Mechanisms of Disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases

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SUMMARY

The primary functions of the gastrointestinal tract have traditionally been perceived to be limited to the digestion and absorption of nutrients and electrolytes, and to water homeostasis. A more attentive analysis of the anatomic and functional arrangement of the gastrointestinal tract, however, suggests that another extremely important function of this organ is its ability to regulate the trafficking of macromolecules between the environment and the host through a barrier mechanism. Together with the gut-associated lymphoid tissue and the neuroendocrine network, the intestinal epithelial barrier, with its intercellular tight junctions, controls the equilibrium between tolerance and immunity to nonself-antigens. When the finely tuned trafficking of macromolecules is dysregulated in genetically susceptible individuals, both intestinal and extraintestinal autoimmune disorders can occur. This new paradigm subverts traditional theories underlying the development of autoimmunity, which are based on molecular mimicry and/or the bystander effect, and suggests that the autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by re-establishing intestinal barrier function. Understanding the role of the intestinal barrier in the pathogenesis of gastrointestinal disease is an area of translational research that encompasses many fields and is currently receiving a great deal of attention. This review is timely given the increased interest in the role of a 'leaky gut' in the pathogenesis of gastrointestinal diseases and the advent of novel treatment strategies, such as the use of probiotics.

KEYWORDS autoimmune, innate immunity, intestinal permeability, tight junction, toll-like receptor

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INTRODUCTION

Autoimmune diseases affect 5–8% of the US population (14–22 million people), which means that they are the third most common category of diseases in the US after cancer and heart disease. They can affect virtually every site in the body, including the gastrointestinal tract. At least 15 diseases are known to be the direct result of an autoimmune response, and circumstantial evidence links more than 80 conditions to autoimmunity.[1]

CLASSICAL THEORIES ON THE PATHOGENESIS OF AUTOIMMUNE DISEASE

Soon after autoimmune diseases were first recognized more than a century ago, researchers began to associate their development with viral and bacterial infections. The connection between infection and autoimmune disease is often explained by a mechanism known as 'molecular mimicry', whereby microbial antigens (or, more specifically, EPITOPES) are postulated to resemble self-antigens.[2] The induction of an immune response to the microbial antigens results in a cross-reaction with the self-antigens and the induction of autoimmunity. According to this theory, once the autoimmune process is activated it becomes independent of continuous exposure to the environmental trigger, and is therefore self-perpetuating and irreversible. Epitope-specific cross-reactivity between microbial antigens and self-antigens has been shown in some animal models to initiate autoimmunity.[3] Conversely, in most human autoimmune diseases, molecular mimicry seems to be a factor in the progression of a pre-existing subclinical autoimmune response, rather than in the initiation of autoimmunity.[3]

Another theory suggests that microorganisms expose self-antigens to the immune system by directly damaging tissues during active infection, and that this leads to the development of autoimmunity. This mechanism has been referred to as the 'bystander effect', and it occurs only when the new antigen is presented with the

orally administered triggering antigen.[4] Whether pathogens mimic self-antigens, release sequestered self-antigens, or both, however, remains to be elucidated.

A fairly new school of thought argues that increased hygiene and a lack of exposure to various microorganisms are responsible for the 'epidemic' of autoimmune diseases that has occurred over the past 30–40 years in industrialized countries, including the US.[5] This so-called 'hygiene hypothesis' is supported by immunological data showing that in neonates microbial antigens can induce a T_H1 IMMUNE RESPONSE that offsets the normally dominant T_H2 IMMUNE RESPONSE; in the absence of microbes, the gut might therefore be predisposed to an exaggerated T_H2 immune response, production of IgE, atopy, and the development of atopic disease.[6] An alternative explanation is that the absence of helminth infections eliminates the normally upregulated T_H2 immune response in childhood, culminating in a more marked T_H1 immune response, which is characteristic of autoimmune and inflammatory diseases.[7,8] Regardless of whether autoimmune diseases are caused by too much or too little exposure to microorganisms, it is now generally believed that adaptive immunity and an imbalance between the T_H1 and T_H2 immune responses are the key elements underlying the pathogenesis of the autoimmune process.[9]

Unfortunately, decades of research that has been carried out based on the assumptions outlined above has not led to successful treatments for these devastating autoimmune diseases.

THE INTESTINAL MUCOSA AS 'PORT OF ENTRY' FOR NONSELF-ANTIGENS

The intestinal epithelium is the largest mucosal surface in the human body, and provides an interface between the external environment and the host. In the gut, two key elements govern the interplay between environmental triggers and the host: intestinal permeability and intestinal mucosal defense.

Intestinal permeability and its regulation

The permeability of the intestinal epithelium depends on the regulation of intercellular TIGHT JUNCTIONS. Tight junctions were originally conceptualized as a secreted extracellular cement forming an absolute and unregulated barrier within the paracellular space. The contribution of the parace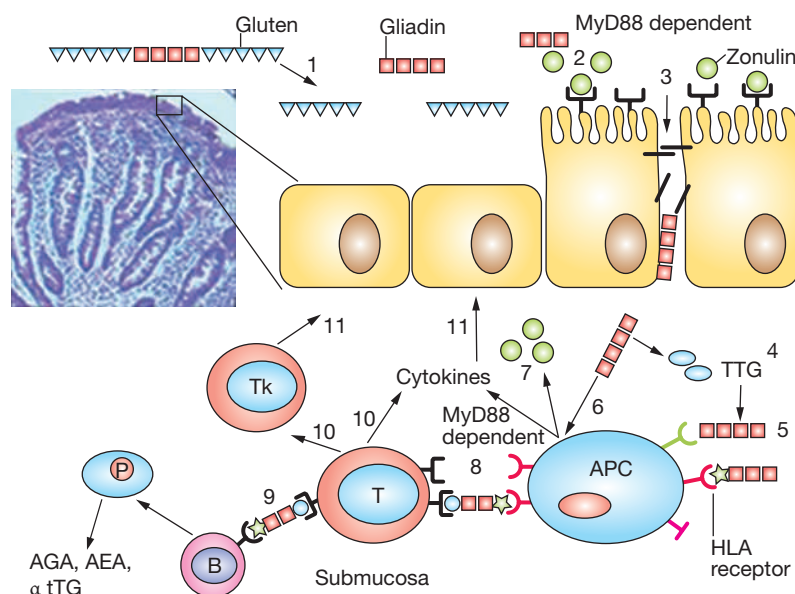llular space of the gastrointestinal tract to the trafficking of macromolecules between the environment and host was therefore judged to be negligible. Research carried out in the last decade has changed this paradigm, and it has been demonstrated that tight junctions are made up of a complex meshwork of proteins, the interaction of which dictates their competency.

To date, multiple proteins that make up the tight junctions strands have been identified: occludin,[10] members of the claudin family,[11] and the junctional adhesion molecule (JAM), a protein belonging to the immunoglobulin superfamily, which has been described as an additional component of tight junction fibrils.[12] Analysis of occludin complementary DNA has revealed that the predicted 504 amino-acid polypeptide

Figure 1 Proposed role of abnormal intestinal permeability in the pathogenesis of celiac disease. Gliadin and its immunomodulatory/inflammatory fragments are present in the intestinal lumen (**1**), which induces MyD88-dependent zonulin release (**2**). Zonulin release causes opening of tight junctions and gliadin passage across the tight junction barriers in subjects with dysregulation of the zonulin system (**3**). After tissue transglutaminase deamidation (**4**), gliadin peptides bind to human leukocyte antigen receptors present on the surface of antigen-presenting cells (**5**). Alternatively, gliadin can act directly on antigen-presenting cells (**6**), causing MyD88-dependent release of both zonulin and cytokines (**7**). Gliadin peptides are then presented to T lymphocytes (**8**), which process is followed by an aberrant immune response, both humoral (**9**) and cell-mediated (**10**), in genetically susceptible individuals. This interplay between innate and adaptive immunity is ultimately responsible for the autoimmune process targeting intestinal epithelial cells, leading to the intestinal damage typical of celiac disease (**11**). AEA, anti-endomysium antibodies; AGA, anti-gliadin antibodies; APC, antigen-presenting cell; α tTG, anti-tissue transglutaminase; B, B lymphocyte; P, plasma cell; T, T lymphocyte; Tk, lymphocyte T killer; TTG, tissue transglutaminase.

(65 kDa) contains four transmembrane spanning domains with two extracellular loops and internal amino and carboxyl termini.[10] The claudins are a group of at least 20 tissue-specific 20–27 kDa proteins that have two extracellular loops, variably charged amino-acid residues among family members and short intracellular tails.[13] Recent studies suggest that claudin 1, the intestine-associated family member,[13] might associate directly with occludin laterally in the same cell membrane, but not intercellularly.[14]

It is now apparent that tight junctions are dynamic structures that are involved in developmental, physiological, and pathological processes. As a result, particular attention is being placed on the role of tight junction dysfunction in the pathogenesis of several diseases, particularly autoimmune diseases.

To meet the many diverse physiological challenges to which the intestinal epithelial barrier is subjected, tight junctions must be capable of rapid and coordinated responses. To achieve such responses, a complex regulatory system orchestrates the assembly and disassembly of the multiprotein tight junction network. Although our knowledge of tight junction ultrastructure and intracellular signaling events governing their modulation has developed significantly during the past decade, relatively little is known about the pathophysiological regulation of tight junctions secondary to extracellular stimuli. *In vitro* studies have suggested that several cytokines (particularly tumor-necrosis factor [TNF]-α and interferon [IFN]-γ), elaborated by immune cells and radicals such as nitric oxide, can cause dysfunction of the intestinal mucosal barrier during the active phase of inflammatory bowel diseases.[15] The discovery of ZONULIN, a molecule that reversibly modulates tight junction permeability, has shed further light on how the intestinal barrier function is regulated in health and disease.[16] The physiological role of the zonulin system remains to be established; however, it is likely that it is involved in several processes, including the movement of fluid, macromolecules, and leukocytes from the bloodstream to the intestinal lumen, and vice versa. Another physiological role of intestinal zonulin is in protecting the proximal intestine against colonization by microorganisms (i.e. innate immunity).[17]

Given the complexity of both the cell-signaling events and intracellular structures that are part of the zonulin system, it is not surprising that the zonulin pathway is affected when the physiological state of epithelial and/or endothelial cells is dramatically changed, as it is in many of the autoimmune diseases in which tight junction dysfunction seems to be the primary defect (see below).

Intestinal mucosal defense
Gut-associated lymphoid tissue
Paracellular passage of macromolecules under either physiological or pathological circumstances is safeguarded by GUT-ASSOCIATED LYMPHOID TISSUE (GALT). GALT serves as a containment system that prevents potentially harmful intestinal antigens from reaching the systemic circulation, and induces systemic tolerance against luminal antigens by a process that involves polymeric IgA secretion and induction of T-regulatory-cell activity. GALT is composed of immune inductive sites (Peyer's patches) and immune effector sites (intraepithelial cells and lamina propria); studies now indicate that GALT is also composed of isolated lymphoid follicles (ILF).

ILF are tertiary lymphoid stuctures that are formed in autoimmune diseases, as well as in several inflammatory pathologies of the gastrointestinal tract.[18] Mature ILF bear a marked resemblance to Peyer's patches in their cellular composition and localization in the distal intestine, as well as in their dependence on the interaction of lymphotoxin with the lymphotoxin β receptor (LTβR) for their formation.[19]

In addition to GALT, the major histocompatibility complex is also an important contributor to intestinal immunological responsiveness. Human leukocyte antigen (HLA) class I and class II genes are located in the major histocompatibility complex on chromosome 6. These genes encode glycoproteins that bind peptides, and the resulting HLA–peptide complex is recognized by certain T-cell receptors in the intestinal mucosa.[20,21] Susceptibility to at least 50 autoimmune diseases is associated with specific HLA class I or class II alleles.

The balance between immunity and tolerance is essential for a healthy intestine; abnormal or inappropriate immune responses can result in inflammatory pathologies. Antigen-presenting M cells—specialized epithelial cells located in the follicle-associated epithelium overlying Peyer's patches and ILF—efficiently take up and transport various microorganisms and present antigen;[22] therefore, ILF are proposed to be local

sites for interactions between lympocytes, antigens, and antigen-presenting cells. Dendritic cells can also capture antigens present in the intestinal lumen by sending dendrites through epithelial tight junctions, while maintaining barrier integrity by modulating the expression of tight junction proteins,[23–25] and then rapidly migrating to other areas such as mesenteric lymph nodes.[26] There is evidence that memory T cells, induced by exposure to an oral antigen, can 'educate' antigen-presenting dendritic cells to instruct naive T cells, through release of soluble factors such as cytokines, to have the same responses as the memory T cell.[27] This evidence supports the notion that dendritic cells have a role in coupling the innate and adaptive immune responses that affect intestinal permeability.

The interplay between innate and adaptive immunity

Recognition of antigens by dendritic cells activates the TOLL-LIKE RECEPTORS (TLRs), and changes the phenotype and function of the dendritic cells. TLRs are the major receptors involved in discriminating between self-antigens and nonself-antigens based on the recognition of conserved bacterial molecular patterns. The systemic T cells that arise after feeding have been called 'T helper type 3' (T_H3), because they drive the production of IgA6, or 'T regulatory 1' ($T_{REG}1$), and they have a strong suppressive effect on the proliferation and IFN-γ production of naive T cells.[7] In intestinal epithelial cells, TLRs have a role in normal mucosal homeostasis and are particularly important in the interaction between the mucosa and luminal flora.[28] As different TLRs present in the gut respond to distinct stimuli, different adaptive immune responses are triggered.[29–31]

TLRs help direct the immune response by activating signaling events that increase expression of factors such as cytokines and chemokines, which recruit and regulate the immune and inflammatory cells that initiate or enhance immune responses. The peripheral memory T-cell response is a critical outcome of adaptive immunity, and TLRs might be required for the generation or maintenance of memory T cells.[32] TLRs are implicated in chronic diseases such as enteric inflammation and infection, and can have both proinflammatory and protective roles. Of interest is that commensal flora, acting through TLR4, positively influence susceptibility to food antigens,[33] and implicate TLRs in the regulation of intestinal permeability. A potential role for TLRs in regulating intestinal permeability is supported by *in vitro* studies using intestinal epithelial cell cultures, which show that TLR2 activates specific protein kinase C isoforms causing the rearrangement of the tight junction protein, ZO-1, leading to an increase in barrier integrity.[29] These data show that bacteria are vital for shaping the immune response, and underscore current interest in the effects of probiotics on intestinal permeability[34–36] that might limit polarization to T_H1 or T_H2 responses and maintain intestinal barrier function.

The intestinal neuroendocrine network

Intestinal homeostasis is coordinated by the responses of different cell types, including both immune and nonimmune cells. The interaction between immune and nonimmune cells is amplified by the influx of inflammatory/immune cells, which increases the exposure of nonimmune cells to soluble mediators (e.g. cytokines) released from immune cells. Macrophages, leukocytes and mucosal mast cells (MMCs) all release several mediators that alter gut function. Of interest is that MMCs seem to have a role in both T_H1-driven and T_H2-driven responses. MMCs release several preformed mediators, such as histamine, serotonin and mast-cell proteases, as well as newly synthesized mediators including leukotrienes, prostaglandins, and platelet-activating factor, in addition to interleukin-4 and TNF-α; many of these mediators affect epithelial permeability.[37–41] This might explain, in part, the increased intestinal permeability that is a feature of both T_H1-mediated and T_H2-mediated pathologies.

A PARADIGM SHIFT IN THE PATHOGENESIS OF AUTOIMMUNE DISEASES

A common denominator in autoimmune diseases is the presence of several pre-existing conditions that lead to an autoimmune process. The first of these conditions is the genetic susceptibility of the host immune system to recognize, and potentially misinterpret, an environmental antigen presented within the gastrointestinal tract. The second is that the host must be exposed to the antigen. Finally, the antigen must be presented to the gastrointestinal mucosal immune system following its paracellular passage from the intestinal lumen to the gut submucosa; this process is normally prevented by competent tight junctions.[42,43] In many cases, increased intestinal permeability seems to precede disease and

causes an abnormality in antigen delivery that triggers the multiorgan process leading to the autoimmune response.[44]

Taking the above information into consideration, we propose that the pathogenesis of autoimmune diseases can therefore now be described by three key points. First, autoimmune diseases involve a miscommunication between innate and adaptive immunity. Second, molecular mimicry or bystander effects alone might not explain entirely the complex events involved in the pathogenesis of autoimmune diseases. Rather, the continuous stimulation by nonself-antigens (environmental triggers) seems to be necessary to perpetuate the process. Contrary to general belief, this concept implies that the autoimmune response can theoretically be stopped and perhaps reversed if the interplay between genes predisposing individuals to the development of autoimmunity and environmental triggers is prevented or eliminated. Third, in addition to genetic predisposition and exposure to triggering nonself-antigens, the loss of the protective function of mucosal barriers that interact with the environment (mainly the gastrointestinal and lung mucosa) is necessary for autoimmunity to develop.

CLINICAL OUTCOMES OF IMPAIRED INTESTINAL PERMEABILITY
Celiac disease
Celiac disease is the best testament to the accuracy of the new paradigm for the pathogenesis of autoimmunity proposed above. This intestinal disorder is a unique model of autoimmunity; in contrast to most other autoimmune diseases, a close genetic association with HLA genes, a highly specific humoral autoimmune response against tissue transglutaminase, and, most importantly, the triggering environmental factor (GLIADIN), are all known factors for celiac disease.

Early in the development of celiac disease, tight junctions are opened,[45,46] most likely secondary to zonulin upregulation,[47] and severe intestinal damage ensues[46] (Figure 1). The upregulation of the zonulin innate immunity pathway is directly induced by exposure to the disease's antigenic trigger, gliadin.[48] Gliadin has been shown to also be a potent stimulus for macrophage proinflammatory gene expression and for cytokine release.[49] Data in mice suggest that both functions are independent of TLR4 and TLR2, but are dependent on MyD88, a key adapter molecule in TLR/interleukin-1

receptor signalling.[50] These data indicate that gliadin initiates intestinal permeability through a MyD88-dependent release of zonulin that enables paracellular translocation of gliadin and its subsequent interaction with macrophages within the intestinal submucosa (Figure 1).

The interaction of gliadin with macrophages initiates signaling through a TLR-like pathway, which results in the establishment of a proinflammmatory (T_H1-type) cytokine milieu and subsequently mononuclear cell infiltration into the submucosa. This, in turn, might permit the interaction of T cells with antigen-presenting cells, including macrophages, ultimately leading to the antigen-specific adaptive immune response seen in patients with celiac disease. Once gluten is removed from the diet, serum zonulin levels decrease, the intestine resumes its baseline barrier function, autoantibody titers are normalized, the autoimmune process shuts off and, consequently, the intestinal damage (which represents the biological outcome of the autoimmune process) heals completely.

Inflammatory bowel disease
The pathogenesis of inflammatory bowel disease (IBD) remains unknown, although there is now convincing evidence to implicate genetic, immunological, and environmental factors in the initiation of the autoimmune process.[51] Several lines of evidence suggest that increased intestinal permeability has a central role in the pathogenesis of IBD. Like celiac disease, IBD might be related to an innate immune deficiency, which leads to the inappropriate access of nonself-antigens to the GALT. In clinically asymptomatic Crohn's disease patients, increased intestinal epithelial permeability precedes clinical relapse by as much as 1 year,[52,53] indicating that a permeability defect might be an early event in disease exacerbation. The hypothesis that abnormal intestinal barrier function is a genetic trait involved in the pathogenesis of IBD is further supported by the observation that clinically asymptomatic first-degree relatives of Crohn's disease patients can have increased intestinal permeability. Although a primary defect in intestinal barrier function might be involved in the early steps of the pathogenesis of IBD, the production of cytokines, including IFN-γ and TNF-α, secondary to the inflammatory process, perpetuates the increased intestinal permeability[40,41] by reorganizing the tight junction proteins, ZO-1, JAM1, occludin, claudin 1, and claudin 4.

Immunohistochemical localization of tight junction proteins in mucosal biopsies from IBD patients shows altered expression of several critical tight junction proteins, including upregulation of claudin 2,[54] which might be due to the disruptive effects of proinflammatory cytokines on the barrier associated with internalization of these transmembrane proteins.[55] In this manner, a vicious circle is created, in which barrier dysfunction allows further leakage of luminal contents, thereby triggering an immune response that in turn promotes further leakiness.

Extraintestinal autoimmune diseases

The 'breach' of the intestinal barrier by nonself-antigens might lead to an immune response targeting extraintestinal organs. These organs include, among others, the skeletal system (ankylosing spondylitis), the pancreas (type 1 diabetes),[56–58] the kidney (IgA nephropathy),[59,60] the liver (nonalcoholic steatohepatitis),[61] and the brain (multiple sclerosis).[62]

CONCLUSIONS

The classical paradigm of autoimmune pathogenesis involving a specific genetic makeup and exposure to environmental triggers has been challenged by the addition of a third element: the loss of intestinal barrier function. Genetic predisposition, miscommunication between innate and adaptive immunity, exposure to environmental triggers, and loss of the intestinal barrier function secondary to dysfunction of intercellular tight junctions, seem to all be key ingredients involved in the pathogenesis of autoimmune diseases. This new theory implies that, once the autoimmune process is activated, it is not self-perpetuating; rather, it can be modulated or even reversed by preventing the continuous interplay between genes and environment. As tight junction dysfunction allows this interaction, new therapeutic strategies aimed at re-establishing the intestinal barrier function offer innovative, unexplored approaches for the treatment of these devastating diseases.

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A Fasano declared competing interests; go to the article online for details. T Shea-Donohue declared she has no competing interests.

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