Potential roles of chitin in mucosal inflammation.

[1]Alan Kamba, [2]Yoshimi Shibata and **[1,3]Emiko Mizoguchi**

[1]Gastrointestinal Unit, Department of Medicine, Massachusetts General Hospital, Boston, MA, USA.
[2]Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA,
[3]Center for the Study of Inflammatory Bowel Disease, Massachusetts General Hospital, Boston, MA, USA.
Correspondence: Emiko Mizoguchi, M.D., Ph.D.
Massachusetts General Hospital, GRJ 825D, 55 Fruit Street, Boston, MA 02114, USA
Tel: (617) 726-7892; Fax: (617) 726-3673; E-mail: emizoguchi@partners.org

Chitin, a polymer of N-acetylglucosamine (GlcNAc) and the second most abundant polysaccharide in nature next to cellulose, is found in many organisms. For example, fungi, crustaceans, insects, amphibians and nematodes include chitin as structural components. In nature, chitin levels are regulated by the balance of chitin biosynthesis (by synthases) and degradation (by chitinases). Although chitin and chitin synthase do not exist in mammals, human chitinase family members (glycosyl hydrolase 18 family) have been identified. It has been well characterized that chitin has a size-dependent effect: Small sized (1-10 μm) but not large (>100 μm) or medium (40-70 μm) sized chitin particles can be recognized and processed by macrophages and dendritic cells, which subsequently enhance interferon γ as well as IL-10 productions in mesenteric lymph nodes and colon [1]. Interferon γ is a T helper type 1 (Th1) cytokine and has an immunoregulatory role while IL-10 is classified as an anti-inflammatory cytokine. These cytokines effectively suppress the over-production of Th2 cytokines such as IL-4/IL-5/IL-13, and appear to have reversed Th2-associated immune responses in animal models of acute/chronic inflammation. Some patients with immunocompromised conditions are vulnerable to infections, which are called opportunistic infections. These infections are caused by various pathogenic organisms, including bacteria, fungi, viruses and parasites. Although bacteria do not contain chitin, it has been previously reported that bacterial chitin-binding proteins play an important role in host-microbial interactions [2]. In addition, through the chitin-binding proteins, bacteria may also efficiently interact with chitin-containing organisms such as fungi and parasites. In particular, bacterial and fungal co-infections sometimes cause chronic inflammation and/or severe septic conditions in immunocompromised hosts and may play a role in the initiation/exacerbation of inflammatory bowel disease and other inflammatory conditions. The study of chitin-mediated innate and acquired immune networks may provide the rational for the development of new therapeutic strategies for infection-based mucosal inflammation.

Keywords chitin; infection, mucosal inflammation

1. Introduction

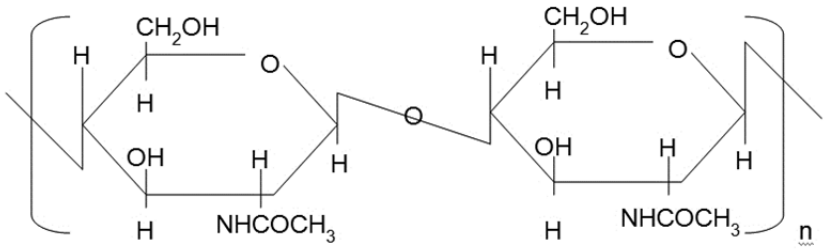
Chitin is a skeletal cell polysaccharide that is a component of the inner cell wall of fungi, and has been the target of anti-fungal drugs [3, 4]. Host defense against chitin-containing pathogens is mediated by the interaction between chitin as a pathogen-associated molecular pattern (PAMP) and the host chitin-binding proteins/receptors as pattern recognition receptors (PRRs), which include mannose receptor family, toll-like receptors (TLRs), and chitinases/chitinase-like proteins. Furthermore, among commensal bacteria and fungi, these chitin-chitinase interactions would also regulate the host defense and inflammatory status. Use of purified chitin has significantly contributed to the understanding of not only the host defense mechanisms against chitin-containing pathogens, but also the development of potential anti-inflammatory therapies with selected chitin forms.

In the mid 80s, a series of studies by Azuma and colleagues suggested that some chitin/chitosan derivatives are able to up-regulate macrophage functions (measured by TNF-α, IL-1, and colony-stimulating factor production) and induce immunologic adjuvant effects [5-7]. In the late 90s, Shibata and colleagues found that chitin microparticles (CMPs) at 1-10 μm diameters, functioned as "mimetic microbes", and induced classically activated (M1) macrophages *in vitro*. Since the M1 activation is mediated by internalization/phagocytosis of CMP, neither unphagocytosable large chitin beads at 40 –100 μm (LCBs), chitosan (de-acetylated form of chitin) microparticles at 1-10 μm (CsMPs), soluble chitosan, nor soluble chitin induce M1 activation *in vitro* [8-10]. Oral and intranasal administrations of CMPs in asthma models in experimental animals proved to be effective in suppressing allergic inflammation [11, 12]. Recently, the oral administration of CMPs ameliorated the histological severity of colitis and increased productions of the Th1 cytokine interferon gamma (IFNγ) in two mouse models of colitis [1]. Mucosal administration by oral gavage of chitin products is an attractive route of choice for inflammatory disease therapy. However, it is of particular importance that recent studies [13-18] have suggested that anti-inflammatory efficacy of chitin treatments seemingly depends on forms of chitin, such as insoluble/soluble, acetylated/de-acetylated, and phagocytosable/non-phagocytosable sizes of particles. Therefore, appropriate chitin preparations provide many new avenues of study that could lead to new methodologies for the modulation of various inflammatory diseases including allergic disorders.

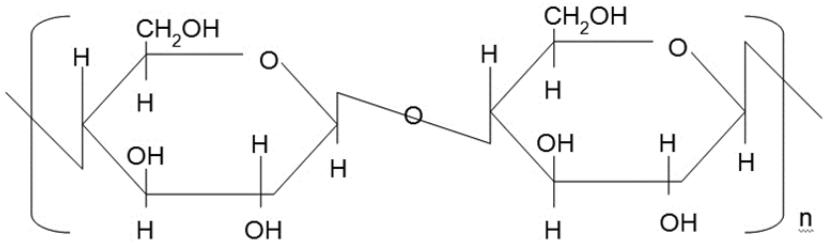
In this chapter, we will summarize the significant progress in this field, which has been made since the 90s studies, and we will update some important findings based on the key publications.

2. What are chitin and chitinases?

Chitin is the second most abundant biologically synthesized polymer found in nature. It is produced as a structural component in fungi, mollusks, cephalopods, and arthropods: including insect and crustacean exoskeletons [19]. Chitin is highly insoluble and forms a hard protective barrier for many organisms. Chitin is a polymer of β-1,4 N-acetylglucosamine, which is a derivative of glucose, and has a chemical structure similar to cellulose. However functionally, it is more comparable to keratin than cellulose. Figure 1 demonstrates the chemical structure of chitin and cellulose for comparison.



Chitin



Cellulose

Fig. 1 Chemical Structure of Chitin and Cellulose

Chitin has a very low immunogenicity even though it is rich in nitrogen [20]. Chitin's physical properties make it useful in medicine as a surgical suture as it accelerates wound healing and the high biodegradability allows the sutures to dissolve over time. Chitin sutures resist degradation by bile, urine, and pancreatic enzymes, which is an issue with other degradable sutures [21]. Chitin is easily obtained from crab and shrimp shells as well as fungal mycelia at a relatively low cost, which makes it an attractive compound.

While chitin synthesis is regulated by chitin synthases, its degradation is a result of enzymatic chitinases. These chitinases break the glycosidic bonds to form monomers of N-acetylglucosamine. Chitinases are used by bacteria to dissolve the chitin found in fungi or insects [22]. This is useful for bacteria in defense against invading fungi, or as an active mechanism of infection. Several plants also possess chitinases as a host defense against invading fungi and herbivorous insects [22].

Although mammals do not possess chitin as a structural component, they can produce several enzymatically active and inactive chitinases. The enzymatically active chitinases include acidic mammalian chitinase (AMCase) and chitotriosidase. Mammalian chitinases play important roles in several diseases including malaria, parasitic, and fungal infections. The enzymatically inactive chitinases, which can attach to chitin but do not possess hydrolytic capabilities, include the molecule chitinase 3-like 1 (CHI3L1), which has been found to be elevated in plasma in several inflammatory diseases including inflammatory bowel diseases (IBD), asthma, and rheumatoid arthritis and is used as a sensitive biomarker in these diseases [23-25]. It has also been found to be elevated in several solid tumors, including breast cancer, colorectal cancer, glioblastoma and malignant melanoma, extracellular myxoid-chondrosarcoma, and Hodgkin's lymphoma and is associated with a poor prognosis [26]. CHI3L1 is quickly becoming an area of research interest as it is being found to play a key inflammatory role in many disease states in several inflammatory disorders and malignant tumors [26].

3. Size dependent effect of chitin and its potential receptors

High molecular weight chitin is known to be non-toxic, nonallergenic, non-immunogenic, biodegradable, and biocompatible. Studies done over 30 years ago demonstrated that preparations of deacetylated chitin and carboxymethyl-chitin induced cytotoxic macrophages, stimulated anti-tumor effects, and conferred non-specific host

resistance to *E. coli* by increasing production of hydrogen peroxide [27]. Chitin was also found to induce inflammatory cytokines including IL-1β and granulocyte-macrophage colony-stimulating factor (GM-CSF), and IFNγ *in vivo* [28].

In contrast, other studies have demonstrated exposures to chitin-containing organisms tend to shift to a Th2 dominant host response. Intrapulmonary administrations of suspended chitin stimulated recruitment of IL-4 positive cells into the lung [17]. IL-4 is known as an inducer of naïve helper T cells into Th2 cells [17]. This study and others have led to speculation of the high incidence of asthma among workers with high environmental exposure to chitin, such as commercial fishers of shrimp and clams [29]. In this regard, larger non-phagocytosable chitin beads (>40 μm) induce innate immunity supporting Th2/allergic responses, when given intraperitoneally [18]. It is well established that mucosal or intra-peritoneal administration of Sephadex beads (cross-linked dextran) at similar sizes (>40 μm) also induces local eosinophilia [30-32]. Since chitin bead- or Sephadex bead- induced innate immune responses are not seen in macrophages cultures in vitro with these beads, additional cells would contribute to the development of *in vivo* innate immune responses. Furthermore, the *in vivo* innate immune response, which supports Th2/allergic response, can be induced by >40 μm beads other than chitin (>40 μm); therefore, the chitin chemical composition is not required for this type of immune response.

This seeming discrepancy has been elucidated through recent studies into different chitin preparations. It has been shown that large (>100μm) sized pieces of chitin are inert. Intermediate sized (40-70μm) chitin stimulated macrophage IL-17A production and receptors, and induced inflammation via the TLR2 MyD88 pathway [33]. Small sized chitin (<40 μm, usually 1-10 μm) induced production of IL-10, an anti-inflammatory cytokine. It was further shown that intermediate chitin binds to and activates the TLR2 pathway cascading to NF-κB and TNFα, while small chitin binds to and activates dectin-1 cascading through spleen tyrosine kinase (Syk) to IL-10 [34]. However, there is some co-activation of TLR2 and Syk pathways by both preparations of chitin. TLR2 is one of the PRRs, which binds to many stimuli including gram positive bacterial lipoteichoic acid (found in the cell wall), fungi, certain viruses including herpes simplex and varicella zoster, and some endogenous molecules. The specific fungal component recognized by TLR2 is yet to be discovered, but it is speculated to be a glycolipid. The TLR2 pathway in hematopoietic cells has been well characterized and is known to be pro-inflammatory, leading to production of NF-κB [35]. Dectin-1 recognizes the β-glucan in fungal cell walls and leads to activation of Syk kinase and NF-κB as well as small amounts of IL-10 [36]. Studies have also demonstrated that TLR2 and dectin-1 work in conjunction to enhance the signals from each receptor [36]. These results suggest that intermediate sized chitin produces a Th2 dominant response, while small chitin creates a Th1-dominant response. However, it is still remained to be studied whether the size-dependent and differential immunological responses to chitin particles require the chitin chemical composition.

It was recently shown that mouse lacking the gene encoding the dectin-1 had increased susceptibility to chemically induced colitis [37]. This result demonstrates the necessity of the dectin-1 receptor in modulating immune responses.

4. Chitin-mediated immuno-biological effects in lung

When mice were injected with chitin (presumably unphagocytosable-sized), IL-4-expressing innate immune cells, including eosinophils and basophils, were induced and had accumulated in lung tissue [17]. This effect was reversed when the chitin was pre-treated with AMCase or when the mice overexpressed AMCase. Overall, unphagocytosable-sized (>40 μm) chitin was described to attract infiltration of innate immune cells involved in immunity to helminth infection and allergies. In sharp contrast, intravenous injection of fractionated CMPs (1-10 μm) had an immune-stimulating effect on alveolar macrophages, inducing production of inflammatory cytokines IL-12, TNF-α, and IL-18, and natural killer cell productions of IFNγ [8].

A study showed when mice were aspirated with intermediate sized chitin, macrophage production of IL-17A and IL-17A receptors was increased in addition to other pro-inflammatory cytokines IL-12p70 and IL-23, which are closely linked with IL-17, chemokine (C-C motif) ligand 5 (CCL5) which is a chemotactic factor that recruits leukocytes into areas of inflammation and activates certain natural killer cells, and chemokine (C-X-C motif) ligand 2 (CXCL2) which is chemotactic for polymorphonuclear leukocytes and hematopoietic stem cells and has been correlated with tumor metastasis and chemotherapy resistance [33, 38]. When mice were aspirated with large sized chitin, no effect was seen.

Oral administration of CMP was also found to inhibit Th2 immunity including IgE production and eosinophil infiltration in lungs of mice [11]. Ragweed (an allergic inducer) stimulation unconjugated with CMP stimulation resulted in significant decreases of Th2-derived cytokines IL-4, IL-5, and IL-10 levels and increased the production of IFNγ in spleen cells, suggesting that the immune responses were redirected toward a Th1 response [11]. Histologically, CMP treatment inhibited the peribronchial, the perivascular, and total lung inflammation as compared with saline control.

While the above study used orally administration method, intranasal administration directly to the lung tissue of CMPs were found to decrease the symptoms of allergic hypersensitivity to various antigens including *Dermatophagoides pteronyssinus* and *Aspergillus furmigatus* [12]. In this study, CMP treatment also reduced IgE serum concentrations, lung inflammation, airway hyperresponsiveness, and eosinophilia in peripheral blood. Cytokine levels of IL-12, IFNγ, and TNFα were increased after CMP treatment while IL-4 was significantly reduced. Chitosan, which is the deacetylated form of chitin and water soluble, also reduced allergen-induced inflammation [39]. These

studies demonstrate that CMP can reduce allergic inflammation in lung tissue, and promote a Th1 immune response, while intermediate sized chitin can induce inflammatory cytokines/chemokines to further promote inflammation. However, the pro-inflammatory effect of chitin particles would not specifically require the chitin chemical composition, since similar sizes of particles with other carbohydrates promote Th2 inflammation [17, 18].

5. Chitin-mediated immunoregulation in colon

In the context of IBD, the oral administration of CMPs improved the histological severity of colitis and increased production of the Th1 cytokine IFNγ in two mouse models of chronic colitis [1]. The dextran sodium sulfate (DSS) colitis model induces acute epithelial injury and ulceration in the colon while the T cell receptor-alpha chain knockout (TCRα KO) model causes spontaneous chronic colitis which resembles ulcerative colitis [40, 41]. In both models, mice were pre-treated with orally gavaged CMPs (1.5 mg/day) once per 3 days, beginning at weaning, for 6 consecutive weeks. The control group was fed phosphate-buffered saline (PBS). Then, the mice were treated with DSS or PBS for 5 days, and their recovery and bodyweight were recorded for an additional 7 days. Mice receiving CMPs regained more bodyweight at days 8-12 and showed improved recovery by clinical scores and histology. It was found that mouse dendritic cells efficiently phagocytosed CMPs after co-incubation and are responsible for their processing. IL-4 production was also significantly reduced when mice were treated with CMPs. The level of TNFα was significantly reduced in the mesenteric lymph nodes of mice from both models of colitis treated with CMPs [1]. Furthermore, CMP-treated mice showed increased production of IL-10 in the colon and marginally enhanced the recruitment of IL-10-producing cells as compared with PBS-treated control. The increase in IL-10-expressing cells was more significantly increased in non-inflamed areas as compared with inflamed regions of the colon. IL-10 may be partially involved in the CMP-mediated immunoregulatory functions in acute intestinal inflammation.

The total bacterial load in the proximal colon was reduced after CMP treatment. *Clostridiales* group of bacteria including *Clostridium, Eubacterium, Epulopicium, Dorea,* and *Ruminococcus* were reduced in mice receiving PBS while their quantity remained unchanged in CMP-treated mice suggesting an improvement in commensal bacterial composition. CMP-treatment improved intestinal barrier function as the level of enteric bacteria which translocated from the colon to various organs, including spleen and liver was much more reduced in the CMP-treated mice as compared to PBS-treated control mice [1].

Taken together, this study suggests chitin can have immune-protective, anti-inflammatory, and anti-microbial effects in the intestines depending on the size of the particles ingested.

6. Fungal infection and intestinal inflammation

Most focus in IBD research has been on the specific bacterial strain(s) involved in the pathogenesis and exacerbation of inflammation. Efforts into identifying fungal pathogens have generally received less attention; however, the topic appears to have gained recent interest with the works of several publications exploring fungi in IBD. The most thoroughly studied involvement is with anti-*Saccharomyces cerevisiae* antibodies (ASCA), which gained considerable attention as levels are significantly increased in CD patients, and as such makes it a useful diagnostic marker [42]. Patients positive for ASCA's and negative for perinuclear antineutrophil cytoplasmic autoantibodies (ANCA), which are used in diagnosing UC, yielded a specificity and positive predictive value of 97% and 96% respectively for CD [43]. Another study showed that ASCA levels differentiate between CD and UC, and correlate with CD disease severity and the need for surgery [44]. In this same study, ASCAs were shown to have a high specificity for CD. However they have a low sensitivity, and may not be very useful alone in differentiating IBD's [44]. While it can be hypothesized that *Saccharomyces cerevisiae* is responsible for the development of ASCAs, it was shown that *C. albicans* is capable of cross-reacting with ASCAs obscuring the true cause [45]. The presence of ASCA, in conjunction with other biomarkers, may predict future disease manifestation. Elevated ASCA levels were found 4.5 and 4.4 years before CD and UC diagnosis respectively [46]. It has been suggested that ASCA's can trigger auto-immunity, as increased levels have been found in several auto-immune disorders such as type 1 diabetes and rheumatoid arthritis. A recent study found a 50%-100% overlap between *Saccharomyces* cell wall component mannan and other well known auto-immune inducing molecules using molecular structure analysis [47].

Genetic affinity analysis of oral fungi in IBD patients demonstrated a 100% match with *C. albicans* found in colonic mucosal biopsy, colonic aspirate, brush smears, and fecal samples, suggesting oral transmission to lower segments of the intestine [48]. Normally *C. albicans* is a commensal fungi when remaining in the yeast form. However, through a change in secreted factors and molecular pathway activations it can convert to a pathogenic form with filamentous hyphae. In an experimental model of colitis, DSS-treatment strongly promoted *C. albicans* colonization compared with controls which received fungi inoculation only [49]. In addition, *C. albicans* exacerbated inflammation as assessed by histology scores and myeloperoxidase/TNF-α productions and TLR-2 expressions in the colon. Interestingly, chitin can bind to TLR-2 and elicit an inflammatory cascade. *C. albicans* colonization also led to generation of ASCAs. Mice

deficient in Galectin-3, which binds to *C. albicans* glycans, had attenuated inflammatory responses and abolished TNF-α and TLR-2 responses in DSS-induced colitis [49].

Other pathogenic fungi including *Candida tropacalis* which is an opportunistic pathogen that induces inflammation in mice deficient in *Clec7a*, which is the gene that codes for the dectin-1 receptor, a receptor that recognizes fungal β-1,3-glucans. Polymorphisms of this gene for this receptor have been associated with UC [37]. Other receptors recognizing fungi include TLR-4 which can bind to O-linked mannan fungal cell components, Galectin-3 receptor that recognizes β-mannosides also found in fungal cell walls, and TLR-9 which can bind to fungal CpG DNA [50]. *C. glabatra* is an opportunistic pathogen found in biofilms in catheters and dentures [51] and has been hypothesized to play a role in IBD as increased levels were found in UC and CD patients [48]. *C. krusei* is also an opportunistic pathogen, causing candidasis and blood infection in immunocompromised and those undergoing chemotherapy [52], and has been hypothesized to play a role in IBD as levels of this fungi were increased in IBD patients [48]. *Trichosporon* is another opportunistic pathogen with increased levels found in colitis-induced *Clec7a* KO mice [37]. We have summarized the fungi that have been associated with IBD in Table 1.

Table 1 Fungal Strains associated with IBD

Fungi	Associated Diseases	References
Candida albicans	UC, CD	48, 49
Candida glabatra	UC, CD	48
Candida krusei	UC, CD	48
Candida tropicalis	UC	37
Saccharomyces cerevisiae	UC, CD	42, 43, 46, 47
Trichsporon	UC	37

7. Bacterial chitinases and chitin-binding proteins

Many bacteria produce chitinases as a way to defend against fungal pathogens, and to digest chitin-containing organisms for food. Some examples of bacteria which produce chitinases include *Serratia marcescens*, one of the most efficient chitin degrading organisms, with different types of chitinases being reported from several strains of this species, such as ChiA, ChiB, and ChiC and a putative chitin-binding protein (CBPs) named CBP21 which is also a part of the chitinases [53]. Chitinases are highly expressed in many marine bacteria as this environment contains large amounts of chitin. Importantly, most chitinase-producing microorganisms contain a gene encoding for the homologue of CBP21, suggesting the potential binding ability of bacteria to chitin. The chitin-binding domains (CBD) interactions with chitin-containing organisms are preserved with mammalian chitinase-like proteins including CHI3L1. Therefore, it is likely that the CBD found within the bacterial chitinase, binds to the human CHI3L1 which then allows for more intimate interactions and ultimately allows the bacteria to enter the cell cytoplasm [54].

Bacterial chitinases and CBPs seem to play a pathogenic role in certain bacterial infections. Recent studies in *Vibrio cholerae* and *Pseudomonas aeruginasa* indicate that CBPs promote the attachment of bacteria to human cells through interactions with mucin during the initial stage of colonization [55-57]. The chitinase found in *Salmonella typhimurium*, whose role in human infection was unknown, was found to interact with endogenously expressed CHI3L1 and enhanced the invasion of bacteria across the intestinal mucosal layer into host cells [2]. When non-pathogenic *E. coli* was substituted with the CBP from *S. marcescens,* it significantly increased the ability of the *E. coli* to adhere to human colon cells, further suggesting this particular CBP is crucial for bacterial pathogenicity [58]. In general for IBD, the microbiome in the gut is shifted to promote more pathogenic bacteria (e.g. *E. coli* and *S. marcescens*) with a reduction in commensal bacteria (e.g. bifidobacteria), this phenomenon is also known as dysbiosis [59]. It is likely that the pathogenic bacteria express chitinases, and may increase their expression under favorable conditions to further promote infection. Adherent invasive *E. coli* (AIEC), which has been associated with IBD and produces a chitinase named ChiA, was also found to bind to colonic epithelial cells, which expressed CHI3L1, and promotes the attachment to those cells [54]. It was also shown that there are 5 critical amino acids necessary for proper ChiA function in interacting with CHI3L1 and disruptions in the amino acid sequence alters the invasion capabilities of AIEC [54]. This suggests that a specific 5 amino acid sequence in ChiA-CBD is partially involved in adhesiveness following invasiveness on/into colonic epithelial cells.

8. Fungal and bacterial co-infections in oral mucosal tissues.

A novel topic in infectious immunology is the presence of fungal-bacterial co-infections in which the two organisms produce a more effective and resistant infection. These infections can evade immune cells and standard anti-biotic treatments by forming a thick impenetrable biofilm. In this mixed environment, anti-biotics can only target exposed bacterial cells, as well as leaving fungal cells unaffected. *Streptococcus gordonii*, which is a normal colonizer of the oral cavity, augments *C. albicans* survival, which contributes to a persistent fungal infection. In the oral cavity, *S. gordonii* adheres to host cells by expressing the conserved SspA and SspB (antigen I/II family) cell wall-anchored proteins that recognize a range of host tissue proteins and cellular receptors [60]. Interestingly, one of the polysaccharides involved in binding of *S. gordonii* and *Streptococcus oralis* to *C albicans* includes N-acetylglucosamine, a monomer of chitin [61]. After the bacteria attach to host cells, *C. albicans* then can adhere to the host-bound bacteria by means of protein-protein interactions [61] or by direct interaction of salivary proteins (basic proline-rich proteins, bPRPs) which had previously attached to *S. gordonii* cells [62]. This aggregation of bacteria and *C. albicans* enhances biofilm formation and consequently the two organisms increase in proximity promoting bacteria-fungal cell communication.. Through a diffusible signal molecule, *S. gordonii* prevents the inhibition of hyphae, which are filamentous formations associated with virulence, therefore altering the phenotype to an invasive form through enhancing biofilm formation of *C. albicans* [59]. In addition, *S. gordonii* promotes *C. albicans* growth by releasing metabolic by-products that can be used as a food source by *C. albicans* [63]. Likewise, *C. albicans* enhances the survival and colonization of *S. gordonii* by reducing the oxygen concentration to more preferable levels and by providing bacterial growth stimulatory factors. [62, 63]. This mutually beneficial environment promotes the formation of mature fungal-bacterial biofilms surrounded by an extracellular matrix. Interestingly, additional bacteria and fungi can attach to this extracellular matrix further promoting infection. We have summarized these interactions in the oral cavity in Figure 2. Interestingly, co-incubation of *S. gordonii* and *C. albicans* also led to the activation of mitogen-activated protein kinase Cek1p, which is involved in morphogenesis [60] demonstrating activation of this pathway which may lead to a more invasive form. These interactions may make oral infections more persistent and harder to treat. [64]

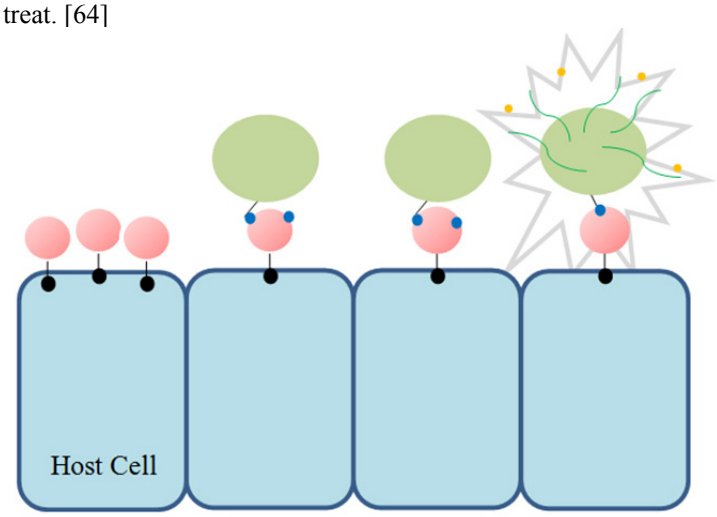


Fig. 2 Demonstration of bacterial fungal co-infection in the oral cavity

First Bacteria such as *S. gordonii* attach to host cells through the use of bacterial cell surface polypeptides which bind to host cellular proteins and receptors (shown as black dots). Next, the bacteria adsorb host salivary proteins (basic pro-line rich proteins, shown as blue dots) which then allow fungi such as *C. albicans* to attach to the bacterial cell forming a chain. Through intimate interactions involving release of diffusible molecules, *S. gordonii* mediates *C. albicans* hyphae formation and subsequent phenotypic changes to an invasive form. This aggregation of bacterial and fungal cells forms a protective layer of extra-cellular matrix which then has the potential to bind other micro-organisms (shown as orange dots). Bacteria are represented by the red circles and fungi by the green circles.

It was also shown in the oral cavity that greater numbers of *Streptococcus mutans* cells bound to *C. albicans* cells with glucans present on their surface than to yeast cells without surface glucans, demonstrating this component as playing a key role in attachment. Surface-bound glucosyltransferases promote the formation of a glucan-rich matrix in situ and may enhance the accumulation of *S. mutans* on the tooth enamel surface, thereby enhancing the development of infectious biofilms. [65]

Overall, fungal-bacterial infections may play a prominent role in persistent infections of the oral cavity and may require anti-fungal/anti-biotic treatments.

9. Fungal and bacterial co-infections in digestive tract and the other organs and a potential involvement of fungal and bacterial co-infections during the development of inflammation.

Fungal-bacterial co-infections have also been implicated in several other mucosal tissues including the lungs, esophagus, urinary tract, and intestines/digestive tract. According to a 2012 review, there have been no *in vivo* models of bacteria-fungi co-infections so this is a research topic that has been minimally investigated. [66]

The presence of *Streptococci oralis/sanguinis/gordonii* increased the ability of *C. albicans* to invade organotypic models of the oral and esophageal mucosa under conditions of salivary flow. The authors developed a novel oral mucosal analogue that was created to simulate the biology of the oral/esophageal cavity. It was observed that *C. albican*s and *streptococci* formed a synergistic partnership where *C. albicans* promoted the ability of *streptococci* to form biofilms on abiotic surfaces or on the surface of an oral/esophageal mucosa analogue. [67].

Fungal-bacterial co-infections have also been implicated in the lung tissue. Critically ill patients receiving mechanical ventilation for > 2 days were assessed for presence of *candida* in lung tissue, and it was found to be associated with prolonged Intensive Care Unit and hospital stays, longer time on mechanical ventilation, and with an increased risk of *P. aeruginosa* ventilator-associated pneumonia. Extra-pulmonary *candida* colonization was also significantly increased in patients exposed to the fungi versus unexposed. The presence of *candida* in lung predisposes the patient for pseudomonas associated pneumonia suggesting the use of anti-fungals in this population [68]. While this study did not prove the presence of co-infection or co-aggregation it strongly supports the notion, although another study did find *P. aeruginosa,* and *S. marcescens* did coaggregrate when added to a *C. albicans* preformed biofilm and increased in number in a polystyrene tube model to simulate patient ventilators [69].

In the context of the colon, it was shown that oral *C. albicans* can travel from the oral cavity to the colonic mucosa and possibly disseminate fungi to sites favorable for multiplication, and may increase colonization in active phase of IBD [48]. Interestingly, it was proven that the *candida* in both oral cavity and colon were genetically identical, therefore it is possible transformed-invasive *C. albicans* can travel from oral cavity through the GI and invade colonic tissues.

It was shown that prior urinary tract infection with *E. coli*, which was found to agglutinate *C. albicans in vitro*, was found to enhance adhesion of inoculated *C. albicans* to bladder mucosa of rats and increased the likelihood of ascending infection by *C. albicans* [70]. This interaction was demonstrated as an adherence of *C. albicans* to preattached *E. coli*.

C. albicans Als1, Als3, and Als5 (Agglutination like Sequence 1,3,5) which are cell surface proteins that are classified as adhesins and play a critical role in biofilm formation [71]. They also are attachment site proteins that mediate aggregation with bacteria and yeasts [72]. Interestingly, these proteins have also been associated with *C. albicans* aggregation and fungal attachment to human embryonic endothelial cells, as well as fungal filamentation, and virulence as assessed by a murine model of haematogenously disseminated candidiasis. To prove the critical function of Als1, mice infected with *candida* containing a defect in Als1 had a significantly higher survival for the first 5 days [73]. Furthermore, in *S. Cerevisae* that were cloned to express Als1 from *candida*, it was proven to mediate cell attachment to oral epithelial cells [74] as well as fungal endocytosis into umbilical endothelial cells [75]. Therefore these attachment proteins may be critical for fungal-bacterial-host cell attachments.

While this chapter has focused on synergistic relationships between fungi and bacteria, it is also likely that adversarial chemical warfare between *candida* and *lactobaccili* occurs in various tissues including in the female reproductive tract, which the *lactobaccili* produce factors such as surfactants, lactic acid, bacteriocin-like substances which inhibit microbial growth, and hydrogen peroxide to combat the fungi [76] these and other secretions may lead to increased toxin production and host inflammation in human disease [64]. Overall, the study of fungal-bacterial co-infections in mucosal tissues has been deficient and its role in human infection and disease unappreciated.

Although fungal-bacterial co-infections have yet to be implicated in IBD, due to the large amount of casual and correlative evidence, it is very likely that they play a role in either the initiation and/or exacerbation of IBD and inflammation. Within this chapter, we have defined the individual fungi and bacteria that have been shown to play a role within IBD, and explained how some of these microorganisms aggregate and form co-infections within other mucosal tissues. Studies in this field have been dampened by the lack of an effective *in vivo* model of co-infections which could be used to investigate their presence within the intestinal tract. To date, the investigation of the role of fungi into IBD has also been lacking. The recent paper by Illiev et al demonstrated the crucial role of Dectin-1 receptor in IBD and characterized some fungi strains including *C. albicans* and *C. tropicalis* that increased under DSS treatment. However in-depth mechanistic studies of fungi interactions in the intestinal mucosa under inflammatory conditions have yet to be performed.

The pathogenesis of IBD is still not completely clear, but is thought to result partially from altered host responses to antigens and gut dysbiosis. A study done on rats demonstrated that mixed lysates of *P. aeruginosa* and *Candida* promoted a Th1 dominant response, which consisted of cell-mediated immunity and a lack of antibody/B cell response, which was associated with neutrophilia and diminished mucus production in the lungs. It was also demonstrated that alone, fungi infection promotes a Th2-response and bacteria promote a Th1-response, so this demonstrates mixed infection also causes a deviation in immune response [77].

Since fungi contain chitin as a structural component it is also likely that this allows for interaction via chitinases and chitinase-like-proteins such as CHI3L1 which is induced through inflammation/cancer and was shown critical in bacterial attachment to host cells. Upregulated expression of CHI3L1 in host may mediate increased interactions with fungi-bacterial biofilms. It is also possible that bacterial chitin-binding proteins can also bind directly with fungi and this plays a role in their interactions.

Two bacteria that have been associated with IBD, *E. coli* and *S. marcescens*, have both been shown to strongly interact with *candida*. Although these studies were done in another mucosal tissue and *in vitro*, these interactions are likely possible to exist within the intestinal tract, too.

No direct link between fungi-bacterial co-infections currently exists for IBD, however circumstantial evidence points to a strong possibility. Further studies in this area including clinical trials would shed light on the prevalence and mechanisms of this complex microbial infection.

10. Current and future clinical utilization of chitin in inflammatory diseases.

Chitin is similar in structure to cellulose comprising the horny substance in the exoskeletons of crabs, shrimp, and insects as well as fungi. Because some of its derivatives are proved to be nontoxic, biodegradable, biocompatible and inexpensive, prostheses (artificial skin matrix, contact lens, and surgical stitch) produced from the chitin derivatives have been developed and widely used in medical practice. For centuries, chitin has been safely used as an effective ingredient in Chinese anti-inflammatory medicine [78, 79]. Chitin has been tested as gel and scaffold forms for tissue engineering [80] and as a carrier for drug delivery [15], and is readily available in health food stores in the US. Global chitin sales are anticipated to exceed $63 billion in the next 3 years based on the information of the Global Industry Analysts in June, 2012. Clinical trials have tested intranasal CMP on allergic rhinitis, intranasal CMP on immunologic effects, and chitin-glucan in cardiovascular diseases (NCT00443495, NCT01508039, and NCT01232309).

Dietary glucosamine supplements (N-acetyl-glucosamine, glucosamine sulfate, glucosamine hydrochloride, and glucosamine), products from which chitin is derived, are occurring anti-inflammatory substances that have been used for arthritis, cardiovascular disease, and cancer prevention [81-83], although the treatment has been reported to cause damage in pancreatic β-cells and increase risk for diabetes [84]. Dietary glucosamine has begun to be measured for its efficacy in IBD [85, 86].

A high rate of asthma is reported in those who work with crabs and shrimps [87]. However, chitin is used in sutures, and soldiers with demonstrated allergies to shellfish had no allergenic response to chitosan bandages applied directly to wounds [88]. It is proposed that the allergy to shellfish is due to meat proteins attached to the shell, rather than to ingested chitin in the shell. Previous studies have shown that CMPs induce an immune response that suppresses allergic responses [11, 89]. Therefore, CMPs treatment may be a potentially safe therapeutic strategy in clinical setting.

11. Conclusions/Future Prospective

Mammalian chitinase and chitin interactions seem to play one of the central roles in the pathogenesis of inflammatory diseases in mucosal tissues. Potentially pathogenic bacteria, which express chitin-binding proteins, can efficiently interact with host cells as well as fungal chitin. Fungal and bacterial co-infections are likely to continue/enhance the solid adhesion of these two distinct organisms on the mucosal surfaces in digestive tract and the other organs. CMP-mediated anti-bacterial and anti-inflammatory effects will be beneficial to suppress the harmful interactions in allergic responses. Fungal infections have been shown to play a role in IBD. So far, fungal-bacterial co-infections have been proven in several epithelial tissues but not yet proven in the intestines. Future *in vivo* studies will enable us to further understand the importance of chitin-chitinase interactions in innate and adaptive immune responses during the development of inflammatory disease and allergic disorders.

Acknowledgements The authors are grateful to Dr. Daren Low for his helpful discussion and advice for preparing this manuscript. This work has been supported by National Institutes 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 E. Mizoguchi.

References

[1] Nagatani K, Wang S, Llado V, Lau CW, Li Z, Mizoguchi A, Nagler CR, Shibata Y, Reinecker HC, Mora JR, Mizoguchi E. Chitin microparticles for the control of intestinal inflammation. Inflamm Bowel Dis. 2012;18:1698-1710.

[2] Mizoguchi E. Chitinase 3-like-1 exacerbates intestinal inflammation by enhancing bacterial adhesion and invasion in colonic epithelial cells. Gastroenterology. 2006;130:398-411.

[3] Debono M, Gordee RS. Antibiotics that inhibit fungal cell wall development. Annu Rev Microbiol. 1994;48:471-497.

[4] Georgopapadakou NH, Tkacz JS. The fungal cell wall as a drug target. Trends Microbiol. 1995;3:98-104.

[5] Nishimura K, Nishimura S, Nishi N, Saiki I, Tokura S, Azuma I. Immunological activity of chitin and its derivatives. Vaccine. 1984;2:93–99.
[6] Nishimura K, Ishihara C, Ukei S, Tokura S, Azuma I. Stimulation of cytokine production in mice using deacetylated chitin. Vaccine. 1986;4:151-156.
[7] Nishimura K, Nishimura S, Seo H, Nishi N, Tokura S, Azuma I. Effect of multiporous microspheres derived from chitin and partially deacetylated chitin on the activation of mouse peritoneal macrophages. Vaccine. 1987;5:136-140.
[8] Shibata Y, Foster LA, Metrger IM, Myrvrk QN. Alveolar macrophage priming by intravenous administration of chitin particles, polymers of N-acetyl-D-glucosamine, in mice. Infect lmmun. 1997;65:1734-1741.
[9] Shibata Y, Metzger WJ, Myrvik QN. Chitin particle-induced cell-mediated immunity is inhibited by soluble mannan: mannose receptor-mediated phagocytosis initiates IL-12 production. J Immunol. 1997;159:2462-2467.
[10] Shibata Y, Foster LA, Kurimoto M, Okamura H, Nakamura RM, Kawajiri K, Justice JP, Van Scott MR, Myrvik QN, Metzger WJ. Immunoregulatory roles of IL-10 in innate immunity: IL-10 inhibits macrophage production of IFN-gamma-inducing factors but enhances NK cell production of IFN-gamma. J Immunol. 1998;161:4283-4288.
[11] Shibata Y, Foster LA, Bradfield JF, Myrvik QN. Oral administration of chitin down-regulates serum IgE levels and lung eosinophilia in the allergic mouse. J Immunol. 2000;164:1314–1321.
[12] Strong P, Clark H, Reid K. Intranasal application of chitin microparticles down-regulates symptoms of allergic hypersensitivity to Dermatophagoides pteronyssinus and Aspergillus fumigatus in murine models of allergy. Clin Exp Allergy. 2002; 32:1794-1800.
[13] Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang MJ, He CH, Takyar S, Elias JA. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011;73:479-501.
[14] Sakai S, Sugawara T, Kishi T, Yanagimoto K, Hirata T. Effect of glucosamine and related compounds on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. Life Sci. 2010;86:337-343.
[15] Muzzarelli RA. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell Mol Life Sci. 1997;53:131-140.
[16] Ormrod DJ, Holmes CC, Miller, TE. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. Atherosclerosis 1998;138: 329-334.
[17] Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, Locksley RM. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 2007;447:92–96.
[18] Kogiso M, Nishiyama A, Shinohara T, Nakamura M, Mizoguchi E, Misawa Y, Guinet E, Nouri-Shirazi M, Dorey CK, Henriksen RA, Shibata Y. Chitin particles induce size-dependent but carbohydrate-independent innate eosinophilia. J Leukoc Biol. 2011;90:167-176.
[19] Tharanathan RN, Kittur FS. Chitin - the undisputed biomolecule of great potential. Crit Rev Food Sci Nutr. 2003;43:61-87.
[20] Ravi Kumar MNV. A review of chitin and chitosan applications. Reactive and Functional Polymers. 2000;46: 1-27.
[21] Nakajima M, Atsumi K, Kifune K, Miura K, Kanamaru H. Chitin is an effective material for sutures. Jpn J Surg. 1986;16:418-424.
[22] Xiao X, Yin X, Lin J, Sun L, You Z, Wang P, Wang F. Chitinase genes in lake sediments of Ardley Island, Antarctica. Appl Environ Microbiol. 2005;71:7904-7909.
[23] Vind I, Johansen JS, Price PA, Munkholm P. Serum YKL-40, a potential new marker of disease activity in patients with inflammatory bowel disease. Scand J Gastroenterol. 2003;38:599-605.
[24] Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret MC, Aubier M, Pretolani M, Elias JA. A chitinase-like protein in the lung and circulation of patients with severe asthma. N Engl J Med. 2007;357:2016-2027.
[25] Kazakova M, Batalov A, Deneva T, Mateva N, Kolarov Z, Sarafian V. Relationship between sonographic parameters and YKL-40 levels in rheumatoid arthritis. Rheumatol Int. 2013;33:341-346.
[26] Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodeling, fibroses and cancer. Dan Med Bull. 2006;53:172-209.
[27] Nishimura K, Nishimura S, Nishi N, Saiki I, Tokura S, Azuma I. Immunological activity of chitin and its derivatives. Vaccine. 1984;2:93–99.
[28] Suzuki K, Okawa Y, Hashimoto K, Suzuki S, Suzuki M. 1984. Protecting effect of chitin and chitosan on experimentally induced murine candidiasis. Microbiol Immunol. 1984;28:903–912
[29] Desjardins A, Malo JL, L'Archevˆ eque J, Cartier A, McCants M, Lehrer SB. 1995. Occupational IgE-mediated sensitization and asthma caused by clam and shrimp. J Allergy Clin Immunol. 1995;96:608–617.
[30] Namovic MT, Walsh RE, Goodfellow C, Harris RR, Carter GW, Bell RL. Pharmacological modulation of eosinophil influx into the lungs of Brown Norway rats. Eur J Pharmacol. 1996;315:81-88.
[31] Oliveira SH, Costa CH, Ferreira SH, Cunha FQ. Sephadex induces eosinophil migration to the rat and mouse peritoneal cavity: involvement of mast cells, LTB4, TNF-alpha, IL-8 and PAF. Inflamm Res. 2002;51:144-153.
[32] Maghni K, Nantel F, Lanoue C, Cloutier S, Cristol JP, Cadieux A, Sirois P. Role of airway eosinophilia and eosinophil activation in Sephadex-induced inflammation. Inflammation. 2004;28:195-206.
[33] Da Silva CA, HartlD, LiuW, LeeCG, Elias JA. TLR-2 and IL-17A in chitin-induced macrophage activation and acute inflammation. J Immunol. 2008;181:4279–4286.
[34] Da Silva CA, Chalouni C, Williams A, Hartl D, Lee CG, Elias JA. Chitin is a size-dependent regulator of macrophage TNF and IL-10 production. J Immunol. 2009;182:3573–3582
[35] Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci USA. 1998;95:588-593.
[36] Ganter BN, Simmons RM, Canavera SJ, Akira S, Underhill DM. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. J Exp Med. 2003;197:1107-1117.
[37] Iliev ID, Funari VA, Taylor KD, Nguyen Q, Reyes CN, Strom SP, Brown J, Becker CA, Fleshner PR, Dubinsky M, Rotter JI,

Wang HL, McGovern DP, Brown GD, Underhill DM. Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. Science. 2012;336:1314-1317.
[38] Acharyya S, Oskarsson T, Vanharanta S, Malladi S, Kim J, Morris PG, Manova-Todorova K, Leversha M, Hogg N, Seshan VE, Norton L, Brogi E, Massagué J. A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell. 2012;150:165-178.
[39] Chen CL, Wang YM, Liu CF, Wang JY. The effect of water-soluble chitosan on macrophage activation and the attenuation of mite allergen-induced airway inflammation. Biomaterials. 2008;29:2173–182
[40] Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher LH, Bhan AK, Tonegawa S. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. Cell. 1993;75:275-282.
[41] Bhan AK, Mizoguchi E, Smith RN, Mizoguchi A. Spontaneous chronic colitis in TCR alpha-mutant mice; an experimental model of human ulcerative colitis. Int Rev Immunol. 2000;19:123-138.
[42] Quinton JF, Sendid B, Reumaux D, Duthilleul P, Cortot A, Grandbastein B, Charrier G, Targan SR, Colombel JF, Poulain D. Antibody to Saccharomyces cerevisiae (bakers' yeast) in Crohn's disease. Br Med J. 1988;297:1105-1106.
[43] Walker LJ, Aldhous MC, Drummond HE, Smith BR, Nimmo ER, Arnott ID, Satsangi J. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut. 1998;42:788–791
[44] Walker LJ, Aldhous MC, Drummond HE, Smith BR, Nimmo ER, Arnott ID, Satsangi J. Anti-Saccharomyces cerevisiae antibodies (ASCA) in Crohn's disease are associated with disease severity but not NOD2/CARD15 mutations. Clin Exp Immunol. 2004;135:490-496.
[45] Schaffer T, Müller S, Flogerzi B, Seibold-Schmid B, Schoepfer AM, Seibold F. Anti-Saccharomyces cerevisiae mannan antibodies (ASCA) of Crohn's patients crossreact with mannan from other yeast strains, and murine ASCA IgM can be experimentally induced with Candida albicans. Inflamm Bowel Dis. 2007;13:1339-1346.
[46] van Schaik FD, Oldenburg B, Hart AR, Siersema PD, Lindgren S, Grip O, Teucher B, Kaaks R, Bergmann MM, Boeing H, Carbonnel F, Jantchou P, Boutron-Ruault MC, Tjønneland A, Olsen A, Crowe FL, Peeters PH, van Oijen MG, Bueno-de-Mesquita HB. Serological markers predict inflammatory bowel disease years before the diagnosis. Gut. 2013;62:683-688.
[47] Rinaldi M, Perricone R, Blank M, Perricone C, Shoenfeld Y. Anti-Saccharomyces cerevisiae Autoantibodies in Autoimmune Diseases: from Bread Baking to Autoimmunity. Clin Rev Allergy Immunol. 2013, in press.
[48] Trojanowska D, Zwolinska-Wcislo M, Tokarczyk M, Kosowski K, Mach T, Budak A. The role of Candida in inflammatory bowel disease. Estimation of transmission of C. albicans fungi in gastrointestinal tract based on genetic affinity between strains. Med Sci Monit. 2010;16:CR451-457.
[49] Jawhara S, Thuru X, Standaert-Vitse A, Jouault T, Mordon S, Sendid B, Desreumaux P, Poulain D. Colonization of mice by Candida albicans is promoted by chemically induced colitis and augments inflammatory responses through galectin-3. J Infect Dis. 2008;197:972-980.
[50] Vega K, Kalkum M. Chitin, chitinase responses, and invasive fungal infections. Int J Microbiol. 2012, Article ID 920459, 10 pages.
[51] Fidel PL Jr, Vazquez JA, Sobel JD. Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. Clin Microbiol Rev. 1999;12:80-96.
[52] Giri S, Kindo AJ. A review of Candida species causing blood stream infection. Indian J Med Microbiol. 2012;30:270-278.
[53] Susuki K, Sugawara N, Susuki M, Uchiyama T, Katouno F, Nikaidou N, and Watanabe T. Chitinases A, B, and C1 of Serratia marcescens 2170 produced by recombinant E. coli: enzymatic properties ad synergism on chitin degradation.Biosci Biotechnol Biochem. 2002; 66:1075-1083.
[54] Low D, Tran HT, Lee IA, Dreux N, Kamba A, Reinecker HC, Darfeuille-Michaud A, Barnich N, Mizoguchi E. Chitin-binding domains of Escherichia coli chiA mediate interactions with intestinal epithelial cells in mice with colitis. Gastroenterol 2013;19:5238-5249.
[55] Kirn TJ, Jude BA, Taylor RK. A colonization factor links Vibrio cholerae environmental survival and human infection. Nature. 2005;438:863–866.
[56] Bhowmick R, Ghosal A, Das B, Koley H, Saha DR, Ganguly S, Nandy RK, Bhadra RK, Chatterjee NS. Intestinal adherence of *Vibrio cholerae* involves a coordinated interaction between colonization factor GbpA and mucin. Infect Immun. 2008;76:4968–4977.
[57] Tran HT, Barnich N, Mizoguchi E. Potential role of chitinases and chitin-binding proteins in host-microbial interactons during the development of intestinal inflammation. Histol Histopathol 2011;26:1543-1464.
[58] Kawada M, Chen CC, Arihiro A, Nagatani K, Watanabe T, Mizoguchi E. Chitinase 3-like-1 enhances bacterial adhesion to colonic epithelial cells through the interaction with bacterial chitin-binding protein. Lab Invest. 2008;88:883-895.
[59] Comito D, Romano C. Dysbiosis in the pathogenesis of pediatric inflammatory bowel diseases. Int J Inflam. 2012; 2012:687143.
[60] Bamford CV, d'Mello A, Nobbs AH, Dutton LC, Vickerman MM, Jenkinson HF. Streptococcus gordonii modulates Candida albicans biofilm formation through intergeneric communication. Infect Immun. 2009;77:3696–3704.
[61] Holmes AR, McNab R, Jenkinson HF. Candida albicans binding to the oral bacterium Streptococcus gordonii involves multiple adhesin-receptor interactions. Infect Immun. 1996; 64:4680-4685.
[62] O'Sullivan JM, Jenkinson HF, Cannon RD. Adhesion of Candida albicans to oral streptococci is promoted by selective adsorption of salivary proteins to the streptococcal cell surface. Microbiology. 2000;146:41-48.
[63] Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: Candida albicans and bacteria. FEMS Microbiol Lett. 2009;299:1–8
[64] Morales DK, Hogan DA. Candida albicans interactions with bacteria in the context of human health and disease. PLoS Pathog. 2010;29:e1000886.

[65] Gregoire S, Xiao J, Silva BB, Gonzalez I, Agidi PS, Klein MI, Ambatipudi KS, Rosalen PL, Bauserman R, Waugh RE, Koo H. Role of glucosyltransferase B in interactions of Candida albicans with Streptococcus mutans and with an experimental pellicle on hydroxyapatite surfaces. Appl Environ Microbiol. 2011;77:6357-6367.
[66] Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirtliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. Clin Microbiol Rev. 2012;25:193-213.
[67] Diaz PI, Xie Z, Sobue T, Thompson A, Biyikoglu B, Ricker A, Ikonomou L, Dongari-Bagtzoglou A. Synergistic interaction between Candida albicans and commensal oral streptococci in a novel in vitro mucosal model. Infect Immun. 2012;80:620-632.
[68] Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, Adrie C, Garrouste-Orgeas M, Cohen Y, Mourvillier B, Schlemmer B; Outcomerea Study Group. Candida colonization of the respiratory tract and subsequent pseudomonas ventilator-associated pneumonia. Chest. 2006;129:110-117.
[69] El-Azizi MA, Starks SE, Khardori N. Interactions of Candida albicans with other Candida spp. and bacteria in the biofilms. J Appl Microbiol. 2004; 96:1067–1073
[70] Levison ME, Pitsakis PG. Susceptibility to experimental Candida albicans urinary tract infection in the rat. J Infect Dis. 1987;155:841-846
[71] Nobile CJ, Schneider HA, Nett JE, Sheppard DC, Filler SG, Andes DR, Mitchell AP. Complementary adhesin function in C. albicans biofilm formation. Curr Biol. 2008;18:1017-1024.
[72] Klotz SA, Gaur NK, De Armond R, Sheppard D, Khardori N, Edwards JE Jr, Lipke PN, El-Azizi M. Candida albicans Als proteins mediate aggregation with bacteria and yeasts. Med Mycol. 2007;45:363-370
[73] Fu Y, Ibrahim AS, Sheppard DC, Chen YC, French SW, Cutler JE, Filler SG, Edwards JE Jr. Candida albicans Als1p: an adhesin that is a downstream effector of the EFG1 filamentation pathway. Mol Microbiol. 2002;44:61-72
[74] Fu Y, Rieg G, Fonzi WA, Belanger PH, Edwards JE Jr, Filler SG. Expression of the Candida albicans gene ALS1 in Saccharomyces cerevisiae induces adherence to endothelial and epithelial cells. Infect Immun. 1998;66:1783-1786.
[75] Sheppard DC, Yeaman MR, Welch WH, Phan QT, Fu Y, Ibrahim AS, Filler SG, Zhang M, Waring AJ, Edwards JE Jr. Functional and structural diversity in the Als protein family of Candida albicans. J Biol Chem. 2004;279:30480-30489
[76] Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect. 2000;2:543–546
[77] Allard JB, Rinaldi L, Wargo MJ, Allen G, Akira S, Uematsu S, Poynter ME, Hogan DA, Rincon M, Whittaker LA. Th2 allergic immune response to inhaled fungal antigens is modulated by TLR-4-independent bacterial products. Eur J Immunol. 2009;39:776-788.
[78] Zhou B, Gao WY Zhang TJ, Chen HX. Application of chitin and its derivatives to pharmaceutical industry for traditional Chinese medicine. Zhongguo Zhong Yao Za Zhi. 2005;30: 1313-1316.
[79] Jull AB, Ni Mhurchu C, Goodfellow C, Harris RR, Carter GW, Bell RL, Rodgers A. Chitosan for overweight or obesity. Cochrane Database Syst Rev. 2008;3:CD003892.
[80] Jayakumar R, Chennazhi KP, Goodfellow C, Harris RR, Carter GW, Bell RL. Chitin scaffolds in tissue engineering. Int J Mol Sci. 2011;12:1876-1887.
[81] Fouladbakhsh J. Complementary and alternative modalities to relieve osteoarthritis symptoms. Am J Nurs. 2011;112:S44-51.
[82] Yeh GY, Davis RB Phillips, RS. Use of complementary therapies in patients with cardiovascular disease. Am J Cardiol. 2006;98:673-680.
[83] Greenlee H, Kwan ML, Goodfellow C, Harris RR, Carter GW, Bell RL. Complementary and alternative therapy use before and after breast cancer diagnosis: the Pathways Study. Breast Cancer Res Treat. 2009;117:653-665.
[84] Pham T, Cornea A, Blick KE, Jenkins A, Scofield RH. Oral glucosamine in doses used to treat osteoarthritis worsens insulin resistance. Am J Med Sci. 2007;333:333-339.
[85] Rawsthorne P, Clara I, Graff LA, Bernstein KI, Carr R, Walker JR, Ediger J, Rogala L, Miller N, Bernstein CN. The Manitoba Inflammatory Bowel Disease Cohort Study: a prospective longitudinal evaluation of the use of complementary and alternative medicine services and products. Gut. 2012;61:521-527.
[86] Yomogida S, Kojima Y, Tsutsumi-Ishii Y, Hua J, Sakamoto K, Nagaoka I. Glucosamine, a naturally occurring amino monosaccharide, suppresses dextran sulfate sodium-induced colitis in rats. Int J Mol Med. 2008;22:317-323.
[87] Brinchmann BC, Bayat M, Goodfellow C, Harris RR, Carter GW, Bell RL. A possible role of chitin in the pathogenesis of asthma and allergy. Ann Agric Environ Med. 2011:18: 7-12.
[88] Waibel KH, Haney B, Moore M, Whisman B, Gomez R. Safety of chitosan bandages in shellfish allergic patients. Mil Med. 2011;176:1153-1156.
[89] Shibata Y, Honda I, Justice JP, Van Scott MR, Nakamura RM, Myrvik QN. Th1 adjuvant N-acetyl-D-glucosamine polymer up-regulates Th1 immunity but down-regulates Th2 immunity against a mycobacterial protein (MPB-59) in interleukin-10-knockout and wild-type mice. Infect Immun. 2001;69:6123-6130.

