

Mechanisms Behind the Leaky Gut

Medical literature on the leaky gut provides useful information that may help unify the picture of how diets used in autism are addressing some issues that have been studied more carefully in celiac disease. This mini-paper will review these mechanisms that may not yet be familiar to many in the autism community.

Most people have heard of the term "leaky gut" but may not realize that this term refers to how larger molecules may get into the blood when structures called tight junctions that are there to seal the gaps between intestinal cells, have instead, opened up. This opening creates a passage that lacks the same type of regulation that happens when substances move through intestinal cells. Scientists like to call this movement of fluid and its solutes through this opening "paracellular transport". Other tissues can be leaky like this, too, like the bladder, kidney cells and even the blood brain barrier.

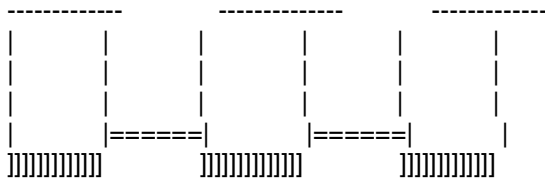
How does paracellular transport work?

Below you'll see a drawing of the epithelial cells that are the absorbing part of the gut. It is important to notice that these cells are very different on the food side versus the blood side where you are trying to shuttle the nutrients that come from digestion.

Think of your gut as a big hose. That hose has an outside and an inside. The picture below is a cross section, as if I cut through the hose with a knife, and we are looking at the open edge of the hose as it would be seen on the top side.

OUTSIDE of the "hose"

This is called the blood side or serosal side
or basolateral side



This is the brush border or apical side or
"lumen" where the food is.

INSIDE of the "hose"

The big rectangles are intestinal cells, and the goods from food travel from the bottom (or food side) to the top (or blood side).

The]]]] represents the highly absorptive side that is touching the food that is going through the intestine, and it has transporters on it that help to absorb particular things across this membrane. The membrane also excludes some things from crossing into cells. You'll hear this side called the brush border, too, because of the villi that make this side look very different. Here is a photograph of the brush border:

http://microvet.arizona.edu/Courses/MIC420/lecture_notes/ecoli/brush_border.html

The dashed line on top is the membrane that helps deliver things that were absorbed into these cells to the circulation. Nutrients move across the cell to get to this membrane that is on the blood side. Transporters within this membrane will move only certain selected things across that membrane in order to deliver them to blood.

In my drawing above, the line of ===== represents tight junctions which keep things from crossing between cells to get to the space that has access to the circulation. When this junction is closed, the nutrients HAVE to go through the cells to get absorbed, and that is a process that is regulated by the transporters on the top and bottom side working together.

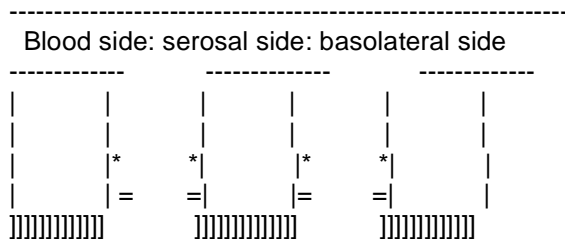
Immediately below, I've put a link to someone else's cartoon of this scene, but this time, we are viewing things from the bottom side of the "hose". The point of the drawing is to show how glucose gets transported into the cell, moves across the intestinal cell, and then crosses that last membrane en route to the blood:

<http://www.rpi.edu/dept/chem-eng/Biotech-Environ/Membranes/bauerp/co.gif>

Another system of regulation exists that has to do with nutrients taking a different route. Instead of going through cells, this time, the nutrients are going around cells using the gaps that exist between these cells that are usually closed off by structures called tight junctions.

The substances that travel this route (called the paracellular route as opposed to the transcellular route) can be peptides from foods (like gluten or casein peptides or peptides from other foods), or non-protein molecules like oxalate. Oxalate is a compound found mainly in plants which is highly reactive and binds to calcium and other molecules.

So, when your tight junctions open up, those cells will change like this:



Brush border or apical side or "lumen" where the food is.

When the gate is open, many molecules pass through those open junctions just as your dog might go through an open gate in your fence. Remember there is a flow of solute-loaded fluid moving through these open gates.

Perhaps we've had the impression that having a leaky gut means something is broken, but that is not necessarily the case. The opening of the gates is regulated, and that regulation can be called upon by systems like the immune system. Certain of the immune system's cytokines open up the "gates" in order to let cells of the immune system in the blood have access to antigens that have been in the gut. Some new work has shown that this opening and closing of the gate also has a lot to do with calcium which makes this system of gatekeeping have an unusual relationship to dietary oxalate.

What have scientists learned about this system of gatekeeping?

I've put a study at the end of this article whose authors discovered that some of this process of opening and closing the tight junctions appeared to be mediated through an interaction with calcium. This did not involve the concentration of calcium that was inside the intestinal cells, but it only involved the calcium that was outside the cell. Removing the calcium from either side of that tight junction could really change things, but changing the level of calcium inside the rectangle (representing the inside of the cell) made no difference at all.

Right next to where that gate is located on the basolateral (or blood side) are some molecules and a "sensor" that picks up calcium that is travelling in the fluid on this basolateral or blood side. I've represented that sensor as an asterisk. If there is adequate calcium at that sensor, then the leaky gut closes, just as if it had been zipped up. In fact, calcium is actually a key ingredient used to close the zipper. When there is not enough calcium present to close the gate, the gate stays open so that calcium from the food side can come in through the gap until there is enough calcium to close the gate again. In fact, at times, there are oscillations that occur as this gate opens and closes in response to calcium.

What happens if the calcium level gets low?

If after the gate opens to let calcium in, there is not enough calcium on the food side to travel through and bind the sensor, then that means there won't be enough calcium to zip up the zipper and close the gate again. The gate will stay open, and until enough calcium comes around to close the gate again, you've got a "leaky gut" that will stay leaky . This means it is important to think about all the circumstances that might cause the supply of calcium to diminish that is travelling from the lumen of the gut or what might cause the calcium on the basolateral side to get low.

What is the connection between calcium and fat maldigestion?

Scientists have figured out that calcium in the lumen can be tied up in fat when one has fat maldigestion and malabsorption. The fat left undigested in the gut binds calcium and makes something of a soap, but this doesn't provide that either the fat or the calcium will get absorbed. This also means that the calcium would fail to make it to the basolateral side when the gate was open, and that would mean the calcium wouldn't be there to interact with the molecules that govern the tight junctions by sensing that there is adequate calcium there. Scientists have done experiments to quantify how this fat effects oxalate absorption, and they also have noted that very often people with celiac disease have this very same maldigestion of fat. This offers one reason people with celiac sprue have a predictable problem with this leaky gut and a condition of excess oxalate absorption that is reflected by high levels of oxalate excretion in the urine called hyperoxaluria.

What is the connection between calcium and oxalic acid that is in the gut?

Undigested fat is not the only way to tie up that needed calcium. Calcium could also be tied up by soluble oxalate that comes from food high in oxalates that were eaten and are present on the inside of the gut.

Food is not the only source of oxalate in the gut. Nature has provided a system to help the body get rid of excess oxalate. Intestinal cells become loaded with oxalic acid when this acid is transferred into them from circulation from the basolateral or blood side, and from there, this acid is actually secreted into the gut. Why does this happen? It is nature's way of ridding the body of a compound that is highly reactive and can be damaging to organs in the body, especially after those organs have already suffered some sort of injury. Oxalates seek injured tissue because they bind to molecules that ordinarily may be hidden from them in healthy tissue.

The secretion of oxalate into the gut happens regardless of the source of those oxalates. That oxalate may have come from the diet, or from chemical or environmental exposure to precursors

to oxalate (such as to glycolic acids), or from excess production of oxalates by our own cells due to vitamin deficiency, genetic defect, or some other reason. Scientists have found our bodies make excess oxalate when deficient in vitamin B6, which is a vitamin that has been under a lot of study in autism. Some people may make excess oxalate from an excess of glycine. There are also genetic defects that produce excess oxalate. If there are times when our bodies produce extra oxalates for a good purpose, it has not been discovered yet, but we will be looking for this good purpose in our oxalate project.

One factor that may determine the level of secretion of oxalate into the intestine is its concentration gradient. Oxalate will try and move from places where it is in a higher concentration to places where it is in lower concentration. An excess level of oxalate on the food side of enteric cells may hamper the secretion of oxalates from the blood side. The body may use signals like angiotensin II to step up oxalate secretion from intestinal cells, but sometimes, even though the level in blood might be higher, the secretion may be disrupted by a biochemical signal. This happened experimentally when the signal from angiotensin II was disrupted....something that might happen with an ACE inhibitor or possibly with a chelating agent. More work needs to be done here.

It makes good sense that the body sends excess oxalate to the gut, because the gut is where calcium from the diet could bind the oxalate and that would keep it from being reabsorbed. The oxalate can just stay in the stool in the form of calcium oxalate because it is only the unbound form that is readily absorbed. There are many studies about this.

How do the microbes in the gut affect oxalate absorption?

A different method of reducing oxalate absorption is provided by microbes inside the gut whose role is to eat oxalates and turn them into something else. Unfortunately, these same microbes are easily killed off by antibiotics. Quite a number of studies have found a lack of these specialized bacteria in people who develop oxalate-related health problems. Trying to address this problem, a biotech company is currently working on a probiotic/enzyme formula to "recolonize" the most capable oxalate-eating bacteria, which is oxalobacter formigenes.

The intestines might feel better when the secretion into the gut of oxalic acid is reduced, because research has shown that oxalic acid is by nature corrosive and burning to tissues. Even so, whenever the intestines lose the ability to get rid of "waste" oxalate (using this secretion coupled with binding calcium or being metabolized by oxalate-eating bacteria), then the oxalate remaining in circulation can cause someone to suffer the consequences of having higher levels of oxalic acid reaching other tissues.

What is the role of zonulin in opening up tight junctions?

A study a number of years ago found that the proteins from wheat and corn could induce a leaky gut in rats which had first been made niacin deficient. Since then, other work has found that there is a relationship between exposure to the wheat protein gliadin and the excess production of a talented disrupter of the tight junction called zonulin.

Zonulin is a physiological molecule which was discovered in 2000. Before that discovery scientists had been studying a mimic of this molecule: a toxin produced by a phage that infected a bacteria that could be infecting a human. This toxin was called Zot, and all its talents at disruption of the tight junction came from its being a mimic of zonulin. By watching what Zot did, scientists learned a whole new set of interactions that were governing paracellular transport in the gut.

Zonulin's presence (similar to lack of calcium) opens up the tight junctions between cells. Scientists found that zonulin was elevated both in serum and in the lumen of the gut in celiac disease. They also learned by monitoring people with skin reactions to gluten called dermatitis

herpetiformis, that this leaky gut/zonulin phenomenon was a part of the disease process that occurred before the flattening of the villi. They learned that the disruption caused by zonulin could be set in motion by a simple exposure to gliadin.

Apparently, zonulin keeps the gate open. I don't think they've figured out exactly why or how it does that, but this may have to do with the fact that the piece of gluten called gliadin mimics part of a molecule called calreticulin that carries a huge load of calcium. Calreticulin is known to be involved in the regulation of oxalate in plants, but any role for calreticulin at this location at the tight junction has not been characterized in animals. There also seems to be one other molecular mimic of gluten at this site in intestinal cells.

Unfortunately, we are stuck with the order in which scientific discovery is taking place because this work on zonulin and the leaky gut is brand new. Antibodies to calreticulin have been found in celiac disease, and also in a lot of other autoimmune diseases associated with a leaky gut that tend to develop alongside celiac disease. This raises several questions:

-- Are the oxalates obtaining access to tissues in the body raising the level of oxidative stress and tissue damage whenever they latch to damaged tissues?

-- Is this what is exposing new antigens from organs like the pancreas, or thyroid or connective tissues to the immune system, and helping to induce autoimmune disease?

-- What happens when oxalates get into the brain if the blood brain barrier is also leaky? Could this explain the origin of calcifications in the brain associated with celiac disease which may be related to seizure?

Why is celiac disease the best model system for understanding these mechanisms at the tight junction?

These new-found mechanisms confirm why hyperoxaluria is a well known component of celiac disease. In fact, celiac disease is the main disease emphasis circling around the study of zonulin. I've put abstracts from three studies below that talk about this connection between zonulin and celiac disease, but there are only thirteen articles on zonulin in the whole of Medline so far.

How does this information relate to the diets that are now currently used in autism?

Certainly, we need to consider that one of the benefits of gf/cf diet might have been an improvement in the barrier function in the gut which would not only reduce the opioid peptide absorption, but would also limit the absorption of oxalates and allergy-provoking food peptides of all sorts. We also need to consider that the introduction of very high oxalate foods as a substitute for gluten and dairy may compromise some of the benefits of removing gluten.

Disaccharidase deficiency apparently appears before villous atrophy shows up in celiac disease. The order to this decline may suggest that something related to the changes at the tight junction that come from exposure to gliadin may furnish the reason that disaccharidase activity falls off so early in celiac disease. Is there a parallel order of things in autism that explains why disaccharidase activity would get low when it does? The lack of disaccharidase activity is the reason for restricting disaccharides in the SCD diet and that restriction does seem to quell the fire in the gut for a significant set of people.

I find myself wishing that the SCD diet as it became used in autism hadn't evolved with such an emphasis on high oxalate foods. There is nothing about restricting disaccharides that means you HAVE to eat high oxalate foods. What might have happened is that parents were seeking more calories for their children, and wanted to make foods that were more like familiar bread products because they couldn't use those complex carbohydrates in cooking on SCD. Perhaps they found that their children really went after these high oxalate foods once the high foods were introduced.

The body does accommodate somewhat to high oxalate foods, but the eagerness of these children for these foods may have also been tied to a somewhat "addictive" quality of oxalates that some parents have reported on our website....addictions which seemed to diminish as the children's exposure to oxalate was lowered.

Because these high oxalate foods were likely included in the SCD in an attempt to enhance nutrition and the calorie count, including them in the diet seemed the right thing to do. Most of the research showing the damage that oxalates can do outside the kidney was and still is not widely known. For this reason the originators of the SCD could not have anticipated what oxalates were capable of doing when a leaky gut allowed these oxalates to be absorbed in a higher than usual quantity.

When we began the oxalate project, we certainly didn't anticipate this issue either, but we've learned this negative side of oxalates together as we saw children improve in very surprising ways after getting off high oxalate foods. Gastrointestinal issues were improving in these children going lower oxalate. Yeast issues were getting better. Dysbiosis seemed to be improving. Some of the children were getting rid of problems with chronic diarrhea or constipation, in addition to having some relief from urinary problems, if those had already developed. These changes were amazing enough, but what really surprised us is when we started hearing about changes in cognition and speech, fine motor and gross motor improvement, and even catch-up growth in children who had not been growing. Is there a chance these absorbed oxalates were also getting past the blood brain barrier, a type of tissue whose barrier regulation is similar to the gut?

Some moms and dads figured out the problem with high oxalate foods on their own because they watched their children's reactions and took them off the nuts and certain vegetables long before anyone started talking about oxalates. These parents are the true scientists who were so very observant! Our oxalate project gave them a vocabulary to describe what they observed, but it also linked them to other parents who were finding that their children were also affected by oxalates.

Do children on the low oxalate diet find improvements in previous intolerances?

We have found that some of the children who previously had horrible reactions to rice and corn and even wheat and dairy are tolerating some of these foods and other starches when sticking to a lower oxalate diet. The increased tolerance to these foods may have come from getting the tight junctions closed which would eliminate the overexposure of the immune system to these food antigens. If this is truly the case, the change might diminish allergic reactions to food and might possibly help restore the disaccharidase activity. All this will have to be studied formally, because right now these improvements are only the reports of parents. Through this project we are getting closer to understanding what it is that should be studied scientifically and what should be measured.

In summary, I hope all this will mean that we are getting closer to understanding something important in the mechanisms that can set into motion, or that will keep in motion, the gut disruption and gastrointestinal pain in children with autism. Maybe a lot of the problem circles around calcium regulation and tight junctions losing their grip, but there may be other players and other places of disruption for these molecules that scientists have not yet identified.

I find it fascinating how much we have been able to learn from celiac disease research. Maybe we should think hard about how and why gastroenterologists tell us that osteoporosis is often the first presenting symptom of celiac disease, for that, of course, is another issue that has a lot to do with calcium.

Our future goals of this project will go beyond the initial management of diet issues or addressing related issues of tissue permeability. We are currently recruiting scientists to specifically study oxalate issues in autism. A further goal will be characterizing the functions of oxalates in animals

in areas that go beyond crystal formation, calcifications, or kidney disease. Obviously, we are still at the very beginning of this project.

Please read the abstracts below. I hope they will help fill in the gaps in this information, especially if you are comfortable with their technical nature.

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A member of the DAN! Thinktank of the Autism Research Institute
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Quote from: FASANO, A. Intestinal zonulin: open sesame! Gut, 2001;49;159-162

It has recently been reported that untreated CD predisposes to autoimmune disorders such as insulin dependent diabetes mellitus, Hashimoto's thyroiditis, autoimmune hepatitis, and connective tissue diseases.²⁸ One could hypothesise that zonulin opens small intestinal TJs during the early stages of CD and permits entry of putative allergens into the intestinal submucosa where an autoimmune response is elicited.

Alterations in intestinal TJ permeability

J Gen Physiol. 1997 Dec;110(6):727-40. Related Articles, Links
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Calcium site specificity. Early Ca²⁺-related tight junction events.

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The molecular mechanisms by which Ca²⁺ and metal ions interact with the binding sites that modulate the tight junctions (TJs) have not been fully described. Metal ions were used as probes of these sites in the frog urinary bladder. Basolateral Ca²⁺ withdrawal induces the opening of the TJs, a process that is abruptly terminated when Ca²⁺ is readmitted, and is followed by a complete recovery of the TJ seal. Mg²⁺ and Ba²⁺ were incapable of keeping the TJ sealed or of inducing TJ recovery. In addition, Mg²⁺ causes a reversible concentration-dependent inhibition of the Ca²⁺-induced TJ recovery. The effects of extracellular Ca²⁺ manipulation on the TJs apparently is not mediated by changes of cytosolic Ca²⁺ concentration. The transition elements, Mn²⁺ and Cd²⁺, act as Ca²⁺ agonists. In the absence of Ca²⁺, they prevent TJ opening and almost immediately halt the process of TJ opening caused by Ca²⁺ withdrawal. In addition, Mn²⁺ promotes an almost complete recovery of the TJ seal. Cd²⁺, in spite of stabilizing the TJs in the closed state and halting TJ opening, does not promote TJ recovery, an effect that apparently results from a superimposed toxic effect that is markedly attenuated by the presence of Ca²⁺. The interruption of TJ opening caused by Ca²⁺, Cd²⁺, or Mn²⁺, and the stability they confer to the closed TJs, might result from the interaction of these ions with E-cadherin. Addition of La³⁺ (2 microM) to the basolateral Ca²⁺-containing solution causes an increase of TJ permeability that fully reverses when La³⁺ is removed. This effect of La³⁺, observed in the presence of Ca²⁺ (1 mM), indicates a high La³⁺ affinity for the Ca²⁺-binding sites. This ability of La³⁺ to open TJs in the presence of Ca²⁺ is a relevant aspect that must be considered when using La³⁺ in the evaluation of TJ permeability of epithelial and endothelial membranes, particularly when used during in vivo perfusion or in the absence of fixatives.

PMID: 9382899 [PubMed - indexed for MEDLINE]

FEBS Lett. 2005 Aug 29;579(21):4851-5. Related Articles, Links

Susan Costen Owens, MAIS, RA

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Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins.

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Coeliac disease is a chronic enteropathy caused by the ingestion of wheat gliadin and other cereal prolamines derived from rye and barley. In the present work, we investigated the mechanisms underlying altered barrier function properties exerted by gliadin-derived peptides in human Caco-2 intestinal epithelial cells. We demonstrate that gliadin alters barrier function almost immediately by decreasing transepithelial resistance and increasing permeability to small molecules (4 kDa). Gliadin caused a reorganisation of actin filaments and altered expression of the tight junction proteins occludin, claudin-3 and claudin-4, the TJ-associated protein ZO-1 and the adherens junction protein E-cadherin.

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Scand J Gastroenterol. 2001 Feb;36(2):163-8. [Related Articles](#), [Links](#)

Intestinal disaccharidase deficiency without villous atrophy may represent early celiac disease.

Murray IA, Smith JA, Coupland K, Ansell ID, Long RG.

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BACKGROUND: Intestinal disaccharidase activities are decreased in untreated celiac disease and also in other conditions without villous atrophy. Of 908 patients examined for suspected malabsorption, 37 (4.1%) had generalized disaccharidase deficiency without villous atrophy. The aim was to determine if generalized disaccharidase deficiency without villous atrophy represented latent celiac disease. **METHODS:** Case notes and histology of the 37 patients were reviewed. History and blood investigations including antigliadin and endomysial antibodies were checked. Where celiac disease was suspected, endoscopic duodenal biopsies for histology and disaccharidase estimation were repeated. **RESULTS:** Of the initial 37 patients, 6 patients had had repeat endoscopic biopsies; one having celiac disease. A further 18 patients were reviewed. The remainder declined further investigation. Eight had repeat endoscopic duodenal biopsies; one had celiac disease. Two with positive celiac serology also had enteroscopy with jejunal biopsies; both had celiac disease. **CONCLUSIONS:** At least 11% of patients with generalized disaccharidase deficiency without villous atrophy develop celiac disease. Enteroscopic biopsies from distal duodenum and proximal jejunum should be considered as the next investigation if endomysial or antigliadin antibodies are positive.

PMID: 11252408 [PubMed - indexed for MEDLINE]

J Cell Sci. 2000 Dec;113 Pt 24:4435-40. [Related Articles](#), [Links](#)

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Human zonulin, a potential modulator of intestinal tight junctions.

Wang W, Uzzau S, Goldblum SE, Fasano A.

Division of Pediatric Gastroenterology and Nutrition, Gastrointestinal Pathophysiology Section, Center for Vaccine Development, Baltimore, MD 21201, USA.

Intercellular tight junctions are dynamic structures involved in vectorial transport of water and electrolytes across the intestinal epithelium. Zonula occludens toxin derived from *Vibrio cholerae* interacts with a specific intestinal epithelial surface receptor, with subsequent activation of a complex intracellular cascade of events that regulate tight junction permeability. We postulated that this toxin may mimic the effect of a functionally and immunologically related endogenous modulator of intestinal tight junctions. Affinity-purified anti-zonula occludens toxin antibodies and the Ussing chamber assay were used to screen for one or more mammalian zonula occludens toxin analogues in both fetal and adult human intestine. A novel protein, zonulin, was identified that induces tight junction disassembly in non-human primate intestinal epithelia mounted in Ussing chambers. Comparison of amino acids in the active zonula occludens toxin fragment and zonulin permitted the identification of the putative receptor binding domain within the N-terminal region of the two proteins. Zonulin likely plays a pivotal role in tight junction regulation during developmental, physiological, and pathological processes, including tissue morphogenesis, movement of fluid, macromolecules and leukocytes between the intestinal lumen and the interstitium, and inflammatory/autoimmune disorders.

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Establishment and characterization of cultured epithelial cells lacking expression of ZO-1.

Umeda K, Matsui T, Nakayama M, Furuse K, Sasaki H, Furuse M, Tsukita S.

Department of Cell Biology, Kyoto University Faculty of Medicine, Yoshida-Konoe, Sakyo-ku, Kyoto 606-8501, Japan.

In well polarized epithelial cells, closely related ZO-1 and ZO-2 are thought to function as scaffold proteins at tight junctions (TJs). In epithelial cells at the initial phase of polarization, these proteins are recruited to cadherin-based spotlike adherens junctions (AJs). As a first step to clarify the function of ZO-1, we successfully generated mouse epithelial cell clones lacking ZO-1 expression (ZO-1^{-/-} cells) by homologous recombination. Unexpectedly, in confluent cultures, ZO-1^{-/-} cells were highly polarized with well organized AJs/TJs, which were indistinguishable from those in ZO-1^{+/+} cells by electron microscopy. In good agreement, by immunofluorescence microscopy, most TJ proteins including claudins and occludin appeared to be normally concentrated at TJs of ZO-1^{-/-} cells with the exception that a ZO-1 deficiency significantly up- or down-regulated the recruitment of ZO-2 and cingulin, another TJ scaffold protein, respectively, to TJs. When the polarization of ZO-1^{-/-} cells was initiated by a Ca²⁺ switch, the initial AJ formation did not appear to be affected; however, the subsequent TJ formation (recruitment of claudins/occludin to junctions and barrier establishment) was markedly retarded. This retardation as well as the disappearance of cingulin were rescued completely by exogenous ZO-1 but not by ZO-2 expression. Quantitative evaluation of ZO-1/ZO-2 expression levels led to the conclusion that ZO-1 and ZO-2 would function redundantly to some extent in junction formation/epithelial polarization but that they are not functionally identical. Finally, we discussed advantageous aspects of the gene knock-out system with cultured epithelial cells in epithelial cell biology.

PMID: 15292177 [PubMed - indexed for MEDLINE]

J Neurochem. 2000 Jan;74(1):320-6. Related Articles, Links
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Affinity purification and partial characterization of the zonulin/zonula occludens toxin (Zot) receptor from human brain.

Lu R, Wang W, Uzzau S, Vigorito R, Zielke HR, Fasano A.

Division of Pediatric Gastroenterology and Nutrition and Center for Vaccine Development, University of Maryland School of Medicine, Baltimore 21201, USA.

The intercellular tight junctions (TJs) of endothelial cells represent the limiting structure for the permeability of the blood-brain barrier (BBB). Although the BBB has been recognized as being the interface between the bloodstream and the brain, little is known about its regulation. Zonulin and its prokaryotic analogue, zonula occludens toxin (Zot) elaborated by *Vibrio cholerae*, both modulate intercellular TJs by binding to a specific surface receptor with subsequent activation of an intracellular signaling pathway involving phospholipase C and protein kinase C activation and actin polymerization. Affinity column purification revealed that human brain plasma membrane preparations contain two Zot binding proteins of approximately 55 and approximately 45 kDa. Structural and kinetic studies, including saturation and competitive assays, identified the 55-kDa protein as tubulin, whereas the 45-kDa protein represents the zonulin/Zot receptor. Biochemical characterization provided evidence that this receptor is a glycoprotein containing multiple sialic acid residues. Comparison of the N-terminal sequence of the zonulin/Zot receptor with other protein sequences by BLAST analysis revealed a striking similarity with MRP-8, a 14-kDa member of the S-100 family of calcium binding proteins. The discovery and characterization of this receptor from human brain may significantly contribute to our knowledge on the pathophysiological regulation of the BBB.

PMID: 10617135 [PubMed - indexed for MEDLINE]

: *Biochim Biophys Acta*. 2005 Nov 30;1762(1):80-93. Epub 2005 Oct 21. Related Articles, Links
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VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for Celiac Sprue probiotics and gluten intolerance.

Angelis MD, Rizzello CG, Fasano A, Clemente MG, Simone CD, Silano M, Vincenzi MD, Losito I, Gobetti M.

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The native structure and distribution of gliadin epitopes responsible for Celiac Sprue (CS) may be influenced by cereal food processing. This work was aimed at showing the capacity of probiotic VSL#3 to decrease the toxicity of wheat flour during long-time fermentation. VSL#3 (10⁹ cfu/ml) hydrolyzed completely the alpha2-gliadin-derived epitopes 62-75 and 33-mer (750 ppm). Two-dimensional electrophoresis, immunological (R5 antibody) and mass spectrometry analyses showed an almost complete degradation of gliadins during long-time fermentation of wheat flour by VSL#3. Gliadins non-hydrolyzed during fermentation by VSL#3 were subjected to peptic-tryptic (PT) digestion and analyzed by CapLC-ESI-Q-ToF-MS (Capillary Liquid Chromatography-Electrospray Ionization-Quadrupole-Time of Flight-Mass Spectrometry). Search for several epitopes showed the only presence of alpha2-gliadin-fragment 62-75 at a very low concentration (sub-ppm range). Compared to IEC-6 cells exposed to intact gliadins extracted from the chemically acidified dough (control), VSL#3 pre-digested gliadins caused a less pronounced reorganization of the intracellular F-actin which was mirrored by an attenuated effect on intestinal mucosa permeability. The release of zonulin from intestinal epithelial cells treated with gliadins was considerably lower when digested with VSL#3. Agglutination test on K 562 (S) cells showed that the PT-digest of wheat flour treated with VSL#3 increased the Minimal Agglutinating Activity of ca. 100 times. Wheat proteins were extracted from doughs and subjected to PT digestion. Compared to PT-digest from chemically acidified dough, celiac jejunal biopsies exposed to the PT-digest from the dough fermented by VSL#3 did not show an increase of the infiltration of CD3(+) intraepithelial lymphocytes. Proteolytic activity by probiotic VSL#3 may have an importance during food processing to produce pre-digested and tolerated gliadins for increasing the palatability of gluten-free products.

PMID: 16311022 [PubMed - in process]

2: Clin Gastroenterol Hepatol. 2005 Apr;3(4):335-41. [Related Articles, Links](#)
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Permeability, zonulin production, and enteropathy in dermatitis herpetiformis.

Smecuol E, Sugai E, Niveloni S, Vazquez H, Pedreira S, Mazure R, Moreno ML, Label M, Maurino E, Fasano A, Meddings J, Bai JC.

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BACKGROUND & AIMS: Dermatitis herpetiformis (DH) is characterized by variable degrees of enteropathy and increased intestinal permeability. Zonulin, a regulator of tight junctions, seems to play a key role in the altered intestinal permeability that characterizes the early phase of celiac disease. Our aim was to assess both intestinal permeability and serum zonulin levels in a group of patients with DH having variable grades of enteropathy. **METHODS:** We studied 18 DH patients diagnosed on the basis of characteristic immunoglobulin (Ig)A granular deposits in the dermal papillae of noninvolved skin. Results were compared with those of classic celiac patients, patients with linear IgA dermatosis, and healthy controls. **RESULTS:** According to Marsh's classification, 5 patients had no evidence of enteropathy (type 0), 4 patients had type II, 2 patients had type IIIb damage, and 7 patients had a more severe lesion (type IIIc). Intestinal permeability (lactulose/mannitol ratio [lac/man]) was abnormal in all patients with DH. Patients with more severe enteropathy had significantly greater permeability ($P < .05$). The serum zonulin concentration (enzyme-linked immunosorbent assay) for patients with DH was $2.1 \pm .3$ ng/mg with 14 of 16 (87.5%) patients having abnormally increased values. In contrast, patients with linear IgA dermatosis had normal histology, normal intestinal permeability, and negative celiac serology. **CONCLUSIONS:** Increased intestinal permeability and zonulin up-regulation are common and concomitant findings among patients with DH, likely involved in pathogenesis. Increased permeability can be observed even in patients with no evidence of histologic damage in biopsy specimens. Patients with linear IgA dermatosis appear to be a distinct population with no evidence of gluten sensitivity.

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3: Lancet. 2000 Apr 29;355(9214):1518-9. [Related Articles, Links](#)
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* Lancet. 2001 Nov 17;358(9294):1729-30.

Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease.

Fasano A, Not T, Wang W, Uzzau S, Berti I, Tommasini A, Goldblum SE.

We identified zonulin, a novel human protein analogue to the *Vibrio cholerae* derived Zonula occludens toxin, which induces tight junction disassembly and a subsequent increase in intestinal permeability in non-human primate intestinal epithelia. Zonulin expression was raised in intestinal tissues during the acute phase of coeliac disease, a clinical condition in which tight junctions are opened and permeability is increased.

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* Letter

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Epitopes of calreticulin recognised by IgA autoantibodies from patients with hepatic and coeliac disease.

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Calreticulin (CRT) was identified as a frequent target of serum autoantibodies (Ab) in various diseases, but anti-CRT Ab of IgA isotype were described only in coeliac (CLD) and some hepatic diseases. Employing ELISA with recombinant CRT we found significantly higher ($P < 0.001$) levels of IgA anti-CRT Ab in sera of patients with primary biliary cirrhosis (PBC) (77.6 ± 8.9 AU/mean \pm SE), autoimmune hepatitis (AIH) (105.1 ± 9.2 AU) and alcoholic liver cirrhosis (ALC) (193.5 ± 21.0 AU) relative to healthy controls (38.6 ± 2.0 AU). The levels of IgG anti-CRT Ab in sera of patients with PBC (59.5 ± 3.4 AU), AIH (89.7 ± 7.9 AU) and ALC (86.4 ± 6.2 AU) were also significantly increased ($P < 0.001$) when compared with controls (38.5 ± 2.1 AU). Pepscan technique with decapeptides of CRT (each overlapping by eight amino acids) revealed antigenic epitopes of this molecule recognised by IgA Ab of almost all tested patients-KGKNVLINKD and QVKSGTIFDNFL. We also identified disease specific antigenic epitopes on CRT molecule, predominantly recognised by IgA Ab of patients suffering from a particular disease: GGYVKLFPNS and YVKLFPNSLD in AIH (83%, 92% of patients), GLQTSQDARF and EQRLKEEED in CLD (both 75%) and ASKPEDWDER in ALC (67%). Identification of disease specific CRT epitopes contributes to clarification of autoreactivity against this molecule.

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Eur J Immunol. 2001 Mar;31(3):918-28. Related Articles, Links

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IgA cross-reactivity between a nuclear autoantigen and wheat proteins suggests molecular mimicry as a possible pathomechanism in celiac disease.

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Celiac disease patients display IgA antibody reactivity to wheat as well as to human proteins. We used serum IgA from celiac patients and, for control purposes, from patients with Crohn's disease, ulcerative colitis and from healthy individuals to identify celiac disease-specific IgA autoantigens in nitrocellulose-blotted extracts from various human cell types (epithelial, endothelial, intestinal cells, fibroblasts). The pattern, recognition intensity and time course of IgA autoreactivity was monitored using serial serum samples obtained from celiac children before and under gluten-free diet. By immunoblot inhibition and subcellular (cytosolic, nuclear) cell fractionation we identified a 55 kDa nuclear autoantigen expressed in intestinal, endothelial cells and in fibroblasts which was recognized by IgA antibodies of approximately half of the celiac disease patients and cross-reacted with wheat proteins. IgA reactivity to the 55 kDa autoantigen disappeared during gluten-free diet and was inhibited after pre-absorption of sera with wheat proteins but not with tissue transglutaminase, previously reported as the unique celiac disease-specific autoantigen. In conclusion, we defined a novel 55 kDa celiac disease-specific nuclear IgA

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autoantigen which shares epitopes with wheat proteins and which is different from tissue transglutaminase and calreticulin. Although the newly defined autoantigen was recognized much less frequently than tissue transglutaminase, our data suggest molecular mimicry between wheat and human proteins as a possible pathomechanism for the induction and/or maintenance of mucosal tissue damage in celiac disease.

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