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DIARRHEA DIGEST

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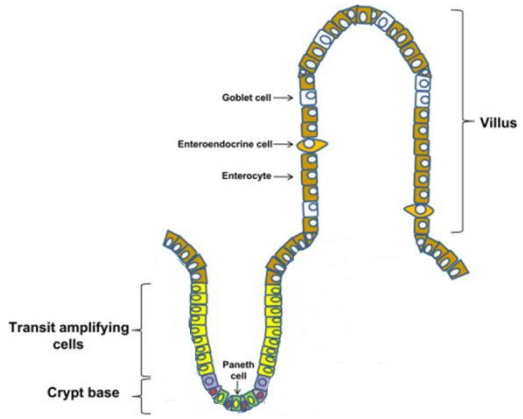
Tales from the crypt: The true role of goblet cells

Have you ever considered the cells that make up your gut? How on earth does the gut house hundreds of species of bacteria, digest food, eliminate waste, *and* inform your immune system of microbial or food-borne problems? You might have a basic idea of the cells of your gut, but a recent *Nature* article draws attention to a slimy cell-type that was previously ignored.

The intestinal epithelium is made up of finger-shaped villi and proliferative crypts (where stem cells of the intestinal epithelium reside) (van der Flier, L. and Clevers, H., 2009). These structures are further divided into enterocytes, goblet cells, entero-endocrine cells, and paneth cells. The enterocyte's role is to absorb nutrients present in the gut. Enteroendocrine cells secrete hormones. Paneth cells reside in the crypts and represent the innate immune system by secreting antimicrobial peptides and lysozymes. Goblet cells are most well-known for secreting mucous, acting as both a protective layer and lubricant for materials in the gut.

As you move through the epithelial layer into the lamina propria, you encounter a number of other cell types, including the dendritic cells which help regulate the adaptive immune system. They are antigen presenting cells (APCs) meaning that they sample antigens, decide if they are a threat or not, and if the antigen is a potential problem dendritic cells, inform the adaptive immune system who then are trained to recognize the antigen and attack it (Coombes, J. and Powrie, F., 2008).

Until recently, researchers believed that dendritic cells simply wedged their dendrites through the epithelial layer and into the lumen to sample antigen. However, researchers from Washington University School of Medicine decided to question this possibility using three dimensional imaging of the intestinal tissue *in vivo.* This type of imaging, called two-photon microscopy allows



(http://www.sciencedirect.com/science/article/pii/S1931524410001180)

researchers to get details of microscopic happenings in live subjects (Denk, W., Strickler, J.H., and Webb, W.W. 1990). Washington University researchers injected sugar into mice intestinal lumen and observed where the model antigen traveled (McDole, et al. 2012). Initially the sugar covered the villi and crypts of the small intestines, but after close inspection researchers watched as the sugar traveled through the villus epithelium layer and into the lumina propria through tiny columns. After further staining McDole et al. determined that these columns were actually mucus secreting goblet cells. The paper goes on to determine that it is actually goblet cells that bring antigens and even sugars through the gut epithelial to the attention of dendritic cells which determine if further action is necessary.

So why is this new delivery role a "*Nature* worthy" discovery? Well, first off it's nice to

know that goblet cells do more than just slime the intestines; they actually present antigen to dendritic cells. But this finding is also major for a number of intestinal diseases and disorders. For instance, people with chronic illnesses that can be associated with food intolerance such as Crohn's disease or Celiac's disease may now have more hope for therapeutics that can easily target the appropriate cells or pathways to calm the overactive immune system.

Previous research has identified that individuals with some of these chronic inflammatory diseases have a reduced number or deficiency in goblet cells. While originally overlooked, the new-found role of goblet cells may shine light on such overlooked findings (Dvorak, A.M. and Dickersin, G.R., 1980). This finding is also very important when it comes to oral vaccine development. As McDole et al. were able to determine the exact interactions which occur between goblet cells and dendritic cells, researchers can screen vaccine candidates for those that will most easily be taken up by and activate the immune system. This allows for easier vaccinations in children, third world countries and other places where needles might not be a desirable option.

R. Easley

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Noted by a messenger on the Message board regarding fecal transplants: Since *C. difficile* only appears to affect people without healthy gut bacteria due to having taken antibiotics, likely anyone with healthy gut bacteria would be a match so almost anyone else could be a donor. I mean, it's not like an organ or something that the body might not like and reject. Besides, it'll be rejecting this transplant soon no matter what.

Not so fast messenger: Don't be surprised if down the road, scientists determine that your gut flora actually may be matched to you, your siblings, and to your offspring much more closely than what we realize. This may be one reason why fecal transplants from siblings work a little better for treating *C. difficile* than transplants from someone who's not a blood relative. Don't get me wrong --- fecal transplants in general work great for treating recurrent *C. difficile* disease. But perhaps a transplant from a sibling offers a little bit more of an advantage.

Importance of Stool Toxin Testing: Are We Coming Full Circle?

In recent years, diagnostic testing for *C. difficile* disease has evolved significantly to include new approaches like the use of molecular assays for detecting the toxin genes (*tcdA* or *tcdB*) and the inclusion of glutamate dehydrogenase (GDH)-based algorithm testing for determining *C. difficile* negative specimens. As with any diagnostic testing, there are pros and cons associated with both approaches. The molecular tests are highly sensitive and offer an assay for determining the presence of toxigenic *C. difficile* in a fecal specimen. For larger institutions that can absorb the increased cost, molecular testing for all incoming specimens is sometimes used as a single test approach. Toxigenic culture, which is the

gold standard for identifying the presence of toxigenic *C.difficile* involves the subculturing of isolates into broth media like brain heart infusion broth (BHI), and then confirming the production of toxin B with tissue culture or by immunoassay. This procedure takes days to complete and requires additional laboratory capabilities that's beyond many clinical labs. The molecular assays provide a result the same day and some studies show a >90% correlation with toxigenic culture.

Algorithm testing uses GDH for determining the presence of *C. difficile,* followed by additional testing using stool toxin or molecular assays to differentiate between toxigenic and nontoxigenic infections. Since most specimens are negative (ca. 80%), this testing approach significantly decreases the high cost of the molecular tests. Algorithm testing can be done using several combinations. First, specimens may be screened for GDH using a standalone ELISA test like the *C. difficile CHEK-60* with only positive specimens being tested using a molecular assay for the toxin genes or immunoassay for stool toxin.

Another approach is using the *C. Diff COMPLETE* test for determining the presence of both GDH and stool toxin at the same time with a single test. With this test, specimens that are GDH and stool toxin-positive can be reported as "positive" and then GDH-positive toxin-negative specimens can be further tested by a molecular test for the presence of toxigenic *C. difficile*. This approach is rapid, eliminates the negative specimens, provides a stool toxin result, and ultimately lowers costs.

With the different options for testing, it's important to consider what these different tests mean and how they impact patient care. A recent study presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) by Planche *et al.* entitled "Clinical Validation of *Clostridium difficile* Infection (CDI) Diagnostics: Importance of Toxin Detection" presented results from the largest *C. difficile* study to-date. In this U.K. study, 12,420 specimens from 10,691 patients were tested using the reference assays: toxigenic culture and tissue culture for stool toxin along with ELISA tests for GDH and toxin and a molecular test. Of the total patients, there were 6,524 inpatients of which 5,927 survived. According to the reference assays, there were 3 groups described: (1) toxigenic culture and tissue culture stool toxin-positive, (2) toxigenic only positive, and (3) *C. difficile* negative. Diagnostic status was evaluated for an association with mortality rate, and a multivariate analysis between groups was done with age, sex, clinic location, white blood cell count, creatinine and albumin as covariates.

There were 435 stool toxin-positive patients (7%), 207 patients positive by only toxigenic culture (3%) and 5,880 patients who were negative for *C. difficile* (90%). Patients with stool toxin had a significantly higher mortality rate (16.6%) compared to the toxigenic only (9.7%; $p<0.022$) and *C. difficile* negative groups (8.6%; $p<0.001$). In addition, the stool toxin-positive patients had significantly higher ($p<0.001$) WBC counts compared to the other two groups. Based on these results, Planche and co-authors concluded that patients having a positive stool toxin have *C. difficile* disease with an increased risk of mortality. In addition, they defined a new diagnostic category: patients who are infected with toxigenic *C. difficile* without stool toxin are "excretors" for whom *C. difficile* disease is unlikely but a risk of transmission should be considered. Since overtreatment may result in additional antibiotic resistance along with potentially putting patients at risk of developing the disease rather than just carrying the organism, many hospitals will take note of this study and again ask the question: Should stool toxin testing be included to optimize their *C. difficile* diagnostic testing? With the continued evolution of *C. difficile* disease, determining the best approach to both diagnosis and treatment continues to be an ongoing dilemma.

J. H. Boone

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Can superbugs such as *C. difficile* be transmitted by air? Check out the following article: http://www.sciencedaily.com/releases/2012/10/121011124436.htm#.UHccdo2m3Y0.email

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Money Down the Toilet --- an informal assessment of the economic impact of foodborne illness

It has happened to us all at least once – something at the restaurant just didn't taste right, but we continued to eat it anyway. Or, perhaps, everything tasted just fine, but you later question the cleanliness of your chosen eating establishment when you awaken at 3:00 AM with a strange feeling in your abdomen, fully aware that in the very near future, something will be coming out of one end or the other. So, as you either sit on or kneel before the porcelain throne, wondering if you will be able to go to work the next day, or if this is a once-and-done phenomenon, know that what impacts your digestive system also impacts the economy.

As you have undoubtedly heard many times before, everything has a monetary cost. This includes diarrhea and other maladies spread through contaminated food. So what does foodborne illness cost the United States economy? Numerous bean counters and statisticians have certainly calculated this statistic many times before, but I thought it would be fun to do anyway, in an informal way, from statistics easily available on the internet. A few assumptions will be made, which may or may not be completely accurate, but nonetheless, we should have at least a very rough estimate of the cost of foodborne illness due to missed working days in the United States.

According to Reuters, the median earnings for a working American are $26,364. Assuming a 5 day work week and two paid weeks off per year, that comes to $106.53 per day. Therefore, we will assume that every day of missed work costs $106.53.

A recent CDC report estimated that 48 million Americans succumbed to some sort of foodborne disease in 2011. The top culprits were Norovirus, *Salmonella spp*., *Clostridium perfringens*, *Campylobacter spp*., and *Staphylococcus aureus*. Of course, it is unlikely that every one of these cases resulted in work being missed. For the purpose of this article we'll assume that in 1/3 of the 48 million cases, or 16 million, the affected individuals were ill enough to stay home from work for at least one day. How many days on average were missed? We'll use the top 5 infections for estimates: Norovirus infections last for an average of 1-3 days; *Salmonella* for 4-7 days; *Campylobacter* for 7 days; *Clostridium perfringens* less than a day; *Staphylococcus aureus* for 1-3 days. Using the top 5 as a guideline, we'll assume that on average, three days of work were missed by an individual affected by a foodborne illness.

How does this all add up? 16 million cases x 3 days/case x $106.53/day = $5,113,440,000.00 - over 5 *billion* dollars – a lot of money down the drain (literally)! What else could be done with $5 billion?

- Provide a 4-year college education to 58,000 kids (at a state school)
- Fund the economy of The Gambia for over 5 years (based off 2009 population and per-capita income estimates)
- Provide a computer (a really nice one) for 5 million households
- Allow the United States government to operate debt-free for a day and a half
- Provide everyone in the city of Richmond, VA with a new mid-sized sedan

- Purchase a Boeing 747 for yourself and 13 of your closest friends
- Purchase a controlling interest in Smucker's Jam, or all of Hasbro Toy
- Provide gasoline for everyone in the city of Chicago for an entire year
- Provide a single-family home for 22,000 families
- Cover the salaries of all NBA players for 2½ years

As most foodborne illnesses can be prevented by thorough hand washing and proper storage, preparation, and cooking of food, a little prevention can go a long way, and lessen the economic impact of foodborne disease.

J. Boone

Shiga Toxin Producers

Shiga toxin-producing *Escherichia coli* (STEC) derive their name from their ability to produce toxins (Stx1 & Stx2) that are similar in structure and function to the Shiga toxin produced by *Shigella dystenteriae.* STEC infections are associated with gastrointestinal disease and have been linked to the development of hemorrhagic colitis and hemolytic uremic syndrome (HUS). Transmission of STEC occurs primarily through the consumption of contaminated foods and causes approximately 100,000 illnesses, 3,000 hospitalizations, and 90 deaths annually in the United States, according to the latest estimate in 1999 (1). According to the CDC and a study conducted by the Michigan Department of Community Health, most STEC isolates are recovered between the months of June and October although the transmission of STEC can happen at any time of the year (2). Those at the greatest risk of developing an STEC infection are young children and elderly persons, although healthy adults may be asymptomatic carriers.

The key virulence factors of STEC are the Shiga toxins. Shiga toxin 1 (Stx1) is incredibly similar to the Shiga toxin produced by *Shigella dystenteriae* in amino acid sequence. It is neutralized by antibodies against Shiga toxin. Shiga toxin 2 (Stx2) is neutralized by homologous antibodies (1, 4). The genes for Stx1 and Stx2 are encoded by temperate bacteriophages. Shiga toxins 1 and 2 are AB_5 toxins consisting of one A subunit linked to five B subunits. These toxins are responsible for the disruption of protein synthesis which can lead to cell death. In humans the B subunit binds to globotriaosylceramide, Gb3, which is expressed on renal tubular and vascular cells in the kidney, brain and in the Paneth cells in the intestine. The A subunit is an N-glycosidase. After the binding and internalization of the toxin the A subunit cleaves ribosomal RNA, thus preventing transcription and overall protein synthesis. (5) Breakdown of protein synthesis most often can lead to cell death which in turn can lead to the damage and loss of function of tissues and organs.

Shiga toxins are not the only virulence factor of importance. STEC isolates from patients who suffered from HUS often carried the virulence gene *eaeA,* which codes for intimin, a protein that enhances attachment and effacement of *E.coli* to intestinal epithelial cells by a type III secretion system (4).

The clinical symptoms of STEC infections include acute and sometimes bloody diarrhea as well as more severe disease such as hemorrhagic colitis and hemolytic-uremic syndrome (HUS) which can be fatal in up to 5% of cases (4). HUS is characterized by thrombocytopenia, hemolytic anemia and renal failure. The time from exposure to onset of diarrhea is around 4 days and the advancement to HUS from onset diarrhea ranges from 1 to 10 days (5).

In the United States most documented STEC infections involve *E.coli* O157:H7, which causes 73,000 cases a year, and six non-O157 serogroups (O26, O45, O103, O111, O121 and O145), which account for the majority of non-O157 STEC infections. (1)

Even though O157:H7 STEC may dominate the headlines in the United States, the CDC has estimated that non-O157 STEC infections may cause twice as many illnesses in the U.S. The large number of undiagnosed non-O157 STEC infections may be attributed to current insufficient testing for non-O157 STEC. Worldwide non-O157 STEC illnesses are as common if not more common than O157 STEC illnesses and as recent as 2011, there was a large outbreak of STEC O104:H4 in Germany linked to the consumption of raw sprouts and secondary transmission. (3)

Approximately 8% of all persons diagnosed with O157 STEC infections develop HUS, with children five years of age and under at greatest risk (1). To make matters worse the infectious dose of O157 STEC and O111 STEC are relatively low at <100 organisms (1).

Although O157 STEC is closely associated with the development of HUS, it is well documented that nonO157 strains of STEC can lead to the development of HUS. Recent research suggest Stx2 positive STEC isolates are 5 times more likely to cause severe disease than an STEC isolate negative for Stx2, and that there is a positive association between the presence of Stx2 and the development of HUS (5). Thus the best indicator of the potential for the development of HUS is accurate detection of Stx2.

Health agencies such the CDC stress the importance of prompt and accurate diagnosis of STEC infections because timely and appropriate treatments are needed to reduce renal damage and improve patient outcome. Prompt appropriate treatment is crucial because it is widely believed that the use of antibiotic therapy with O157 STEC infections can lead to more severe disease such as HUS due to increased toxin production (1).

Because rapid detection of STEC is key in preventing unnecessary treatment that can cause further severity of disease and renal damage, enzyme immunoassays (EIA) may represent a standard practice along with culture. Prompt results of non-culture EIA that test for the presence of Shiga toxins offer additional benefits such as having the ability to detect all serotypes of STEC (1, 2). Toxin differentiation assays provide an even greater diagnostic tool because of the positive association between Stx2 and the development of HUS.

The CDC recommends non-O157 STEC and O157 STEC testing for all stool specimens from patients with an acute onset of community-acquired diarrhea as well as all patients suspected of having HUS (1). U.S. studies have shown STEC were detected in 0%-4% of all stools submitted for testing at clinical laboratories. These rates are similar to those of *Salmonella* species, *Shigella* species and *Campylobacter* species (1).

In conclusion STEC presents itself as a serious public health concern and with increases in epidemiologic knowledge and advancements in culturing technique and rapid enzyme immunoassays, we will be better able to provide more prompt and accurate diagnosis and treatment that may prevent further illness and unnecessary financial costs.

J. Heptinstall

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On average, 4 out of 5 people in the U.S. "SUFFER" from diarrhea during the course of a year. However, we don't think this means that the other person enjoys it!

Current Methods for the Detection of Celiac Disease

Close to two million Americans are suffering from undiagnosed celiac disease. Celiac disease (CD), one of the most common autoimmune disorders of the small intestine, is triggered by the ingestion of wheat gluten and similar proteins in rye and barley in genetically susceptible individuals. The classical symptoms of CD are diarrhea, abdominal distension, and failure to thrive. However, >80% of patients screened either have no symptoms or display atypical symptoms of CD. Atypical symptoms can include anemia, osteoporosis, arthritis, infertility, peripheral neuropathy, and even liver failure.[1,2] A 2012 study estimated that the prevalence of CD in the general population of the United States was 0.71%, similar to that found in several European countries. Since the symptoms are non-specific, diagnosis of CD is difficult, and many patients are unaware that they have the disease. In the 2012 prevalence study, 86% of the identified CD patients were undiagnosed before the study began. The prevalence of CD varies by race/ethnicity with the highest prevalence among non-Hispanic whites.[3] Long term untreated CD can result in an increased risk for developing T-cell lymphoma, small bowel adenocarcinoma, and other cancers of the gastrointestinal tract.[2]

CD is triggered and sustained by the entry of gluten peptides into the lamina propria of the intestine after crossing the epithelial barrier. Gluten is a mixture of storage proteins found in wheat grains. The elastic properties of gluten are needed for the formation of dough from wheat flour and give bread its texture and taste. Gluten contains large stretches of glutamine and proline that make it highly resistant to breakdown by gastrointestinal enzymes. Undigested peptides are capable of crossing the intestinal epithelium where they trigger inflammation in susceptible individuals.[1,4] Much of the genetic susceptibility is linked to two alleles of the human leukocyte antigen complex: HLA-DQ2 and HLA-DQ8. In CD patients, gluten-derived peptides are presented by HLA-DQ2 or HLA-DQ8 to trigger a T-cell mediated immune response. However, only 2-5% of HLA-DQ2+ and HLA-DQ8+ individuals develop CD indicating that other factors contribute to the manifestation of the disease. Possible factors include early exposure of infants to dietary gluten, enteropathic viral infection, and changes in intestinal bacterial flora.[2]

CD is treated by strict adherence to a gluten-free diet. When patients are on a gluten-free diet for 5 years or more, the risk of developing gastrointestinal cancer returns to that of the normal population. Adherence to a strict gluten-free diet can be highly difficult for CD patients. The daily intake of gluten is high in the Western diet and maintaining strict avoidance can limit social activities, nutritional variety, be expensive, and make travel difficult. Also, a portion of CD patients are highly sensitive to gluten. Daily consumption of as little as 50 mg of gluten, the equivalent of 1/100th of a slice of bread, may contribute to the persistence of mucosal damage.[2,5] Consequently, even after long term maintenance of a gluten-free diet, many patients still have symptoms and/or mucosal damage. Therefore, alternative therapies are needed to supplement a gluten-free diet to improve the quality of life for patients.

Traditionally, duodenal biopsy has been the gold standard to detect CD. The major antigen involved in the immune response to CD is tissue transglutaminase (TTG), a ubiquitous calcium dependent enzyme that can crosslink proteins via a glutamine-lysine bond.[6] TTG can also convert glutamine to glutamic acid resulting in peptides that have a high affinity for HLA-DQ2 and HLA-DQ8. Serological screening tests for CD include tests for the detection of antibodies against TTG, detection of anti-endomysial antibodies (EMA), and the detection of antibodies

against deamidated gliadin peptide (DGP), an antigen obtained by the modification of fragments of the ethanol-soluble fraction of gluten. The EMA test is a complex indirect immunofluorescent assay using a smooth muscle substrate. The TTG and DGP assays are available in ELISA format. Both the EMA and TTG tests have a higher sensitivity than the DGP test (93% for TTG and EMA versus 88% sensitivity with DGP) as well as higher specificities (99% for EMA, 97% for TTG, and 94% for DGP). However, due to the complexity of the EMA test, detection of TTG is the preferred screening test for CD.[1]

S. Doyle

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