

A microscopic view of various bacteria, including rod-shaped and spherical forms, set against a light blue background. The bacteria are rendered in a semi-transparent, glowing style.

Bacterial Triggering of Autoimmunity: The How and Why.

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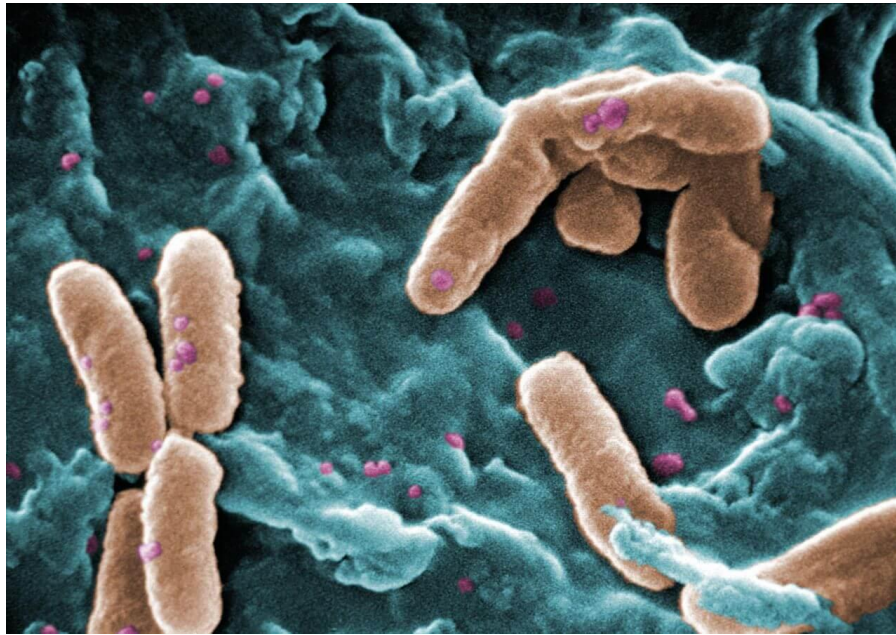


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Dr. Brady has 27 years of experience as an integrative practitioner and over 23 years in health sciences academia. He is a licensed naturopathic medical physician in Connecticut and Vermont, is board certified in functional medicine and clinical nutrition, and completed his initial clinical training as a doctor of chiropractic. Dr. Brady has been the chief medical officer of DFH for over 16 years and has been a guiding presence in product formulation and design, clinical education and support, quality control oversight and strategic partnerships for the company. He also currently serves as the chief medical officer for Diagnostic Solutions Labs, and is the director of the Human Nutrition Institute, and associate professor of clinical sciences at the University of Bridgeport in Connecticut. He has appeared on the plenary speaking panel of some of the largest and most prestigious conferences in the field including; IFM, ACAM, A4M, ACN, IHS, AANP and many more. He is in clinical practice at Whole Body Medicine in Fairfield, CT, specializing in functional, nutritional and metabolic medicine.



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Introduction

With the steady increase in the incidence of virtually every autoimmune disease occurring in the Western industrialized world, and standard treatment still relying mainly on symptom control using overt immune suppressing medications which carry significant side-effects, clinicians are rightly looking for any advantage in the prevention and upstream management of autoimmune disorders¹⁻². With the concomitant explosion of research into the microbiome, and more specifically the gastrointestinal microbiota (GM), showing linkages between specific aberrant patterns (signatures) of dysbiosis and greater prevalence of specific chronic complex metabolic diseases, including autoimmune conditions, there is a natural desire to understand why these relationships may exist, whether they are simply associations or causal, and what mechanisms may underlie such relationships. This article will review some of the known mechanisms by which bacterial organisms in the GM may contribute to immune dysregulation and potentially the development of an autoimmune disorder in an individual.

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Selected Relationships Between GI Microbes and Autoimmune Diseases

There are now multitudes of associations that have been firmly established between the overgrowth of certain commensal, opportunistic and pathogenic gastrointestinal bacteria, and the increased prevalence of specific autoimmune disorders (See table 1)³. While some of these associations have been known for quite some time, mechanisms of causality have also rapidly been being established in the research.

GUT MICROBES AND SYSTEMIC PATHOLOGY

Examples of Epidemiologic Associations Between GI Microbes and Systemic Autoimmune Pathology	
<i>Klebsiella</i>	Ankylosing Spondylitis
<i>Citrobacter, Klebsiella, Proteus, Prevotella</i>	Rheumatoid Arthritis
<i>Bacteroidetes</i> spp	Arthritis in general
<i>Fusobacterium</i>	Systemic sclerosis
<i>Mycobacteria</i>	Psoriasis & Crohn's Dz.
<i>Yersinia</i>	Grave's Disease & Hashimoto's Dz.
<i>Streptococcus</i>	PANDAS
<i>Chlamydia, Salmonella, Shigella, Yesrsinia</i>	Reactive arthritis (ReA)
<i>S. Pyogenes</i>	Rheumatic Fever
<i>Camphylobacter jejuni</i>	Gullian Barre Syndrome
<i>E. coli, Proteus</i>	Autoimmunity in general

Table 1: Selected Associations of Microbial Overgrowth and Autoimmune Disorders

Modified from: Mayes MD. Epidemiologic studies of environmental agents and systemic autoimmune diseases. *Environ Health Perspect* 1999;107 (suppl. 5):743-748

Molecular Mimicry

The concept of molecular mimicry is comparatively a simple one to understand, and one that has been appreciated the longest, attracting considerable research related to the genesis of autoimmune disorders. Simply stated, environmental exposure to specific antigens (including dietary peptides and those expressed by microbes) can, in genetically susceptible individuals, induce cross-reactions with structurally similar amino acid motifs associated with specific host tissues proteins. (See Figure 1).

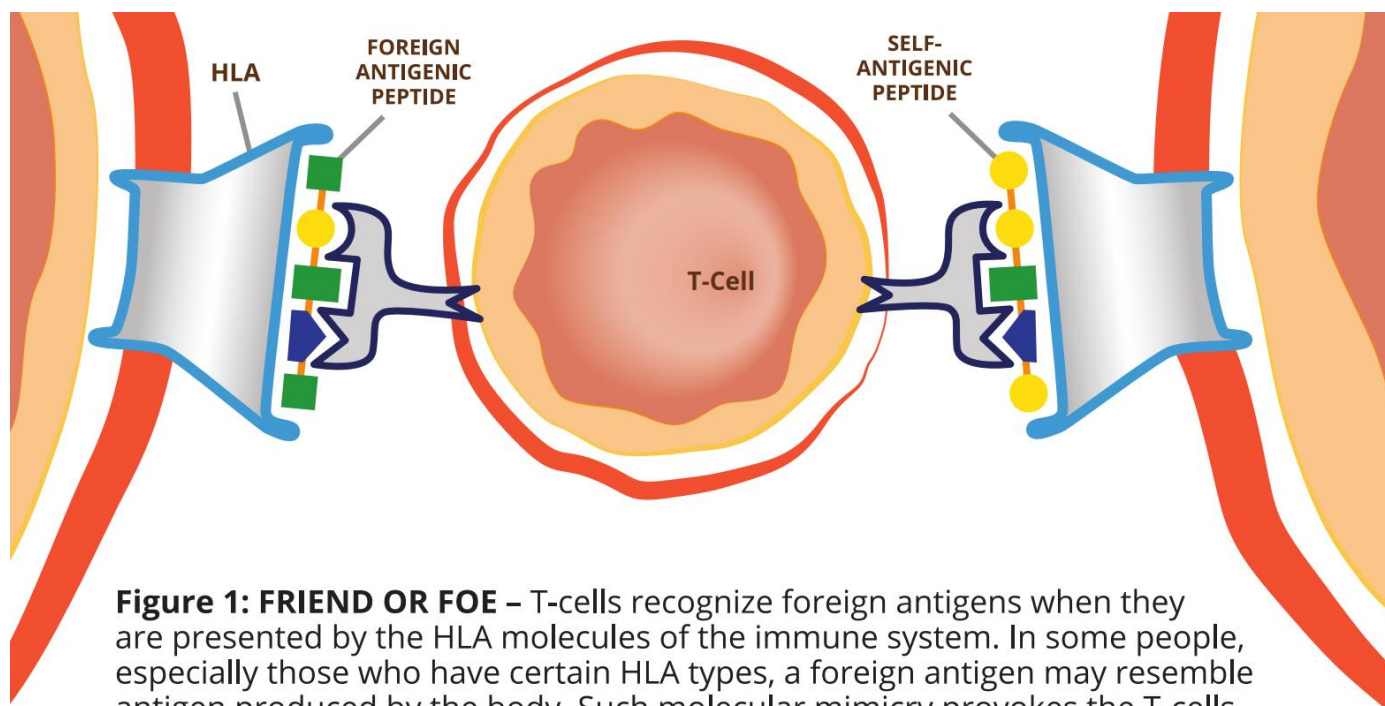
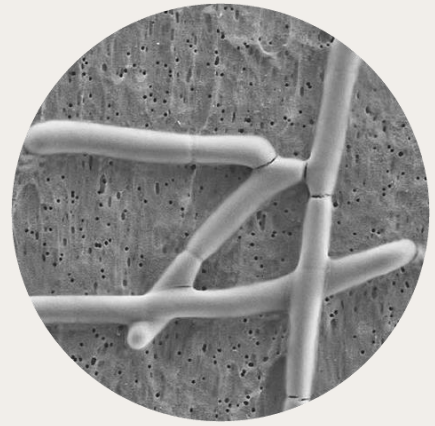


Figure 1: FRIEND OR FOE – T-cells recognize foreign antigens when they are presented by the HLA molecules of the immune system. In some people, especially those who have certain HLA types, a foreign antigen may resemble antigen produced by the body. Such molecular mimicry provokes the T-cells to attack body tissues that contain the self-antigens.

Source: Unknown

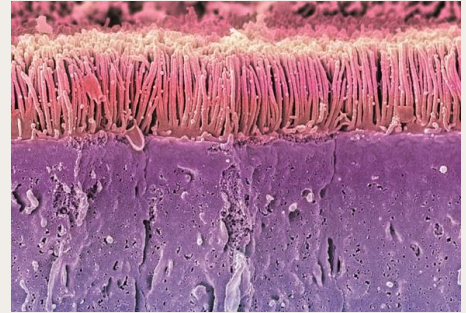
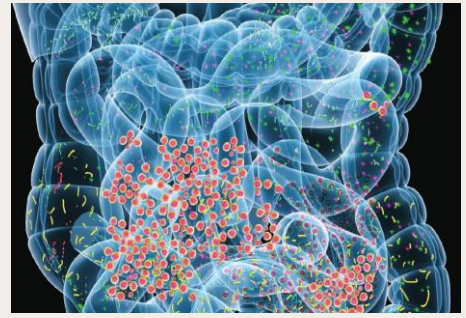
Molecular Mimicry

Pishak et al demonstrated that the mucous membrane of healthy people is colonized by *Bifidobacteria*, *Lactobacilli*, *Bacteroides*, *Escherichia* and *Enterococci*; while the mucous membrane in rheumatoid arthritis (RA) subjects is mainly colonized by aerobic opportunistic conventionally pathogenic enterobacteria (i.e., enteropathogenic *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, etc.), *Staphylococci*, *Enterococci* and other anaerobic bacteria (*Bacteroides*, *Peptococci*, *Peptostreptococci*, etc.).⁴ They have also reported the observed phenomenon that as RA exacerbates and then enters remission, which is a common occurrence across the spectrum of autoimmune disorders, the composition of the subject's GI microbiota correspondingly also changes between the aberrant pattern detailed above and the one typical of normal subjects. The data of Tiwana et al suggests an increased immune response to *Klebsiella* in patients with ankylosing spondylitis (AS), ulcerative colitis (UC), and Crohn's disease (CD) and to *Proteus* in patients with RA.⁵ Alan Ebringer and his group in the United Kingdom have established over the course of many years that a substantial percentage of patients diagnosed with RA have chronic stealth infection with *Proteus mirabilis* in the upper urinary tract, and overabundance has also been documented in the GI tract.⁶ His group also established the specific amino acid motifs of cross-reaction between the *Proteus* expressed hemolysin and the RA-associated HLA-DR molecules, as well as those between the *Proteus* expressed urease enzyme and host hyaline cartilage containing type XI collagen, the type only found in the small joints affected in RA. This specific association to type XI collagen structures may finally, at least partially, explain why only specific joints are involved in RA, while others are typically spared.



Molecular Mimicry

Ebringer's generally successful treatment protocol includes antibiotic therapy, such as ciprofloxacin (sometimes in combination with NSAIDs, disease modifying anti-rheumatic drugs [DMARDs], and other immunosuppressive agents as needed), with the added use of natural blocking agents such as cranberry juice, vitamin C for urine acidification, and plenty of fluids (diuresis).⁷ Interestingly enough, DMARD medications, such as sulfasalazine, exhibit antimicrobial properties against non-sporing anaerobes, such as *Clostridia* and *Enterobacteria*, as well as having anti-inflammatory properties to include suppression of inflammatory cytokines and induction of apoptosis in inflammatory cells. It may very well be the antimicrobial properties of these medications which represent their main mechanism of action in the treatment of RA. Doxycycline and minocycline, two other relatively safe and moderately effective antibiotic DMARDs, inhibit matrix metalloproteinase and nitric oxide synthase, suppress adaptive immune cells, and increase IL-10 (an anti-inflammatory cytokine) production⁸.



Modification of Host Proteins and Immune Responses by Bacteria and Their Metabolites

Oral bacterial infection with *Porphyromonas gingivalis*, the primary cause of periodontal disease, may also play a role in peptide citrullination, theorized to be involved in the loss of self-tolerance and development of autoimmunity in RA, according to Liao et al.⁹ *P. gingivalis* secretes the enzyme peptidyl arginine deiminase (PPAD4) and can facilitate the conversion of arginine to citrulline at the C-terminus of proteins (See Figure 2). Citrullinated protein and anti-citrullinated antibodies play an important role in the pathogenesis of rheumatoid arthritis, at least in many cases. PAD4 gene encoding and PAD4 protein is one of the risk genes associated with protein citrullination, while PAD4 antibodies are specific markers of RA¹⁰. It is worth noting that *P. gingivalis* and citrullination of host proteins, such as fibrinogen, have begun to be implicated in accelerated atherosclerosis¹¹, as has citrullination of glial fibrillary acidic protein (GFAP) in the progression of neurodegenerative diseases, such as Alzheimer's disease (AD)¹².

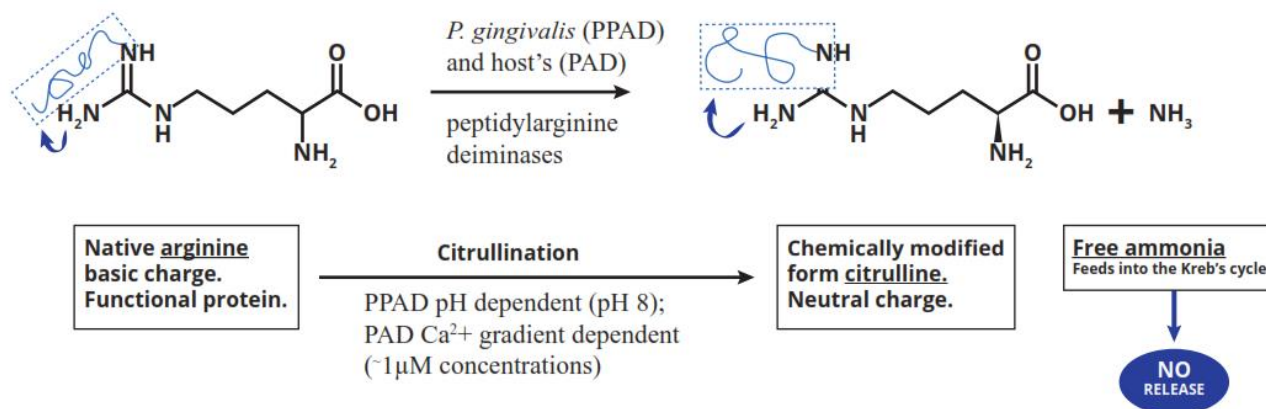


Figure 2: Chemical modification of arginine amino acid residue by *P. gingivalis* and host-mediated peptidylarginine deiminases resulting in the conversion of arginine on functional peptide to citrulline (defective protein). This posttranslational modification alters the spatial arrangement of the original 3D-structure and function of the protein peptide as indicated by arrows (arginine-peptide and citrulline-peptide). Ammonia released during the chemical reaction is beneficial for PPAD activation.

From: Olsen I, Singhrao SK, Potempa J. Citrullination as a plausible link to periodontitis, rheumatoid arthritis, atherosclerosis and Alzheimer's disease. *Journal Oral Microbio*, 2018, VOL. 10, 1487742. <https://doi.org/10.1080/20002297.2018.1487742>

Modification of Host Proteins and Immune Responses by Bacteria and Their Metabolites

Researchers have continued to go beyond establishing mere associations between the presence of various microbes and autoimmune disorders. Some have actually experimentally induced autoimmune disease by infecting animals with specific pathogens. Mazmanian et al inoculated a wild-type mouse with the bacterium *Helicobacter hepaticus* to create an experimental mouse version of the autoimmune disorder inflammatory bowel disease (IBD).¹³ *H. hepaticus* activates Th17 cells which release cytokines associated with inflammation, such as IL-17, which cause symptoms of the disease. They then introduced *Bacteroides fragilis*, expressing the polysaccharide A (PSA), to the gut of the animals where the PSA molecule was taken up by dendritic cells and presented on their surface, activating CD4 T cells and regulatory T cells (Tregs). The Tregs released IL-10 which suppresses the inflammatory action of IL-17, alleviating the IBD in mice. In summary, the researchers induced autoimmune disease by introducing one specific bacteria to the GM composition of these animals, and resolved it by introducing another, making a compelling argument for a causal relationship between the GI microbiota and autoimmune activity. Similarly, Maeda et al have shown direct evidence that *Prevotella copri* can aggravate arthritis, inducing severe synovitis in mouse models when the animals were recolonized with feces from RA subjects or *P.copri*¹⁴. They also elucidated pathogenic cellular mechanisms by which *P. copri* can induce arthritis progression, including increasing the Th17 cell population, Th17 cell related cytokines (i.e., IL-6 and IL-23), and Th17-biased responses to the arthritis-related autoantigen RPL23A. Several research teams have also shown the potential of gut dysbiosis to mediate leakage of the mucosal and immune barrier, leading to penetration of bacteria and/or their components directly into the entire body, as such bacterial components have been detected in the synovium of RA patients, leading to inflammation and pannus formation in the joints^{8,15}. Other bacterial toxins, especially endotoxins such as lipopolysaccharides (LPS), might also be important causative agents in the pathogenesis of rheumatoid arthritis (RA), as molecular associations between LPS/TLR4/collagen type II in chondrocytes upregulate the NF- κ B and PI-3K signaling pathways and activate pro-inflammatory activity¹⁶.

Modification of Host Proteins and Immune Responses by Bacteria and Their Metabolites

Autoimmune thyroid disorders also have been linked to bacterial infections, including GI overgrowth of the pathogenic organism *Yersinia enterocolitica*. Petru et al state; "*Yersinia shows on its surface saturable binding sites for TSH. TSH receptor antibodies could be produced in selected individuals having been infected with bacteria showing TSH receptors. It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases.*"¹⁷ Other researchers have shown a much higher prevalence of *Yersinia* serum antibodies in patients with thyroid disease versus controls. However, once again, there is no universal causality established, as autoimmune phenomena is a complex issue and seems to be potentially fueled by a multitude of potential antecedents, triggers, and mediators. For example, ubiquitous viral infections, such as EBV, HSV, HTLV-1, enterovirus, etc., have long been implicated in the genesis of many cases of autoimmune thyroiditis¹⁸. Specific dietary antigens have also been linked to autoimmune thyroid disease. Celiac patients have approximately 10 times the rate of auto-immune thyroid diseases (such as Hashimoto's thyroiditis and Grave's disease) as non-celiac individuals, reflective of the affinity of gluten-gliadin antigen-antibody complexes for thyroid tissue.¹⁹ It may be no coincidence that the emergence of an apparent epidemic of autoimmune diseases has corresponded with the ever-increasing consumption of poor-quality modern processed foods known to both negatively alter the GI microbiome and to contain a constant (often hidden) stream of offending dietary antigens, including gluten-containing grains. More recently, there have been discussions in the literature regarding additional ways that bacterial organisms present in some individual's GI microbiota can induce complex metabolic changes in the host. One such is the bacterial production of dipeptidyl-peptidase-4 (DPP-4) homologs, which can mimic DPP-4 produced by human host lymphocytes, endothelial and epithelial cells. DPP-4 facilitates proteolytic modification of proteins, including key hormones, neuropeptides, and chemokines. Modification of such critical bioactive proteins can induce changes in digestion, intestinal function, behavior, and immune function and have been postulated to have the potential to lead ultimately to autoimmune phenomena in the host²⁰.

The Perfect Storm Effect in Autoimmunity

Fassano has written extensively about the “triad of autoimmunity”, referring to the observation that in order to have any autoimmune disorders manifest there must be three critical factors in-play; 1) An environmental trigger/antigen exposure, 2) An individual genetic susceptibility, and 3) The presence of intestinal hyper-permeability (aka: “leaky gut syndrome”)²¹. While there are certainly models of autoimmunity related to other phenomenon, such as host viral infection (i.e., EBV, CMV, HSV, HTLV-1) and the resultant tissue inflammation and destruction caused by the immune response (i.e., the “bystander effect”), which are not mediated through the gut and would require no GI mucosal hyper-permeability issues, it still presents an intriguing hypothesis in those situations where the disease appears to be triggered by either bacterial or food proteins, both present in the gut¹⁸. One such situation where there seems to be a pathway to the development of RA, CD, and type-1 diabetes (T1D), which involves an interplay between an infectious environmental exposure, genetic susceptibility, and likely gut hyper-permeability is the case of *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The association of MAP with these disorders is based on shared genetic predisposition and molecular mimicry with environmental antigens. MAP infection in subjects with genetic predisposition via single nucleotide polymorphisms (SNPs) in the negative regulator *protein tyrosine phosphatase non-receptor type 2 and 22* (PTPN2/22) may lead to immune dysregulation by stimulating hyper-proliferative T-cells, production of pro-inflammatory cytokines (overexpression of IFN- γ), and through molecular mimicry the production of autoantibodies²².

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Summary

While all of these associations may be interesting to researchers, what does this really mean to a clinician? Some critics would argue that there is a lack of interventional data to suggest eradication of these associated organisms, improvement of the GM in general, and avoidance of potential dietary antigens positively affects patient outcomes. This may be true in many instances, but it has been well established, for example, by Ebringer that successful treatment of Proteus clinically helps those with RA⁷, and dietary elimination of gluten-containing grains is entirely accepted as the most viable intervention in Celiac disease. One potential issue in play is that by the time a patient is diagnosed with autoimmune disease there is often already substantial host-tissue damage. However, what if potential triggers were routinely screened for and removed by health care providers, particularly in those with a family history of autoimmune disorders? The entire course of the disorder might be favorably altered, and many of these disorders might potentially never emerge clinically. In the functional, integrative and naturopathic medicine models, there is a strong emphasis on both early detection and interventions that target the underlying pathophysiologic basis and underlying dysfunction of a disease process. Therefore, in these models the goal is to take clinical actions to reduce the potential for the disease process to progress, or ideally ever even begin. This also seems to intuitively make sense even in those subjects who already have established disease, as even though you may not be able to undo the damage already done, you can likely - if nothing else- slow down the pathophysiologic process. This is particularly true since the interventions required pose little or no risk and are also relatively inexpensive; including pro- and prebiotics, antimicrobial botanicals and volatile oils, mucosal-supporting nutrients and botanicals, and dietary modulation. Substantially improved testing to assess the GI microbiota utilizing quantitative PCR and next-generation sequencing molecular methods are also now widely available to clinicians at relatively low cost with rapid turn-around time, further enhancing the clinicians ability to objectively and accurately screen for risky patterns in an individual's GM composition and to intervene in ways that may favorably alter the landscape and the behavior of the patient's immune response, and ultimately the reduction in autoimmune disease occurrence, morbidity, and in some cases mortality.

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Disclosures

David M. Brady is the chief medical officer of Diagnostic Solutions Labs, LLC (GI-MAP™) and Designs for Health, Inc.

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