Autoimmune Disease:

Leaving the Era of Reaction and Entering the New Era of Prediction

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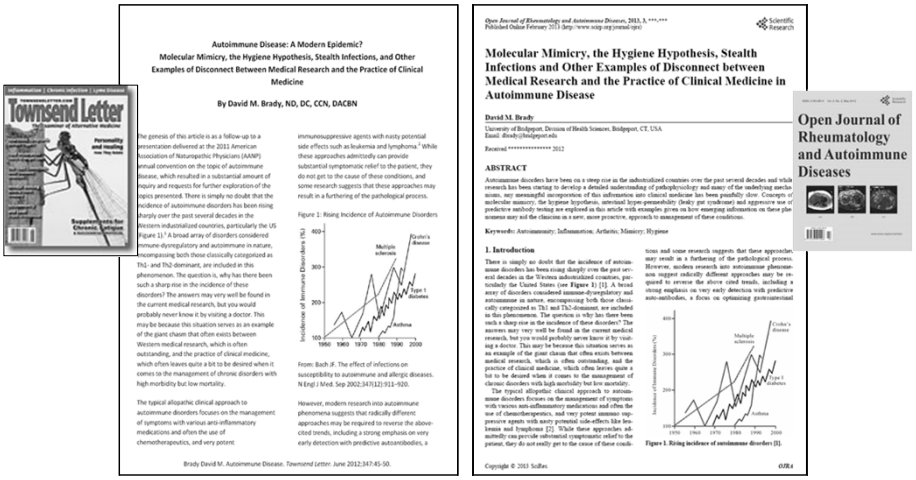

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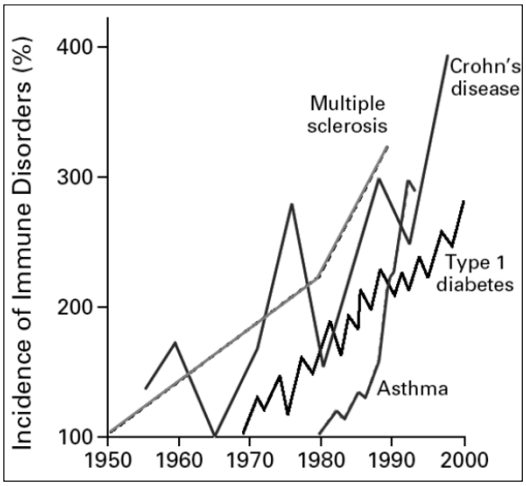
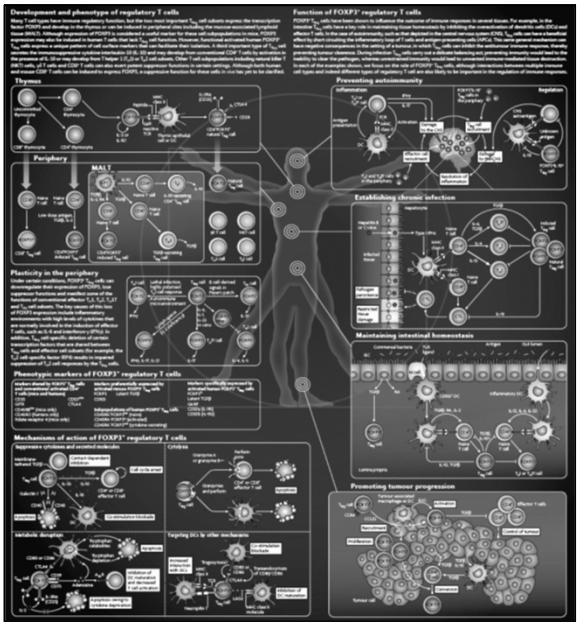
Increasing Incidence of Immune Regulatory Disorders



Bach JF: (2002) 347:911-920

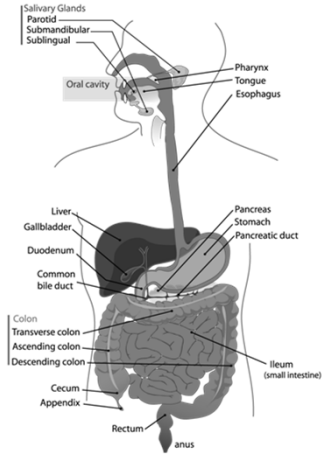
How do we see illness?

Autoimmune Disease



The Importance of Mucosal Immunity

"The dominating part of the immune defense, even if flora is excluded, is localized in the gut—no less than 75% of the immune cells of the body are suggested to be found in the GI tract."



Bengmark S. Acute and "chronic" phase reaction--a mother of disease, ClinNutr, Vol. 23, No. 6, pp. 1256-1266, December 2004

"Death begins in the colon"

- E. E. Metchnicoff
  - Russian Pathologist,
  - 6th Ever Nobel Laureate (1908)
  - Father of "orthobiosis" theory and probiotics

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Nat Rev Genet. ; 13(4): 260–270. doi:10.1038/nrg3182.

The Human Microbiome: at the interface of health and disease

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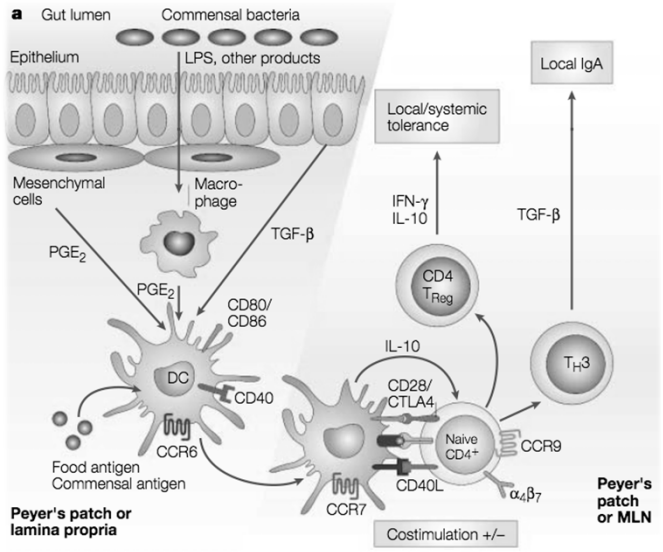
Abstract

Interest in the role of the microbiome in human health has burgeoned over the p
advent of new technologies for interrogating complex microbial communities. T
dynamics of the microbiome can be described by many of the tools and observa
study of population ecology. Deciphering the metagenome and its aggregate ge
also can be used to understand the functional properties of the microbial commu
microbiome and metagenome probably have important functions in health and d
exploration is a frontier in human genetics.

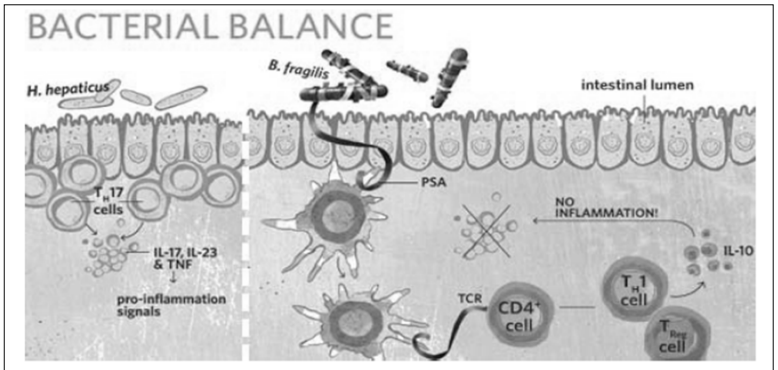
Until recently, the properties of the microbiota of humans (formerly call
were largely a black box. Cultivation *in vitro*, which has been the cor
microbiology since the 19th century, cannot be applied to many of the
populated microbial communities[1]. However, DNA-based analyses h
horizon, by generating enormous new data sets that can be mined for
composition and functional properties of vastly greater numbers of m
For example, the Human Microbiome Project (HMP) by the NIH has
terabyte 16S rRNA metagenomic dataset of over 35 billion reads taken fr
300 U.S. subjects, across 15 body sites. Large-scale endeavors (e.g. t
European project, MetaHit[2]) provide a preliminary understanding of t
significance of the human microbiome and its collective genes (the metag

The aim of these projects, particularly the HMP, is to characterize the
of the 'normal' microbiome of healthy individuals. Important questio
commonalities and differences between healthy individuals in both m
functional pathways are being addressed. The presence of major chis
sites such as the vagina[3] and the gastrointestinal tract[4] provide new w

- The human microbiome and its relationship to disease is a new and rapidly evolving field of study.
- Co-evolution of hosts and their microbiomes has led to cooperative interactions in metabolism and homeostasis.
- Concepts from community ecology such as resilience, community disturbances, and extinction are useful in understanding the microbiome.
- New computational and statistical tools are being actively developed to analyze the large sequence datasets generated by the increasingly powerful technologies.
- The taxonomic composition and functional characteristics of the microbiome may allow individuals to be categorized into different microbial patterns, called "enterotypes", in the gastrointestinal tract. Although low-level taxonomy varies substantially among individuals, higher level taxonomy and functional characteristics appear largely preserved.
- Many factors affect the composition of the microbiome over the course of a human lifetime. These include inheritance, mode of infant delivery, diet, and age-related changes in adults.
- The relationships between the microbiome and several human diseases are being intensively studied for conditions that include colorectal cancer, inflammatory bowel disease, and immunologically-mediated skin diseases.
- Causal relationships for many of the associations between the microbiome and disease states have yet to be proven.
- Understanding the links between the microbiome and human disease may provide prophylactic or therapeutic tools to improve human health.



ANATOMICAL BASIS OF TOLERANCE AND IMMUNITY TO INTESTINAL ANTIGENS Allan McI.Mowat
NATURE REVIEWS, IMMUNOLOGY VOLUME 3, APRIL 2003, 331-341



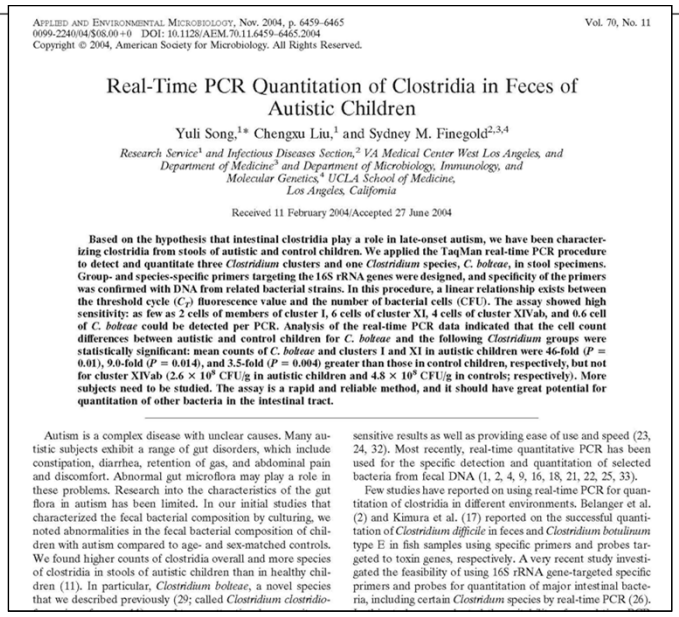
We inoculated a wild-type mouse with the bacterium *H. hepaticus* to create an experimental mouse version of the autoimmune disorder inflammatory bowel disease (IBD). *H. hepaticus* activates Th17 cells which release cytokines asociated with inflammation, like IL-17, causing symptoms of disease. But once *B. fragilis* expressing the polysaccharide A (PSA) is added to the gut, dendritic cells take up and present the PSA molecule on their surface, activating CD4 T cells and regulatory T cells(Tregs). The Tregs release IL-10 which suppresses the inflammatory action of IL-17, in effect alleviating IBD in mice.

The Scientist
Volume 23 | Issue 8 | Page 34
By Sarkis Mazmanian as told to Sara McBride

Disorders Associated with Dysbiosis and Intestinal Hyperpermeability

Inflammatory Bowel Disease	**Dermatitis Herpetiformis**
Irritable Bowel Syndrome	**Autism**
Celiac Disease	**Childhood Hyperactivity**
Infectious Enterocolitis	**Spondyloarthropathies**
Cystic Fibrosis	**Pancreatic Insufficiency**
Chronic Fatigue Immune Deficiency Syndrome	**Weight Gain**
Acne	**Neoplasia Treated with Cytotoxic Drugs**
Eczema	**Hepatic Dysfunction**
Psoriasis	**Alcoholism**
Urticaria	**Environmental Illness**

Unno N, Fink MP. Intestinal epithelial hyperpermeability. Mechanisms and relevance to disease. *Gastroenterol Clin North Am. 1998;27(2):289-307*.



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2004, p. 6459–6465 Vol. 70, No. 11
0099-2240/04/$08.00+0 DOI: 10.1128/AEM.70.11.6459–6465.2004

Real-Time PCR Quantitation of Clostridia in Feces of Autistic Children

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Based on the hypothesis that intestinal clostridia play a role in late-onset autism, we have been characterizing clostridia from stools of autistic and control children. We applied the TaqMan real-time PCR procedure to detect and quantitate three *Clostridium* clusters and one *Clostridium* species, *C. bolteae*, in stool specimens. Group- and species-specific primers targeting the 16S rRNA genes were designed, and specificity of the primers was confirmed with DNA from related bacterial strains. In this procedure, a linear relationship exists between the threshold cycle (C_T) fluorescence value and the number of bacterial cells (CFU). The assay showed high sensitivity: as few as 2 cells of members of cluster I, 6 cells of cluster XI, 4 cells of cluster XIVab, and 0.6 cell of *C. bolteae* could be detected per PCR. Analysis of the real-time PCR data indicated that the cell count differences between autistic and control children for *C. bolteae* and the following *Clostridium* groups were statistically significant: mean counts of *C. bolteae* and clusters I and XI in autistic children were 46-fold ($P = 0.01$), 9.0-fold ($P = 0.014$), and 3.5-fold ($P = 0.004$) greater than those in control children, respectively, but not for cluster XIVab (2.6×10^6 CFU/g in autistic children and 4.8×10^5 CFU/g in controls; respectively). More subjects need to be studied. The assay is a rapid and reliable method, and it should have great potential for quantitation of other bacteria in the intestinal tract.

Autism is a complex disease with unclear causes. Many autistic subjects exhibit a range of gut disorders, which include constipation, diarrhea, retention of gas, and abdominal pain and discomfort. Abnormal gut microflora may play a role in these problems. Research into the characteristics of the gut flora in autism has been limited. In our initial studies that characterized the fecal bacterial composition by culturing, we noted abnormalities in the fecal bacterial composition of children with autism compared to age- and sex-matched controls. We found higher counts of clostridia overall and more species of clostridia in stools of autistic children than in healthy children (11). In particular, *Clostridium bolteae*, a novel species that we described previously (29; called *Clostridium clostridio-*

sensitive results as well as providing ease of use and speed (23, 24, 32). Most recently, real-time quantitative PCR has been used for the specific detection and quantitation of selected bacteria from fecal DNA (1, 2, 4, 9, 16, 18, 21, 22, 25, 33).

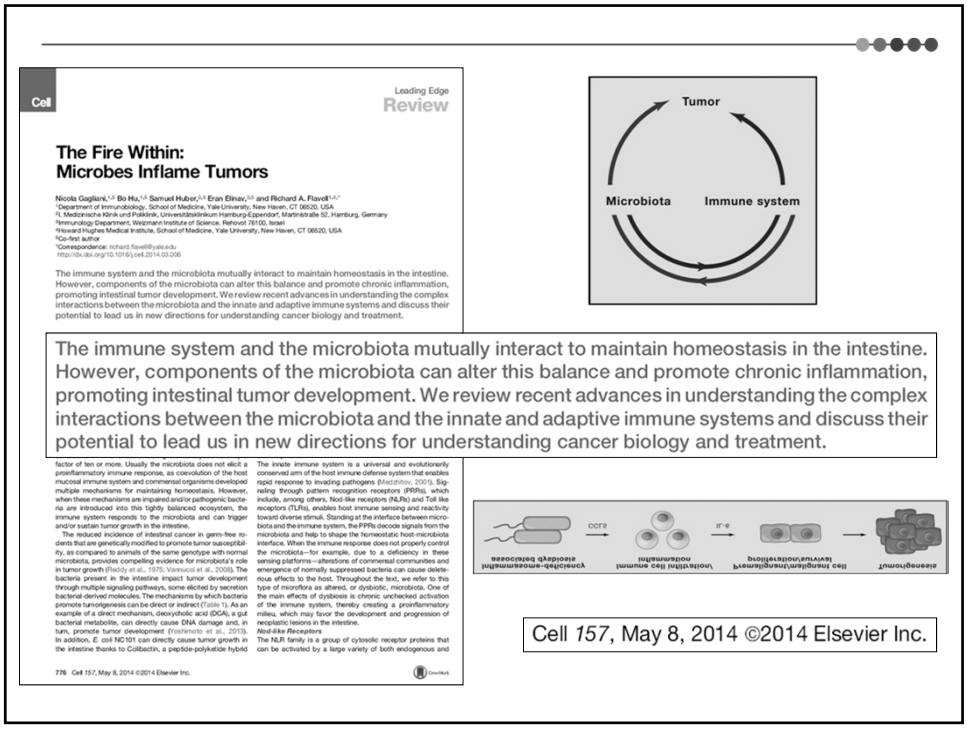
Few studies have reported on using real-time PCR for quantitation of clostridia in different environments. Belanger et al. (2) and Kimura et al. (17) reported on the successful quantitation of *Clostridium difficile* in feces and *Clostridium botulinum* type E in fish samples using specific primers and probes targeted to toxin genes, respectively. A very recent study investigated the feasibility of using 16S rRNA gene-targeted specific primers and probes for quantitation of major intestinal bacteria, including certain *Clostridium* species by real-time PCR (26).

Clostridia in Autism by Real Time PCR

	C. bolteae	*Clostridium cluster I*	*Clostridium cluster XI*	*Clostridium cluster XIVab*
Control (N=8)	$(3.9 \pm 0.3)\ 10^3$	$(4.1 \pm 0.3)\ 10^5$	$(4.0 \pm 0.4)\ 10^6$	$(2.6 \pm 0.2)\ 10^8$
Autistic (N=15)	$(1.8 \pm 0.1)\ 10^5$	$(3.7 \pm 0.4)\ 10^6$	$(1.4 \pm 0.1)\ 10^7$	$(4.8 \pm 0.6)\ 10^8$

- Group I *(Clostridium cluster I)*
  - Forward primer, CI-F1 TACCHRAGGAGGAAGCCAC 54.6
- Group II *(Clostridium cluster XI)*
  - Forward primer, CXI-F1 ACGCTACTTGAGGAGGA 46.5
- Group III *(Clostridium cluster XIVab)*
  - Forward primer, CXIV-F1 GAWGAAGTATYTCGGTATGT 46.2

Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol.* 2004;70(11):6459-6465.



Cell

Leading Edge
Review

The Fire Within: Microbes Inflame Tumors

Nicola Gagliani,[1,2] Bo Hu,[1,2] Samuel Huber,[1,2] Eran Elinav,[3,4] and Richard A. Flavell[1,4,*]
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The immune system and the microbiota mutually interact to maintain homeostasis in the intestine. However, components of the microbiota can alter this balance and promote chronic inflammation, promoting intestinal tumor development. We review recent advances in understanding the complex interactions between the microbiota and the innate and adaptive immune systems and discuss their potential to lead us in new directions for understanding cancer biology and treatment.

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factor of ten or more. Usually the microbiota does not elicit a proinflammatory immune response, as coevolution of the host mucosal immune system and commensal organisms developed multiple mechanisms for maintaining homeostasis. However, when these mechanisms are impaired and/or pathogenic bacteria are introduced into this tightly balanced ecosystem, the immune system responds to the microbiota and can trigger and/or sustain tumor growth in the intestine.

The reduced incidence of intestinal cancer in germ-free rodents that are genetically modified to promote tumor susceptibility, as compared to animals of the same genotype with normal microbiota, provides compelling evidence for microbiota's role in tumor growth (Reddy et al., 1975; Vannucci et al., 2008). The bacteria present in the intestine impact tumor development through multiple signaling pathways, some elicited by secretion bacterial-derived molecules. The mechanisms by which bacteria promote tumorigenesis can be direct or indirect (Table 1). As an example of a direct mechanism, deoxycholic acid (DCA), a gut bacterial metabolite, can directly cause DNA damage and, in turn, promote tumor development (Yoshimoto et al., 2013). In addition, *E. coli* NC101 can directly cause tumor growth in the intestine thanks to Colibactin, a peptide-polyketide hybrid

The innate immune system is a universal and evolutionarily conserved arm of the host immune defense system that enables rapid response to invading pathogens (Medzhitov, 2001). Signaling through pattern recognition receptors (PRRs), which include, among others, Nod-like receptors (NLRs) and Toll like receptors (TLRs), enables host immune sensing and reactivity toward diverse stimuli. Standing at the interface between microbiota and the immune system, the PRRs decode signals from the microbiota and help to shape the homeostatic host-microbiota interface. When the immune response does not properly control the microbiota—for example, due to a deficiency in these sensing platforms—alterations of commensal communities and emergence of normally suppressed bacteria can cause deleterious effects to the host. Throughout the text, we refer to this type of microflora as altered, or dysbiotic, microbiota. One of the main effects of dysbiosis is chronic unchecked activation of the immune system, thereby creating a proinflammatory milieu, which may favor the development and progression of neoplastic lesions in the intestine.

Nod-like Receptors

The NLR family is a group of cytosolic receptor proteins that can be activated by a large variety of both endogenous and

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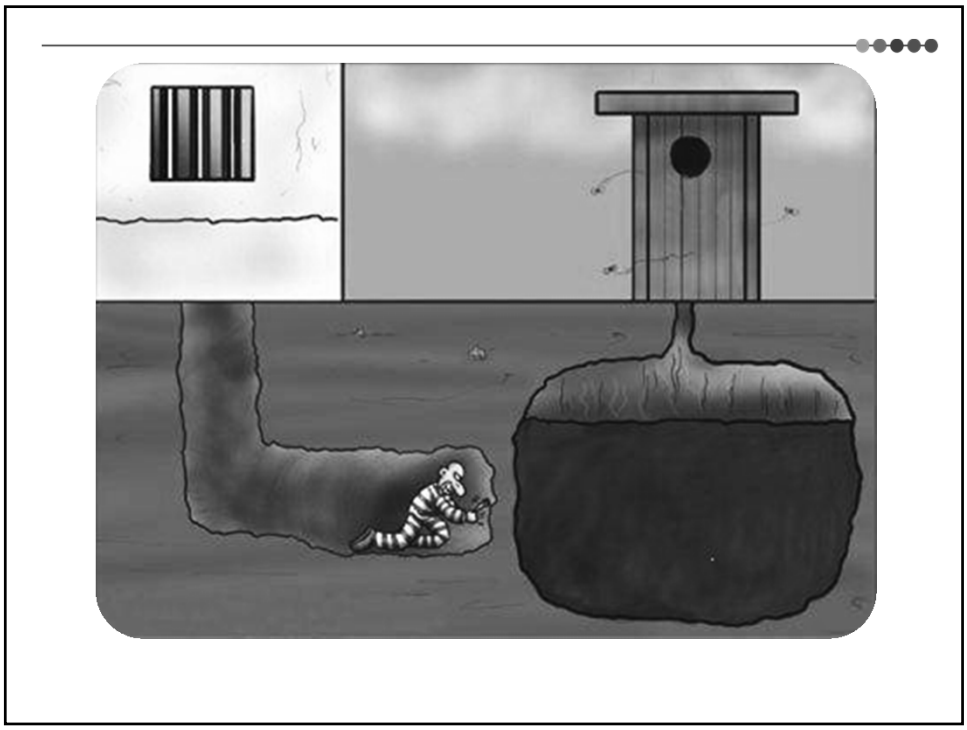


LEADING ARTICLE

A molecular revolution in the study of intestinal microflora

E Furrie

Gut 2006;**55**:141–143. doi: 10.1136/gut.2005.081695

Bacterial colonisers of the colon comprise several hundred bacterial species that live in a complex ecosystem. Study of this complex ecosystem has been carried out, until recently, by traditional culture techniques with biochemical methods to identify organisms. The development of molecular techniques to investigate ecological microbial communities has provided the microbiologist with a vast array of new techniques to investigate human intestinal microflora. Metagenomics, the science of biological diversity, combines the use of molecular biology and genetics to identify and characterise genetic material from complex microbial environments. The combination of metagenomics and subsequent quantitation of each identified species using molecular techniques allows the relatively rapid analysis of whole bacterial populations in human health and disease

Bacteria permanently colonise the whole length of the gastrointestinal tract with by far the highest concentration of organisms

disease (IBD) has focused on the search for a causative bacterial agent, with many and varied candidates being proposed.[5–9] It has now been generally accepted that analysis of the microbial ecosystem and changes in the balance of organisms at initiation and during disease yields far more relevant information than hunting for the proverbial "needle in the haystack". This change has partly been driven by the general ineffectiveness of targeted antibiotic therapy to treat IBD[10–14] and the potential of probiotics as therapy for IBD, allowing re-establishment of homeostasis present in healthy gut.[15–17]

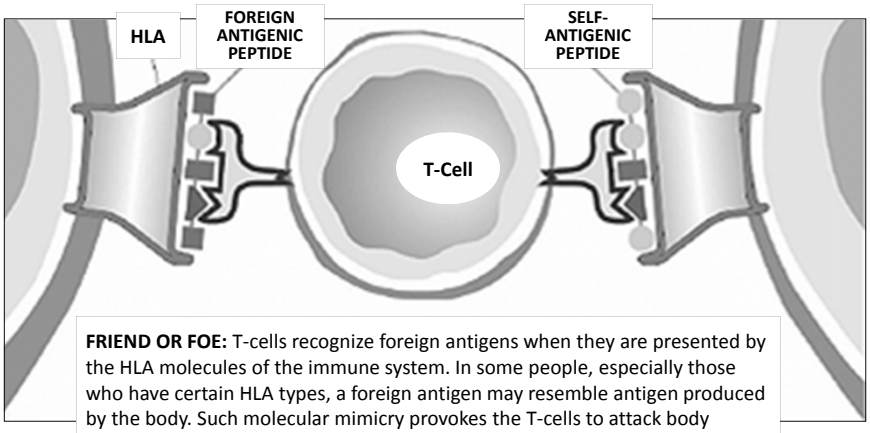
In order to develop these alternative therapies it is essential to determine what comprises a healthy colonic ecosystem and how this balance of organisms is altered during various states and stages of IBD. As a large majority of bacterial species present in the colon are effectively unculturable,[18 19] it is impossible for detailed examination of the colonic microflora to be achieved using traditional culture techniques. The increased ease in which molecular analysis can be carried out by most microbiologists has led to an explosion in sequencing of ribosomal DNA (rDNA) from different bacterial species and

Gut Microbes and Systemic Pathology

- Examples of epidemiologic associations between GI microbes and systemic autoimmune pathology:
  - *Klebsiella*: Ankylosing Spondylitis
  - *Citrobacter, Klebsiella,* Proteus Rheumatoid Arthritis
  - *Yersinia*: Grave's Disease & Hashimoto's Dz.
  - *S. Pyogenes*: Rheumatic Fever
  - *Camphylobacter jejuni*: Gullian Barre Syndrome
  - *E. coli*, Proteus: Autoimmunity in general

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

Bugs/Foods: Friend or Foe?

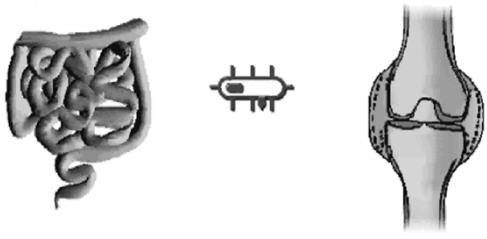


FRIEND OR FOE: T-cells recognize foreign antigens when they are presented by the HLA molecules of the immune system. In some people, especially those who have certain HLA types, a foreign antigen may resemble antigen produced by the body. Such molecular mimicry provokes the T-cells to attack body tissues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

Gut-Autoimmune Connection

- Maldigestion
- Leaky Gut
- GI Infections

Immune Complex Formation



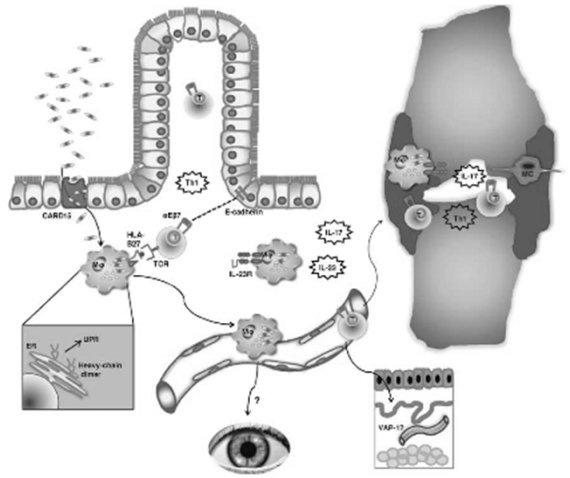


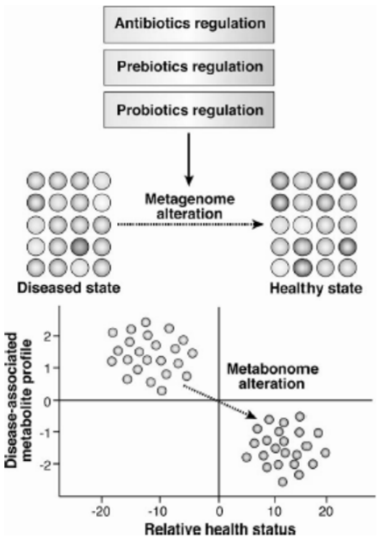
Figure 2 A model linking gut to joint inflammation in spondyloarthritis. (1) Bacteria attach to and invade the intestinal epithelium and the lamina propria. HLA-B27 or CARD15 polymorphisms can result in altered recognition and handling of bacterial antigens. (2) Invading bacteria infect, or are taken up by, macrophages (M) in the lamina propria and survive intracellularly. (3) HLA-B27 can present bacterial or autologous antigens to T cells (T). Furthermore, the heavy chain easily misfolds, leading to an unfolded protein response (UPR) and stress. (4) Bacterial infection induces Th1 and Th17 responses, and IL-23R susceptibility variants, expressed on macrophages and other antigen-presenting cell types, may modulate the Th17 response. (5) More T cells and other immune mediators are recruited, releasing proinflammatory cytokines. (6) Activated T cells and macrophages carrying bacterial components migrate via blood vessels to the target joint or eventually to other sites such as skin and eye. (7) In the target joint, gut-derived macrophages and T cells recruit other immune cells and result in the activation of mesenchymal cells (MCs), which further enhance and sustain inflammation. HLA-B27, human leukocyte antigen B27; IL-23R, IL-23 receptor.

Jaques P, Elewaut D. Joint expedition: linking gut inflammation to arthritis. Mucosal Immunology. Vol 1,No 5, Sept. 2008

The Colonization Resistance of the Mucous Membrane of the Large Intestine in Patients with Rheumatoid Arthritis in a Period of Exacerbation

"The mucous membrane of healthy people is colonized by bifidobacteria, *lactobacilli, Bacteroides, Escherichia* and *enterococci*. The mucous membrane in RA subjects is mainly colonized by aerobic opportunistic conventionally pathogenic enterobacteria (*enteropathogenic Escherichia, Citrobacter, Enterobacter, Klebsiella,* etc.), *staphylococci, enterococci* and anaerobic bacteria (*Bacteroides, peptococci, peptostreptococci*, etc.). Taking into account significant changes of colonization resistance in the colon mucous membrane in remission period of RA, it is necessary to apply bacteriotherapy, using bacterial drugs containing bifidobacteria and lactobacteria."

Pishak OV. Bukovinian State Medical Academy, Public Health Ministry of Ukraine. Mikrobiol Z. 1999 Sep-Oct;61(5):41-7.



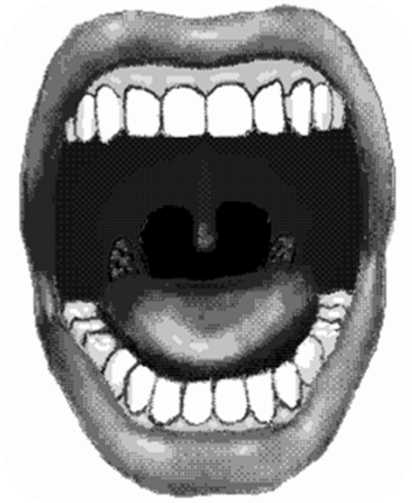
Predominant Bacteria		E+007	
Obligate Anaerobes			
Bacteroides spp.	3.9	1.6 — 6.7	>= 1.3
Clostridia spp.	7.6	1.5 — 6.2	>= 1.0
Prevotella spp.	2.5	1.6 — 6.2	>= 1.1
Fusobacteria spp.	7.1	1.6 — 7.4	>= 1.1
Streptomyces spp.	2.6	1.6 — 5.8	>= 1.0
Mycoplasma spp.	4.3	1.7 — 6.2	>= 1.2
Facultative Anaerobes			
Lactobacillus spp.	3.4	1.8 — 7.8	>= 1.2
Bifidobacter spp.	4.9	2.3 — 7.6	>= 1.8
Escherichia coli (E. coli)	6.7	1.7 — 7.7	>= 1.1

Predominant Bacteria play major roles in health. They provide colonization resistance against potentially pathogenic organisms, aid in digestion and absorption, produce vitamins and SCFA's, and stimulate the GI immune system. DNA probes allow detection of multiple species (spp.) within a genus, so the genera that are reported cover many species.

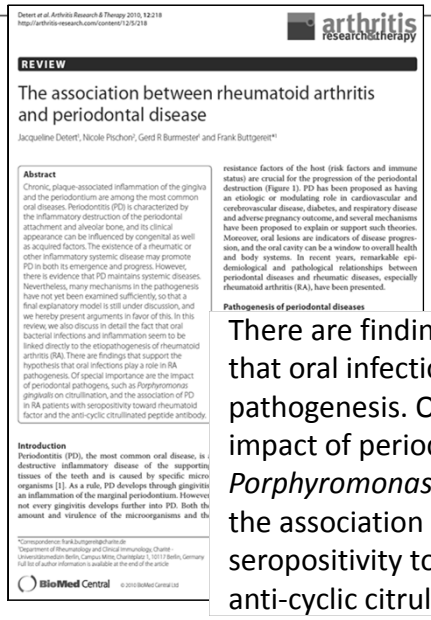
Organisms are detected by DNA analysis. One colony forming unit (CFU) is equivalent to one bacterium. Each genome detected represents one cell, or one CFU. Results are expressed in scientific notation, so an organism reported as 2.5 E+007 CFU/gram is read as 25 million colony forming units per gram of feces.

Opportunistic Bacteria	Expected Value	
Citrobacter spp.	Positive	Negative
Klebsiella oxytoca	Positive	Negative

Opportunistic Bacteria may cause symptoms and be associated with disease. They can affect digestion and absorption, nutrient production, pH and immune state. Antibiotic sensitivity tests will be performed on all opportunistic bacteria found, although clinical history is usually considered to determine treatment since the organisms are not generally considered to be pathogens.



Infectious agents like *Porphyromonas gingivalis*, a bacterial strain driving periodontal disease, are able to produce peptidyl arginine deiaminase 4 (PAD4), an enzyme that mediates the citrullination of proteins like vimentin, collagen and fibrinogen, which serve as autoantigens in RA.



Detert et al. Arthritis Research & Therapy 2010, 12:218
http://arthritis-research.com/content/12/5/218

arthritis research&therapy

REVIEW

The association between rheumatoid arthritis and periodontal disease

Jacqueline Detert[1], Nicole Pischon[2], Gerd R Burmester[1] and Frank Buttgereit*[1]

Abstract

Chronic, plaque-associated inflammation of the gingiva and the periodontium are among the most common oral diseases. Periodontitis (PD) is characterized by the inflammatory destruction of the periodontal attachment and alveolar bone, and its clinical appearance can be influenced by congenital as well as acquired factors. The existence of a rheumatic or other inflammatory systemic disease may promote PD in both its emergence and progress. However, there is evidence that PD maintains systemic diseases. Nevertheless, many mechanisms in the pathogenesis have not yet been examined sufficiently, so that a final explanatory model is still under discussion, and we hereby present arguments in favor of this. In this review, we also discuss in detail the fact that oral bacterial infections and inflammation seem to be linked directly to the etiopathogenesis of rheumatoid arthritis (RA). There are findings that support the hypothesis that oral infections play a role in RA pathogenesis. Of special importance are the impact of periodontal pathogens, such as *Porphyromonas gingivalis* on citrullination, and the association of PD in RA patients with seropositivity toward rheumatoid factor and the anti-cyclic citrullinated peptide antibody.

Introduction

Periodontitis (PD), the most common oral disease, is a destructive inflammatory disease of the supporting tissues of the teeth and is caused by specific microorganisms [1]. As a rule, PD develops through gingivitis, an inflammation of the marginal periodontium. However, not every gingivitis develops further into PD. Both the amount and virulence of the microorganisms and the

resistance factors of the host (risk factors and immune status) are crucial for the progression of the periodontal destruction (Figure 1). PD has been proposed as having an etiologic or modulating role in cardiovascular and cerebrovascular disease, diabetes, and respiratory disease and adverse pregnancy outcome, and several mechanisms have been proposed to explain or support such theories. Moreover, oral lesions are indicators of disease progression, and the oral cavity can be a window to overall health and body systems. In recent years, remarkable epidemiological and pathological relationships between periodontal diseases and rheumatic diseases, especially rheumatoid arthritis (RA), have been presented.

Pathogenesis of periodontal diseases

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Full list of author information is available at the end of the article

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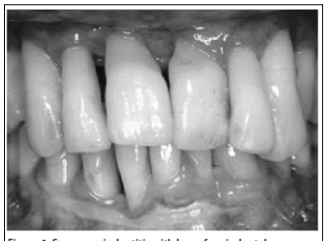


Figure 1. Severe periodontitis with loss of periodontal attachment and alveolar bone.

There are findings that support the hypothesis that oral infections play a role in RA pathogenesis. Of special importance are the impact of periodontal pathogens, such as *Porphyromonas gingivalis* on citrullination, and the association of PD in RA patients with seropositivity toward rheumatoid factor and the anti-cyclic citrullinated peptide antibody.

Detert *et al. Arthritis Research & Therapy* 2010, **12**:218
http://arthritis-research.com/content/12/5/218

Oral-Hematogenous Spread

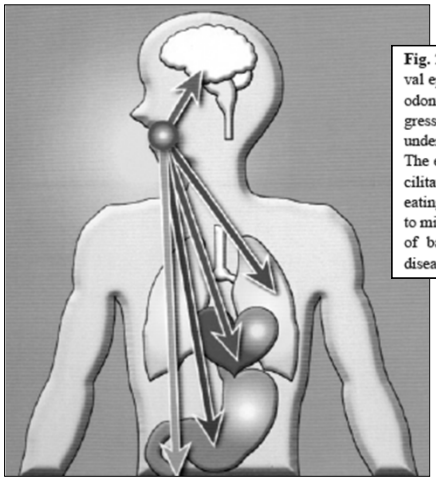
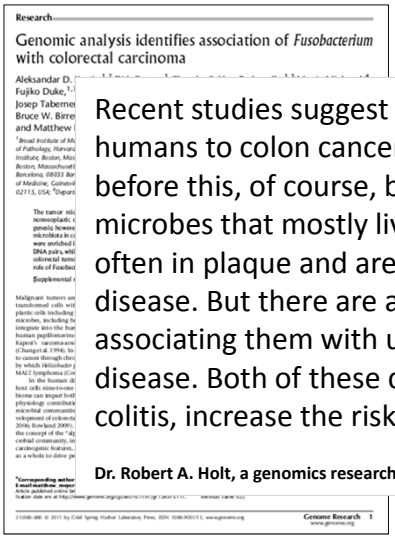


Fig. 2. Cause of periodontitis-related systemic diseases. The gingival epithelium functions as an innate physical barrier to protect periodontal tissues from bacterial invaders. However, with disease progression, local inflammation ulcerates the epithelium to expose the underlying connective tissues and blood capillaries to plaque biofilm. The exposed ulcerative area (8 – 20 cm^2 in affected oral cavities) facilitates direct entry of biofilm pathogens into the circulation during eating and tooth brushing. Eventually, periodontal pathogens are able to migrate throughout the entire body. This oral-hematogenous spread of bacteria is a primary cause of periodontitis-derived systemic diseases.

Leaky Mouth?

J Pharmacol Sci **113**, 103 – 109 (2010)

Oral Bacterium and Colon Cancer?



Research

Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma

Recent studies suggest that Fusobacteria predispose humans to colon cancer. Fusobacteria were known before this, of course, but were thought of as microbes that mostly live in the mouth — they are often in plaque and are associated with periodontal disease. But there are also recent reports associating them with ulcerative colitis and Crohn's disease. Both of these diseases, especially ulcerative colitis, increase the risk of colon cancer.

Dr. Robert A. Holt, a genomics researcher at the British Columbia Cancer Agency

Clinical & Developmental Immunology, March 2006; 13(1): 41–48

Taylor & Francis

Rheumatoid arthritis is an autoimmune disease triggered by *Proteus* urinary tract infection

ALAN EBRINGER & TAHA RASHID

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Abstract

Rheumatoid arthritis (RA) is a chronic and disabling polyarthritic disease, which affects mainly women in middle and old age. Extensive evidence based on the results of various microbial, immunological and molecular studies from different parts of the world, shows that a strong link exists between *Proteus mirabilis* microbes and RA. We propose that sub-clinical *Proteus* urinary tract infections are the main triggering factors and that the presence of molecular mimicry and cross-reactivity between these bacteria and RA-targeted tissue antigens assists in the perpetuation of the disease process through production of cytopathic auto-antibodies.

Patients with RA especially during the early stages of the disease could benefit from *Proteus* anti-bacterial measures involving the use of antibiotics, vegetarian diets and high intake of water and fruit juices such as cranberry juice in addition to the currently employed treatments.

Keywords: *Humoral autoimmunity*, Proteus mirabilis, *rheumatoid arthritis*, *urinary tract infection*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease, which affects millions of people all around the world, with a prevalence rate ranging from 0.5 to 1% (Lawrence et al. 1998). The disease in the majority of patients takes a mild to moderate course, whilst in others it has a more disabling consequence, which might have a great effect on the socio-economic status of the patient (Cooper 2000).

RA affects individuals of middle age groups and occurs three times more frequently in women than in men.

Aetiopathogenesis

A general scientific consensus exists, which considers RA as an immune-mediated disease that could possibly be triggered by an environmental (microbial) factor in a genetically susceptible individual.

Extensive evidence supports the role of cellular and humoral autoimmunity in the development of RA, and some of these are listed as follows:

1. Predominant role of B lymphocytes in the pathogenesis of RA (Weyand et al. 2005) and signs of accumulations of immunoglobulins and other inflammatory products such as complements at the site of synovial pathological lesions in RA patients (Low and Moore 2005).
2. Detection of elevated levels of auto-antibodies in the serum and/or synovial fluid of patients with RA (Rantapaa-Dahlqvist 2005).
3. Significant improvements in RA disease parameters following B cell depletion therapy, e.g. with the use of anti-CD20 antibodies (Perosa et al. 2005).

Role of HLA genes in RA

The role of genetics in development of RA has been examined mainly through family, twin and molecular analytical studies. For instance, familial distribution of RA among first-degree relatives (Deighton et al. 1992a) and twins (MacGregor et al. 2000), indicates that RA runs in some families, basically supporting the

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Scand J Rheumatol 2003;32:2–11

REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer[1,2], Taha Rashid[1], and Clyde Wilson[1]

[1]Division of Life Sciences, Infection and Immunity Group, King's College London, [2]Department of Rheumatology, Middlesex Hospital, University College, London, UK

Rheumatoid arthritis (RA) is a chronic disease, affecting women more than men, especially in those possessing the "shared epitope" (EQK/RRAA) amino acid sequences present in HLA-DR1/4 molecules. *Proteus mirabilis* carries sequences showing molecular mimicry to the "shared epitope" and to type XI collagen of hyaline cartilage. Elevated levels of antibodies to *P.mirabilis* have been reported from 14 different countries involving 1375 RA patients and the microbe has been isolated from urine cultures of such patients. Our working hypothesis is that the disease develops as a result of repeated episodes of *Proteus* upper urinary tract infections. Prospective studies involving the trial of anti-*Proteus* measures in RA patients should be evaluated in the management of this disease. Antibiotics, high fluid intake, and fruit extracts, such as cranberry juice, have all been found to be effective in the treatment of urinary tract infections. Such measures could be used as possible additional adjuncts to the standard therapy with NSAIDs and DMARDs.

Key words: rheumatoid arthritis, *Proteus* urinary tract infections, diet

Rheumatoid arthritis (RA) is the most common inflammatory joint disease, affecting millions of people all around the world. It usually takes a progressive course with characteristic exacerbations and remissions. Virtually any joint can be involved in RA, but the disease predominantly affects the small joints of the hands and feet, and less frequently the large joints including the cervical spine. (1)

RA is three times more common in women than men, affecting mainly middle aged and elderly people. (2) Although the world-wide distribution of the disease ranges between 0.2% and 5.3%, (3) the incidence, however, is showing a noticeable decline during the last 4 decades, especially among Western World populations. (4)

Genetic backgrounds

Familial aggregation has been observed, but no strong pattern of inheritance has been established among relatives of patients with RA. (5) In a study on two groups of twins with RA from Finland and UK, it was shown that genetic factors have a substantial contribution to RA, the heritability in each groups was observed to be 53% and 65%, respectively. (6)

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More than 25 years ago a significant discovery was made when the HLA-DR4/Dw4 genetic marker was found in 70% of the patients with RA, using both mixed lymphocyte culture assay (7) and serological methods. (8) Subsequently it was observed that only certain types and subtypes of HLA-DR molecules were associated with RA. The third hypervariable regions of all RA-associated HLA-DRβ1 alleles were found to share a hexameric amino acid sequence homology involving positions 69–74 and consisting of glutamic acid, glutamine, lysine or arginine, arginine, alanine and alanine "EQK/RRAA". This sequence similarity in RA, which has been given the name of "shared epitope" (SE) (9) motif, was not found in other HLA-DR molecules, like HLA-DR4 subtypes (DRβ1*0402 and 0403), which are not associated with RA.

Regardless of the difference in the distribution of HLA genes among various ethnic groups, it has been reported that more than 95% of patients possess one or another of the RA-linked HLA-DR molecules (10) which contain the SE motif. These include HLA-DR4 subtypes (DRβ1*0401, *0404, 0405 and 0408), HLA-DR1 subtypes (DRβ1*0101 and 0102), HLA-DR6 (DRβ1*1402) and HLA-DR10 (DRβ1*1001). (11, 12)

The importance of SE amino acid sequences in conferring susceptibility to RA is not only confined to humans, but also to animals such as the canine species. (13) The mere association of the SE moiety with RA, however, is not necessarily sufficient to trigger the disease. In spite of a positive correlation between the presence of DR4 and SE homozygosity

Antibodies to Proteus in RA

Table I. Worldwide distribution of elevated *Proteus* antibodies in patients with RA.

No.	Location (country)	RA patients	Year	Reference
1.	London (**England**)	**30**	1985	17
2.	Winchester (**England**)	**162**	1988, 1997	104, 105
3.	Newcastle (**England**)	**142**	1992	106
4.	Epsom (**England**)	**27**	1999	107
5.	Stevenage (**England**)	**140**	1995, 1995, 1996	30, 108, 34
6.	Dundee (**Scotland**)	**176**	1995, 1999	109, 40
7.	Dublin (**Ireland**)	**29**	1988	110
8.	Toulouse (**France**)	**15**	1994	111
9.	Brest (**France**)	**50**	1995	112
10.	Hamilton (**Bermuda**)	**34**	1995	108
11.	Oslo (**Norway**)	**53**	1995	100
12.	Otsu (**Japan**)	**80**	1997	113
13.	Chandigarh (**India**)	**70**	1997	114
14.	Amsterdam (**Holland**)	**25**	1998	115
15.	Taichung (**Taiwan**)	**39**	1998	116
16.	Barcelona (**Spain**)	**34**	1999	107
17.	Moscow (**Russia**)	**27**	2000	117
18.	Bethesda & Philadelphia (**USA**), Montreal (**Canada**)	**113**	2000	19
19.	Tokyo (**Japan**)	**30**	Submitted	
20.	Helsinki (**Finland**)	**99**	Submitted	
		1375	**Total numbers of RA patients**	

Alan Ebringer, MD, King's College, London

Scand J Rheumatol 2003;32:2–11

REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer[1,2], Taha Rashid[1], and Clyde Wilson[1]

Rheumatoid arthritis and microbial therapy

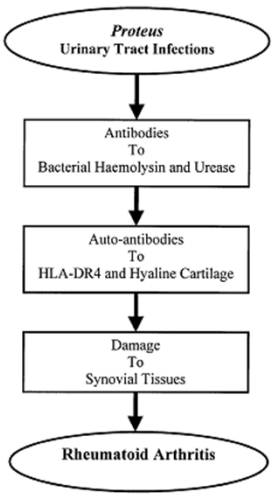
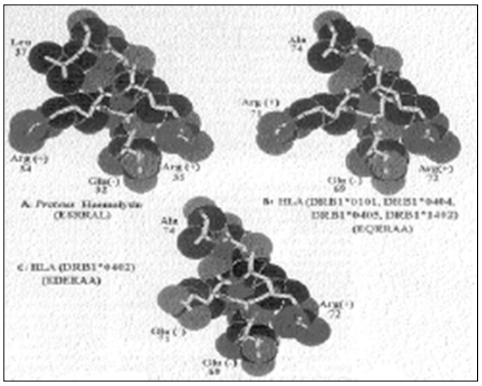


Fig. 1. Suggested etiopathogenetic sequence from *Proteus* urinary infections to RA

Molecular Mimicry: Proteus and HLA-DR1/DR4



Alan Ebringer, MD
King's College, London

A: ESRRAL sequence of *Proteus mirabilis* haemolysin
B: EQRRAA sequence within DRB1*0101 (HLA-DR1)
C: EDERAA sequence of DRB1*0402 (HLA-DR4/Dw10)
(predicted from known crystallographic structure)

Table II. Possible explanation for some commonly encountered features in RA.

Associated RA Features	Suggested Explanations
Female preponderance 3:1	Increased incidence of **UTIs** in females
Disease onset in **30–50 years**	Increased incidence of **UTIs** among middle and older age groups
Exacerbation **after pregnancy**	Increased incidence of **UTIs** in the **puerperium**
Low concordance rate in identical twins and fluctuating course of the disease	Involvement of non-genetic **environmental factors** in the pathogenesis of the disease
Presence of **rheumatoid factors** in high proportions of RA patients	A **secondary phenomenon** due to B cell stimulation and presence of antigen-antibody complexes
Presence of "**EQRRAA**" amino acid motif in over 95% of patients possessing the RA-associated **HLA-DR** molecules	Cross-reactivity with "**ESRRAL**" amino acid sequences present in the ***Proteus* hemolysins**
High proportion of **small joints** involvement, having hyaline cartilage which contains type XI **collagen**, possessing the "**IRRET**" amino acid sequence	Cross-reactivity with "**LRREI**" amino acid motif present in the ***Proteus* urease** enzyme

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REVIEW

Rheumatoid arthritis: proposal for the use of anti-microbial therapy in early cases

Alan Ebringer[1,2], Taha Rashid[1], and Clyde Wilson[1]

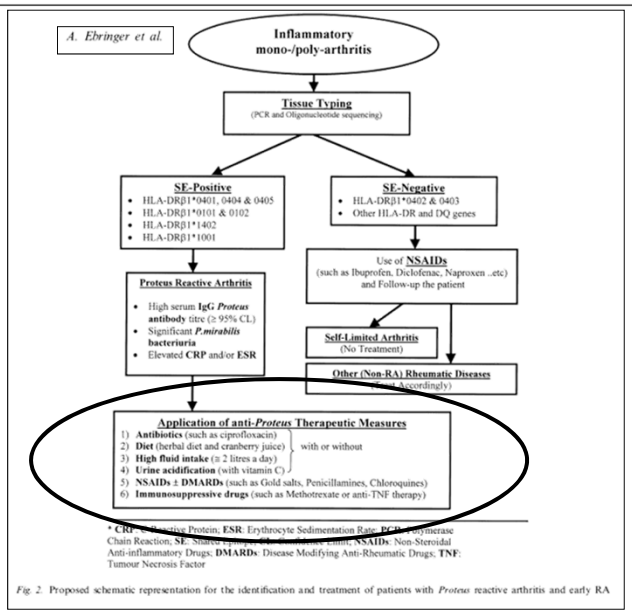
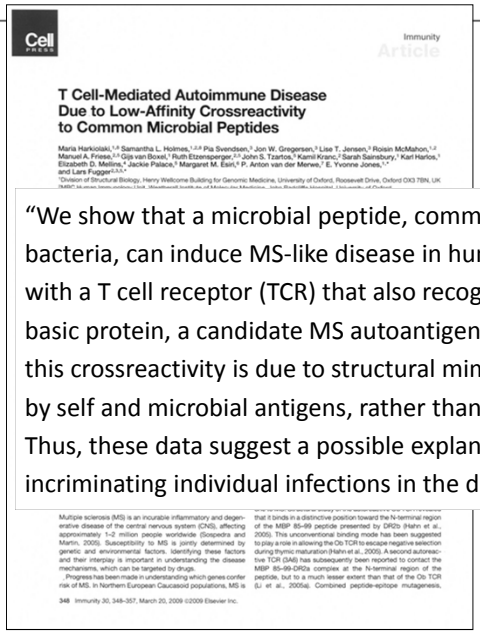


Fig. 2. Proposed schematic representation for the identification and treatment of patients with *Proteus* reactive arthritis and early RA



Immunity

Article

T Cell-Mediated Autoimmune Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides

Maria Harkiolaki,[1,8] Samantha L. Holmes,[1,2,8] Pia Svendsen,[3] Jon W. Gregersen,[3] Lise T. Jensen,[3] Roisin McMahon,[1,2] Manuel A. Friese,[2,4] Gijs van Boxel,[2] Ruth Etzensperger,[2,4] John S. Tzartos,[5] Kamil Kranc,[2] Sarah Sainsbury,[1] Karl Harlos,[1] Elizabeth D. Mellins,[6] Jackie Palace,[2] Margaret M. Esiri,[2] P. Anton van der Merwe,[7] E. Yvonne Jones,[1,*] and Lars Fugger[2,3,*]

[1]Division of Structural Biology, Henry Wellcome Building for Genomic Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

"We show that a microbial peptide, common to several major classes of bacteria, can induce MS-like disease in humanized mice by crossreacting with a T cell receptor (TCR) that also recognizes a peptide from myelin basic protein, a candidate MS autoantigen. Structural analysis demonstrates this crossreactivity is due to structural mimicry of a binding hotspot shared by self and microbial antigens, rather than to a degenerate TCR recognition. Thus, these data suggest a possible explanation for the difficulty in incriminating individual infections in the development of MS."

Harkiolaki M, Holmes SL, Svendsen P, et al. T-Cell-Mediated Autoimmune Disease Due to Low-Affinity Crossreactivity to Common Microbial Peptides. *Immunity* 30, 348-357, March 20, 2009

Thyroid Disorders, Autoimmunity and The GI Environment

("Microbes")

Virology Journal

BioMed Central

Review

Open Access

Viruses and thyroiditis: an update

Rachel Desailloud*[1,2] and Didier Hober[1]

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Virology Journal 2009, **6**:5 doi:10.1186/1743-422X-6-5

This article is available from: http://www.virologyj.com/content/6/1/5

Abstract

Viral infections are frequently cited as a major environmental factor involved in subacute thyroiditis and autoimmune thyroid diseases This review examines the data related to the role of viruses in the development of thyroiditis.

Our research has been focused on human data. We have reviewed virological data for each type of thyroiditis at different levels of evidence; epidemiological data, serological data or research on circulating viruses, direct evidence of thyroid tissue infection. Interpretation of epidemiological and serological data must be cautious as they don't prove that this pathogen is responsible for the disease. However, direct evidence of the presence of viruses or their components in the organ are available for retroviruses (HFV) and mumps in subacute thyroiditis, for retroviruses (HTLV-1, HFV, HIV and SV40) in Graves's disease and for HTLV-1, enterovirus, rubella, mumps virus, HSV, EBV and parvovirus in Hashimoto's thyroiditis. However, it remains to determine whether they are responsible for thyroid diseases or whether they are just innocent bystanders. Further studies are needed to clarify the relationship between viruses and thyroid diseases, in order to develop new strategies for prevention and/or treatment.

severe thyroid pain); iii/Autoimmune thyroid disease which includes Hashimoto's thyroiditis (and painless thyroiditis also known as silent thyroiditis or subacute lym-

emphasize some mechanisms and to support such a possibility in humans. We have reviewed virological data at different levels of evidence: epidemiological data, serolog-

5: *Acta Med Austriaca* 1987;14(1):11-4

[Antibodies to Yersinia enterocolitica in immunogenic thyroid diseases].

[Article in German]

Petru G, Stunzner D, Lind P, Eber O, Mose JR

"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"

(19.6%). The antibody titres were mainly directed towards Yersinia subtypes 8 and 3. It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases such as Graves' disease or Hashimoto thyroiditis.

PMID: 3618088, UI: 87294986

5: *Acta Med Austriaca* 1987;14(1):11-4

[Antibodies to Yersinia enterocolitica in immunogenic thyroid diseases].

[Article in German]

Petru G, Stunzner D, Lind P, Eber O, Mose JR

In 1976 Shenkman et al. revealed that in patients with thyroid disorders antibodies

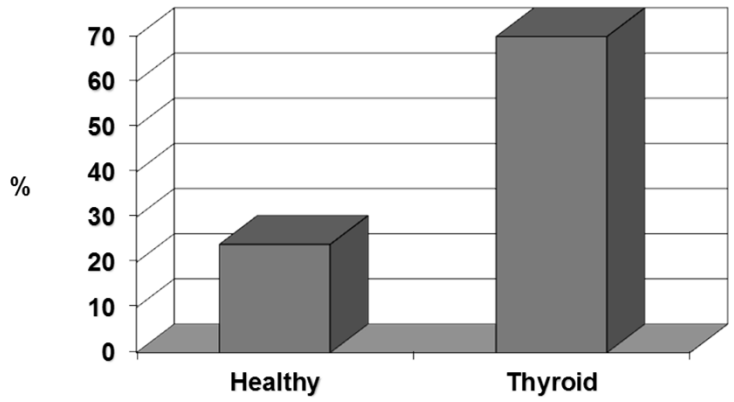
"It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases…"

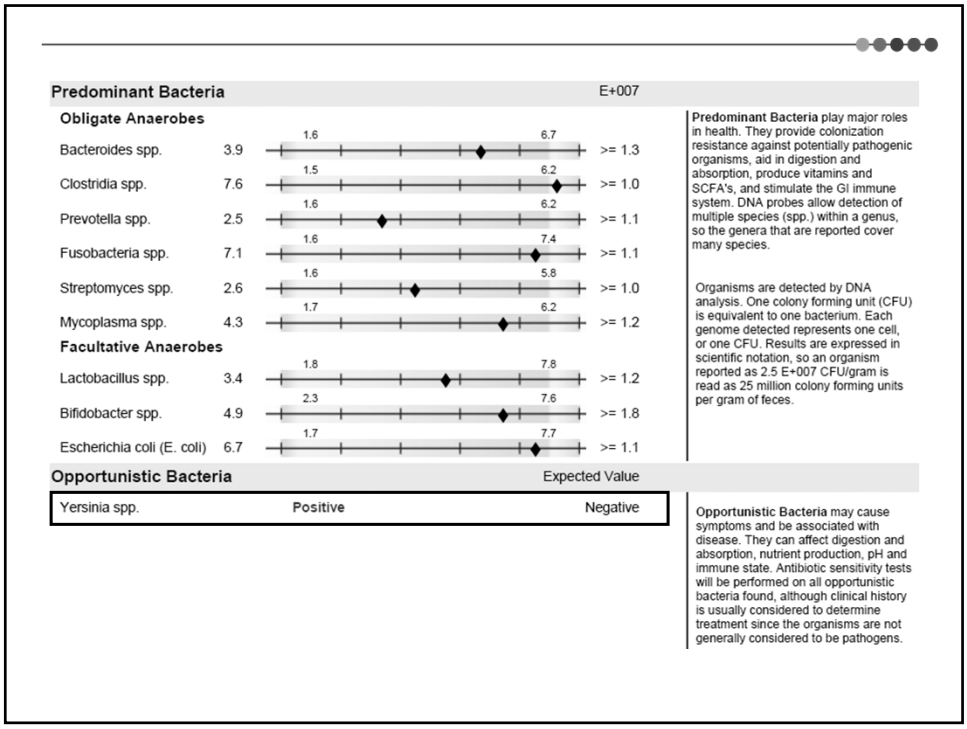
from immunogenic than in patients with non-immunogenic thyroid disorders (19.6%). The antibody titres were mainly directed towards Yersinia subtypes 8 and 3. It may, therefore, be assumed that the gram-negative bacterium Yersinia enterocolitica may have an active part in triggering immunogenic thyroid diseases such as Graves' disease or Hashimoto thyroiditis.

PMID: 3618088, UI: 87294986

Stunzner et al, Acta Med Austriaca 1987;14(1):11-4

Prevalence of Yersinia Antibodies in Thyroid Disorder Patients





Predominant Bacteria E+007

Obligate Anaerobes

Bacteroides spp.	3.9	1.6	6.7	>= 1.3
Clostridia spp.	7.6	1.5	6.2	>= 1.0
Prevotella spp.	2.5	1.6	6.2	>= 1.1
Fusobacteria spp.	7.1	1.6	7.4	>= 1.1
Streptomyces spp.	2.6	1.6	5.8	>= 1.0
Mycoplasma spp.	4.3	1.7	6.2	>= 1.2

Facultative Anaerobes

Lactobacillus spp.	3.4	1.8	7.8	>= 1.2
Bifidobacter spp.	4.9	2.3	7.6	>= 1.8
Escherichia coli (E. coli)	6.7	1.7	7.7	>= 1.1

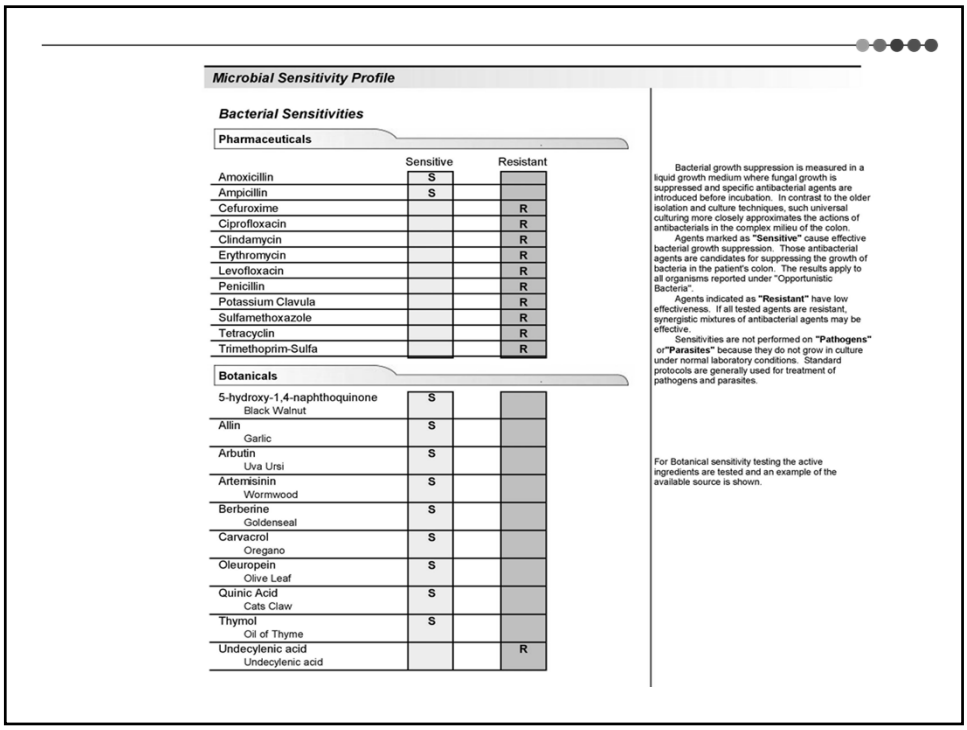
Predominant Bacteria play major roles in health. They provide colonization resistance against potentially pathogenic organisms, aid in digestion and absorption, produce vitamins and SCFA's, and stimulate the GI immune system. DNA probes allow detection of multiple species (spp.) within a genus, so the genera that are reported cover many species.

Organisms are detected by DNA analysis. One colony forming unit (CFU) is equivalent to one bacterium. Each genome detected represents one cell, or one CFU. Results are expressed in scientific notation, so an organism reported as 2.5 E+007 CFU/gram is read as 25 million colony forming units per gram of feces.

Opportunistic Bacteria Expected Value

Yersinia spp.	Positive	Negative

Opportunistic Bacteria may cause symptoms and be associated with disease. They can affect digestion and absorption, nutrient production, pH and immune state. Antibiotic sensitivity tests will be performed on all opportunistic bacteria found, although clinical history is usually considered to determine treatment since the organisms are not generally considered to be pathogens.



Microbial Sensitivity Profile

Bacterial Sensitivities

Pharmaceuticals

	Sensitive	Resistant
Amoxicillin	S	
Ampicillin	S	
Cefuroxime		R
Ciprofloxacin		R
Clindamycin		R
Erythromycin		R
Levofloxacin		R
Penicillin		R
Potassium Clavula		R
Sulfamethoxazole		R
Tetracyclin		R
Trimethoprim-Sulfa		R

Botanicals

5-hydroxy-1,4-naphthoquinone (Black Walnut)	S	
Allin (Garlic)	S	
Arbutin (Uva Ursi)	S	
Artemisinin (Wormwood)	S	
Berberine (Goldenseal)	S	
Carvacrol (Oregano)	S	
Oleuropein (Olive Leaf)	S	
Quinic Acid (Cats Claw)	S	
Thymol (Oil of Thyme)	S	
Undecylenic acid (Undecylenic acid)		R

Bacterial growth suppression is measured in a liquid growth medium where fungal growth is suppressed and specific antibacterial agents are introduced before incubation. In contrast to the older isolation and culture techniques, such universal culturing more closely approximates the actions of antibacterials in the complex milieu of the colon.

Agents marked as **"Sensitive"** cause effective bacterial growth suppression. Those antibacterial agents are candidates for suppressing the growth of bacteria in the patient's colon. The results apply to all organisms reported under "Opportunistic Bacteria".

Agents indicated as **"Resistant"** have low effectiveness. If all tested agents are resistant, synergistic mixtures of antibacterial agents may be effective.

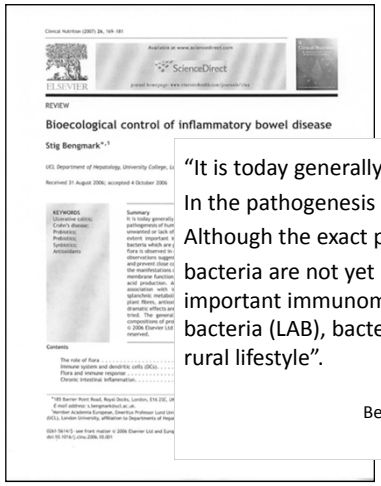
Sensitivities are not performed on **"Pathogens"** or **"Parasites"** because they do not grow in culture under normal laboratory conditions. Standard protocols are generally used for treatment of pathogens and parasites.

For Botanical sensitivity testing the active ingredients are tested and an example of the available source is shown.

Sample Natural GI Antimicrobial

	Amounts per serving
Serving size	2 caps
Number of servings per container	30
Number of capsules per container	60
Tribulus terrestris (standardized to 40% furostanolsaponins)	400 mg
Chinese Wormwood (Artemisia annua/apiacea) (standardized to >10% artemisinin)	300 mg
Berberine sulfate (from Berberus aquifolius)	200 mg
Barbery (Berberis vulgaris) (standardized to 6% berberine)	100 mg
Bearberry Arctostaphylos uva ursi)	100 mg
Grapefruit/Citrus Seed Extract	200 mg
Magnesium Caprylate *(Yielding 267 mg of Caprylic Acid)	300 mg
Black Walnut (Juglans nigra)	100 mg
Suggested Dose: Take 2 capsules, one to three times daily, in between meals as directed by your health care practitioner.	

Probiotics as Inflammatory Modulators


ScienceDirect

REVIEW

Bioecological control of inflammatory bowel disease

Stig Bengmark*,1

"It is today generally accepted that intestinal flora is deeply involved In the pathogenesis of human inflammatory bowel diseases (IBDs), Although the exact presence of unwanted or lack of specific crucial bacteria are not yet known. Westerners lack to large extent important immunomodulatory and fibre-fermenting lactic acid bacteria (LAB), bacteria which are present in all with a more primitive rural lifestyle".

Bengmark S. Bioecological control of inflammatory bowel disease. ClinNutr, 26, 169-181 (2007).

Thyroid Disorders, Autoimmunity and The GI Environment

("Food Antigens")

Autoimmune Thyroid Disease and Celiac 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

Ansaldi N et al, Autoimmune thyroid disease and celiac disease in children (Abstract), *J Pediatr Gastroenterol Nutr*, Vol. 37, No. 1, pp. 63-6, July 2003.

2100 Gastrointestinal Function Profile

Methodology: DNA Analytsis, GC/MS, Microscopic, Colorimetric, Automated Chemistry, ELISA

	Results		Reference Range	
Beneficial SCFA				
Total SCFA	169.8		=> 35.0	mM/g
n-Butyrate	22.6		=> 4.6	mM/g
Acetate %	64.7		50.0 - 72.0	%
Butyrate %	13.3		8.0 - 28.0	%
Propionate %	21.2		15.0 - 22.0	%
Valerate %	0.8	L	1.2 - 4.0	%
Inflammation				
Lactoferrin	0.1		<= 7.3	ug/mL
WBCs	Neg		Neg	
Mucus	Neg		Neg-Rare	
Immunology				
Fecal sIgA	19	L	40 - 204	mg/dL
Anti-gliadin sIgA	5		<= 20	mg/dL

Mycology

Yeast in small amounts is considered normal. Yeast overgrowth has been linked to many chronic conditions, in part because of antigenic responses in some patients to even low rates of yeast growth. Potential symptoms include diarrhea, headache, bloating, atopic dermatitis and fatigue.

Parasitology

Parasite infections are a major cause of non-viral diarrhea. Symptoms may include constipation, gas, bloating, increased allergy response, colitis, nausea and distention.

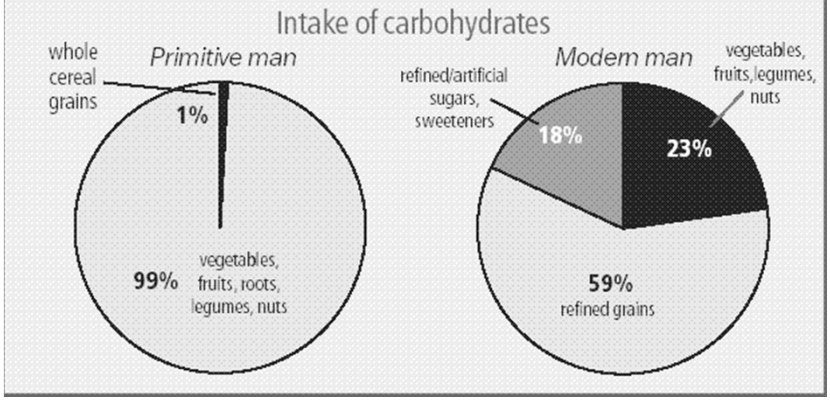
The **Adiposity Index** is derived by using DNA probes that detect multiple genera of the phyla Firmicutes and Bacteroidetes. Abnormalities of these phyla may be associated with increased caloric extraction from food.

Beneficial SCFA

Short chain fatty acids (SCFA) are produced by bacterial fermentation of dietary polysaccharides and fiber. The product, N-butyrate, is taken up and used to sustain the normal activity of colonic epithielial cells. Butyrate has been shown to lower the risk of colitis and colorectal cancer. A healthy balance of GI microbes depends on production of SCFA by one bacterium to allow the normal growth of another one in a complex cross-feeding network.

Figure 3: Changing carbohydrate intake patterns

The two pie charts compare the carbohydrate intake patterns of primitive and modern man. Note the extremely high intake of essential sugar-rich fruits, roots, nuts and legumes of the former [1].



Eur J ClinNutr 1997; 51(4):207-216

Salt Intake and Autoimmunity

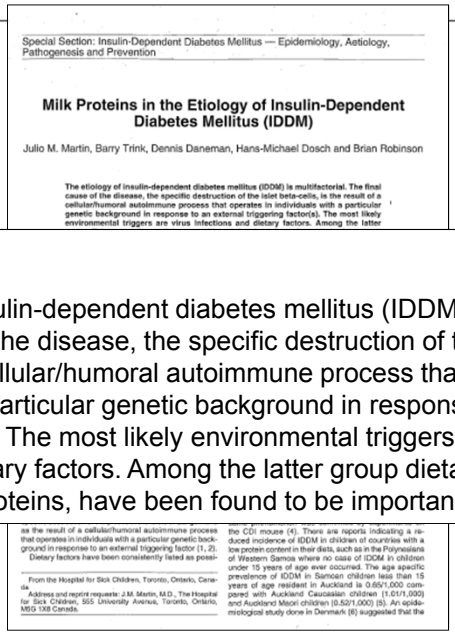
Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells.

Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA, Muller DN, Hafler DA.
1] Departments of Neurology and Immunobiology, Yale School of Medicine, 15 York Street, New Haven, Connecticut 06520, USA [2] Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, Massachusetts 02142, USA.

Abstract

Here we show that increased salt (sodium chloride, NaCl) concentrations found locally under physiological conditions in vivo markedly boost the induction of murine and human TH17 cells. The TH17 cells generated under high-salt conditions display a highly pathogenic and stable phenotype characterized by the upregulation of the pro-inflammatory cytokines GM-CSF, TNF-α and IL-2. Moreover, mice fed with a high-salt diet develop a more severe form of EAE, in line with augmented central nervous system infiltrating and peripherally induced antigen-specific TH17 cells. Thus, **increased dietary salt intake might represent an environmental risk factor for the development of autoimmune diseases through the induction of pathogenic TH17 cells.**

PMID: 23467095 [PubMed - as supplied by publisher]



Special Section: Insulin-Dependent Diabetes Mellitus — Epidemiology, Aetiology, Pathogenesis and Prevention

Milk Proteins in the Etiology of Insulin-Dependent Diabetes Mellitus (IDDM)

Julio M. Martin, Barry Trink, Dennis Daneman, Hans-Michael Dosch and Brian Robinson

The etiology of insulin-dependent diabetes mellitus (IDDM) is multifactorial. The final cause of the disease, the specific destruction of the islet beta-cells, is the result of a cellular/humoral autoimmune process that operates in individuals with a particular genetic background in response to an external triggering factor(s). The most likely environmental triggers are virus infections and dietary factors. Among the latter

Abstract

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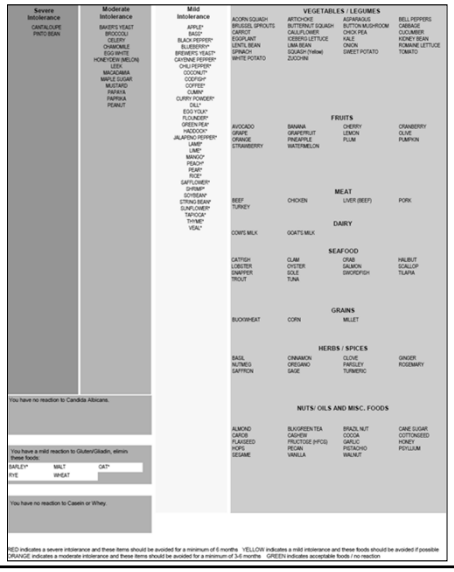
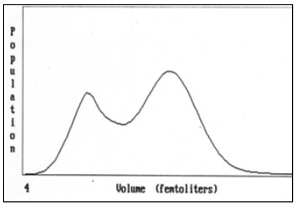
Ann Med. 1991 Oct;23(4):447-52: Martin JM, Trink B, Daneman D, et al.

Food Sensitivity Testing

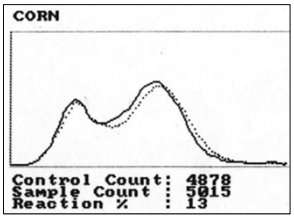
ALCAT WORLDWIDE



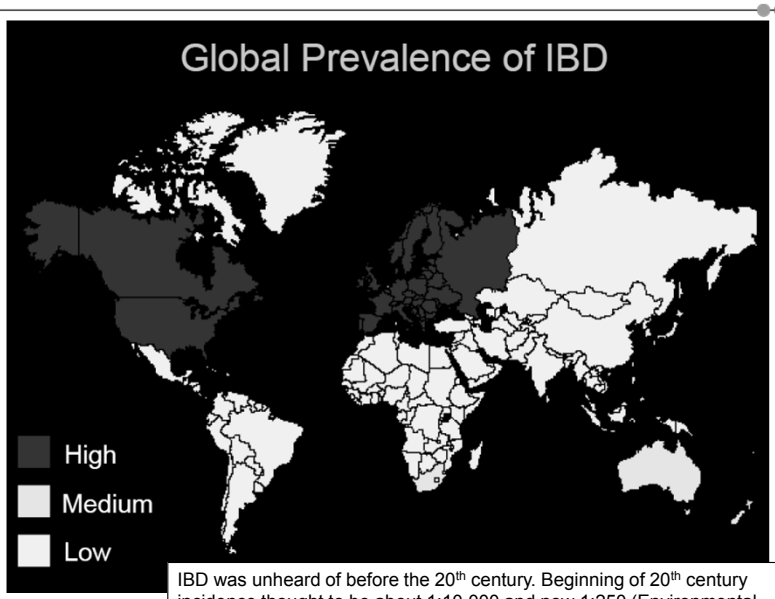
Pre-antigen exposure



Post-antigen exposure



Global Prevalence of IBD



IBD was unheard of before the 20th century. Beginning of 20th century incidence thought to be about 1:10,000 and now 1:250 (Environmental factors at play!). Similar data exists with asthma, hay fever, DM, MS, etc.

Weinstock J: IFM Annual Symposium (2011)

The New England Journal of Medicine

Editorials

Eat Dirt — The Hygiene Hypothesis and Allergic Diseases

THERE has been an epidemic of both autoimmune diseases (in which the immune response is dominated by type 1 helper T [Th1] cells, such as type 1 diabetes, Crohn's disease, and multiple sclerosis) and allergic diseases (in which the immune response is dominated by type 2 helper T [Th2] cells, such as asthma, allergic rhinitis, and atopic dermatitis), as documented in the article by Bach in this issue of the *Journal*.[1] The occurrence of these diseases is higher in more affluent, Western, industrialized countries. One theory proposed to explain this increase in the prevalence of autoimmune and allergic diseases is that it results from a decrease in the prevalence of childhood infection. Although this theory dates back to at least the mid-1960s in relation to Th1-mediated diseases, Strachan first proposed in 1989 that this theory might also explain the increase in Th2-mediated diseases,[2] and it has subsequently come to be called the hygiene hypothesis. A gradual change in the frequency of childhood infection has been occurring for a long time, affected by the introductions of indoor plumbing in the 19th century, antibiotics in the middle of the 20th century, and cleaner, more energy-efficient homes at the end of the 20th century.

Bach details a number of potential mechanisms by which the decrease in the frequency of childhood infections might influence the frequency of autoimmune diseases. In the light of the article by Braun-Fahrländer and coworkers,[3] also in this issue of the *Journal*, two mechanisms deserve special attention. The first is that the decrease in antigenic stimulation related to the decrease in the frequency of childhood infections has resulted in a decrease in the levels of regulatory cytokines — specifically, interleukin-10 and possibly transforming growth factor β (TGF-β). CD25-positive T cells and other regulatory T cells produce interleukin-10 and TGF-β and act to down-regulate both Th1-mediated responses and Th2-mediated responses. It is unclear how interleukin-10 and TGF-β

Fahrländer et al. is that stimulation of the innate immune system by endotoxin may be important in the ontogeny of the normal immune system.

A series of epidemiologic reports suggests that there has been a decrease in the frequency of allergy and asthma among children of farmers in Western, industrialized countries.[4,5] The current study by Braun-Fahrländer et al.[3] is a cross-sectional study involving 812 children between 6 and 13 years of age from farming and nonfarming households in rural areas of central Europe. The investigators measured endotoxin levels in mattress dust and found a relation between higher levels of endotoxin in the dust and a decreased frequency of hay fever, allergic asthma, and allergic sensitization in these children.

Endotoxin is a lipopolysaccharide that forms the outer layer of the cell membrane of all gram-negative bacteria. Endotoxin levels vary widely but tend to be highest in environments where there are farm animals such as cows, horses, and pigs, because the fecal flora of larger mammals is a major source of endotoxin. Endotoxin is also found in the dust in houses and outdoors in dirt and can be measured in dust or air. In its airborne form, endotoxin can be inhaled or swallowed and acts as a potent immunostimulatory molecule through its lipid A moiety, which signals, through CD14 and toll-like receptor 4 (TLR4), other molecules (MyD88 and toll-like receptor 9 [TLR9]) of the innate immunity pathway.

How does endotoxin decrease Th2-mediated diseases such as allergies and allergic asthma? At low levels, lipopolysaccharide is a potent inducer of interleukin-12 and interferon-γ, which are cytokines that stimulate Th1-mediated immunity, and also decreases the production of Th2 inflammatory cytokines such as interleukin-4, interleukin-5, and interleukin-13. Finally, lipopolysaccharide increases defensins and collectins, such as surfactant protein A in the lungs, which enhance the developing immune response of a neonate. The effects of endotoxin are dose-dependent; at high doses, endotoxin produces hypersensitivity pneumonitis and stimulates the release of inflammatory mediators.[6] Even at low doses, endotoxin is associated with wheezing during the first year of life.[7] Given these potential opposing effects of endotoxin exposure, greater knowledge about what dose of endotoxin is protective and what dose is a risk factor is needed.

PERSPECTIVES

SCIENCE AND SOCIETY

Farm living: effects on childhood asthma and allergy

Erika von Mutius and Donata Vercelli

Abstract | Numerous epidemiological studies have shown that children who grow up on traditional farms are protected from asthma, hay fever and allergic sensitization. Early-life contact with livestock and their fodder, and consumption of unprocessed cow's milk have been identified as the most effective protective exposures. Studies of the immunobiology of farm living point to activation and modulation of innate and adaptive immune responses by intense microbial exposures and possibly xenogeneic signals delivered before or soon after birth.

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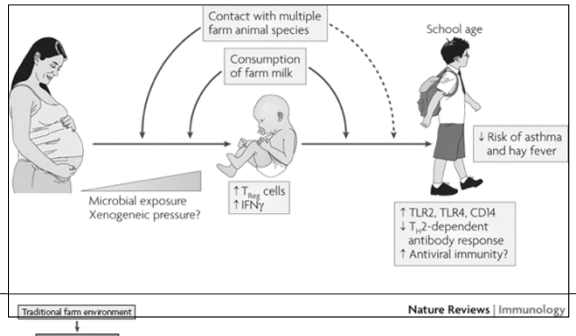
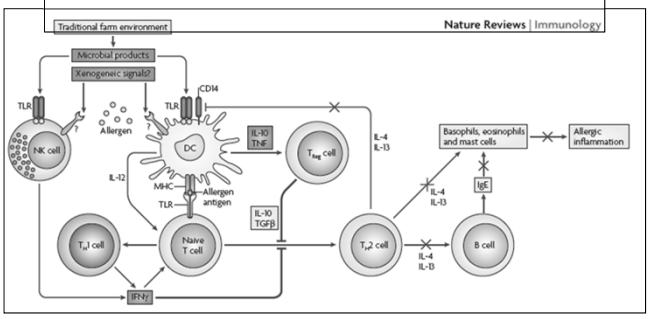
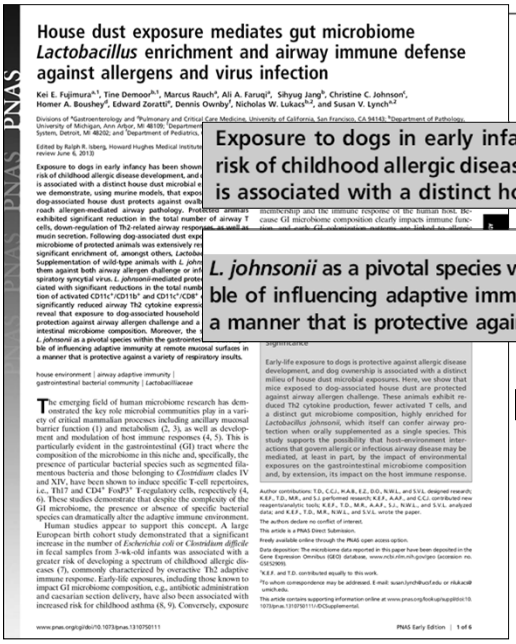
NATURE REVIEWS | IMMUNOLOGY
VOLUME 10 | DECEMBER 2010 | 861







House dust exposure mediates gut microbiome *Lactobacillus* enrichment and airway immune defense against allergens and virus infection

Kei E. Fujimura[a,1], Tine Demoor[b,1], Marcus Rauch[a], Ali A. Faruqi[a], Sihyug Jang[b], Christine C. Johnson[c], Homer A. Boushey[d], Edward Zoratti[e], Dennis Ownby[f], Nicholas W. Lukacs[b,2], and Susan V. Lynch[a,2]

Exposure to dogs in early infancy has been shown to reduce the risk of childhood allergic disease development, and dog ownership is associated with a distinct house dust microbial exposure.

Moreover, the study identifies *L. johnsonii* as a pivotal species within the gastrointestinal tract capable of influencing adaptive immunity at remote mucosal surfaces in a manner that is protective against a variety of respiratory insults.

Fujimura et al.

www.pnas.org/cgi/doi/10.1073/pnas.1310750111




Parasites in Your Gut Actually Help Protect You From Allergies

by David Gutierrez, staff writer

(NaturalNews) Humans and gastrointestinal parasites might have co-evolved in such a way that the parasites actually help regulate to human immune system to prevent against allergies, according to a study conducted by researchers from the University of Nottingham.

Researchers believe that over the course of millions of years, gastrointestinal parasites have evolved an ability to suppress the human immune system as a survival mechanism. Because parasitic infestation has been so common throughout human evolutionary history, the human immune system has in turn evolved to compensate for this effect.

This means that if the parasites are removed, the immune system may actually function too strongly, resulting in maladaptive immune responses such as asthma, eczema and other allergies.

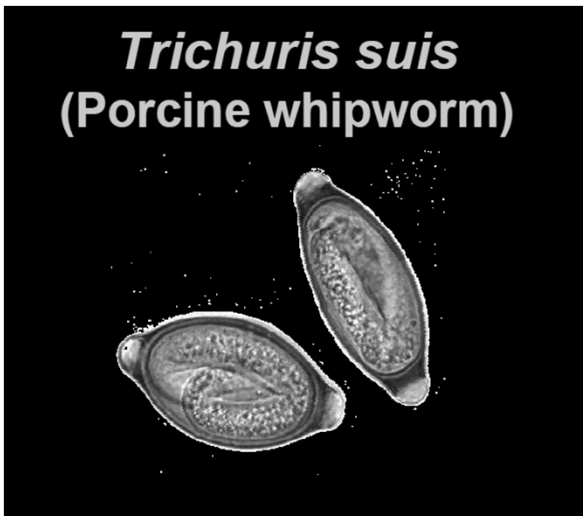
To test this hypothesis, researchers used drugs to eliminate hookworm infection in a 1,500 children between the ages of six and 17 who were living in a rural village in central Vietnam. This region was selected for its very low rates of allergies and high parasitic infestation rate. Two-thirds of all children in the area are infested with hookworm or other gastrointestinal parasites.

The researchers found that once the children were no longer infected with parasites, their rates of dust mite allergies significantly increased. This supports the hypothesis that parasites help regulate immune responses.

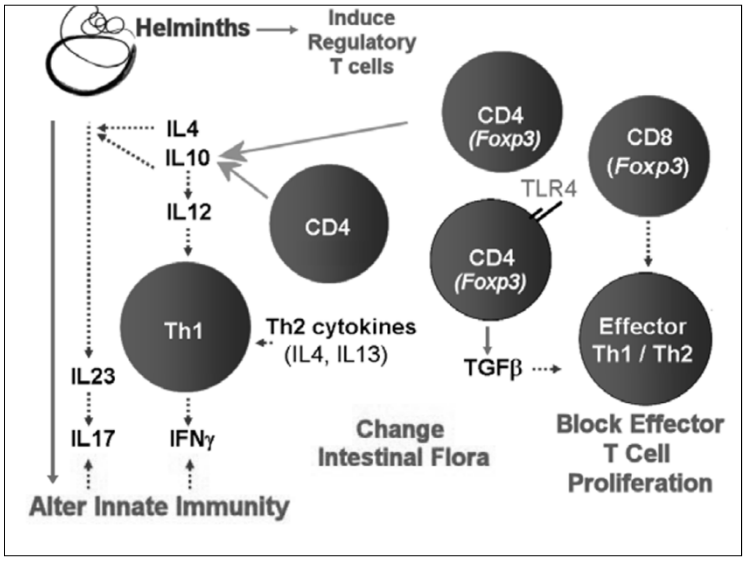
"The next step is to understand exactly how and when gut parasites program the human immune system in a way that protects against allergies, and for such studies, follow-up from birth will be essential," said researcher Carsten Flohr.

Researchers hope that understanding the relationship between parasites and the human immune system could lead to a better overall understanding of allergies.

"The prospects of further studies in this area are very exciting, as we could see groundbreaking treatments for asthma and other allergies developed as a result," said Elaine Vickers of Asthma UK, which funded the study.



Weinstock J: IFM Annual Symposium (2011)



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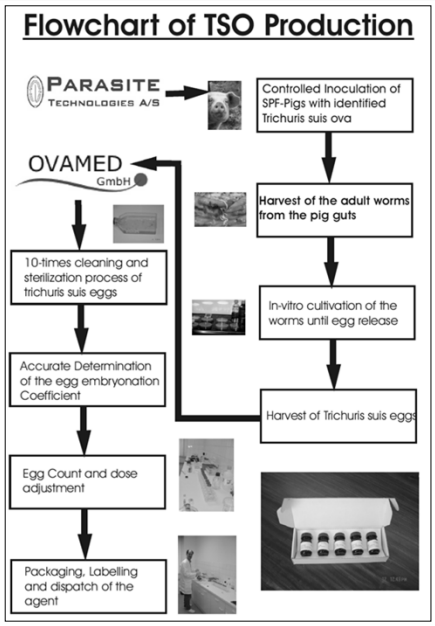


www.ovamed.org

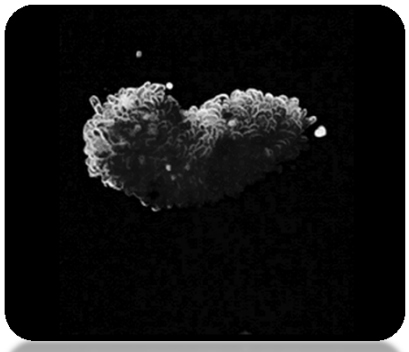
Gastroenterology
Worms Flop in Crohn's Disease
Published: Nov 8, 2013 (MedPageToday)

The German partner of Coronado Biosciences has terminated a clinical trial of *Trichuris suis*ova -- a whipworm parasite of pigs -- for Crohn's disease because of a lack of efficacy. This action was taken because of a recommendation of an independent data monitoring committee, which noted that no safety concerns had arisen during the study, known as TRUST-2. The committee conducted a second interim analysis of 240 patients who had been treated for 3 months in a phase II study conducted by Dr. Falk of Pharma GmbH. Coronado chief executive officer Harlan Weisman acknowledged that the company wasn't surprised at the disappointing results, because its own double-blind trial, TRUST-1, of helminth treatment in Crohn's also had shown inadequate efficacy. TRUST-1 had not met its primary endpoint of response, which was defined as a decrease of 100 points on the Crohn's Disease Activity Index, or a secondary endpoint of remission, or a score on the disease activity index of 150 or lower. "We believe [*Trichuris suis* ova] has therapeutic potential in other diseases and will continue to work diligently to advance its development for the treatment of autoimmune diseases," Weisman said in a statement. Parasitic helminths have evolved to live in their mammalian hosts, which respond with the release of several cytokines of the interleukin family and other immune cells such as eosinophils and mast cells. The overall response is similar to the Th2 component of the immune response. Epidemiologic studies have found that the prevalence of inflammatory bowel disease is highest in locales where helminthic infections no longer exist, and animal studies of initial clinical studies have suggested that induced infection might be protective against autoimmunity.

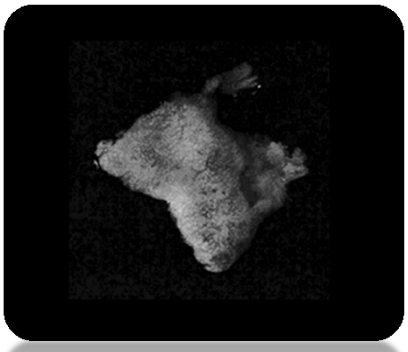
Healthy Villi of Small Intestine

IBD and Mucosal Health

Normal



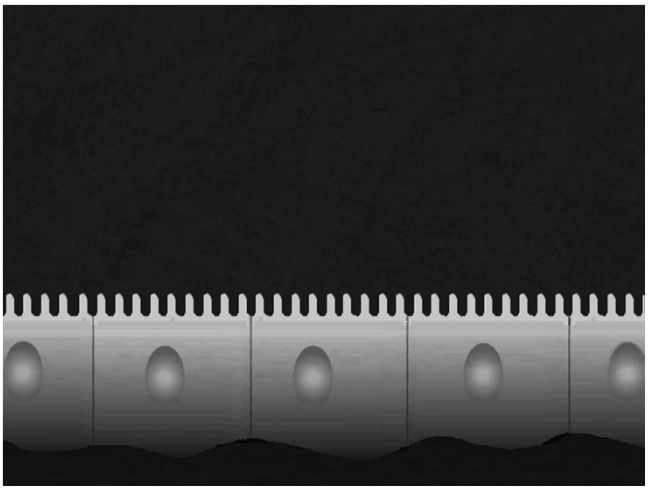
Inflammatory (Celiac)



Healthy Gut

**Healthy Villi –
Good Absorption**

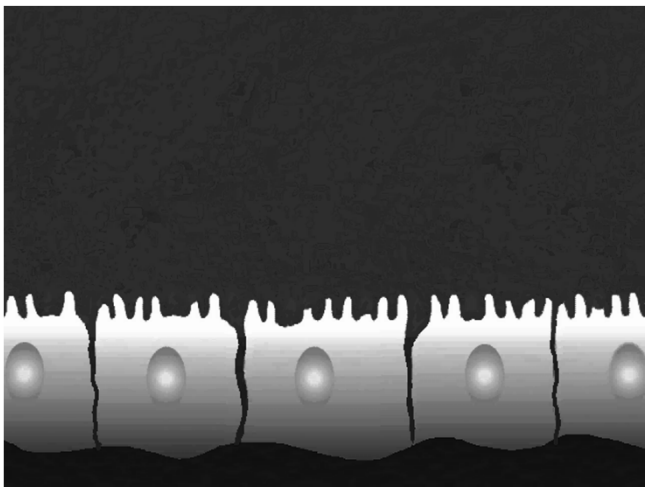
**Healthy
Cell junctions**

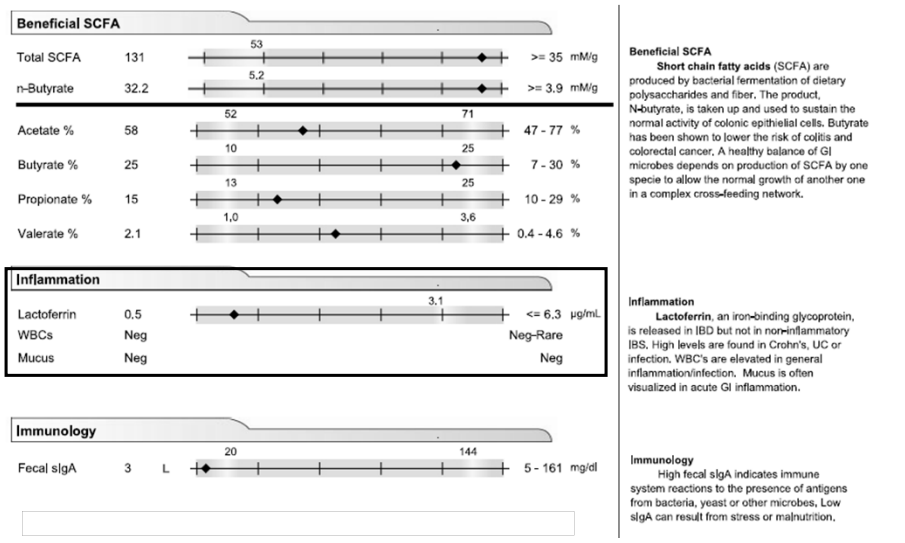


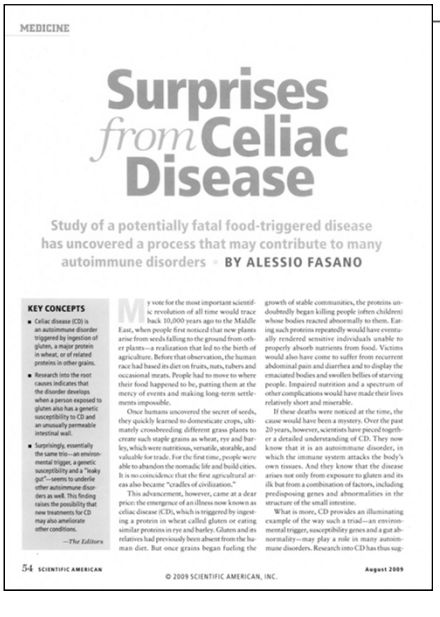
Leaky Gut & Malabsorption

**Damaged Villi –
Poor Absorption**

**Damaged
Cell junctions**






MEDICINE

Surprises from Celiac Disease

Study of a potentially fatal food-triggered disease has uncovered a process that may contribute to many autoimmune disorders • BY ALESSIO FASANO

KEY CONCEPTS
- Celiac disease (CD) is an autoimmune disorder triggered by ingestion of gluten, a major protein in wheat, or of related proteins in other grains.
- Research into the root causes indicates that the disorder develops when a person exposed to gluten also has a genetic susceptibility to CD and an unusually permeable intestinal wall.
- Surprisingly, essentially the same trio—an environmental trigger, a genetic susceptibility and a "leaky gut"—seems to underlie other autoimmune disorders as well. This finding raises the possibility that new treatments for CD may also ameliorate other conditions.
—The Editors

My vote for the most important scientific revolution of all time would trace back 10,000 years ago to the Middle East, when people first noticed that new plants arise from seeds falling to the ground from other plants—a realization that led to the birth of agriculture. Before that observation, the human race had based its diet on fruits, nuts, tubers and occasional meats. People had to move to where their food happened to be, putting them at the mercy of events and making long-term settlements impossible.

Once humans uncovered the secret of seeds, they quickly learned to domesticate crops, ultimately crossbreeding different grass plants to create such staple grains as wheat, rye and barley, which were nutritious, versatile, storable, and valuable for trade. For the first time, people were able to abandon the nomadic life and build cities. It is no coincidence that the first agricultural areas also became "cradles of civilization."

This advancement, however, came at a dear price: the emergence of an illness now known as celiac disease (CD), which is triggered by ingesting a protein in wheat called gluten or eating similar proteins in rye and barley. Gluten and its relatives had previously been absent from the human diet. But once grains began fueling the growth of stable communities, the proteins undoubtedly began killing people (often children) whose bodies reacted abnormally to them. Eating such proteins repeatedly would have eventually rendered sensitive individuals unable to properly absorb nutrients from food. Victims would also have come to suffer from recurrent abdominal pain and diarrhea and to display the emaciated bodies and swollen bellies of starving people. Impaired nutrition and a spectrum of other complications would have made their lives relatively short and miserable.

If these deaths were noticed at the time, the cause would have been a mystery. Over the past 20 years, however, scientists have pieced together a detailed understanding of CD. They now know that it is an autoimmune disorder, in which the immune system attacks the body's own tissues. And they know that the disease arises not only from exposure to gluten and its ilk but from a combination of factors, including predisposing genes and abnormalities in the structure of the small intestine.

What is more, CD provides an illuminating example of the way such a triad—an environmental trigger, susceptibility genes and a gut abnormality—may play a role in many autoimmune disorders. Research into CD has thus sug-

54 SCIENTIFIC AMERICAN © 2009 SCIENTIFIC AMERICAN, INC. August 2009

[THE AUTHOR]

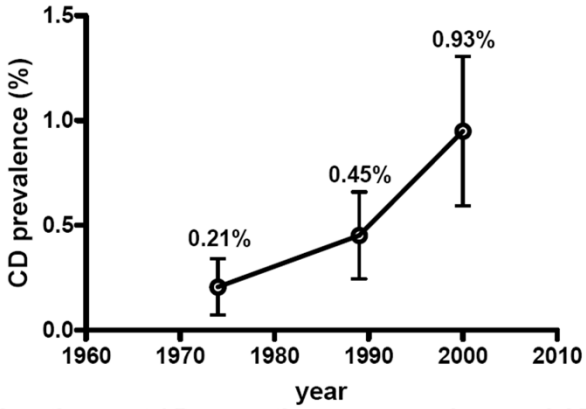
Alessio Fasano is professor of pediatrics, medicine and physiology and director of the Mucosal Biology Research Center and the Center for Celiac Research at the University of Maryland School of Medicine. Much of his basic and clinical research focuses on the role of intestinal permeability in the development of celiac disease and other autoimmune disorders.

The theory that a leaky gut contributes to CD and autoimmunity in general was initially greeted with skepticism.

Fasano A. Surprises from Celiac Disease. Scientific American, August 2009

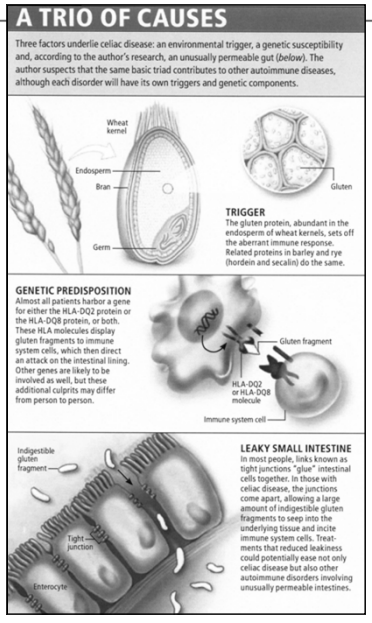
Increased Prevalence Over Time in U.S.A.

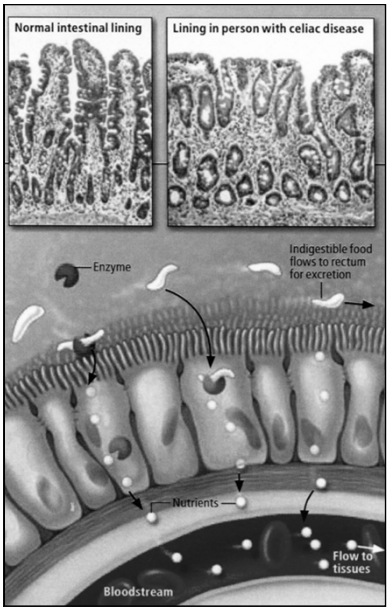
(in Line with Other Autoimmune Diseases)

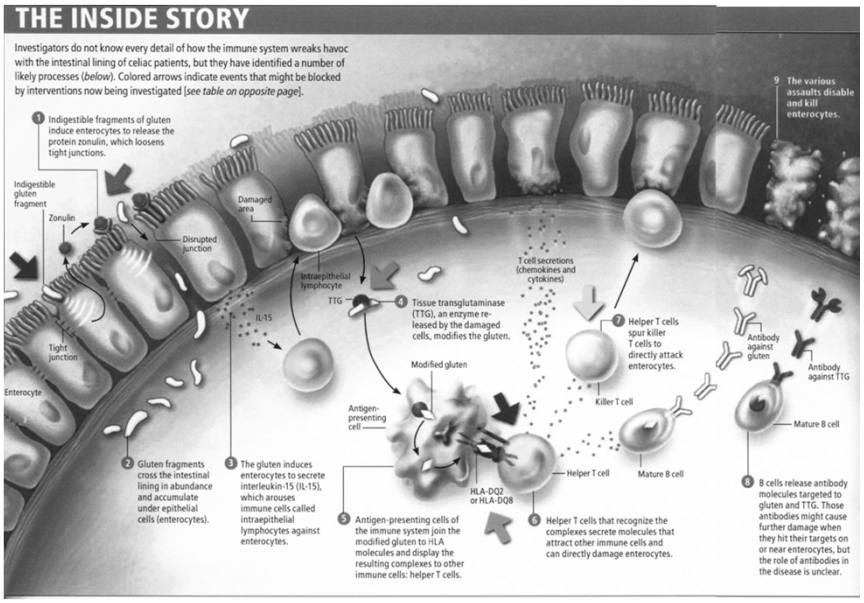


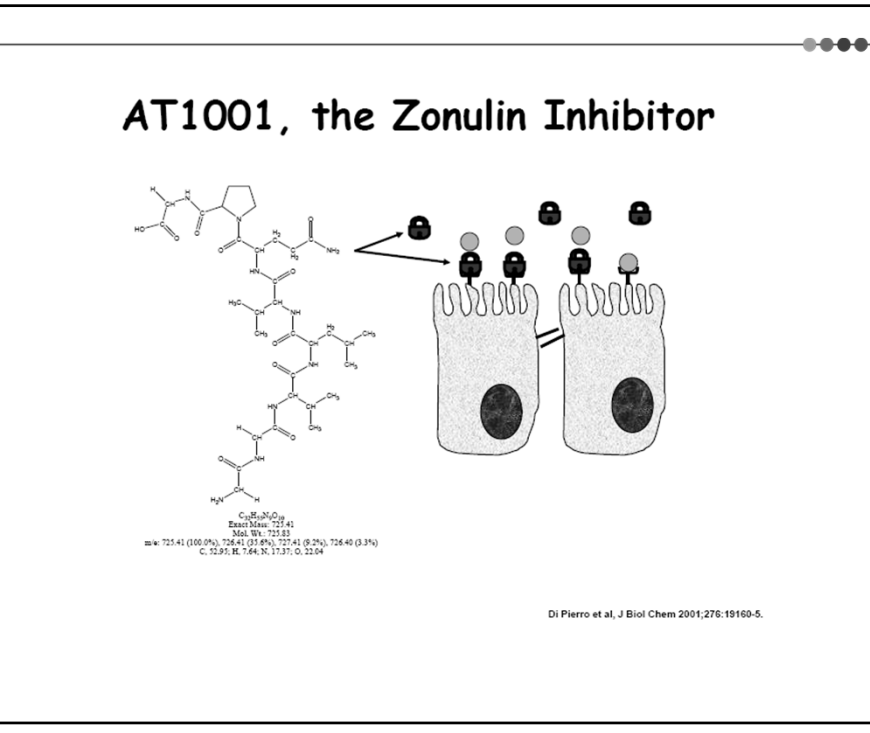
During the past 35 years the true prevalence of CD in USA doubled every 15 years.

C. Catassi et al, Annal Med 2011









Alba Clinical Trial Summary in Celiac Disease with Larazotide Acetate – Tight Junction Regulator

- Phase Ib - Single Dose (CLIN1001-002)
  - 21 Celiac disease subjects
  - Double blind, placebo controlled
  - 3 days QD, single gluten challenge on day 2
  - In-patient study
  - Completed March 2006
- Phase IIa - Multiple Dose (CLIN1001-004)
  - 86 celiac disease subjects
  - Double blind, placebo controlled
  - 2 weeks TID dosing and gluten challenge
  - Dose ranging - 7 arms
  - Multi-center Outpatient Study
  - Completed March 2007
- Phase IIb - Multiple Dose (CLIN1001-006)
  - 184 celiac disease subjects
  - Double blind, placebo controlled
  - 6 weeks TID dosing and gluten challenge
  - Dose ranging - 4 arms
  - Multi-center Outpatient Study

0% Bioavailability

No Adverse Safety Trends

- **Larazotide acetate acts locally in the gastrointestinal tract**
- **No systemic exposure, no measurable plasma drug levels in any clinical study**
- **No immunogenicity, no antibody development in any clinical study**
- **No toxicity observed to date in 24 completed animal toxicology studies**
- **No safety signals in ~500 celiac subjects exposed to larazotide acetate up to 8 weeks**
- **To date, safety comparable to placebo**

The June 2014 issue of Elle contains an article on chronic GI problems in women and discusses "leaky gut syndrome" and its association to autoimmune disorders and features interviews with and quotes from Dr. Alessio Fasano, Dr. David M. Brady, and others.

Example GI Mucosal Repair Formulation

	Amounts per serving
Serving size	1 tsp. (6 g)
Number of servings per container	40
L-Glutamine	1500 mg
N-Acetyl Glucosamine	1000 mg
PepZin GI (Zinc-Carnosine)	75 mg
Deglycyrrhizinated Licorice (DGL)(Glycyrrhiza glabra)	400 mg.
Aloe vera (Aloe barbadensis)	300 mg
Slippery Elm (Ulmus fulva)	200 mg
Marshmallow (Althea officinalis)	100 mg
Chamomile (Matricaria chamomilus)	100 mg
Okra (Hibiscus esulentus)	100 mg
Cat's Claw (Unicaria tomentosa-TOA free)	100 mg
Mucin	200 mg
MSM	100 mg
Quercitin	100 mg
Prunus (concentrate)	100 mg
Citrus pectin	1000 mg
Stevia	
Natural Flavors	
Suggested Dose: Take 1/2-1 tsp., one to two times daily or as directed by your health care practitioner.	

1: Aliment Pharmacol Ther. 2000 Dec;14(12):1567-79.

A pilot study of N-acetyl glucosamine, a nutritional substrate for glycosaminoglycan synthesis, in paediatric chronic inflammatory bowel disease.

Salvatore S, Heuschkel R, Tomlin S, Davies SE, Edwards S, Walker-Smith JA, French I, Murch SH.

University Department of Paediatric Gastroenterology, Royal Free, London, UK.

BACKGROUND: The breakdown of glycosaminoglycans is an important consequence of inflammation at mucosal surfaces, and inhibition of metalloprotease activity may be effective in treating chronic inflammation. AIM: To report an alternative approach, using the nutriceutical a… …and glycoproteins, as a substrate fo… …djunct therapy to 12 children with s… …en of these children suffered from … children with distal ulcerative colit… …liable (nine cases), histochemical … Eight of the children given oral Glc… …nn's stricture, only 3 of 7 have require… …etected in the others. Rectal adminis… biopsied there was evidence of hi… …s and intracellular GlcNAc. CONC… bowel disease, with a mode of actio… …g disease. However, controlled trial… …h indications for use.

CONCLUSIONS: GlcNAc shows promise as an inexpensive and nontoxic treatment in chronic inflammatory bowel disease, with a mode of action which is distinct from conventional treatments. It may have the potential to be helpful in stricturing disease.

Sugar supplement may treat immune disease

07 June 2007 by Aria Pearson
Magazine issue 2607. Subscribe and get 4 free issues.

A sugar supplement may sweeten the overactive immune cells responsible for autoimmune diseases such as multiple sclerosis (MS) and type 1 diabetes and stop them attacking the body's tissues.

Crowd control

Enlarge image

Autoimmune diseases are triggered when receptors on the outside of immune cells called T-helper 1 (Th1) cells start binding "self" antigens rather than pieces of foreign invaders. Anything that decreases the amount of binding should suppress the autoimmune response.

Previous studies suggested that glucosamine, a dietary supplement commonly taken by people with osteoarthritis, has some immunosuppressive effects. This led Michael Demetriou and colleagues at the University of California, Irvine, to investigate a similar but more potent compound called N-acetylglucosamine (GlcNAc).

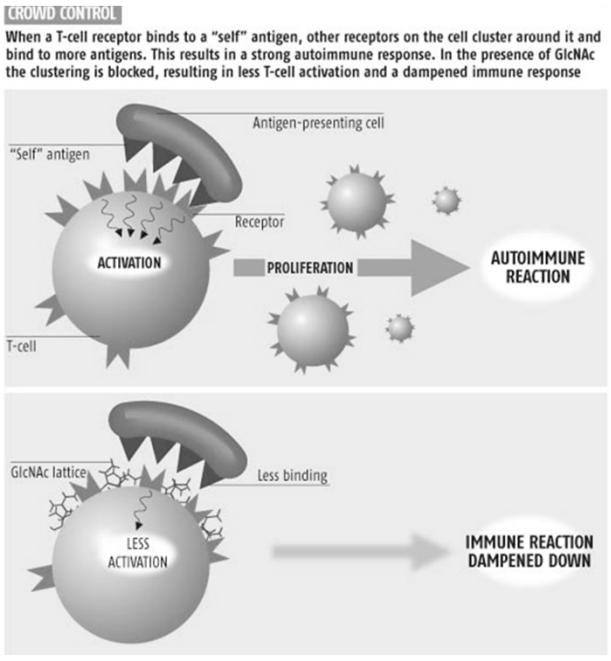
A large number of proteins in the body are modified by the attachment of sugar molecules to their surface through a process called glycosylation, and altered glycosylation has been implicated in some autoimmune diseases. Demetriou's team found that naturally occurring GlcNAc molecules attach to T-cell receptors and these GlcNAc "branches" form a lattice on the cell surface that prevents the receptors from clustering near where the antigens are located (see Diagram). Less clustering means less antigen binding, and less activation of Th1 cells, reducing the autoimmune reaction.

Mice given oral GlcNAc supplements had twice as much GlcNAc branching on their T-cell receptors as untreated mice. The researchers also found that T-cells engineered to cause the mouse equivalent of MS failed to do so if they had been incubated in GlcNAc first. A daily oral dose of GlcNAc also prevented type 1 diabetes in mice genetically engineered to develop the disease (*The Journal of Biological Chemistry*, DOI: 10.1074/jbc.M701890200).

T-cells engineered to cause the mouse equivalent of multiple sclerosis failed to do so if they had been incubated in GlcNAc

The researchers found that naturally occurring GlcNAc molecules attach to T-cell receptors and these GlcNAc "branches" form a lattice on the cell surface that prevents the receptors from clustering near where the antigens are located… less clustering means less antigen binding, and less activation of Th1 cells, reducing the autoimmune reaction.

The Journal of Biological Chemistry, DOI: 10.1074/jbc.M701890200).



Curcumin

Curcumin is a polyphenolic compound extracted from the spice turmeric. In Ayurvedic medicine, turmeric and curcumin have been used, among other things, for their anti-inflammatory properties. Curcumin possesses inhibitory effects on cyclooxegenase-1 (COX-1), cyclooxegenase-2 (COX-2), lipoxygenase (LOX), TNF- α, interferon gamma (IFN-gamma), inducible nitric oxide synthase (iNOS), and NF-κB, in addition to demonstrating powerful antioxidant effects. Additionally, by modulating cytokine and chemokine production, curcumin consequently impacts the balance of Th-1 and Th-2 T helper cells further downstream.

It is primarily through these mechanisms of action that curcumin has been shown in human and animal studies to positively impact the signs and symptoms of those suffering from a variety of autoimmune conditions including colitis, RA, SLE and Sjogren's syndrome.[16-20]

Andrographis

ParActin® is a special extract of the medicinal herb *Andrographis paniculata*. This annual herb has been widely used as part of Indian folk medicine and Ayurveda for centuries. In low doses (25-30mg) ParActin® acts as an immune stimulant, but at higher doses (150-250mg) it activates the peroxisome proliferator activated receptor gamma (PPARγ) nuclear receptor. When activated, PPARγ not only stimulates the expression of genes involved in energy homeostasis, specifically the metabolism of glucose and fatty acids, but also key regulators of the immune and inflammatory responses.[8] By activating PPARγ, inhibition of NF-κβ takes place which includes the reduced production of various downstream inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β.[9]

In models of multiple sclerosis (MS), Paractin® was shown to act as an inhibitor of the T cell-mediated immune response. Inappropriate T cell activity (CD4+, CD8+ cells) and its associated myelin-specific autoimmune responses have been shown to be part of the pathogenesis of MS. Paractin® was shown to modulate and reduce disease-associated cytokines such a NF-κB, inflammatory markers commonly associated with MS pathophysiology.[10] In human case studies, individuals given Paractin® were found to experience a decrease in a variety of symptoms associated with MS, including speech ataxia, fatigue, depression and restless leg syndrome.[11]

Supplement Facts

Serving Size 4 capsules
Servings Per Container 30

Amount Per Serving		% Daily Value
Curcumin C3 Complex® (*Curcuma longa*)(rhizomes)(containing three curcuminoids: curcumin, bisdemethoxy curcumin, demethoxy curcumin)[standardized to contain 95% curcuminoids]	750 mg	*
N-Acetyl Glucosamine	750 mg	*
ParActin® (Bioactive 14-Neo-Andro Compound) (*Andrographis paniculata*)(stem and leaf) [standardized to contain 50% andrographolides]	250 mg	*

Am J Physiol Gastrointest Liver Physiol 294: G208–G216, 2008.
First published October 25, 2007; doi:10.1152/ajpgi.00398.2007.

Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier

Juan Kong,[1] Zhongyi Zhang,[1] Mark W. Musch,[1] Gang Ning,[2] Jun Sun,[2] John Hart,[4] Marc Bissonnette,[3] and Yan Chun Li[1]

Departments of [1]Medicine and [2]Pathology, The University of Chicago, Chicago, Illinois; [3]The Huck Institutes for Life Sciences, The Pennsylvania State University, University Park, Pennsylvania; and [4]Gastroenterology and Hepatology Division, Department of Medicine, University of Rochester Medical Center, Rochester, New York

Submitted 31 August 2007; accepted in final form 23 October 2007

Kong J, Zhang Z, Musch MW, Ning G, Sun J, Hart J, Bissonnette M, Li YC. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol* 294: G208–G216, 2008. First published October 25, 2007; doi:10.1152/ajpgi.00398.2007.—Emerging evidence supports a pathological link between vitamin D deficiency and the risk of inflammatory bowel disease (IBD). To explore the mechanism we used the dextran sulfate sodium (DSS)-induced colitis model to investigate the role of the vitamin D receptor (VDR) in mucosal barrier homeostasis. While VDR$^{+/+}$ mice were mostly resistant to 2.5% DSS, VDR$^{-/-}$ mice developed severe diarrhea, rectal bleeding, and marked body weight loss, leading to death in 2 wk. Histological examination revealed extensive ulceration and impaired wound healing in the colonic epithelium of DSS-treated VDR$^{-/-}$ mice. Severe ulceration in VDR$^{-/-}$ mice was preceded by a greater loss of intestinal transepithelial electric resistance (TER) compared with VDR$^{+/+}$ mice. Confocal and electron microscopy (EM) revealed severe disruption in epithelial junctions in VDR$^{-/-}$ mice after 3-day DSS treatment. Therefore, VDR$^{-/-}$ mice were much more susceptible to DSS-induced mucosal injury than VDR$^{+/+}$ mice. In cell cultures, 1,25-dihydroxy-vitamin D$_3$ [1,25(OH)$_2$D$_3$] markedly enhanced tight junctions formed by Caco-2 monolayers by increasing junction protein expression and TER and preserved the structural integrity of tight junctions in the presence of DSS. VDR knockdown with small interfering (si)RNA reduced the junction proteins and TER in Caco-2 monolayers. 1,25(OH)$_2$D$_3$ can also stimulate epithelial cell migration in vitro. These observations suggest that VDR plays a critical role in mucosal barrier homeostasis by preserving the integrity of junction complexes and the healing capacity of the colonic epithelium. Therefore, vitamin D deficiency may compromise the mucosal barrier, leading to increased susceptibility to mucosal damage and increased risk of IBD.

tight junction; inflammatory bowel disease; dextran sulfate sodium

THE INTESTINAL EPITHELIAL barrier consists of epithelial cells and the intercellular junctions. The barrier regulates macromolecule trafficking between the lumen and the internal milieu and protects the host by preventing harmful solutes, microorganisms, toxins, and luminal antigens from entering the body (40). Compromise or disruption of the intestinal barrier function causes deleterious effects and results in exposure of the host to luminal antigens and bacteria, leading to inflammation. Impaired barrier functions have been described in a number of common gastrointestinal disorders, including inflammatory bowel disease (IBD) (7).

The integrity of the intestinal mucosal barrier is preserved by the enormous regenerating capacity of the mucosal epithelium. The intestinal stem cells, located at the base of the crypt, are responsible for replenishing the epithelium through cell division and differentiation. After extensive destruction, rapid resealing of the surface epithelium is accomplished by epithelial cell restitution, proliferation, and differentiation (6). Another important component of the mucosal barrier is the apical and subapical intercellular junctions between the epithelial cells, namely tight junctions and adherens junctions (18). These junction structures seal the paracellular space and regulate the permeability of the mucosal barrier.

IBD, including Crohn's disease and ulcerative colitis, is a major chronic disorder affecting the gastrointestinal tract in humans. Although the etiopathogenesis of IBD has not been clearly elucidated, it is thought to involve a complex interplay among genetic, environmental, microbial, and immune factors (33). One potential pathogenic factor is impaired mucosal barrier function, and intestinal hyperpermeability is common in IBD patients (11). A relatively high number of first degree relatives of patients with Crohn's disease have increased intestinal permeability in the absence of clinical symptoms (32, 42), suggesting barrier dysfunction precedes, or is at least a very early defect, in the disease process that might require genetic predisposition and environmental triggers. Indeed, previous studies have demonstrated decreased expression and differential localization of junction complex proteins in the mucosa of patients with IBD (10, 16, 29). Therefore, dysregulation of junction proteins is an important pathogenic mechanism underlying the increased permeability seen in the intestinal epithelium of IBD patients.

Previous studies have suggested a link between vitamin D deficiency and IBD risk (23). The prevalence of IBD exhibits a north-south gradient (24), paralleling sunlight exposure, an important source of vitamin D. Populations near the equator are at relatively lower risk for developing IBD. Seasonal variations in the onset and exacerbation of IBD have also been reported (27, 36) with high incidence in the winter. Early studies have reported a high prevalence of vitamin D deficiency in patients with established Crohn's disease (12, 38). Decreased vitamin D levels have also been detected in patients with newly diagnosed IBD (17, 19, 35). In the IL-10$^{-/-}$ mouse model of intestinal inflammation, vitamin D deficiency or vitamin D receptor (VDR) deficiency exacerbates the symp-

Vitamin D and the Gut

In vitro experiments demonstrate that VDR mediates the activity of 1,25(OH)2D3 that induces junction protein expression and strengthens the tight junction complex. These data are consistent with, and explain at least in part, the observation reported in the literature that vitamin D deficiency is linked to increased incidence of IBD in human population.

Vitamin D: An Antimicrobial?

www.medscape.com

From Future Microbiology

The Vitamin D–antimicrobial Peptide Pathway and Its Role in Protection against Infection

Adrian F Gombart

Posted: 12/11/2009; Future Microbiology. 2009;4(9):1151-1165. © 2009

Abstract and Introduction

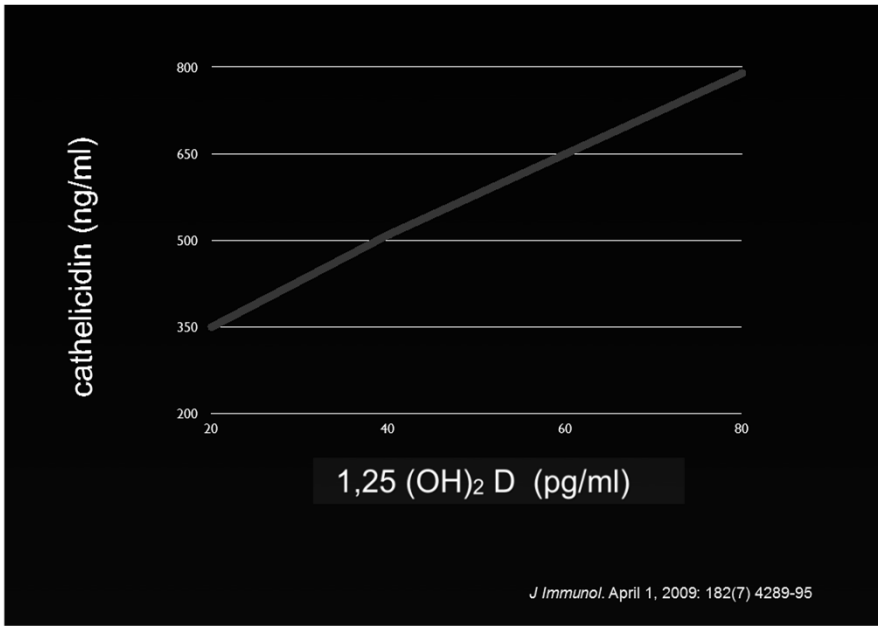
Abstract

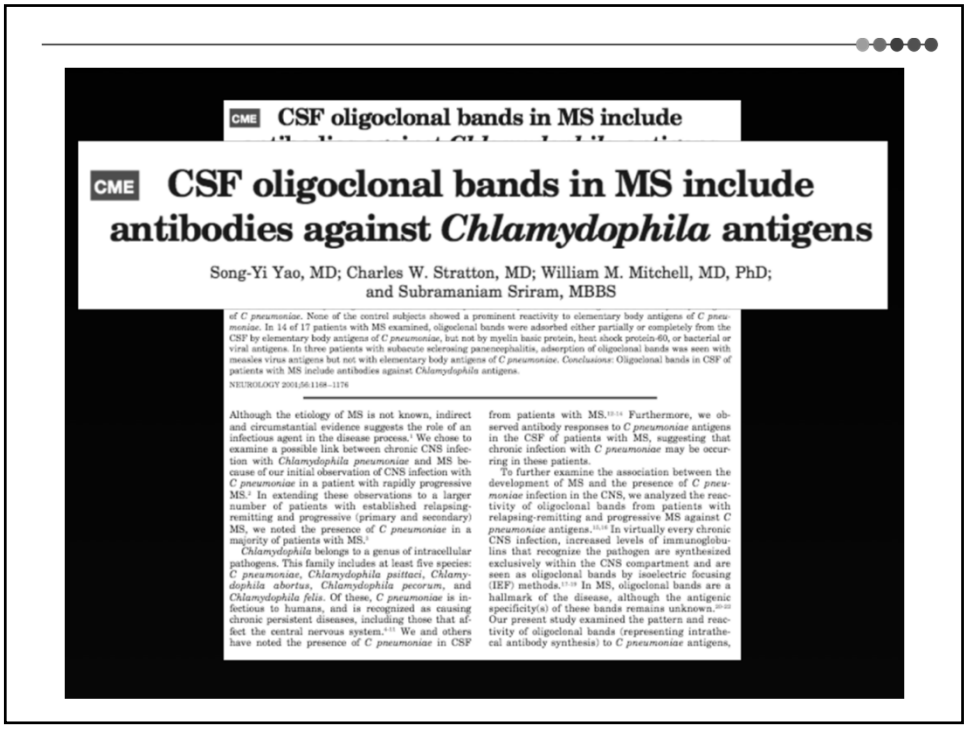
Vitamin D deficiency has been correlated with increased rates of infection. Since the early 19th century, both environmental (i.e., sunlight) and dietary sources (cod liver) of vitamin D have been identified as treatments for TB. The recent discovery that vitamin D induces antimicrobial peptide gene expression explains, in part, the 'antibiotic' effect of vitamin D and has greatly renewed interest in the ability of vitamin D to improve immune function. Subsequent work indicates that this regulation is biologically important for the response of the innate immune system to wounds and infection and that deficiency may lead to suboptimal responses toward bacterial and viral infections. The regulation of the cathelicidin antimicrobial peptide gene is a human/primate-specific adaptation and is not conserv… low-affinity recepto… selection to place f… xenobiotic respons… regulation and lead…

Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D_3

Adrian F. Gombart,*,[1] Niels Borregaard,[†] and H. Phillip Koeffler*

*Department of Medicine, Division of Hematology/Oncology, Cedars-Sinai Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, California, USA; and [†]The Granulocyte Research Laboratory, Department of Hematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark





CME CSF oligoclonal bands in MS include

CME

CSF oligoclonal bands in MS include antibodies against *Chlamydophila* antigens

Song-Yi Yao, MD; Charles W. Stratton, MD; William M. Mitchell, MD, PhD; and Subramaniam Sriram, MBBS

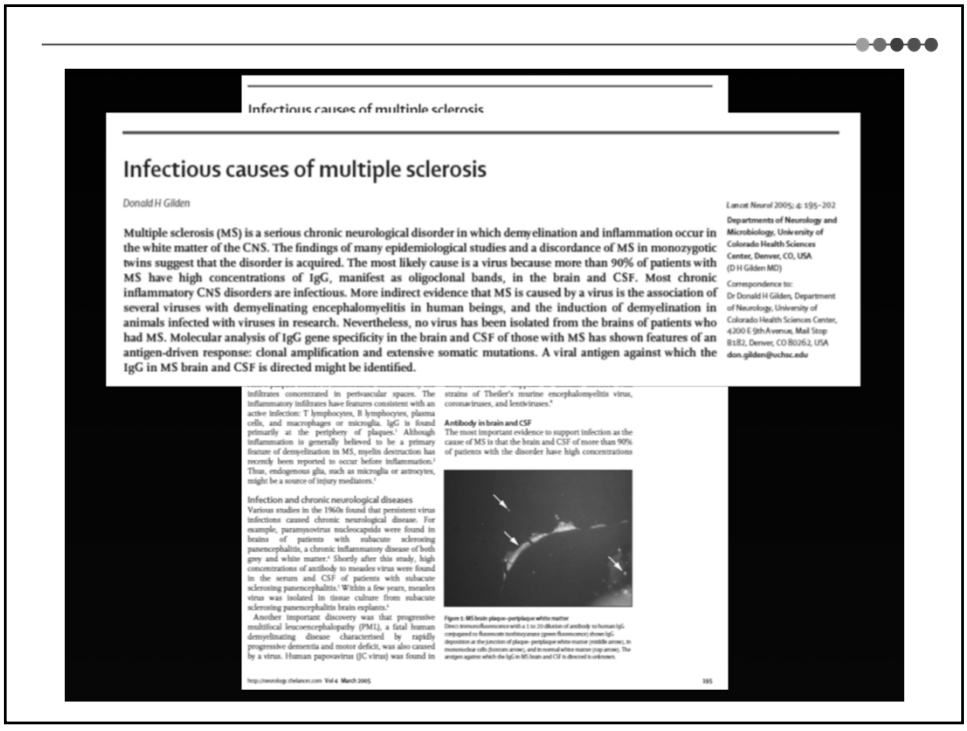
of *C pneumoniae*. None of the control subjects showed a prominent reactivity to elementary body antigens of *C pneumoniae*. In 14 of 17 patients with MS examined, oligoclonal bands were adsorbed either partially or completely from the CSF by elementary body antigens of *C pneumoniae*, but not by myelin basic protein, heat shock protein-60, or bacterial or viral antigens. In three patients with subacute sclerosing panencephalitis, adsorption of oligoclonal bands was seen with measles virus antigens but not with elementary body antigens of *C pneumoniae*. *Conclusions:* Oligoclonal bands in CSF of patients with MS include antibodies against *Chlamydophila* antigens.

NEUROLOGY 2001;56:1168–1176

Although the etiology of MS is not known, indirect and circumstantial evidence suggests the role of an infectious agent in the disease process.[1] We chose to examine a possible link between chronic CNS infection with *Chlamydophila pneumoniae* and MS because of our initial observation of CNS infection with *C pneumoniae* in a patient with rapidly progressive MS.[2] In extending these observations to a larger number of patients with established relapsing-remitting and progressive (primary and secondary) MS, we noted the presence of *C pneumoniae* in a majority of patients with MS.[3]

Chlamydophila belongs to a genus of intracellular pathogens. This family includes at least five species: *C pneumoniae*, *Chlamydophila psittaci*, *Chlamydophila abortus*, *Chlamydophila pecorum*, and *Chlamydophila felis*. Of these, *C pneumoniae* is infectious to humans, and is recognized as causing chronic persistent diseases, including those that affect the central nervous system.[4–11] We and others have noted the presence of *C pneumoniae* in CSF from patients with MS.[12–14] Furthermore, we observed antibody responses to *C pneumoniae* antigens in the CSF of patients with MS, suggesting that chronic infection with *C pneumoniae* may be occurring in these patients.

To further examine the association between the development of MS and the presence of *C pneumoniae* infection in the CNS, we analyzed the reactivity of oligoclonal bands from patients with relapsing-remitting and progressive MS against *C pneumoniae* antigens.[15,16] In virtually every chronic CNS infection, increased levels of immunoglobulins that recognize the pathogen are synthesized exclusively within the CNS compartment and are seen as oligoclonal bands by isoelectric focusing (IEF) methods.[17–19] In MS, oligoclonal bands are a hallmark of the disease, although the antigenic specificity(s) of these bands remains unknown.[20–22] Our present study examined the pattern and reactivity of oligoclonal bands (representing intrathecal antibody synthesis) to *C pneumoniae* antigens,



Infectious causes of multiple sclerosis

Infectious causes of multiple sclerosis

Donald H Gilden

Lancet Neurol 2005; 4: 195–202

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Multiple sclerosis (MS) is a serious chronic neurological disorder in which demyelination and inflammation occur in the white matter of the CNS. The findings of many epidemiological studies and a discordance of MS in monozygotic twins suggest that the disorder is acquired. The most likely cause is a virus because more than 90% of patients with MS have high concentrations of IgG, manifest as oligoclonal bands, in the brain and CSF. Most chronic inflammatory CNS disorders are infectious. More indirect evidence that MS is caused by a virus is the association of several viruses with demyelinating encephalomyelitis in human beings, and the induction of demyelination in animals infected with viruses in research. Nevertheless, no virus has been isolated from the brains of patients who had MS. Molecular analysis of IgG gene specificity in the brain and CSF of those with MS has shown features of an antigen-driven response: clonal amplification and extensive somatic mutations. A viral antigen against which the IgG in MS brain and CSF is directed might be identified.

infiltrates concentrated in perivascular spaces. The inflammatory infiltrates have features consistent with an active infection: T lymphocytes, B lymphocytes, plasma cells, and macrophages or microglia. IgG is found primarily at the periphery of plaques.[3] Although inflammation is generally believed to be a primary feature of demyelination in MS, myelin destruction has recently been reported to occur before inflammation.[4] Thus, endogenous glia, such as microglia or astrocytes, might be a source of injury mediators.[5]

Infection and chronic neurological diseases

Various studies in the 1960s found that persistent virus infections caused chronic neurological disease. For example, paramyxovirus nucleocapsids were found in brains of patients with subacute sclerosing panencephalitis, a chronic inflammatory disease of both grey and white matter.[6] Shortly after this study, high concentrations of antibody to measles virus were found in the serum and CSF of patients with subacute sclerosing panencephalitis.[7] Within a few years, measles virus was isolated in tissue culture from subacute sclerosing panencephalitis brain explants.[8]

Another important discovery was that progressive multifocal leucoencephalopathy (PML), a fatal human demyelinating disease characterised by rapidly progressive dementia and motor deficit, was also caused by a virus. Human papovavirus (JC virus) was found in strains of Theiler's murine encephalomyelitis virus, coronaviruses, and lentiviruses.[9]

Antibody in brain and CSF

The most important evidence to support infection as the cause of MS is that the brain and CSF of more than 90% of patients with the disorder have high concentrations

Figure 1: MS brain plaque–periplaque white matter

Direct immunofluorescence with a 1 to 20 dilution of antibody to human IgG conjugated to fluorescein isothiocyanate (green fluorescence) shows IgG deposition at the junction of plaque–periplaque white matter (middle arrow), in mononuclear cells (bottom arrow), and in normal white matter (top arrow). The antigen against which the IgG in MS brain and CSF is directed is unknown.

Autoimmune Disease

Where are we going?

NEW

PREDICTORS

of DISEASE

Molecules called predictive autoantibodies appear in the blood years before people show symptoms of various disorders. Tests that detected these molecules could warn of the need to take preventive action.

Abner Louis Notkins, Scientific American, 296(3):72-79, 2007

Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. J Clin Invest 2001 ; 108 : 1417 -22

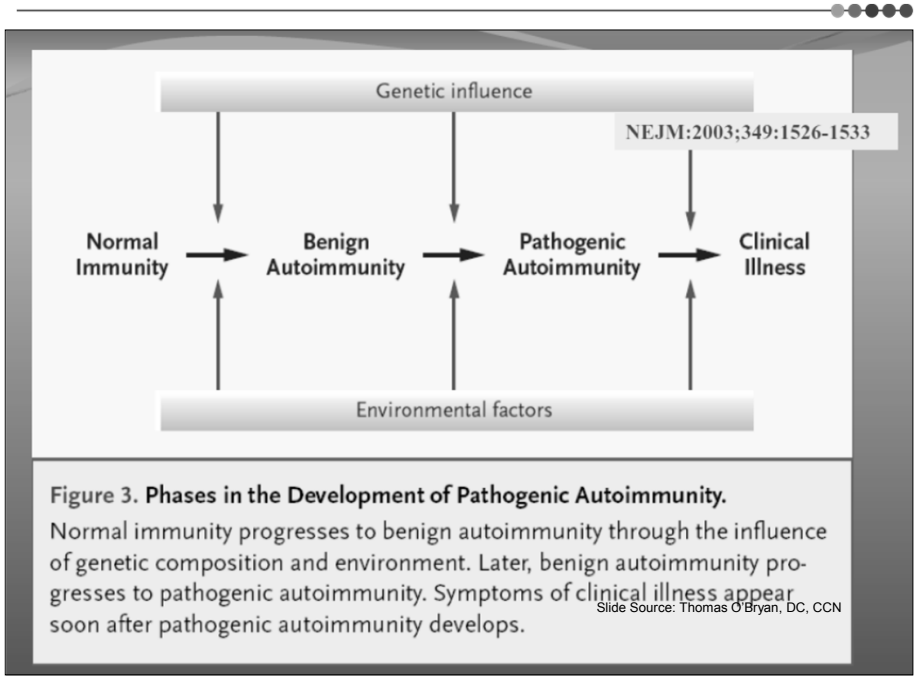


Figure 3. **Phases in the Development of Pathogenic Autoimmunity.**
Normal immunity progresses to benign autoimmunity through the influence of genetic composition and environment. Later, benign autoimmunity progresses to pathogenic autoimmunity. Symptoms of clinical illness appear soon after pathogenic autoimmunity develops.

Slide Source: Thomas O'Bryan, DC, CCN

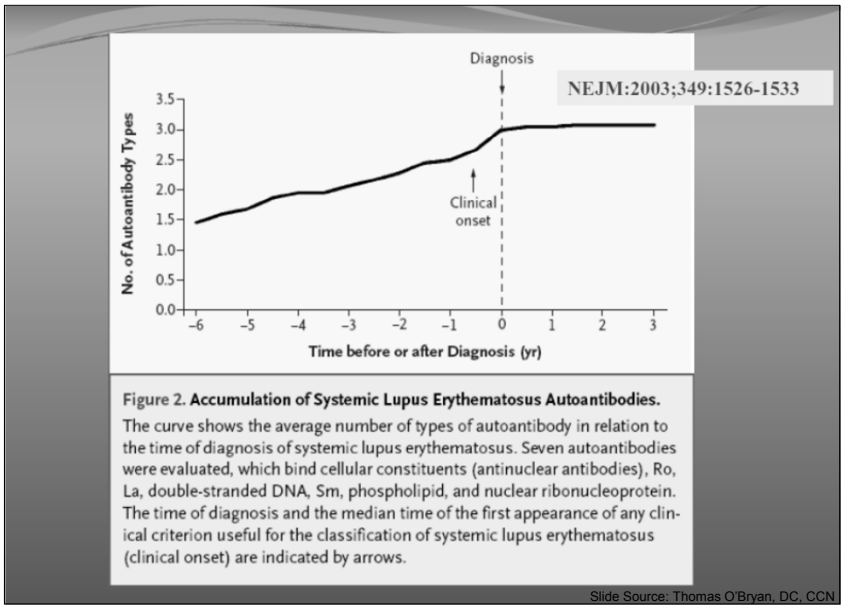
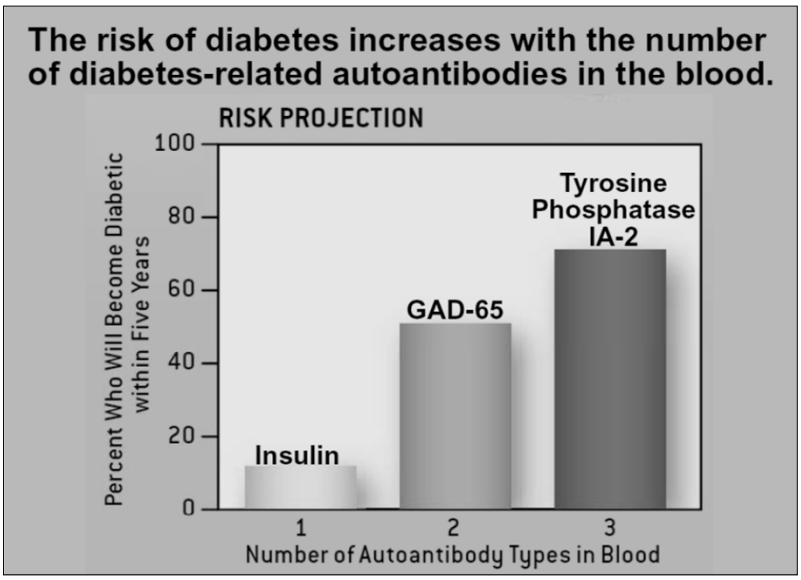


Figure 2. **Accumulation of Systemic Lupus Erythematosus Autoantibodies.**
The curve shows the average number of types of autoantibody in relation to the time of diagnosis of systemic lupus erythematosus. Seven autoantibodies were evaluated, which bind cellular constituents (antinuclear antibodies), Ro, La, double-stranded DNA, Sm, phospholipid, and nuclear ribonucleoprotein. The time of diagnosis and the median time of the first appearance of any clinical criterion useful for the classification of systemic lupus erythematosus (clinical onset) are indicated by arrows.

Slide Source: Thomas O'Bryan, DC, CCN



Slide Source: Aristo Vojdani, PhD

Predictivity of Autoimmunity

Organ specific autoimmune diseases

Disease	Antibodies	PPV	Years before Clinical Dx
Hashimoto's thyroiditis *	Anti-thyroid peroxidase antibodies (postpartum)	92%	7-10
Primary biliary cirrhosis *	Anti-mitochondrial antibodies	95%	25
Type I diabetes**	Pancreatic islet cell, insulin, 65 kD glutamic acid decarboxylase, tyrosine phosphatase-like protein	43, 55, 42, and 29%	14

* Shoenfeld Y, Blank M, Abu-Shakra M, et al. The Mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune disease – 2008. *IMAJ* 2008;10:13-19

** Lindberg B, Ivarsson SA, et al. Islet autoantibodies in cord blood from children who developed Type I (insulin-dependent) diabetes mellitus before 15 years of age *Diabetologia* 1999 42: 181–187

Slide source: Thomas O'Bryan, DC, CCN

Predictivity of Autoimmunity

Organ specific autoimmune diseases

Disease	Antibodies	PPV	Years before Clinical Dx
Addison's disease	Adrenal cortex antibodies	70	10
Crohn's colitis	Anti- *Saccharomyces cerevisae* antibodies	100%	3
Celiac disease	Anti-tissue transglutaminase Anti-endomysial antibodies (HLA-DQ2 or DQ8 antigens)	50-60% (100%)	7

Shoenfeld Y, Blank M, Abu-Shakra M, et al. The Mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune disease – 2008. *IMAJ* 2008;10:13-19

Slide source: Thomas O'Bryan, DC, CCN

Systemic autoimmune diseases

Disease	Antibodies	PPV	Years before Clinical Dx
SLE	RNP, Sm, dsDNA, Ro, La, and cardioliptin antibodies	94-100%	7-10
Scleroderma	Anti-centromere antibodies Anti-topoisomerase I antibodies	100%	11
RA	Rheumatoid factor Anti-cyclic citrullinated peptide	52-88% 97%	14
Sjögren's	Anti-Ro and anti-La antibodies	73%	5

Slide source: Thomas O'Bryan, DC, CCN

POTENTIAL USES OF AUTOANTIBODIES

Autoantibodies could:

- **Predict the risk of falling ill**
- **Project the probability of contracting a particular disease so that the potential patient could consider preventive therapy:**
  - **Primary prevention – Remove environmental factors that trigger the disease**
  - **Secondary prevention – Modulate the destructive process before the onset of clinical symptoms**
- **Anticipate the timing of a disorder, revealing how soon a disease is likely to cause symptoms**
- **Project the course of a disease**
  - **Predict the severity and probable rate of progression of a disease**
- **Classify the disease**
  - **In a patient with an established disease autoantibodies can help define the nature of the markers to classify the disease as autoimmune or not autoimmune**

If inexpensive tests for predictive antibodies can be developed, they could become a standard part of a routine checkup.

Slide source: Aristo Vojdani, PhD

Healthy Subject

	1	2	3	4	5	6	7	8	9	10	11	12
P	Parietal Cell 0.51	ATPase 0.27	H. pylori 0.51	LAMP-2 ADH 0.34	LPS 0.91	Gliadin 0.36	Trans glutaminase 0.37	Synapsin 0.27	Rotavirus 0.28	Thyroid Peroxidase 0.25	TSH-RAP 0.30	Thyroxine Bind-Pre 0.36
P	Parietal Cell 0.49	ATPase 0.28	H. pylori 0.55	LAMP-2 ADH 0.32	LPS 0.88	Gliadin 0.32	Trans glutaminase 0.35	Synapsin 0.24	Rotavirus 0.30	Thyroid Peroxidase 0.28	TSH-RAP 0.33	Thyroxine Bind-Pre 0.32
C	Parietal Cell 0.47	ATPase 0.32	H. pylori 0.34	LAMP-2 ADH 0.21	LPS 0.52	Gliadin 0.33	Trans glutaminase 0.22	Synapsin 0.25	Rotavirus 0.26	Thyroid Peroxidase 0.20	TSH-RAP 0.28	Thyroxine Bind-Pre 0.29
C	Parietal Cell 0.48	ATPase 0.31	H. pylori 0.32	LAMP-2 ADH 0.20	LPS 0.51	Gliadin 0.33	Trans glutaminase 0.20	Synapsin 0.23	Rotavirus 0.29	Thyroid Peroxidase 0.19	TSH-RAP 0.27	Thyroxine Bind-Pre 0.30
P	Intrinsic Factor 0.42	Tropomyosin 0.29	C. difficile 0.35	Occludin 0.29	Wheat 0.45	Gluteomorphin 0.36	Gliadin Transglut 0.28	Cerebellar 0.35	Thyroglobulin 0.29	TSH Receptor 0.36	Thyroxine Bind-Glob 0.25	Thyroid Diiodinase 0.34
P	Intrinsic Factor 0.39	Tropomyosin 0.32	C. difficile 0.33	Occludin 0.31	Wheat 0.41	Gluteomorphin 0.34	Gliadin Transglut 0.31	Cerebellar 0.32	Thyroglobulin 0.34	TSH Receptor 0.31	Thyroxine Bind-Glob 0.21	Thyroid Diiodinase 0.37
C	Intrinsic Factor 0.35	Tropomyosin 0.25	C. difficile 0.30	Occludin 0.36	Wheat 0.40	Gluteomorphin 0.29	Gliadin Transglut 0.24	Cerebellar 0.31	Thyroglobulin 0.22	TSH Receptor 0.32	Thyroxine Bind-Glob 0.33	Thyroid Diiodinase 0.35
C	Intrinsic Factor 0.37	Tropomyosin 0.26	C. difficile 0.33	Occludin 0.35	Wheat 0.43	Gluteomorphin 0.27	Gliadin Transglut 0.26	Cerebellar 0.29	Thyroglobulin 0.21	TSH Receptor 0.28	Thyroxine Bind-Glob 0.36	Thyroid Diiodinase 0.33

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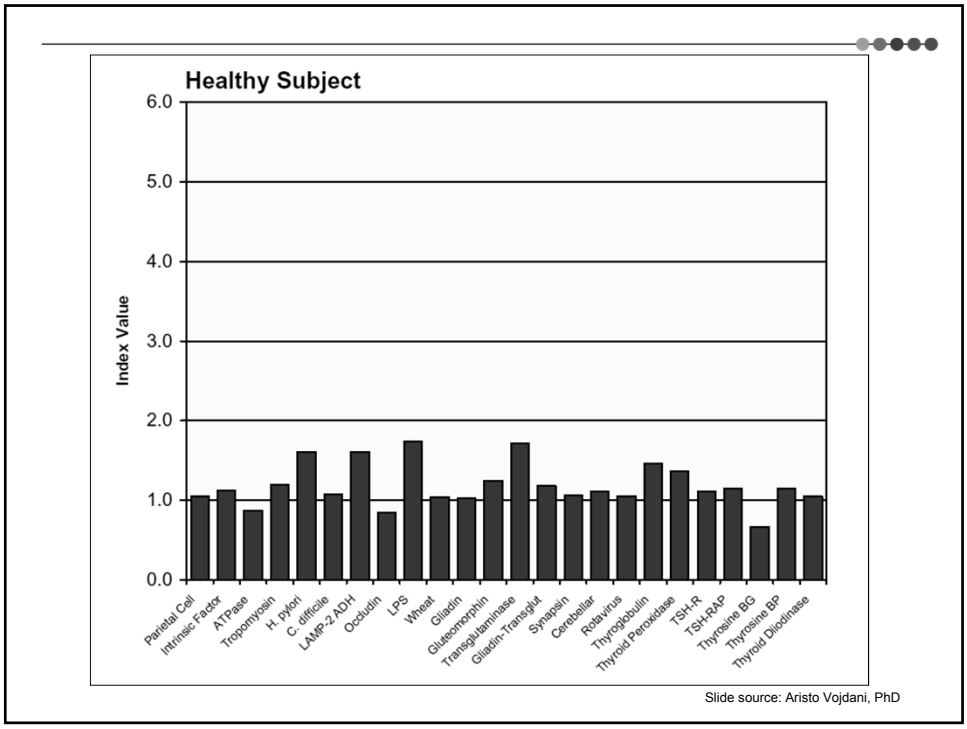
Slide source: Aristo Vojdani, PhD



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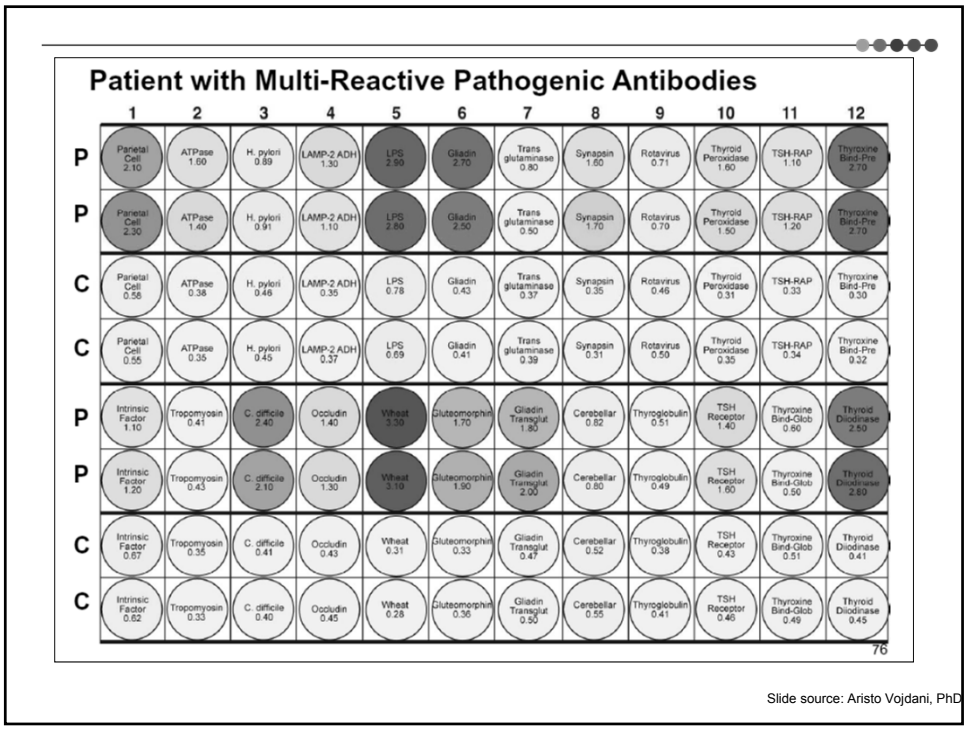

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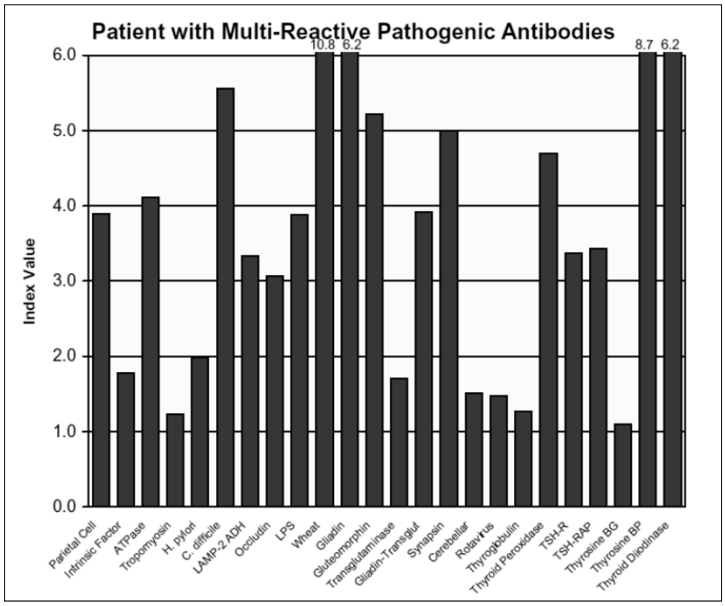

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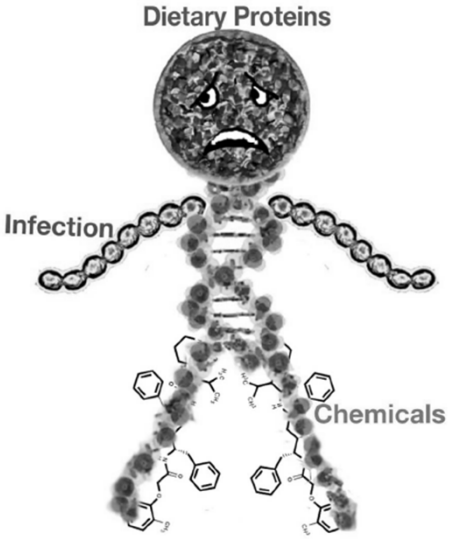
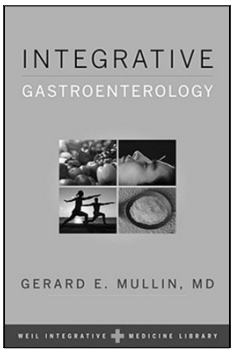
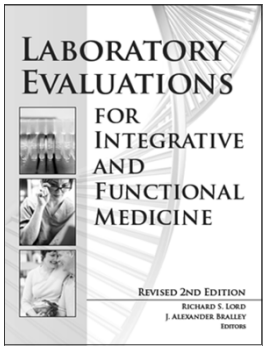
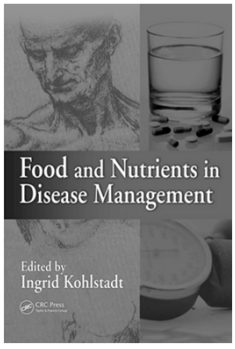


Assessments & Interventions for Autoimmune Disease

- Detect and remove opportunistic and pathogenic GI bugs
- Detect and eliminate food sensitivities
- Predictive autoantibody testing
- Check for toxins & support detoxification
- Vitamin D status optimization
- Quench excess inflammation & oxidative stress
- Nutritional interventions (anti-inflammatory diet, Low AA)
- Gastrointestinal restoration (4R program)
- Stress Reduction







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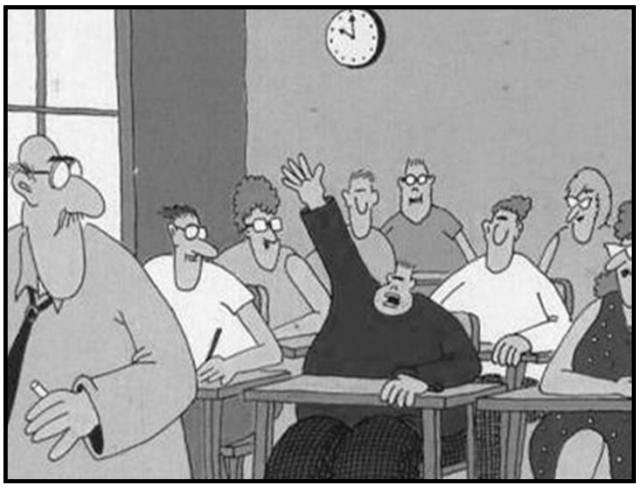

"Dr. Brady, may I be excused? My brain is full."