activity of breaking down chitin. In addition to being attached to enzymes as domains, chitin-binding domain has also been found as an independent, non-catalytic, CBP (Henrissat and Davies, 1997, Vaaje-Kolstad et al., 2005a; Vaaje-Kolstad et al., 2005b). In the CAZy databases (http://www.cazy.org) these independent CBPs are categorized as three families: 14, 18 and, 33 (CBM 14, CBM18, and CBM33). Families 14 and 18 CBPs are found in insect, fungi, and yeast, whereas family 33 CBPs is found mostly in bacteria and viruses (Vaaje-Kolstad et al., 2005b). Family 33 CBPs are called Chitin-bind-3 in the PFAM database (http://pfam.sanger.ac.uk/family?id=Chitin_bind_3), and is called ChtBD3 in SMART database http://smart.embl-heidelberg.de. Bacterial CBPs have been studied extensively in species that can use chitin as a sole source of carbon. CBPs play an important role, together with chitinase, to degrade chitin efficiently, or to bind to environmental surface containing chitin (Vaaje-Kolstad et al., 2005a). In a recent work, Vaaje-Kolstad et al have elegantly shown that CBP21 has also enzymatic activity which enables it to degrade crystal chitin (Vaaje-Kolstad et al., 2010). As we will discuss later, besides having a role in degrading chitin and binding to chitinous material to survive in the environment, bacterial CBPs also play a role as a virulence factor.

Most of the previously performed studies focus on the association between the chitinase domains and their chitinase activities. It is quite possible that such chitinase domains may play a role in interacting with other molecules within the cell or from other cells with biological significance instead of chitinolytic activities. In fact, CRD has been shown to bind chitin, but it has no effect on enzymatic activity or anti-fungal activity of tobacco class I chitinase (Iseli et al., 1993), suggesting that CRD regulates the cellular processes rather than the catalytic activity within the chitinase.

Biological roles of chitinases in normal conditions

Chitinases have diverse physiological and biological roles depending on the organisms. They also play an important part in maintaining the chitin balance in the natural environment as well as having potential applications in agricultural, biological and environmental controls (Kasprzewska, 2003; Dahiya et al., 2006; Duo-Chuan, 2006). Chitinases are utilized by many bacteria and Archaea to degrade chitin for C and N sources to support their growth (Gao et al., 2003; Bhattacharya et al., 2007). In fungi and insects, chitinases are needed to break down chitin components of cell walls and old cuticle respectively; these processes are essential for molting, growth and development of cell walls and cuticle (Duo-Chuan, 2006, Arakane and Muthukrishnan, 2010). Plant chitinases are also associated with stress responses and regulate their growth and development (Kasprzewska, 2003). In mammals, chitinases are believed to play a role in digesting the chitinous material or defending mammals from any chitin-contained pathogens including fungi and insects (Boot et al., 2001).

Biological roles of bacterial chitinases and CBPs in pathogenic conditions

In addition to the functions of regulating cellular growth and proliferation, chitinases also play an important biological role in defending the host cells or by increasing interaction with pathogens due to their ability to hydrolyze and/or interact with chitin present in pathogens or host cells. The roles of plant chitinases in defending from the infectious pathogens have been well understood (Duo-Chuan, 2006; Arakane and Muthukrishnan, 2010; Kasprzewska, 2003). Plant chitinases efficiently defend against pathogens including viruses, bacteria, fungi and insects (Kasprzewska, 2003). Fungi use chitinases to hydrolyze chitin, a major component of insect cuticle which allows the fungus to penetrate and invade the insect efficiently, is considered one of the virulence factors of fungi (Duo-Chuan, 2006). Chitinases are produced by various bacteria which are unable to use chitin as a sole carbon source. Of note, numerous reports suggest that bacterial chitinases seem to be involved in the pathogenesis of bacteria. Therefore, we will summarize the potential role of some selected bacterial chitinases in the following section.

Listeria monocytogenes

Listeria monocytogenes, a Gram-positive bacteria found in soil, water, and decaying vegetation, is the causative effect of a severe food-borne infection (so called listeriosis) that can lead to meningitis, meningo-encephalitis, septicemia, perinatal infections and gastroenteritis (Vazquez-Boland et al., 2001). This bacteria has two genes, which encode chitinases (ChiA and ChiB) and a CBP (Leisner et al., 2008). *L. monocytogenes* is able to hydrolyze chitin, and it was found that both ChiA and ChiB contribute to this function (Leisner et al., 2008). Chitinases and CBP appear to play an important role in the pathogenesis of *L. monocytoge*nes in mice models of infection. Deletion of *chiA*, *chiB* or *cbp* genes in *L. monocytogenes* leads to the reduction in bacterial translocation to the liver and spleen in infected mice (Chaudhuri et al., 2010). ChiA expression of *L. monocytogenes*, but not of non-invasive bacteria, was upregulated in the cytosol of *L-monocytogenesis*-infected macrophages (Chatterjee et al., 2006). This result suggests that ChiA is required for

bacterial survival and growth in the host cells. In contrast, ChiB and CBP may play a role in the initial colonization of the host cells. These studies suggest that chitinases and CBP of *L. monocytogenes* seem to have dual functions: 1) to help the bacterium live in the natural environment and 2) to help the bacterium to colonize and survive in the host cells. With respect to the host cell pathogenesis, it is speculated that since chitin was not synthesized by the mammals that *L. monocytogenes* chitinases and CBPs may have been interacting with chitin-like components such as glycoproteins or other carbohydrate moieties expressing on the surface of host cells (Chaudhuri et al., 2010).

Vibrios

Vibrios are Gram-negative marine bacteria, which are often associated with chitinous exoskeleton of copepods, can utilize chitin as the sole source of carbon and nitrogen (Colwell and Sprira, 1992). Chitinases and CBPs play an important role in *V. cholerae* and other *Vibrionaceae* during the process of chitin utilization (Nalin et al., 1979; Svitil et al., 1997; Meibom et al., 2004). *V. cholerae* causes cholera, a severe disease associated with large amounts of watery diarrhea, in humans upon ingestion. This symptom is caused by a cholera toxin secreted by the infected *V. cholerae* after it has colonized in the intestinal epithelium (Kaper et al., 1995). Therefore, the attachment of *V. cholerae* to the surface of host to the intestinal epithelia is essential for their pathogenesis. The genome of *V. cholera*e harbors 4 gene encoding chitinases and CBP (Bhowmick et al., 2007; Kirn et al., 2005). CBP and chitinases were induced when *V. cholerae* was grown in a medium containing the chitin monomer (GlcNAc), oligomers $(GlcNAc)_{2-6}$ or chitin (polymer of GlcNac) suggesting that CBP and chitinases can efficiently bind to and hydrolyze these glycans, respectively (Meibom et al., 2004). The CBP of *V. cholerae*, named as GbpA, was demonstrated to be an essential factor for bacterial binding to sugar components of environmental chitinous surfaces as well as human intestine. Furthermore, GbpA is considered to play an important role in bacterial colonization in the environment and in human epithelial cells for bacteria (Kirn et al., 2005). Bhowmick et al demonstrated that (Bhowmick et al., 2008) there was a coordinated interaction between GbpA and mucin both *in vitro* and *in vivo* (in an infectious mouse model). Recombinant protein GbpA can specifically bind to mucin in a concentration dependent manner. In addition, mucin and GbpA appear to have a mutual effect on up-regulateing their expressions in each other: mucin upregulates the expression of GbpA, and also mucin expression was increased after infection with *V. cholerae*. The result strongly suggests the important role of bacterial CBPs in the processes of pathogenesis (e.g. adhesion, colonization) to the host cells. It also indicates that CBPs not only bind to hydrolyzed chitin products (e.g $GlcNAc_{1-6}$), but that it also binds to GlcNAc, a component in mucin and other N-glycosylated proteins, presumably.

According to a recent study, bacterial gene regulation in response to bacterial cell-population density (so called quorum sensing) plays a key role in regulating GbpA expression and biofilm formation of *V. cholerae* (Hammer and Bassler, 2003; Jude et al., 2009). The data showed that GbpA was upregulated in low- as compare to high-density of *V. cholerae* (Jude et al., 2009). It is predicted that GbpA may be involved in the initial attachment to the host cells but may not be required for biofilm formation.

Although there is no data available for the biological function of the four chitinases produced by *V. cholerae*, a study from *Vibrio harveyi*, a close relative of *V. cholerae*, demonstrated that *V. harveyi* chitinases expression was also regulated by quorum sensing (Defoirdt et al., 2010). The data showed that quorum sensing negatively regulates the expression of chitinases in *V. harveyi*. Therefore bacterial chitinases may contribute during the initial steps of bacterial attachment to the host cells.

Legionella pneumophila

L. pneumophila, a Gram-negative aquatic bacterium, is often found in fresh waters, protozoan hosts, potable water systems and cooling towers. *L. pneumophila* is the major *Legionella* species that causes Legionnaires' disease, a severe pneumonia particularly affecting immunocompromised hosts (Fields et al., 2002). In humans, the disease may be transmitted by inhaling aerosole containing *L. pneumophila*. The bacterium then colonizes in the respiratory tract, and invades alveolar macrophages, which eventually induces significant tissue-damage in the infected lungs (DebRoy et al., 2006). By the blast analysis for genome of *L. pneumophila*, it has been identified that this bacteria contains a chitinase (ChiA), and a CBP (gil54296231).

Deb Roy et al. reported that deletion of the *chiA* gene in *L. pneumophila* had no effect on growth in an intracellular cell culture, but it did reduce the bacterial persistence in the lungs of infected mice (DebRoy et al, 2006). The study strongly suggests that chitinase enhances bacterial survival in mammalian host cells. Furthermore, it was also speculated that ChiA may breakdown chitin-like components in the lung (e.g. N-acetylated chitinous proteins) that promote bacterial persistence and survival in the host cells (DebRoy et al, 2006). Alternatively, ChiA-mediated bacterial persistence in the lung may be not due to the chitinolytic activity of ChiA but due to the host response against ChiA. The compromised host along with the presence of bacteria with ChiA enables bacterial survival within the host cells, whereas the hosts can exert more effective immune responses to exclude the bacteria without the existence of ChiA (DebRoy et al., 2006).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic pathogen in humans, and is the major cause of infection in patients with cystic fibrosis (CF) (Stover et al., 2000). Although *P. aeruginosa* is unable to use chitin as a sole carbon source (Folders et al., 2001; Jagmann et al., 2010), CBP and chitinase were isolated and characterized in some clinical isolates of *P. aeruginosa*. However, these proteins were not identified in the strains, which were isolated from soil (Folders et al., 2000; Folders et al., 2001). A CBP of *P. aeruginosa*, called CbpD, appears to be differently expressed at distinct stages of diseases. CbpD was highly produced in strains that caused initial and acute infection as compared to strains isolated from the chronic phases in the patients with CF. This result suggests that CBP may play an important role in the initial adhesion of bacteria to the lung epithelial cells but not the later chronic phase of pulmonary infection mediated by *P. aeruginosa*. This finding is consistent with our recent finding that non-pathogenic *E. coli* overexpressed CBP21 (derived from *Serratia marcescens*) has an increased rate of adhesion to colonic epithelial cells (Kawada et al., 2008). Although there is no direct evidence showing that CBP and chitinase of *P. aeruginosa* play a role as virulence factors during pulmonary infection, the above findings strongly suggest that *P. aeruginosa* utilizes these proteins during the initial phases of pulmonary infections in CF patients.

The DNA microarray analysis by Salunkhe et al revealed that the expression of CBP in *P. aeruginosa* was significantly upregulated in the CF-associated strains as compared to the laboratory strain, suggesting the potentially pathogenic role of CBP as a virulent factor of the *P. aeruginosa* infection in CF patients (Salunkhe et al., 2005). Since the *P. aeruginosa* strain, isolated from the affected areas with burn-wounding, shows significantly less CBP expression as compared to the strain isolated from the lungs with CF patients, CBP in *P. aeruginosa* may play a distinct and specific role in the types and/or areas of disease and inflammation (Sriramulu et al., 2005).

Mucin was considered to be one of the factors that could regulate gene expression in *P. aeruginosa*: the expression of ChiC (one of chitinases in *P. aeruginosa*) was significantly upregulated when the bacteria was grown in a medium containing CF-like sputum (which contains mucin) as compared to growth in a medium without CF sputum (Fung et al., 2010). This result is consistent with data that mucin significantly upregulated CBP expression on *V. cholerae* (Bhowmick et al., 2008).

A recent study revealed that both CBP and chitinase in the CF-related strain of *P. aeruginosa* have higher transcriptional level compared to the non-CF-related strain when they are grown with planktonic cells (Manos et al., 2009), suggesting that both proteins play a pivotal role in the pathogenesis of *P. aeruginosa* infection. Furthermore, the study showed that chitinase and CBP in CF-related *P. aeruginosa* strains are significantly down-regulated in the biofilm compartment compared to planktonic cells. This data suggests that CBP and chitinase are likely to play an important role in the initial biofilm formation of *P. aeruginosa* rather than maintaining the biofilm as a stable condition.

Escherichia coli

E. coli, a Gram-negative, rod-shaped bacteria, which is a commonly found resident in the human intestine, and is unable to use chitin as a sole carbon source. Our group recently found that chitinase ChiA has been encoded in the genome of 13 groups strains of *E. coli* strains, including both pathogenic and non-pathogenic strains. The ChiA protein in *E. coli* strain contains a catalytic domain belong to the family 18 glycoside hydrolase, and the 7 chitin-binding domains (Fig. 1). Although the physiological regulation of ChiA protein was previously studied (Francetic et al., 2000), the biological function of this gene is still understudied. Our laboratory is currently investigating the biological function of *chiA gene* in AIEC by generating a deletion mutant of chiA in AIEC reference strain LF82. Our preliminary and unpublished data suggests that the *chiA* deletion in the AIEC LF82 strain significantly reduces their adhesion to IECs as compare to that of wild-type AIEC LF82 bacteria. This data indicates that ChiA plays a role as a virulence-factor in AIEC, and may promote bacteria adhesion to mucosal tissues.

Bacterial chitinases and CBPs seem to play a pathogenic role in certain strains of bacterial infection. Recent studies in *V. cholerae* and *P. aeruginasa* indicate that CBPs promote the attachment of bacteria to the host cells through the interaction with mucin during the initial stage of colonization (Kirn et al., 2005; Bhowmick et al., 2008; Jude et al., 2009). The microarray and proteomics analyses of *P. aeruginosa* show that CBP is differently regulated, depending on the growing environment conditions and the state of disease. CBPs may play a crucial role in the initial attachment process to the host cells not only in *P. aeruginosa* but also in *V. cholera* (Salunkhe et al., 2005; Sriramulu et al., 2005; Manos et al., 2009; Fung et al., 2010). Data from *L. pneumophila* and *L. monocytogenes* also support that bacterial chitinases are involved in the process of bacterial infection (Leisner et al., 2008; Chaudhuri et al., 2010). However, it is still unknown whether the chitinase-mediated bacterial infection is mediated by the catalytic domain and/or the other associated domains of bacterial chitinases. It is possible that chitinases use their glycohydrolase activity to break down chitin-like components (which contain GlcNAc) in the host cells. It might be possible also that associated domains, including Fn3 or ChtBD3, act as an adherent molecule to enhance the accessibility of bacteria to the host cells. At this stage, the possibility that bacterial chitinases may affect the immune responses of the host cells to make a better condition for the bacteria as seen in the upregulated expression of ChiA during the infectious process of *L. monocytogenes* in the host macrophages

cannot be ruled out (Chatterjee et al., 2006).

The selected candidate molecules which play key roles in host-microbial interactions in IBD

Bacterial adhesion to IECs is the first step in the pathogenesis of many bacteria involved in gut infectious diseases. Adhesion enables the bacteria to colonize in the gut, thus limiting clearance from the intestine. In this section, we will summarize some key molecules, which effectively enhance the potentially pathogenic bacterial adhesion to IECs during the inflammatory conditions.

Bacterial chitinases and CBPs

To identify host components, which enhance bacterial adhesion and invasion provide us a great opportunity to understand the mechanisms in host-microbe interactions and the pathogenesis of intestinal inflammation. As described in the above section, CBP in *V. cholerae* plays an important role in the initial adhesion process to the host cells (Kirn et al., 2005). Other groups also demonstrated that CBP plays an important role as an adhesion molecule that enables bacteria to initiate their attachment to the intestine, which is an essential step for bacterial colonization (Bhowmick et al., 2008; Jude et al., 2009). Bhowmick et al showed that the presence of CBP lead to a quicker and better chance of bacterial adhesion to intestinal mucin, which was identified as a host molecule interacting with CBP-expressed in *V. cholerae* (Bhowmick et al., 2008). Interestingly, *in vivo* data indicates that infection with CBP-expressing *V. cholera* significantly enhances mucin production as well as fluid secretion of host cells, which were characteristic features of cholera disease (Bhowmick et al., 2008). In contrast to cholera disease, which commonly generates the formation of a thick mucous layer, IBD generally shows the disrupted mucous layer of the intestine. Thus, mucin is unlikely to be the major factor leading to the bacterial accumulation in the intestine during the development of IBD. Inducible molecules under inflammatory conditions, other than mucin, may play a key role in interacting with luminal bacteria adhesion to IECs. As described previously, CBP-expressed *V. cholera* specially enhances the production of mucin by host cells, and the CBP has also been known to bind with mucin (Bhowmick et al., 2008). Therefore, it must be worthwhile to examine whether bacterial CBPs/chitinases in the bacterial flora of IBD patients also stimulate the production of mucin or any other molecules in the hosts.

Mammalian chitinases

Our group previously identified that CHI3L1 is significantly upregulated in the animal models of colitis and in human IBD (Mizoguchi, 2006). CHI3L1, an N-glycosylated chitin-binding protein, contains a conserved catalytic domain, which is classified as a member of family 18 glycosyl hydrolases. However, unlike enzymatic-active mammalian chitinases (e.g. acid mammalian chitinase, chitotriosidase), CHI3L1 has no chitinolytic enzyme activity, due to point mutations in selected amino acids in the catalytic domains (Kzhyshkowska et al., 2007). Crystal structure revealed that CHI3L1 is N-glycosylated with two molecules of GlcNAc at the 60th asparagine residue (Fusetti et al., 2003). It has been speculated that glycosylated CHI3L1 may play a crucial role in interacting with bacterial components including chitinases and CBPs, but the exact mechanisms have not been completely revealed.

Our group also showed that CBP of *S. marcescens* (CBP21) promotes bacterial adhesion to CHI3L1-expressing in CECs (Kawada et al., 2008). This data indicates that bacterial CBP potentially may be the molecule directly or indirectly interacting with CHI3L1, probably in a similar manner as observed in *V. cholera* (Bhowmick et al., 2008).

Enzymatic-active mammalian chitinases, such as acidic mammalian chitinase and chitotriosidase, may be potential proteins also which enable to interact with bacterial CBPs and/or chitinases. In fact, it has been previously reported that the increased expression of chitotriosidase in neonates is detected after bacterial infection (Labadaridis et al., 2005). Of note, the bacteria generally do not contain chitin as a structural component. Thus the induction of chitotriosidase in host cells may not due to the need for chitionolytic activity of this protein. In addition, Hartl et al reported that the biological function of acidic mammalian chitinase is independent of chitinolytic activity (Hartl et al., 2009). Unlike CHI3L1, chitotriosidase is not N-glycosylated but contains sites for O-glycosylation (Boot et al., 1995). Therefore N- or O-glycosylation of mammalian chitinases may act as one of the binding factors for interaction with bacterial CBPs or chitinases.

Carcinoembryonic antigen-related cell-adhesion molecules

Members of the CEACAM family serve as receptors for a variety of Gram-negative bacteria that live on mucosal surfaces of the human body. In an example of convergent evolution, these microbes have evolved distinct CEACAM-binding adhesins that seem to promote the colonization of the mucosa. CEACAM-binding adhesins from pathogenic bacteria display a high selectivity for human CEACAMs and do not associate with orthologues from non-primate mammalian species. The carcinoembryonic antigen (CEA) gene family may represent a paradigm for such gene families since it is highly divergent in humans, mice and dogs, and its members have functions in both immune regulation and reproduction (Zebhauser et al., 2005; Kammerer et al., 2007). The CEA family is a member of the immunoglobulin superfamily (IgSF), which consists of the CEA-related cell adhesion molecule (CEACAM) and the pregnancy specific glycoprotein (PSG) subgroups. They are composed basically of a leader, one N-terminal immunoglobulin variable (IgV)-like domain (N domain),

followed by a variable number of two different types of Ig constant (IgC)-like domains (named A and B) with a few notable exceptions. Homotypic and heterotypic adhesion is the most prominent function of these extracellular domains. CEACAM family members are typically cell membrane associated glycoproteins and are part of the immunoglobulin superfamily. CEACAM1 (BGP), CEACAM5 (CEA) and CEACAM6 (NCA) are expressed on IECs. CEACAM1 contains a hydrophobic transmembrane domain followed by either a short or long cytoplasmic domain, whereas CEACAM5 and CEACAM6 attach to the cell membrane via a GPI-anchor. In humans, CEACAM1 is the target of several Gram-negative pathogenic bacteria that inhabit the nasopharyngeal, intestinal or urogenital mucosa. In particular, *Neisseria gonorrhoeae, N. lactamica, N. meningitidis, N. subflava, Haemophilus influenzae, Moraxella catarrhalis,* and *E. coli* strains have been found to associate with the protein core or carbohydrate structures of this glycoprotein (Sauter et al., 1993; Virji et al., 1993; Chen and Gotschlich, 1996; Hill et al., 2001; Toleman et al., 2001; Hill and Virji, 2003). These bacterial species utilize distinct surface proteins (adhesins) to engage CEACAMs. For example, the neisserial colony opacity associated (Opa) proteins allow gonococci and meningococci to bind several CEACAM family members.

AIEC that persist within macrophages and epithelial cells selectively colonize the ileum of CD patients (Darfeuille-Michaud et al., 2004) Barnich et al., 2007). Such pathogenic strains are achieving increasing relevance since various studies performed in France (Darfeuille-Michaud et al., 2004), the United Kingdom (Martin et al., 2004), Spain (Martinez-Medina et al., 2009b) and the USA (Sasaki et al., 2007; Eaves-Pyles et al., 2008; Baumgart et al., 2007) have reported them to be more prevalent in CD patients than in controls. The presence of AIEC in healthy subjects suggests that AIEC strains are facultative pathogens that cause disease in susceptible hosts. AIEC strains were found to be highly associated with ileal mucosa in CD patients (Darfeuille-Michaud et al., 2004). CD-associated AIEC strains adhere to the brush border of primary ileal enterocytes isolated from CD patients but not controls without IBD (Barnich et al., 2007), suggesting that there are specific alterations of the ileal epithelial cells in patients with CD which allow AIEC strains to adhere to a greater extent. AIEC adhesion is dependent on type 1 pili expression on the bacterial surface, organelles that are critical for mediating the colonization of epithelial surfaces. Among the various candidate proteins known to enable bacterial adhesion, both CEACAM6 and CEACAM5 are overexpressed in the ileal epithelial cells of patients with CD, as compared with those of controls. Interestingly, several pathogens in humans and mice exploit these domains to adhere to and infect their target cells. An example, CD-associated AIEC colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM (Carvalho et al., 2009) and CEACAM6 acts as a receptor for AIEC adhesion and colonization and plays a key role in the development of gut inflammation. Indeed pre-treatment of primary ileal enterocytes isolated from CD-patients with an anti-CEACAM6 monoclonal antibody strongly decreased AIEC adhesion and intraperitoneal administration of anti-CEACAM6 monoclonal antibody prevented colonization and clinical symptoms of colitis in AIEC-infected CEABAC10 transgenic mice (Barnich et al., 2007; Carvalho et al., 2009). The presence of AIEC induces an increase in CEACAM6 expression by cultured IECs, as does with interferon-γ (IFN-γ) or tumor necrosis factor α (TNF-α), two pro-inflammatory mediators of which increased levels are typically found in the intestine of patients with CD. Thus, AIEC may directly, by adhering to host cells and indirectly via increased secretion of TNF-α from AIEC-infected macrophages (Glasser et al., 2001; Bringer et al., 2006), induce IECs to up-regulate CEACAM6, thereby enabling their own adhesion to these cells. Patients expressing a basal level of CEACAM6 in ileum would be predisposed to develop ileal CD, and the presence of AIEC bacteria would lead to a secretion of IFN-γ or TNF-α amplifying a loop of colonization and inflammation. Since AIEC are critical to the initiation and/or maintenance of inflammation in CD, blocking of the interaction between the type 1 pili (on AIEC) and CEACAM6 expressed by epithelial cells (e.g. with the use of monosaccharides, oligosaccharides, or anti-CEACAM6 antibodies) might serve as a specific and effective strategy to disrupt the inflammatory amplification loop in patients with CD in the future.

Others molecules involved in host-microbial interaction

Numerous others molecules are described to allow bacteria/host cell interactions. In this review, we will introduce two other examples: 1) concern that bacteria which make their own receptors and 2) the concern is M cells (microfold cells), which can be a portal entry for gut pathogens.

In the 1990s it was believed that the binding of enteropathogenic *E. coli* (EPEC) to epithelial cells triggered tyrosine phosphorylation of a protein in a host cell intimin receptor of 90 kDa, termed Hp90. However, Hp90 was not a host cell protein but was a LEE (the locus of enterocyte effacement)-encoded type III secretion system effecter protein termed Tir (translocated intimin receptor) (Kenny et al., 1997). This discovery, which introduced a revolutionary concept of a bacterium that makes its own receptor, appears to be unique to attach effacing bacteria. Following translocation, cytosolic Tir spontaneously inserts into the plasma membrane of the eukaryotic cell in a hairpin loop topology, which is presented on the external face of the plasma cell membrane and functions as the receptor for intimin (Garmendia et al., 2005).

Since the initial lesions in CD occur at Peyer's

patches and lymphoid follicles (Fujimura et al., 1996), knowledge of the interaction of M cells with bacteria is particularly important. The apical microfold membranes of M cells facilitate adherence and uptake of microorganisms and microbial antigens for presentation to lymphoid or antigen-presenting cells in the sub-epithelial tissue. M cells are an epithelial cell phenotype of the follicle-associated epithelium (FAE) that translocate 'foreign' material from the gut lumen to lymphoid tissue within the intestinal mucosa. There is evidence that M cells represent the portal of entry for most gut pathogens (Neutra et al., 1999). Overtly invasive pathogens such as *Shigella* and *Salmonella* are unable to invade via normal colonic cells and enter via M cells (Phalipon and Sansonetti, 1999; Sansonetti and Phalipon, 1999). Recent studies have reported that glycoprotein 2 (GP2), specifically expressed on the apical plasma membrane of M cells among enterocytes (Terahara et al., 2008), is recognized by FimH, the adhesin of type 1 pili on bacteria (Hase et al., 2009). Entry of type 1 piliated bacteria, such as *E. coli* and *Salmonella typhimurium*, into M cells could occur by means of the GP2-FimH recognition. More recently, we have reported that AIEC bacteria could interact with M cells located in human Peyer's patches via long polar fimbriae (LPF), demonstrating that CD-associated AIEC bacteria by expressing LPF can use Peyer's patches as an open gate to induce early stages of the disease (Chassaing et al., 2011).

Conclusions

Bacterial CBPs and chitinases are found in the various kinds of bacterial species. Previous reports suggest that bacterial CBPs and chitinases have a biological function as virulence factors. The expressions of these proteins are regulated by the host bacteria themselves and/or their environment, and have been predicted to be involved in the biofilm formation or colonization of bacteria to the host. In addition, they seem to play an important role for bacterial survival in the host cells, suggesting their potential to modify the host immune responses.

Bacterial CBPs may play a critical role in enhancing the bacterial adhesion to host cells, which express mammalian chitinases and chitinase-like proteins including CHI3L1. In addition, CEACAM6 on CECs is one of the key molecules to contribute host/microbial inteactions under inflammatory conditions. It is also worthwhile to further examine other key molecules on host cells, which may be induced to initiate and perpetuate interactions with bacterial CBPs/ chitinases.

Acknowledgements. We would like to thank Mr. Terry Danford Lott and Ms. Cindy W. Lau for their excellent assistance in preparing this manuscript. This work has been supported by National Institute of Health (DK 80070, DK74454, DK64289 and DK43351), and grants from the Eli and Edythe L. Broad Medical Foundation and American Gastroenterological Association Foundation to EM.

References

Arakane Y. and Muthukrishnan Y. (2010). Insect chitinase and chitinase-like proteins. Cell. Mol. Life Sci. 67, 201-216.

Barnich N., Carvalho F.A., Glasser A.L., Darcha C., Jantscheff P., Allez M., Peeters H., Bommelaer G., Desreumaux P., Colombel J.F. and Darfeuille-Michaud A. (2007). CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. J. Clin. Invest. 117, 1566-1574.

Baumgart M., Dogan B., Rishniw M., Weitzman G., Bosworth B., Yantiss R., Orsi R.H., Wiedmann M., McDonough P., Kim S.G., Berg D., Schukken Y., Scherl E. and Simpson K.W. (2007). Culture independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. Isme J. 1, 403-418.

Bhattacharya D., Nagpure A. and Gupta R.K. (2007). Bacterial chitinases: Properties and potential. Crit. Rev. Biotechnol. 27, 21-28.

Bhowmick R, Ghosal A. and Chatterjee N.S. (2007). Effect of environmental factors on expression and activity of chitinase genes of vibrios with special reference to *Vibrio cholerae*. J. Appl. Microbiol. 103, 97-108.

Bhowmick R., Ghosal A., Das B., Koley H., Saha D.R., Ganguly S., Nandy R.K., Bhadra R.K. and Chatterjee N.S. (2008). Intestinal adherence of *Vibrio cholerae* involves a coordinated interaction between colonization factor GbpA and mucin. Infect. Immun. 76, 4968-4977.

Boot R.G., Blommaart E.F.C., Swart E., Ghauharali-van der Vlugt K., Bijl N., Moe C., Place A. and Aerts J. (2001). Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J. Biol. Chem. 276, 6770-6778.

Boot R.G., Renkema G.H., Strijland A., Vanzonneveld A.J. and Aerts J. (1995). Cloning of a cDNA-encoding chitotriosidase, a human chitinase produced by macrophages. J. Biol. Chem. 270, 26252-26256.

Bringer M.A., Glasser A.L., Tung C.H., Meresse S. and Darfeuille-Michaud A. (2006). The Crohn's disease-associated adherent-invasive *Escherichia coli* strain LF82 replicates in mature phagolysosomes within J774 macrophages. Cell Microbiol. 8, 471-484.

Carvalho F.A., Barnich N., Sivignon A., Darcha C., Chan C.H., Stanners C.P. and Darfeuille-Michaud A. (2009). Crohn's disease adherent-invasive *Escherichia coli* colonize and induce strong gut inflammation in transgenic mice expressing human CEACAM. J. Exp. Med. 206, 2179-2189.

Chassaing B., Rolhion N., de Vallee A., Salim S.Y., Prorok-Hamon M., Neut C., Campbell B.J., Soderholm J.D., Hugot J.P., Colombel J.F. and Darfeuille-Michaud A. (2011). Crohn disease-associated adherent-invasive *E. coli* bacteria target mouse and human Peyer's patches via long polar fimbriae. J. Clin. Invest. 121, 966-975.

Chatterjee S.S., Hossain H., Otten S., Kuenne C., Kuchmina K., Machata S., Domann E., Chakraborty T. and Hain T. (2006). Intracellular gene expression profile of *Listeria monocytogenes*. Infect. Immun. 74, 1323-1338.

Chaudhuri S., Bruno J.C., Alonzo F., Xayarath B., Cianciotto N.P. and Freitag N.E. (2010). Contribution of chitinases to *Listeria monocytogenes* pathogenesis. Appl. Environ. Microbiol. 76, 7302-7305.

Chen T. and Gotschlich E.C. (1996). CGM1a antigen of neutrophils, a

receptor of gonococcal opacity proteins. Proc. Natl. Acad. Sci. USA 93, 14851-14856.

Colwell R.R. and Sprira W.M. (1992). The ecology of *Vibrio cholerae*. In: Cholera. Barua D. and Greenough III (eds). Plenum Press, New York. pp107-127.

Dahiya N., Tewari R. and Hoondal G.S. (2006). Biotechnological aspects of chitinolytic enzymes: a review. Appl. Microbiol. Biotechnol. 71, 773-782.

Darfeuille-Michaud A., Neut C., Barnich N., Lederman E., Di Martino P., Desreumaux P., Gambiez L., Joly B., Cortot A. and Colombel J.F. (1998). Presence of adherent *Escherichia coli* strains in ileal mucosa of patients with Crohn's disease. Gastroenterology 115, 1405-1413.

Darfeuille-Michaud A., Boudeau J., Bulois P., Neut C., Glasser A.L., Barnich N., Bringer M.A., Swidsinski A., Beaugerie L. and Colombel J.F. (2004). High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. Gastroenterology 127, 412-421.

DebRoy S., Dao J., Soderberg M., Rossier O. and Cianciotto N.P. (2006). *Legionella pneumophila* type II secretome reveals unique exoproteins and a chitinase that promotes bacterial persistence in the lung. Proc. Natl. Acad. Sci. USA 103, 19146-19151.

Defoirdt T., Ruwandeepika H.A.D., Karunasagar I., Boon N. and Bossier P. (2010). Quorum sensing negatively regulates chitinase in *Vibrio harveyi*. Environ. Microbiol. Rep. 2, 44-49.

Duo-Chuan L. (2006). Review of fungal chitinases. Mycopathologia 161, 345-360.

Eaves-Pyles T., Allen C.A., Taormina J., Swidsinski A., Tutt C.B., Jezek G.E., Islas-Islas M. and Torres A.G. (2008). *Escherichia coli* isolated from a Crohn's disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. Int. J. Med. Microbiol. 298, 397-409.

Fields B.S., Benson R.F. and Besser R.E. (2002). Legionella and Legionnaires' disease: 25 Years of Investigation. Clin. Microbiol. Rev. 15, 506-526.

Folders J., Tommassen J., van Loon L.C. and Bitter W. (2000). Identification of a chitin-binding protein secreted by *Pseudomonas aeruginosa*. J. Bacteriol. 182, 1257-1263.

Folders J., Algra J., Roelofs M.S., van Loon L.C., Tommassen J. and Bitter W. (2001). Characterization of *Pseudomonas aeruginosa* chitinase, a gradually secreted protein. J. Bacteriol. 183, 7044-7052.

Francetic O., Badaut C., Rimsky S. and Pugsley A.P. (2000). The ChiA (YheB) protein of *Escherichia coli* K-12 is an endochitinase whose gene is negatively controlled by the nucleoid-structuring protein H-NS. Mol. Microbiol. 35, 1506-1517.

Frank D.N., Amand A.L.S., Feldman R.A., Boedeker E.C., Harpaz N. and Pace N.R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA 104, 13780-13785.

Fujimura Y., Kamoi R. and Iida M. (1996). Pathogenesis of aphthoid ulcers in Crohn's disease: Correlative findings by magnifying colonoscopy, electron microscopy, and immunohistochemistry. Gut 38, 724-732.

Fukamizo T. (2000). Chitinolytic Enzymes: Catalysis, substrate binding, and their application. Curr. Protein Pept. Sci. 1, 105-124.

Fung C., Naughton S., Turnbull L., Tingpej P., Rose B., Arthur J., Hu H.H., Harmer C., Harbour C., Hassett D.J., Whitchurch C.B. and Manos J. (2010). Gene expression of *Pseudomonas aeruginosa* in a mucin-containing synthetic growth medium mimicking cystic fibrosis lung sputum. J. Med. Microbiol. 59, 1089-1100.

Fusetti F., T Pijning, Kalk K.H., Bos E. and Dijkstra B.W. (2003). Crystal structure and carbohydrate-binding properties of the human cartilage glycoprotein-39. J. Biol. Chem. 278, 37753-37760.

Gao J., Bauer M.W., Shockley K.R., Pysz M.A. and Kelly R.M. (2003). Growth of hyperthermophilic *Archaeon Pyrococcus* futiosus on chitin involves two family 18 chitinases. Applied and Environmental Microbiology 69, 3119-3128.

Garmendia J., Frankel G. and Crepin V.F. (2005). Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: Translocation, translocation, translocation. Infect. Immun. 73, 2573-2585.

Glasser AL., Boudeau J., Barnich N., Perruchot M.H., Colombel J.F. and Darfeuille-Michaud A. (2001). Adherent invasive *Escherichia coli* strains from patients with Crohn's disease survive and replicate within macrophages without inducing host cell death. Infect Immun 69, 5529-5537.

Hammer B.K. and Bassler B.L. (2003). Quorum sensing controls biofilm formation in *Vibrio cholerae*. Mol. Microbiol. 50, 101-114.

Hartl D., He C.H, Koller B., Da Silva C.A., Kobayashi Y., Lee C.G., Flavell R.A. and Elias J.A. (2009). Acidic mammalian chitinase regulates epithelial cell apoptosis via a chitinolytic-independent mechanism. J. Immunol. 182, 5098-5106.

Hase K., Kawano K., Nochi T., Pontes G.S., Fukuda S., Ebisawa M., Kadokura K., Tobe T., Fujimura Y., Kawano S., Yabashi A., Waguri S., Nakato G., Kimura S., Murakami T., Iimura M., Hamura K., Fukuoka S.I., Lowe A.W., Itoh K., Kiyono H. and Ohno H. (2009). Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. Nature 462, 226-U101.

Hashimoto M., Ikegami T., Seino S., Ohuchi N., Fukad H., Sugiyama J., Shirakawa M. and Watanabe T. (2000). Expression and characterization of the chitin-binding domain of chitinase A1 from *Bacillus circulans* WL-12. J. Bacteriol. 182, 3045-3054.

Henrissat B. and Davies G. (1997). Structural and sequence-based classification of glycoside hydrolases. Curr. Opin. Struct. Biol. 7, 637-644.

Hill D.J. and Virji M. (2003). A novel cell-binding mechanism of *Moraxella catarrhali*s ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. Mol. Microbiol. 48, 117-129.

Hill D.J., Toleman M.A., Evans D.J., Villullas S., van Alphen L. and Virji M. (2001). The variable P5 proteins of typeable and non-typeable *Haemophilus influenzae* target human CEACAM1. Mol. Microbiol. 39, 850-862.

Iseli B., Boller T. and Neuhaus J.M. (1993). The N-terminal cysteine-rich domain of tobacco class-I chitinase is essential for chitin-binding but not for catalytic or antifungal activity. Plant Physiol. 103, 221-226.

Jagmann N., Brachvogel H.P. and Philipp B. (2010). Parasitic growth of *Pseudomonas aeruginosa* in co-culture with the chitinolytic bacterium *Aeromonas hydrophila*. Environ. Microbiol. 12, 1787-1802.

Johnson J.R. (1991). Virulence factors in *Escherichia coli* urinary tract infection. Clin. Microbiol. Rev. 4, 80-128.

Jude B.A., Martinez R.M., Skorupski K. and Taylor R.K. (2009). Levels of the secreted *Vibrio cholerae* attachment factor GbpA are modulated by quorum-sensing-induced proteolysis. J. Bacteriol. 191, 6911-6917.

Kammerer R., Popp T., Hartle S., Singer B.B. and Zimmermann W. (2007). Species-specific evolution of immune receptor tyrosine based activation motif-containing CEACAM1-related immune receptors in the dog. BMC Evol. Biol. 7, 196.

Kaper J.B., Morris J.G. and Levine M.M. (1995). Cholera. Clin. Microbiol. Rev. 8, 48-86.

Kasprzewska A. (2003). Plant chitinases - Regulation and function. Cell. Mol. Biol. Lett. 8, 809-824.

Kawada M., Chen C.C., Arihiro A., Nagatani K., Watanabe T. and Mizoguchi E. (2008). Chitinase 3-like-1 enhances bacterial adhesion to colonic epithelial cells through the interaction with bacterial chitin-binding protein. Lab. Invest. 88, 883-895.

Kenny B., DeVinney R., Stein M., Reinscheid D.J., Frey E.A. and Finlay B.B. (1997). Enteropathogenic *E-coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell 91, 511-520.

Kirn T.J., Jude B.A. and Taylor R.K. (2005). A colonization factor links Vibrio cholerae environmental survival and human infection. Nature 438, 863-866.

Kzhyshkowska J., Gratchev A. and Goerdt S. (2007). Human chitinases and chitinase-like proteins as indicators for inflammation and cancer. Biomark Insights 2, 128-146.

Labadaridis I., Dimitriou E., Theodorakis M., Kafalidis G., Velegraki A. and Michelakakis H. (2005). Chitotriosidase in neonates with fungal and bacterial infections. Arch. Dis. Child.-Fetal Neonatal Ed. 90, F531-F532.

Lee C.G., Da Silva C., Dela Cruz C.S., Ahangari F., Ma B., Kang M.J., He C.H, Takyar S. and Elias J.A. (2010). Role of chitin, chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu. Rev. Physiol. 73, 479-501.

Leisner J.J., Larsen M.H., Jorgensen R.L., Brondsted L., Thomsen L.E. and Ingmer H. (2008). Chitin hydrolysis by *Listeria* spp., including L. monocytogenes. Appl. Environ. Microbiol. 74, 3823-3830.

Manos J., Arthur J., Rose B., Bell S., Tingpej P., Hu H., Webb J., Kjelleberg S., Gorrell M.D., Bye P. and Harbour C. (2009). Gene expression characteristics of a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* during biofilm and planktonic growth. FEMS Microbiol. Lett. 292, 107-114.

Martin H.M., Campbell B.J., Hart C.A., Mpofu C., Nayar M., Singh R., Englyst H., Williams H.F. and Rhodes J.M. (2004). Enhanced *Escherichia coli* adherence and invasion in Crohn's disease and colon cancer. Gastroenterology 127, 80-93.

Martinez-Medina M., Aldeguer X., Lopez-Siles M., Gonzalez-Huix F., Lopez-Oliu C., Dahbi G., Blanco J.E., Blanco J., Garcia-Gil L.J. and Darfeuille-Michaud A. (2009a). Molecular diversity of *Escherichia coli* in the human gut: New ecological evidence supporting the role of adherent-invasive *E. coli* (AIEC) in Crohn's disease. Inflamm. Bowel Dis. 15, 872-882.

Martinez-Medina M., Mora A., Blanco M., Lopez C., Alonso M.P., Bonacorsi S., Nicolas-Chanoine M.H., Darfeuille-Michaud A., Garcia-Gil J. and Blanco J. (2009b). Similarity and divergence among adherent-invasive *Escherichia coli* and extraintestinal pathogenic *E. coli* strains. J. Clin. Microbiol. 47, 3968-3979.

Meibom K.L., Li X.B.B., Nielsen A.T., Wu C.Y., Roseman S. and Schoolnik G.K. (2004). The *Vibrio cholerae* chitin utilization program. Proc. Natl. Acad. Sci. USA 101, 2524-2529.

Mizoguchi A., Mizoguchi E. and Bhan A.K. (2003). Immune networks in animal models of inflammatory bowel disease. Inflamm. Bowel Dis. 9, 246-259.

Mizoguchi E. (2006). Chitinase 3-like-1 exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells. Gastroenterology 130, 398-411.

Morimoto K., Karita S., Kimura T., Sakka K. and Ohmiya K. (1997). Cloning, sequencing, and expression of the gene encoding *Clostridium paraputrificum* chitinase ChiB and analysis of the functions of novel cadherin-like domains and a chitin-binding domain. J. Bacteriol. 179, 7306-7314.

Nalin D.R., Daya V., Reid A., Levine M.M. and Cisneros L. (1979). Adsorption and growth of *Vibrio cholerae* on chitin. Infect. Immun. 25, 768-770.

Nell S., Suerbaum S. and Josenhans C. (2010). The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nat. Rev. Microbiol. 8, 564-577.

Neutra M.R., Mantis N.J., Frey A. and Giannasca P.J. (1999). The composition and function of M cell apical membranes: Implications for microbial pathogenesis. Semin. Immunol. 11, 171-181.

Orikoshi H., Nakayama S., Hanato C., Miyamoto K. and Tsujibo H. (2005). Role of the N-terminal polycystic kidney disease domain in chitin degradation by chitinase A from a marine bacterium, *Alteromonas* sp strain O-7. J. Appl. Microbiol. 99, 551-557.

Phalipon A. and Sansonetti P.J. (1999). Microbial-host interactions at mucosal sites. Host response to pathogenic bacteria at mucosal sites. Curr. Top. Microbiol. Immunol. 236, 163-189.

Rath H.C., Herfarth H.H., Ikeda J.S., Grenther W.B., Hamm T.E., Balish E., Taurog J.D., Hammer R.E., Wilson K.H. and Sartor R.B. (1996). Normal luminal bacteria, especially bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta(2) microglobulin transgenic rats. J. Clin. Invest. 98, 945-953.

Sadlack B., Merz H., Schorle H., Schimpl A., Feller A.C. and Horak I. (1993). Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene Cell 75, 253-261.

Salunkhe P., Smart C.H.M., Morgan J.A.W., Panagea S., Walshaw M.J., Hart C.A., Geffers R., Tummler B. and Winstanley C. (2005). A cystic fibrosis epidemic strain of *Pseudomonas aeruginosa* displays enhanced virulence and antimicrobial resistance. J. Bacteriol. 187, 4908-4920.

Sansonetti P.J. and Phalipon A. (1999). M cells as ports of entry for enteroinvasive pathogens: Mechanisms of interaction, consequences for the disease process. Semin. Immunol. 11, 193-203.

Sasaki M., Sitaraman S.V., Babbin B.A., Gerner-Smidt P., Ribot E.M., Garrett N., Alpern J.A., Akyildiz A., Theiss A.L., Nusrat A. and Klapproth J.M.A. (2007). Invasive *Escherichia coli* are a feature of Crohn's disease. Lab. Invest. 87, 1042-1054.

Sauter S. L., Rutherfurd S.M., Wagener C., Shively J.E. and Hefta S.A. (1993). Identification of the specific oligosaccharide sites recognized by type 1-firmbriae from *Escherichia-coli* on nonspecific cross-reacting antigen, ACD66 cluster granulocyte glycoprotein. J. Biol. Chem. 268, 15510-15516.

Sriramulu D.D., Nimtz M. and Romling U. (2005). Proteome analysis reveals adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis lung environment. Proteomics 5, 3712-3721.

Stover C.K., Pham X.Q., Erwin A.L., Mizoguchi S.D., Warrener P., Hickey M.J., Brinkman F.S.L., Hufnagle W.O., Kowalik D.J., Lagrou M., Garber R.L., Goltry L., Tolentino E., Westbrock-Wadman S., Yuan Y., Brody L.L., Coulter S.N., Folger K.R., Kas A., Larbig K., Lim R., Smith K., Spencer D., Wong G.K.S., Wu Z., Paulsen I.T., Reizer J., Saier M.H., Hancock R.E.W., Lory S. and Olson, M.V. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature 406, 959-964.

Suarez V., Staehelin C., Arango R., Holtorf H., Hofsteenge J. and Meins F. (2001). Substrate specificity and antifungal activity of recombinant tobacco class I chitinases. Plant Mol. Biol. 45, 609-618.

Suzuki K., Taiyoji M., Sugawara N., Nikaidou N., Henrissat B. and Watanabe T. (1999). The third chitinase gene (chiC) of *Serratia marcescens* 2170 and the relationship of its product to other bacterial chitinases. Biochem. J. 343, 587-596.

Svitil A.L., Chadhain S.M.N., Moore J.A. and Kirchman D.L. (1997). Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. Appl. Environ. Microbiol. 63, 408-413.

Swidsinski A., Ladhoff A., Pernthaler A., Swidsinski S., Loening-Baucke V., Ortner M., Weber J., Hoffmann U., Schreiber S., Dietel M. and Lochs H. (2002). Mucosal flora in inflammatory bowel disease. Gastroenterology 122, 44-54.

Terahara K., Yoshida M., Igarashi O., Nochi T., Pontes G.S., Hase K., Ohno H., Kurokawa S., Mejima M., Takayama N., Yuki Y., Lowe A.W. and Kiyono H. (2008). Comprehensive gene expression profiling of Peyer's patch M cells, villous M-like cells, and intestinal epithelial cells. J. Immunol. 180, 7840-7846.

Toleman M., Aho E. and Virji M. (2001). Expression of pathogen-like Opa adhesins in commensal *Neisseria*: genetic and functional analysis. Cell Microbiol. 3, 33-44.

Vaaje-Kolstad G., Horn S.J., van Aalten D.M.F., Synstad B. and Eijsink V.G.H. (2005a). The non-catalytic chitin-binding protein CBP21 from *Serratia marcescens* is essential for chitin degradation. J. Biol. Chem. 280, 28492-28497.

Vaaje-Kolstad G., Houston D.R., Riemen A.H.K., Eijsink V.G.H. and van Aalten D.M.F. (2005b). Crystal structure and binding properties of the *Serratia marcescens* chitin-binding protein CBP21. J. Biol. Chem. 280, 11313-11319.

Vaaje-Kolstad G., Westereng B., Horn S.J., Liu Z.L., Zhai H., Sorlie M. and Eijsink V.G.H. (2010). An oxidative enzyme boosting the enzymatic conversion of recalcitrant polysaccharides. Science 330, 219-222.

Vazquez-Boland J.A., Kuhn M., Berche P., Chakraborty T., Dominguez-Bernal G., Goebel W., Gonzalez-Zorn B., Wehland J. and Kreft J. (2001). *Listeria* pathogenesis and molecular virulence determinants. Clin. Microbiol. Rev. 14, 584-640.

Virji M., Makepeace K., Ferguson D.J.P., Achtman M. and Moxon E.R. (1993). Meningococcal opa and opc proteins- Their role in colonization and invasion of human epithelial and endothelial cells. Mol. Microbiol. 10, 499-510.

Watanabe T., Ito Y., Yamada T., Hashimoto M., Sekine S. and Tanaka H. (1994). The roles of the C-terminal domain and type III domains of chitinase A1 from *Bacillus circulans* WL-12 in chitin degradation. J. Bacteriol. 176, 4465-4472.

Watanabe T., Kobori K., Miyashita K., Fujii T., Sakai H., Uchida M. and Tanaka H. (1993). Identification of glutamic acid-204 and aspartic acid-200 in chitinase-A1 of *Bacillus circulans* WL-12 as essential residues for chitinase activity. J. Biol. Chem. 268, 18567-18572.

Xu J., Mahowald M.A., Ley R.E., Lozupone C.A., Hamady M., Martens E.C., Henrissat B., Coutinho P.M., Minx P., Latreille P., Cordum H., Van Brunt A., Kim K., Fulton R.S., Fulton L.A., Clifton S.W., Wilson R.K., Knight R.D. and Gordon J.I. (2007). Evolution of symbiotic bacteria in the distal human intestine. PLoS. Biol. 5, 1574-1586.

Zebhauser R., Kammerer R., Eisenried A., McLellan A., Moore T. and Zimmermann W. (2005). Identification of a novel group of evolutionarily conserved members within the rapidly diverging murine Cea family. Genomics 86, 566-580.

Accepted June 6, 2011