

Assessment of Intestinal Barrier Permeability to Large Antigenic Molecules

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LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

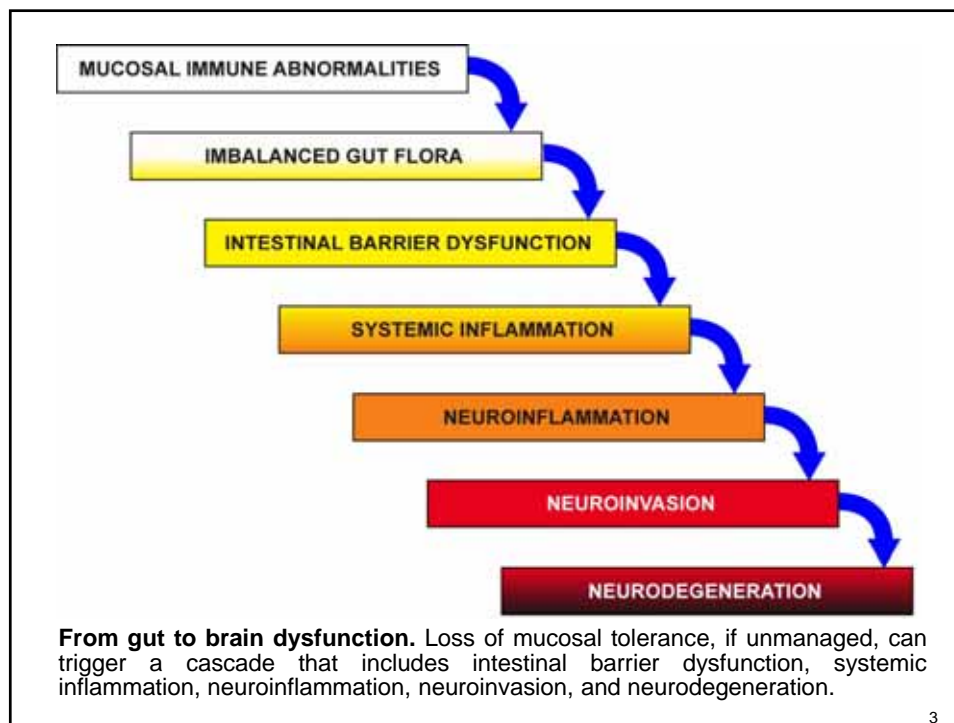
- Explain why testing for intestinal barrier function should be performed with large (antigenic) molecules and not with very small probes such as lactulose manitol test.
- In assessment of intestinal integrity consider all components of intestinal antigens including: dietary proteins, yeast, aerobic bacteria and anaerobic bacteria.
- Understand why measurements of IgG, IgM and IgA against dietary proteins, yeast, aerobic and anaerobic bacteria can assist in detection of food allergy, intestinal imbalance, candidiasis, intestinal barrier dysfunction and mucosal/ humoral immune deficiency.
- Understand that intestinal barrier function can be measured in oral fluid and its abnormality may indicate the loss of mucosal regulatory mechanisms.

LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

- Be able to analyse IBF test results and classify patients according to the detected abnormalities.
- Learn more factors affecting mucosal immune system, intestinal barrier function, autoimmunity, and nervous system abnormalities.
- Learn the role different enzymes, in particular dipeptidyl peptidase (DPP IV) play in the GI tract as well as in the immune system.
- Learn why binding environmental factors to DPP IV can result in autoimmunity.
- Learn more about the importance of regulatory T cells, regulatory cytokines in TH-17 cell in protection against infection.
- Understand that when barriers are broken by different factors, the result can be food intolerance, immune disorders and autoimmunity.

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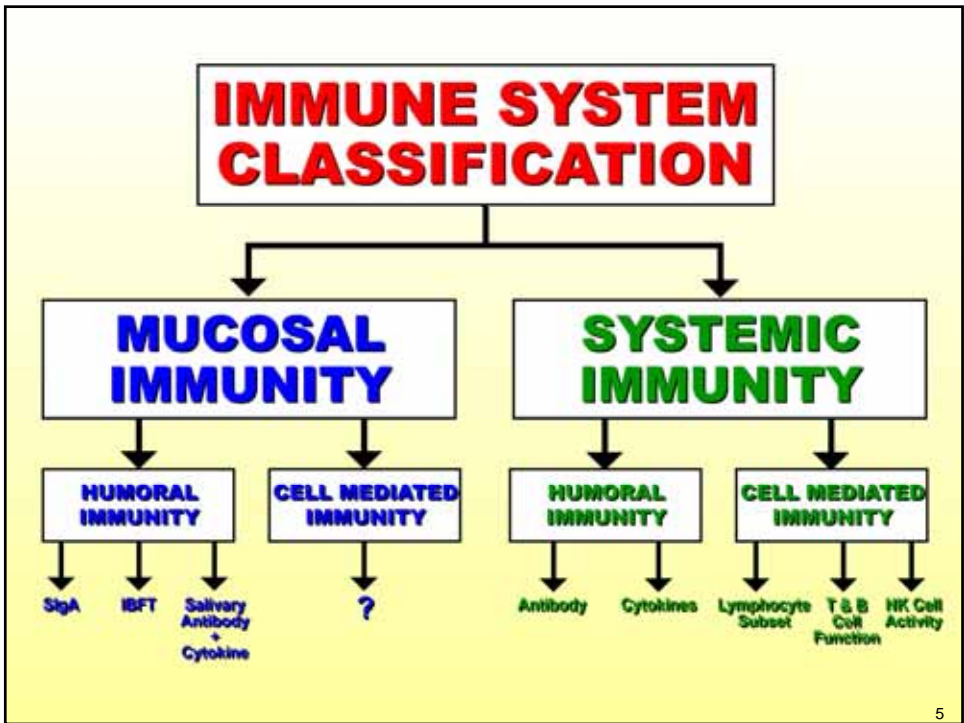
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The road to health and happiness begins, unexpectedly, in the intestinal tract, where 70% of one's immune system resides. Unfortunately, this road is also home to the bad bacteria that multiply with life's stresses; which not only compromises one's immune system, it can cause gas, bloating, and really uncomfortable blue jeans.



She who possesses an enlightened digestive system will be blessed with stronger immunity and happiness.

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Four major barriers in the human body

- Skin barrier
- Lung barrier
- Intestinal barrier
- Blood-Brain Barrier

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

Fasano, A., and Shea-Donohue, T., *Nature Clin Practice,
Gastroenterol & Hepatol*, 2005, 2(9):416-422

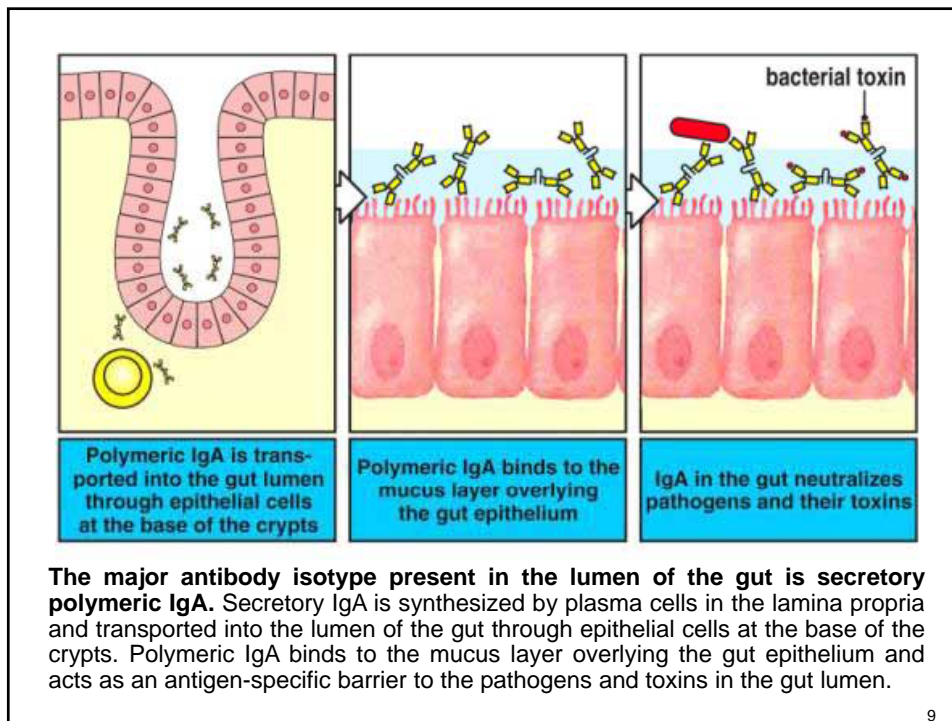
CONCLUSIONS: The classical paradigm of autoimmune pathogenesis involving a specific genetic makeup and exposure to environmental triggers has been challenged by the addition of a third element: the loss of intestinal barrier function. This new theory implies that, once the autoimmune process is activated,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

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The Importance of IgA in Mucosal Immunity

- ◆ Two different mechanisms work against ingested antigens, which manage to escape immune exclusion. These ingested antigens, if not controlled, may induce:
 1. active secretory immune response
 2. oral tolerance to soluble antigens
- ◆ IgA plays a major role by disposing of microbial and dietary antigens locally and preventing them from entering the blood.
- ◆ IgA, as an anti-inflammatory immunoglobulin, blocks complement-mediated immune effector mechanisms and functions.
- ◆ Mucosal IgA neutralizes viruses.
- ◆ IgA blocks the attachment of pathogens to mucosal tissue and cells.
- ◆ **A clinical counterpart of immune exclusion is the IgA deficient individuals who have high levels of serum antibodies to food antigens, particularly bovine milk proteins. These individuals experience chronic hyperabsorption of macromolecules and have a tendency to develop autoantibodies and even autoimmune disease.**

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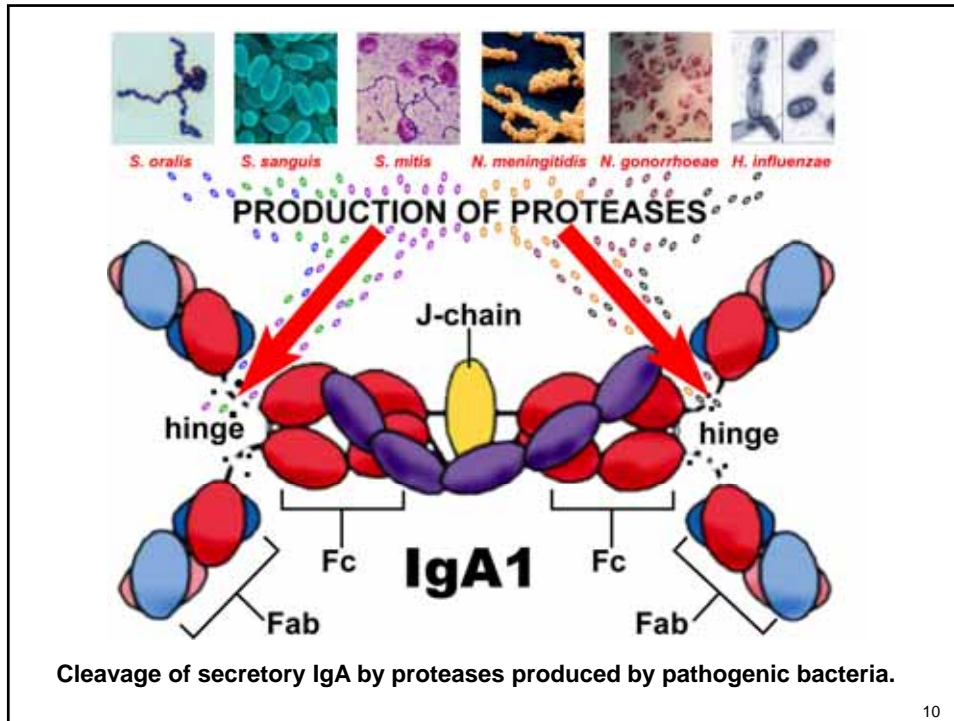


Table 1 – Conditions that can change the level of secretory IgA in oral fluid	
Increased secretory IgA level	Decreased secretory IgA level
• Acute stress	• Chronic stress
• Chronic oral infection	• Recurrent tonsillitis
• Heavy smoking	• Adenoid hyperplasia
• Periodontitis	• Bacterial colonization on molar surfaces
• Oropharyngeal carcinoma	• Asthmatic with recurrent respiratory tract infection
• Chronic GI infection	• Cutaneous candidiasis
• Some medications	• Some medications
• Alcoholism	• Adrenal insufficiencies

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- Aristo Vojdani, Ph.D., M.T.**

Table 2– Specific secretory IgA antibodies found in oral fluid		
Bacteria	Viruses	Fungi
<i>Streptococcus mutans</i>	Respiratory syncytial virus	<i>Candida albicans</i>
<i>Porphyromonas gingivalis</i>	Influenza A	<i>Saccharomyces</i>
<i>Actinobacillus</i>	Parainfluenza	Protozoa
Group A <i>Streptococcus</i>	Rotavirus	<i>Giardia</i>
<i>Bordetella pertussis</i>	Coxsackie virus	<i>Entamoeba histolytica</i>
<i>E. Coli O, K antigens, enterotoxin</i>	Cytomegalovirus	Dietary Proteins
<i>Shigella</i>	Arboviruses – Semliki forest	Milk proteins
<i>Salmonella</i>	Ross river, Japanese B	Soy lectins and proteins
<i>Campylobacter</i>	Dengue	Wheat gluten, gliadin
<i>Bacteroides fragilis</i>	HIV	Peanut lectins and proteins
<i>Clostridium difficile</i>	Rhinovirus	Corn proteins
<i>Klebsiella pneumoniae</i>	Poliovirus 1,2,3	Egg
<i>Pseudomonas aeruginosa</i>	Echovirus	Others

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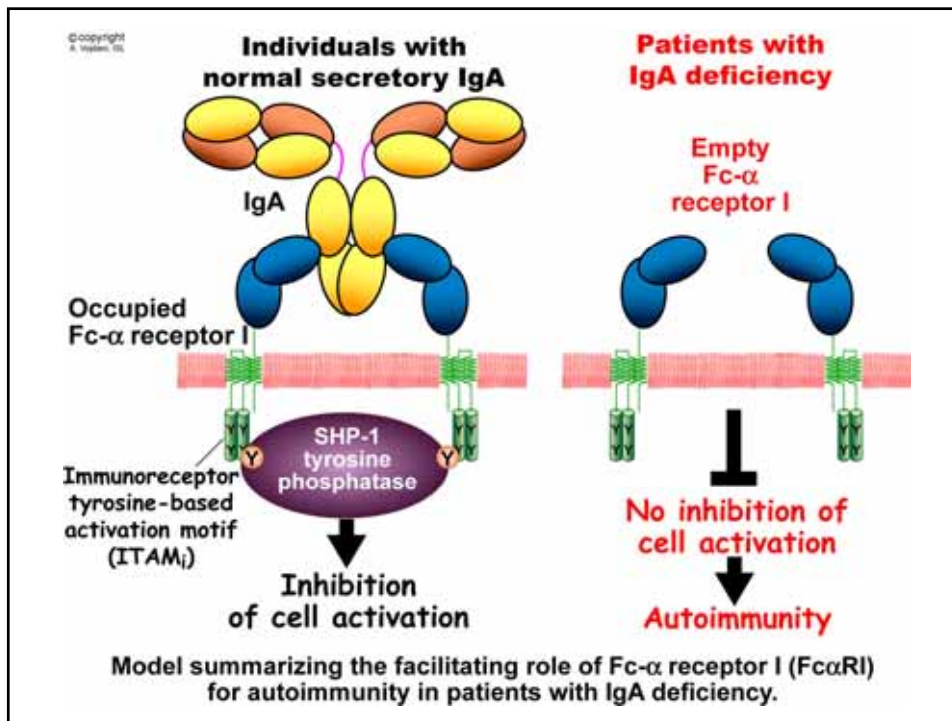
Table 3– Diseases associated with low secretory IgA	
Conditions	Disease
●Allergy	●Asthma, atopy, eczema
●Autoimmunity	●Rheumatoid arthritis ITP, hemolytic anemia, pernicious anemia, systemic lupus erythematosus, Still's disease, transfusion reactions due to anti-IgA antibody, dermatomyositis, vitiligo, Sjogren's syndrome, Henoch-Schonlein syndrome, primary biliary cirrhosis, autoimmune hepatitis
●Respiratory tract	●Recurrent sinopulmonary infections, sarcoidosis, pulmonary hemosiderosis
●Gastrointestinal diseases	●Giardiasis, Crohn's disease, ulcerative colitis, nodular lymphoid hyperplasia, celiac disease, lactose intolerance, malabsorption villous atrophy, achlorhydria, cholelithiasis
●Neurological	●Seizures, migraine, sensory neuropathy, myasthenia gravis, cerebral vasculitis
●Familial history of hypogammaglobulinemia	●Common variable immunodeficiency
●Endocrinopathy	●Thyroiditis. Graves disease, idiopathic Addison's disease, diabetes mellitus, 21-hydroxylase deficiency
●Chromosomal abnormalities	●Chromosome 14
●Malignancy	●Gastric carcinoma and lymphoma

Autoimmunity in IgA Deficiency: Revisiting the Role of IgA as a Silent Housekeeper

Jacob CMA, Pastorino AC, Fahl K *et al.*
J Clin Immunol (2008) 28 (Suppl 1):S56–S61

Abstract: Both systemic and organ-specific autoimmune diseases are major manifestations of IgA deficiency (IgAD), the most common primary immunodeficiency. In addition, to discuss the clinical findings of IgAD patients, we proposed a hypothesis to explain the high association with autoimmune phenomena.

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Measurement of Secretory IgA in Saliva
Secretory IgA is an important factor in mucosal immunity and in intraluminal microbial defense system.


	<u>INDICATION</u>
■ Normal Level	1. Good Mucosal Immunity
■ Low Level	1. Mucosal Immune Deficiency 2. Serum Antibody to Food Antigens (Food Allergy) 3. Autoimmune Disease
■ High Level	1. Bacterial Overgrowth 2. Enterotoxins 3. Viral Infection 4. Serum Antibody to Food Antigens (Food Allergy) 5. Autoimmune Disease

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SECRETORY IgA IN ORAL FLUID

Total Secretory IgA



Specimen Requirements:
4 mL of saliva collected over a 24-hr period

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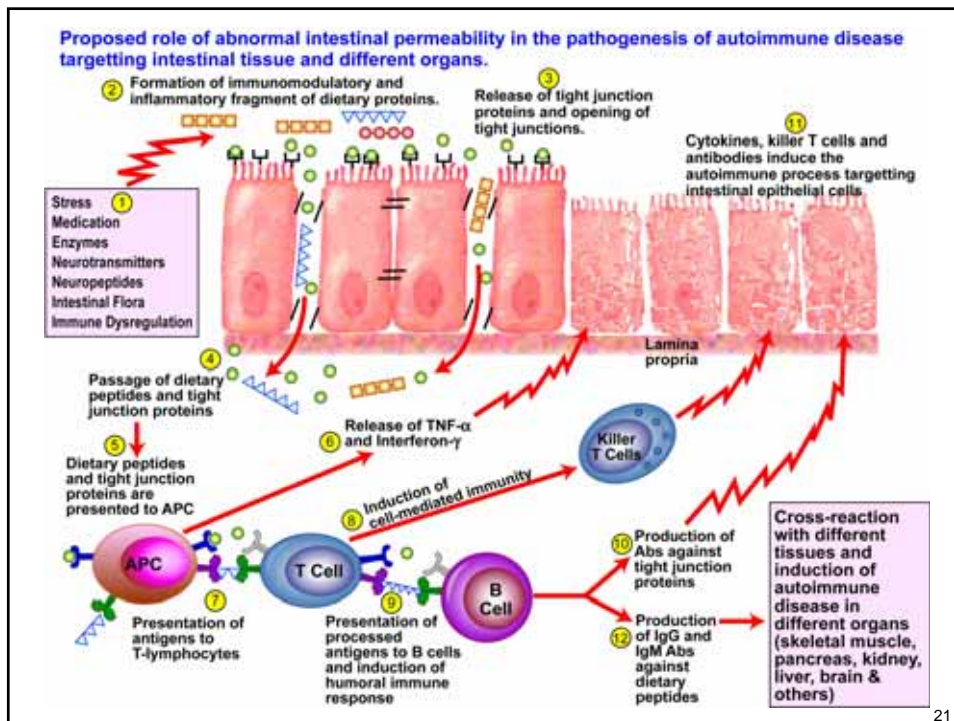
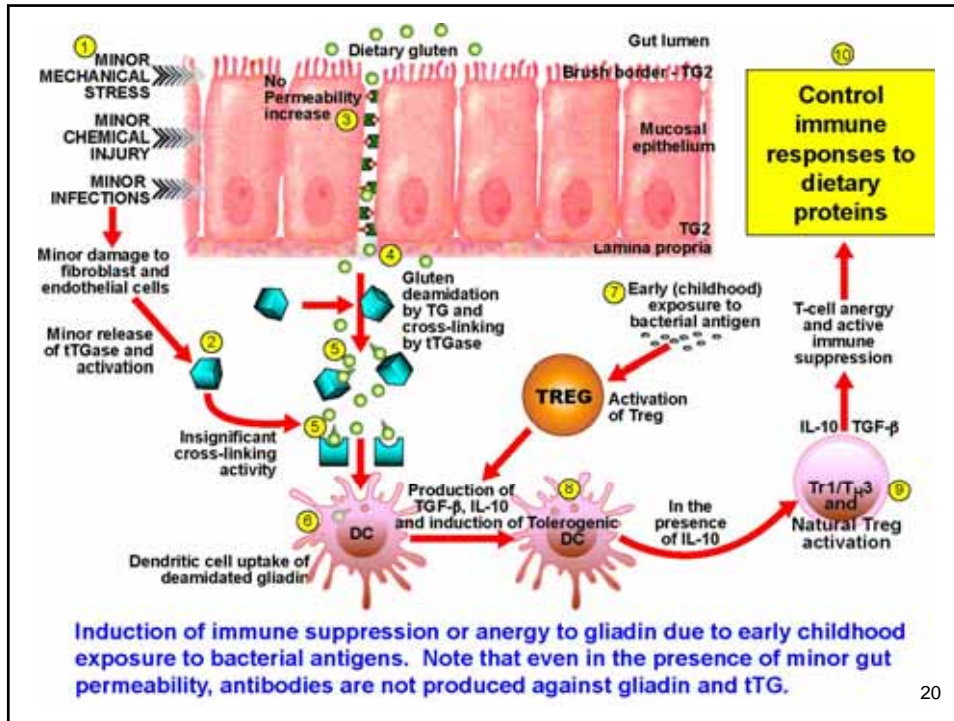
MOLECULAR ANALYSIS OF HOST-MICROBIAL RELATIONSHIPS IN THE INTESTINE

- ◆ Human beings harbor an incredibly complex and abundant ensemble of microbes.
- ◆ These resident bacteria shape our physiology in many ways.
- ◆ Germ-free mice were colonized with bacteroides and intestinal transcriptional responses were measured using DNA microarrays.
- ◆ Colonized bacteria modulated expression of genes involved in important intestinal function including:
 - 1 Nutrient Absorption
 - 2 Lipid Absorption Capacity
 - 3 Mucosal Barrier Fortification
 - 4 Xenobiotic Metabolism
 - 5 Angiogenesis
 - 6 Postnatal Intestinal Maturation

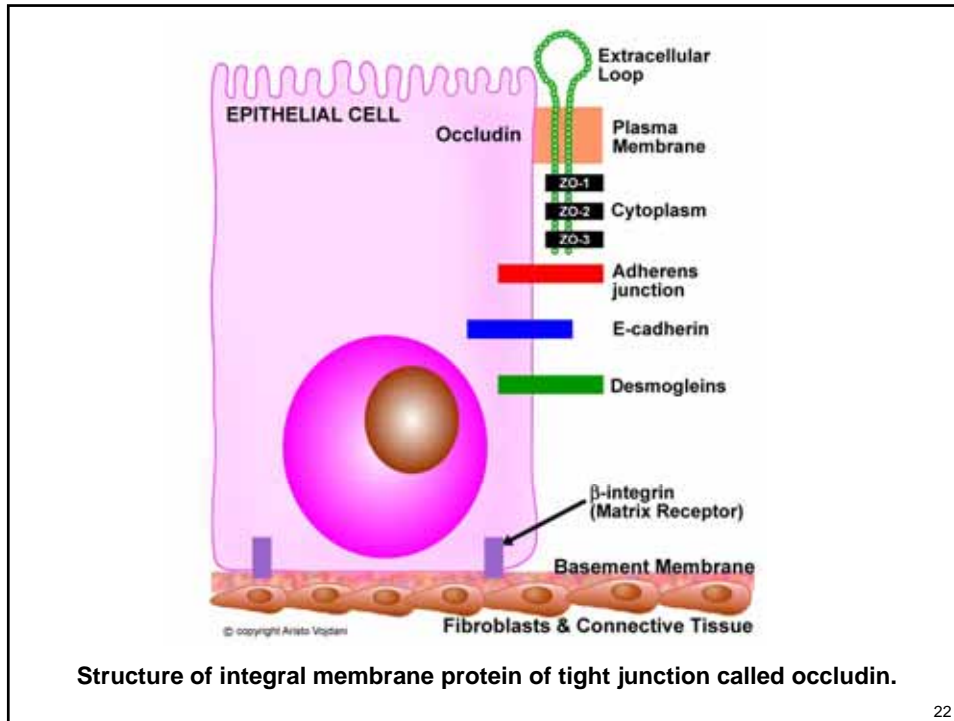
Hooper, L.V., et al. *Science* 291: 881-84, 2 Feb. 2001.

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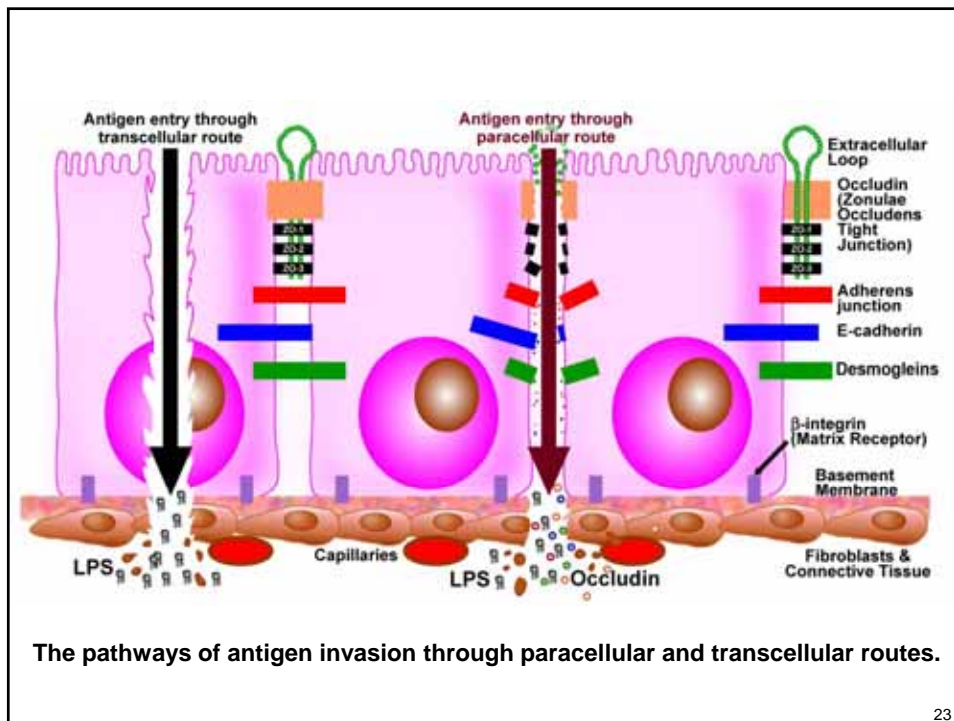
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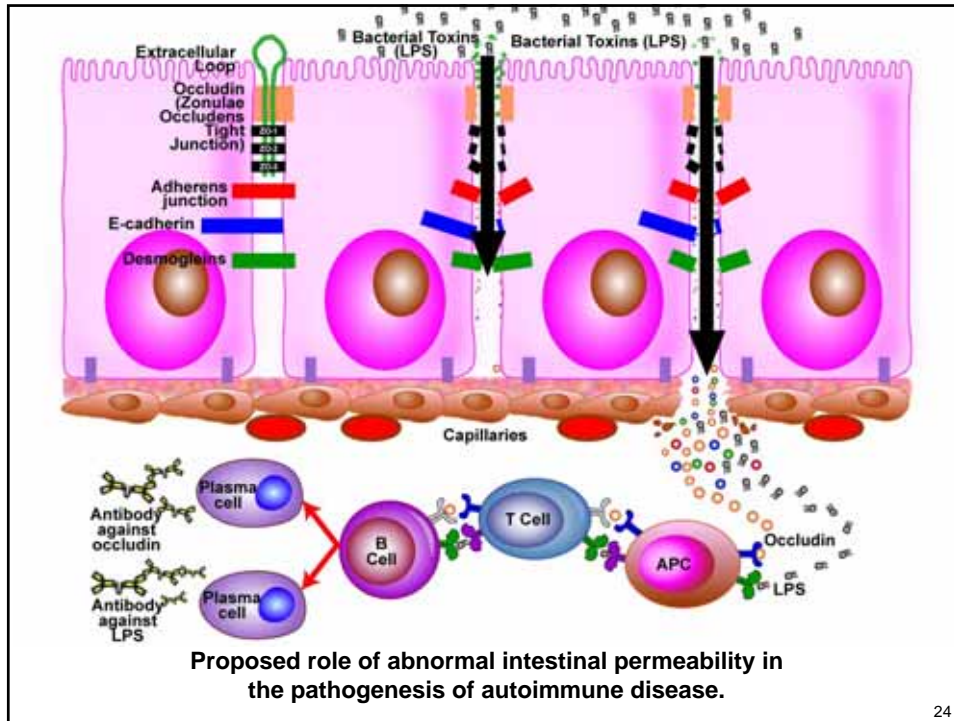


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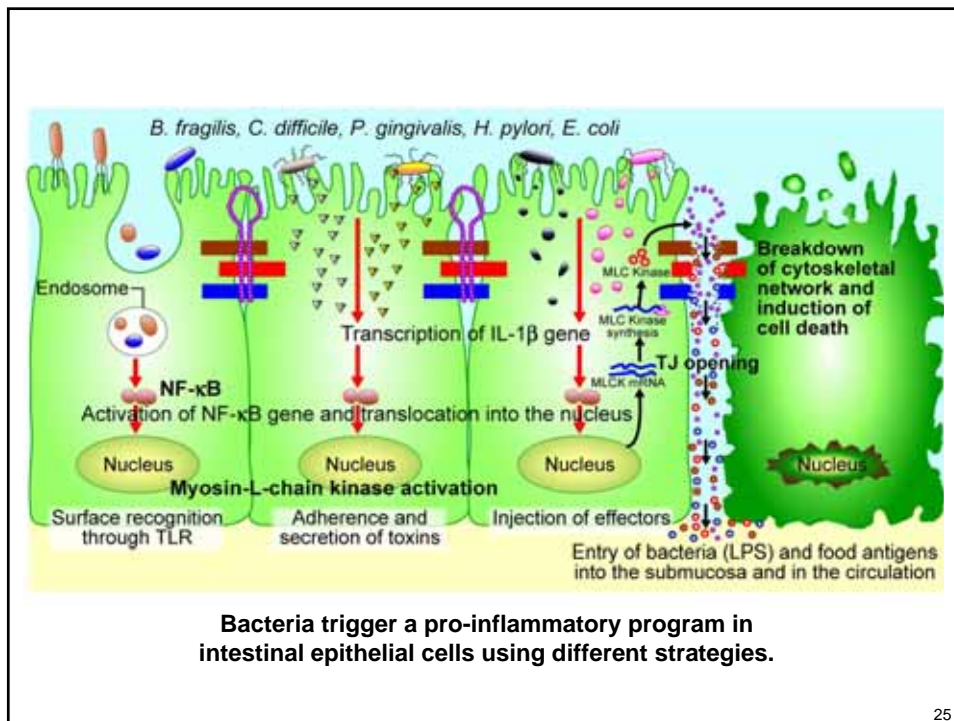


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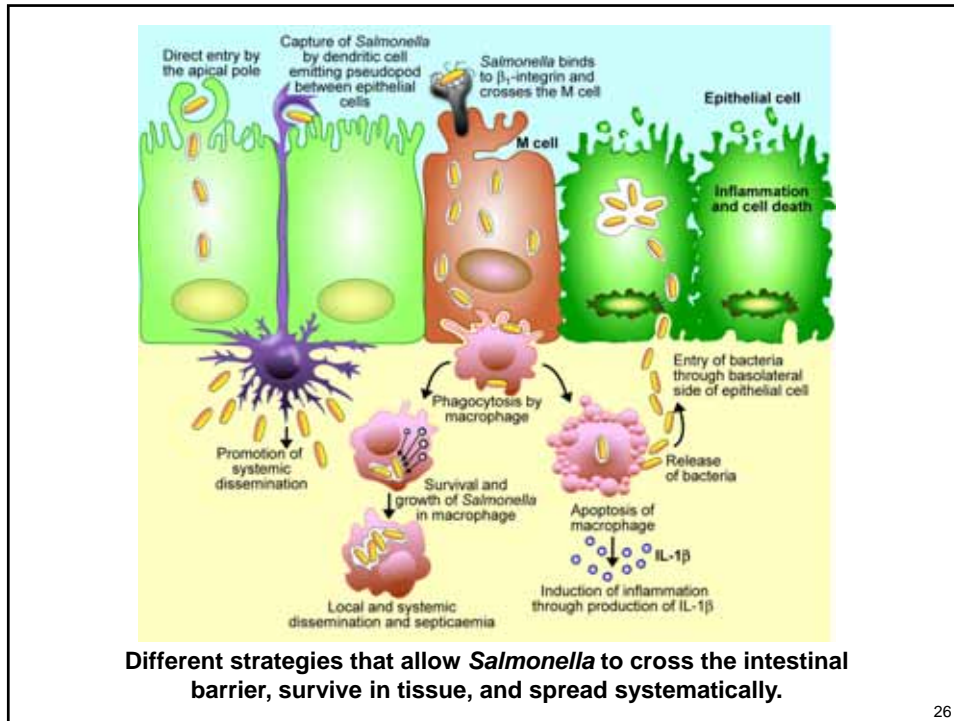
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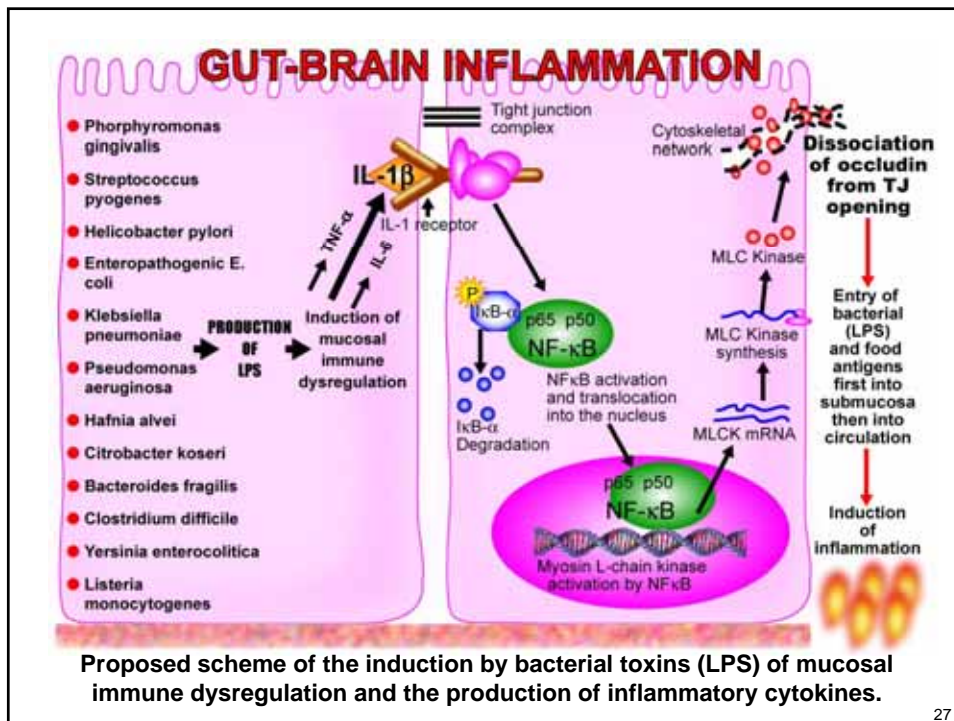
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Evaluation of the gut mucosal barrier: Evidence for increased antigen transfer in children with atopic eczema

Heli Majamaa, MD, and Erika Isolauri, MD Tampere, Finland

Background: Intestinal antigen handling determines subsequent immune response to the antigen. Antigens are absorbed across epithelium along two functional pathways. The main pathway is degradative, which reduces the immunogenicity of the antigen. A minor pathway allows the transport of intact proteins, which is crucial for antigen-specific immune responses. The Ussing chamber method allows the quantitative measurement of protein transfer across the intestinal mucosa.

Objective: This study was designed to explore the theory that altered antigen transfer across the intestinal mucosa is a factor in the pathogenesis of atopic eczema, characterized by hyperreactivity to environmental antigens.

Methods: The absorption and degradation of horseradish peroxidase (molecular weight, 40,000 d) were studied in vitro in Ussing chambers. Eighteen biopsy specimens of upper small intestinal mucosa from 14 patients (aged 0.5 to 8 years) with atopic eczema and 18 specimens from 15 age-matched control subjects were examined.

Results: The mean (95% confidence interval) absorption of intact horseradish peroxidase was significantly higher in children with atopic eczema than in control subjects: 242 (81-404) $\text{pmol} \cdot \text{hr}^{-1} \cdot \text{cm}^{-2}$ versus 23 (12-33) $\text{pmol} \cdot \text{hr}^{-1} \cdot \text{cm}^{-2}$; $t = 2.86$, $p = 0.007$. The

Conclusions: Our results may reflect a primarily altered antigen transfer in patients who have atopic eczema, which may initiate and perpetuate prompt immune responses to common environmental antigens, including foods."

environmental antigens, including foods. (J ALLERGY CLIN IMMUNOL 1996;97:985-90.)

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INCREASED FOOD ANTIGENS TRANSFER IN ATOPIC ECZEMA

- ◆ Dietary antigens are macromolecules with molecular weight in the range of 10,000 – 70,000 dalton.
- ◆ These antigens are absorbed across the epithelial layer by transcytosis along two functional pathways.
- ◆ The main-degradative pathway entails lysosomal processing of the protein to smaller peptide fragments, and is important in host defense to diminish the antigen load.
- ◆ A minor pathway allows transport of intact proteins, which results in antigen-specific immune responses.
- ◆ Immature absorptive functions increase antigen exposure which results in priming for immune responses.
- ◆ In food allergy, disturbances in intestinal permeability and antigen transfer occur when an allergen comes into contact with intestinal mucosa.

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INCREASED FOOD ANTIGENS TRANSFER IN ATOPIC ECZEMA

- ◆ In active cow's milk allergy, the absorption of both intact and degraded horse radish peroxidase (a secondary antigen) is increased in untreated cases.
- ◆ After complete avoidance of cow's milk, horse radish peroxidase transport returns to normal.
- ◆ It is important to measure the intestinal permeability to a high molecular weight probe such as HRP than low molecular probe such as lactulose manitol test.
- ◆ This recommendation is based on findings that a low molecular weight probe suffers from a high degree of false positivity.

Majamaa, et al., *Clin. Immunol.* **97**: 985, 1996.

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Escherichia coli Antibody: A Screening Test for Immunodeficiency

Webster A.D.B. et al., *British Medical Journal*, 1976, 3:16-18

“Summary

Six patients suffering from recurrent chest infections were found to lack antibodies to a pooled antigen obtained from six different serotypes of commensal *Escherichia coli* bacteria. All had normal serum IgG concentrations, but five subsequently benefited from regular gammaglobulin injections. **We suggest that the absence of such *E. coli* antibodies usually indicates a clinically significant defect in antibody production. This simple screening test is of use in the diagnosis of primary and secondary immunodeficiency disorders.**”

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MEASUREMENT OF ANTIBODIES TO *CANDIDA ALBICANS* AS A SCREENING TEST FOR HUMORAL IMMUNODEFICIENCY

Phillips and Matthew: *J. Immunological Methods*. 105:127, 1987.

In screening for defects in humoral immunity, as well as measuring serum immunoglobulins, it is advisable to have some measure of antibody production, e.g., immunizing with a test antigen and measuring the antibody response some days later. However, this delay may be inconvenient and requires a second blood sample. An alternative is to test for antibody to a widespread commensal organism to which the patient must have been repeatedly exposed. The measurement of antibody to *E. coli* by haemagglutination has been previously used for this purpose.

We report here that an equally good and simpler alternative is to measure antibodies to the commensal *Candida albicans* by immunofluorescence. Using a polyvalent conjugate, all 114 blood donors tested had antibody titres > 8 to *C. albicans*; similar responses were noted in 20 children (aged 6 months – 16 years) without recurrent infections. In contrast, anti-candida responses were low or absent as expected in patients with hypogammaglobulinaemia but also in patients with other immunodeficiency diseases.

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Testing for Intestinal Barrier Function

- Lactulose
- Manitol
- Polysugars
- Large Antigens

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Human α -lactalbumin as a marker of macromolecular absorption

Jakobsson *et al.* GUT, 1986, Vol 27: pages 1029-1034

- The molecules used in most permeability studies are not proteins but – for example, lactulose and polyethylene glycoles with relatively low molecular weights. The transfer of these substances through the gut membranes does not reflect the situation for transfer of food protein.
- **Therefore intestinal barrier should be assessed using large molecules, which are antigenic.**

Intestinal Barrier Function Test Utilizes All Components of the Intestinal Flora Including

- Dietary proteins (milk, wheat, soy, egg, corn).
- Yeast (*Candida albicans*, *C. tropicalis* and *C. krusei*).
- Aerobic bacteria (*E. coli*, Lactobacillus, Enterococcus)
- Anaerobic bacteria (*Bacteroides fragilis*, *Clostridium perfrengens*).

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Determination of IgG, IgM and IgA Against Dietary Proteins, Yeast, Aerobic and Anaerobic Bacteria May Allow Detection of the Following Clinical Conditions:

- **Food Allergy**
- **Intestinal Imbalance**
- **Candidiasis**
- **Gut Barrier Dysfunction**
- **Bacterial Translocation**
- **Autoimmunity**
- **Immunodeficiencies**

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The Intestinal Barrier
Function Test in Oral
Fluid is covered by
U.S. Patent
#6,689,569
awarded to
A. Vojdani
and
Immunosciences Lab.

**INTESTINAL BARRIER FUNCTION
SALIVA**

Intestinal Barrier Function Test, Saliva

- Combined Dietary Proteins Antibodies
Wheat, Corn, Soy, Milk, Egg IgA
- Yeast Antibodies IgA
- Aerobic Bacteria Antibodies IgA
- Anaerobic Bacteria Antibodies IgA
- Secretory IgA

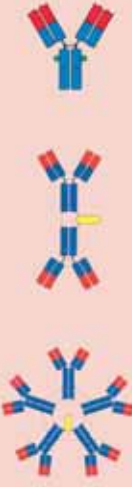
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Saliva test for detection of food allergy, candidiasis, microflora imbalance, intestinal barrier dysfunction and humoral immunodeficiencies

Awarded 2/10/04

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INTESTINAL BARRIER FUNCTION TEST IN SERUM

Intestinal Barrier Function Test, Serum

- Combined Dietary Proteins Antibodies
Wheat, Corn, Soy, Milk, Egg (IgG, IgM, IgA)
- Yeast Antibodies (IgG, IgM, IgA)
- Aerobic Bacteria Antibodies (IgG, IgM, IgA)
- Anaerobic Bacteria Antibodies (IgG, IgM, IgA)

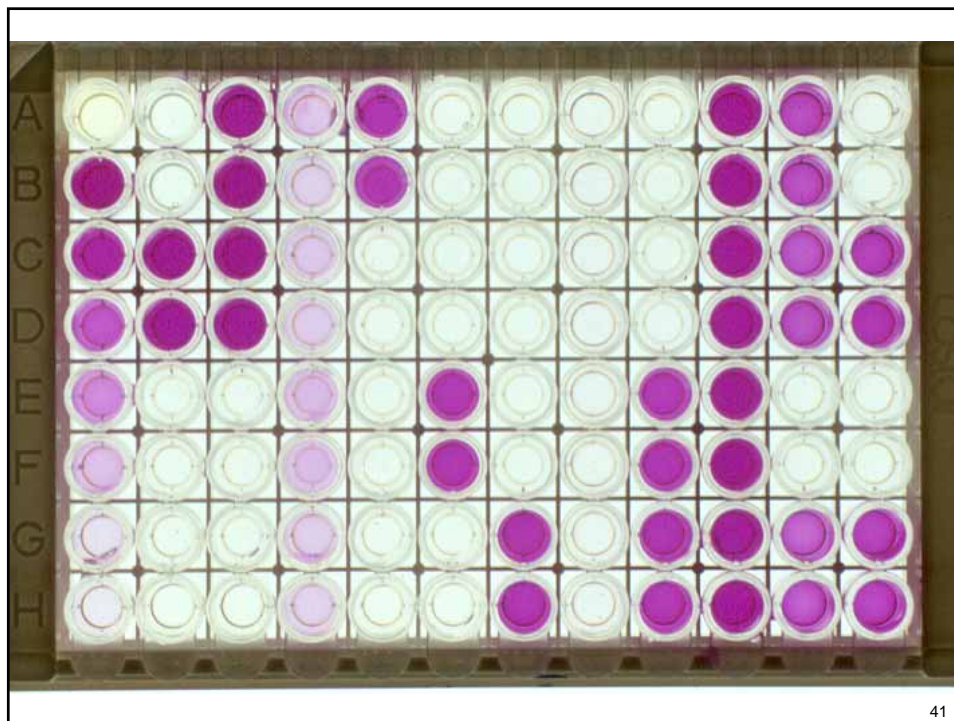
Specimen Requirements:
Collect 5 mL red top or tiger top



The Intestinal Barrier Function Test in Serum is covered by U.S. Patent #6,103,400 awarded to A. Vojdani and Immunoscience Lab.

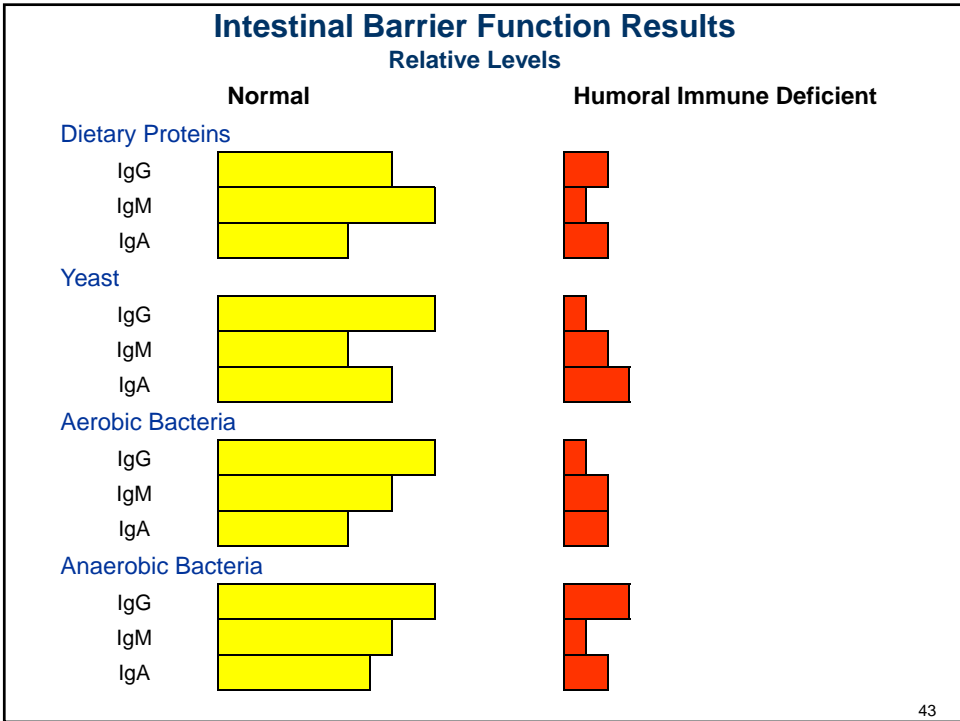
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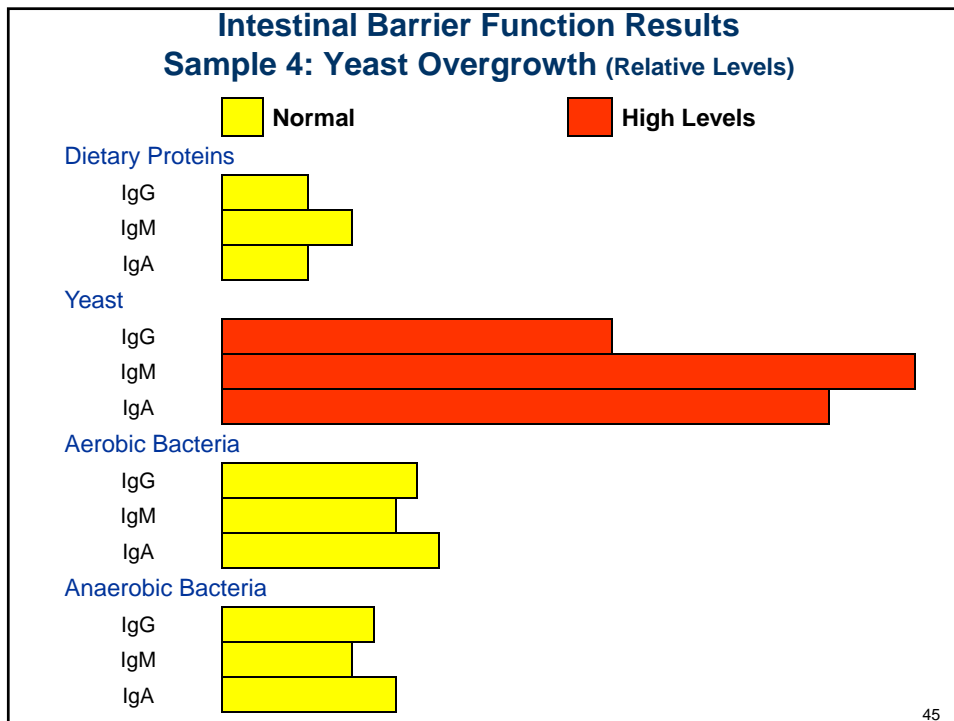
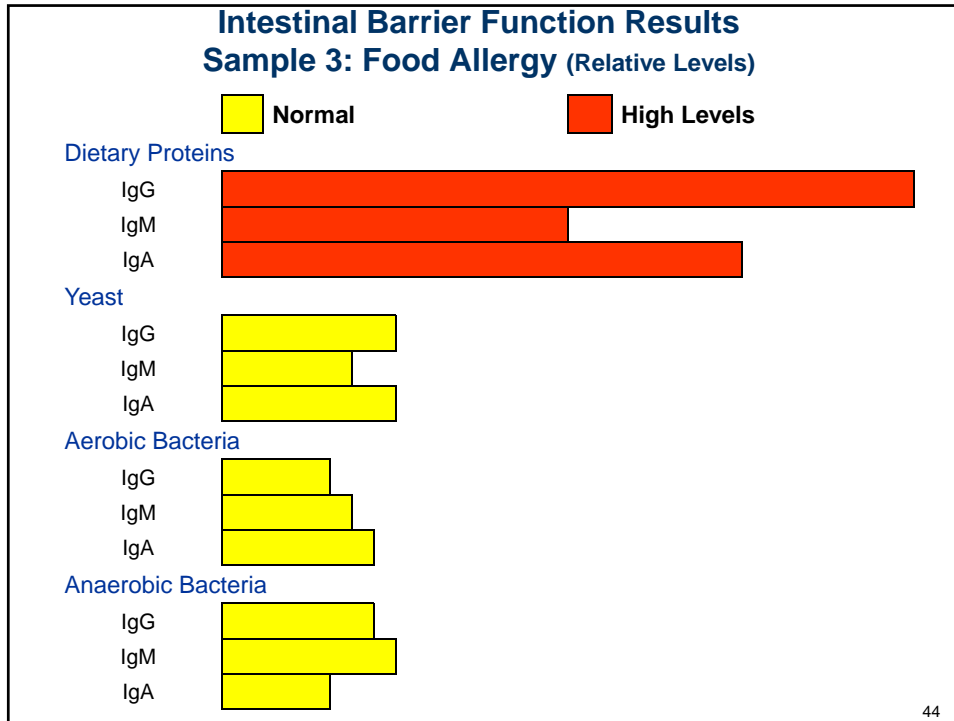


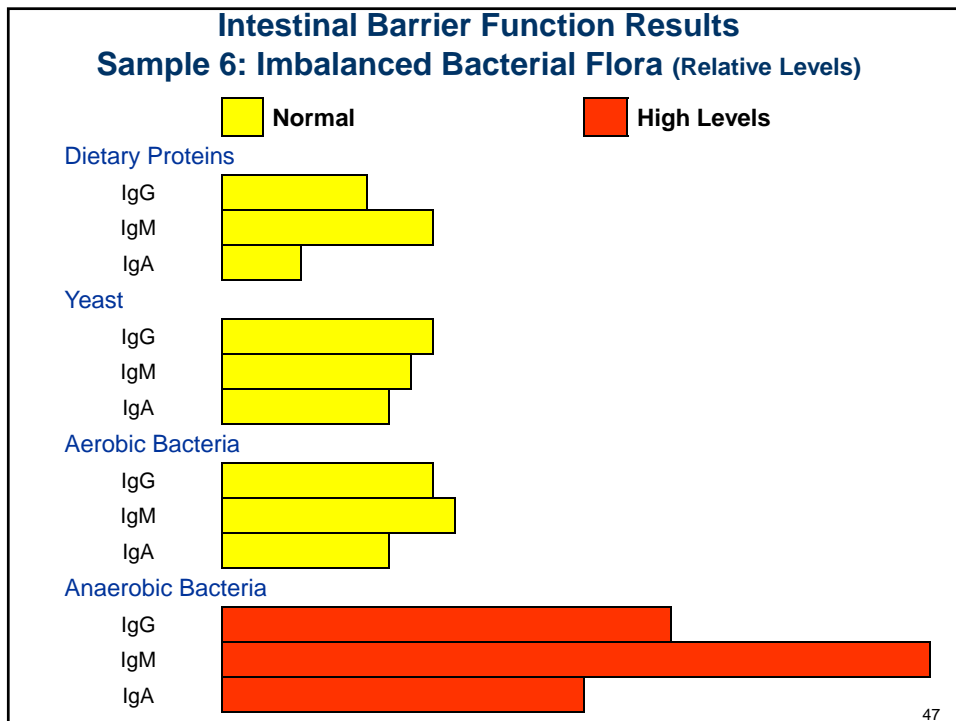
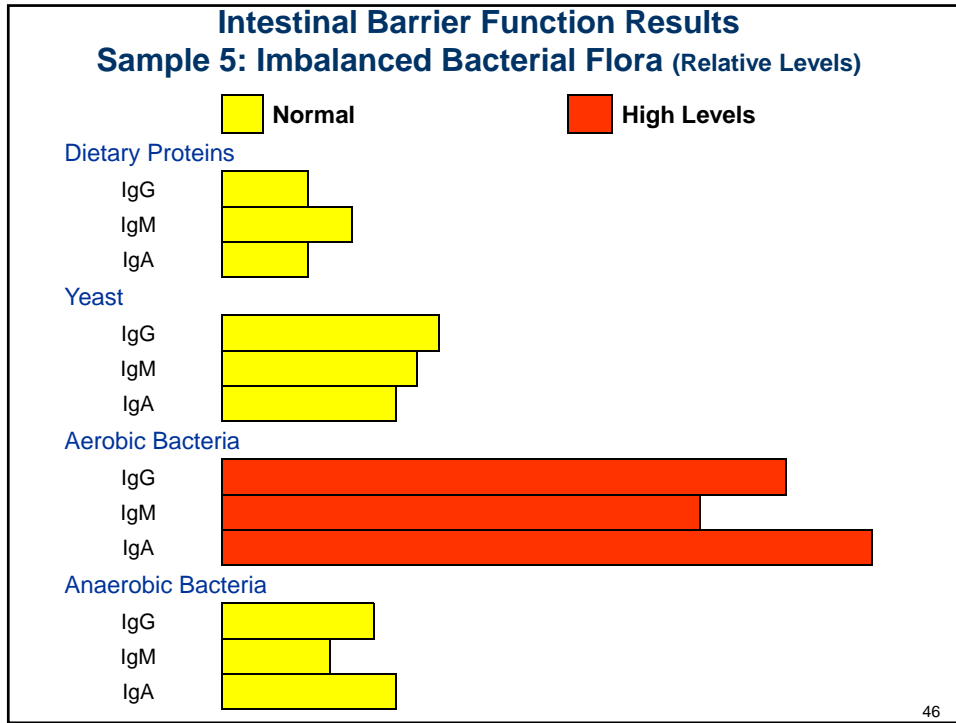
*LABORATORY TEST
RESULTS OF
CLINICAL
SPECIMENS WITH
DIFFERENT
GASTROINTESTINAL
DISORDERS*

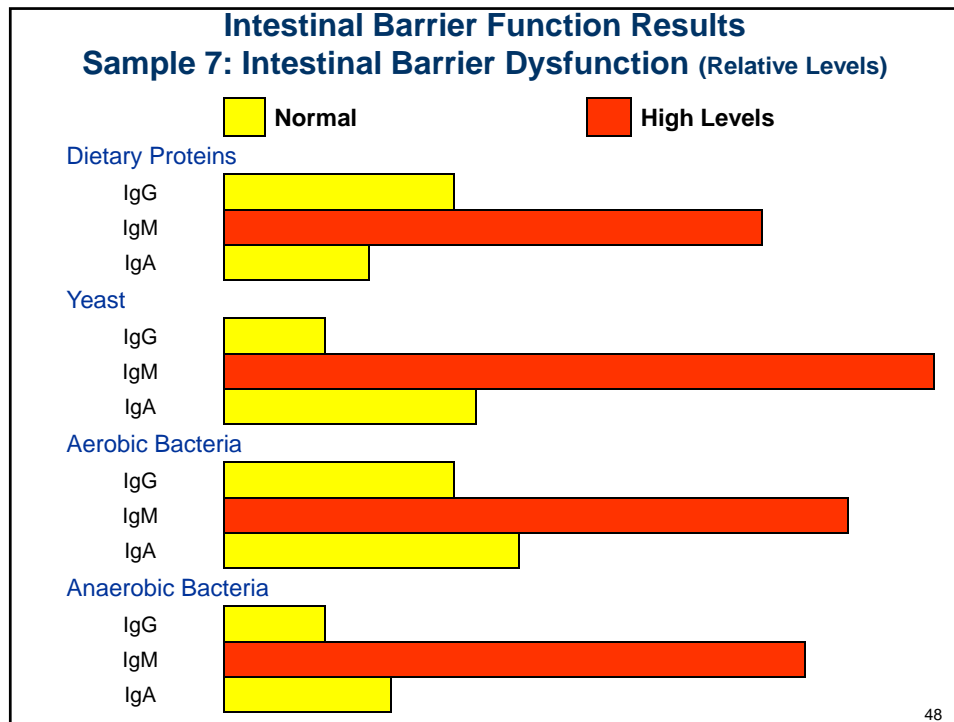
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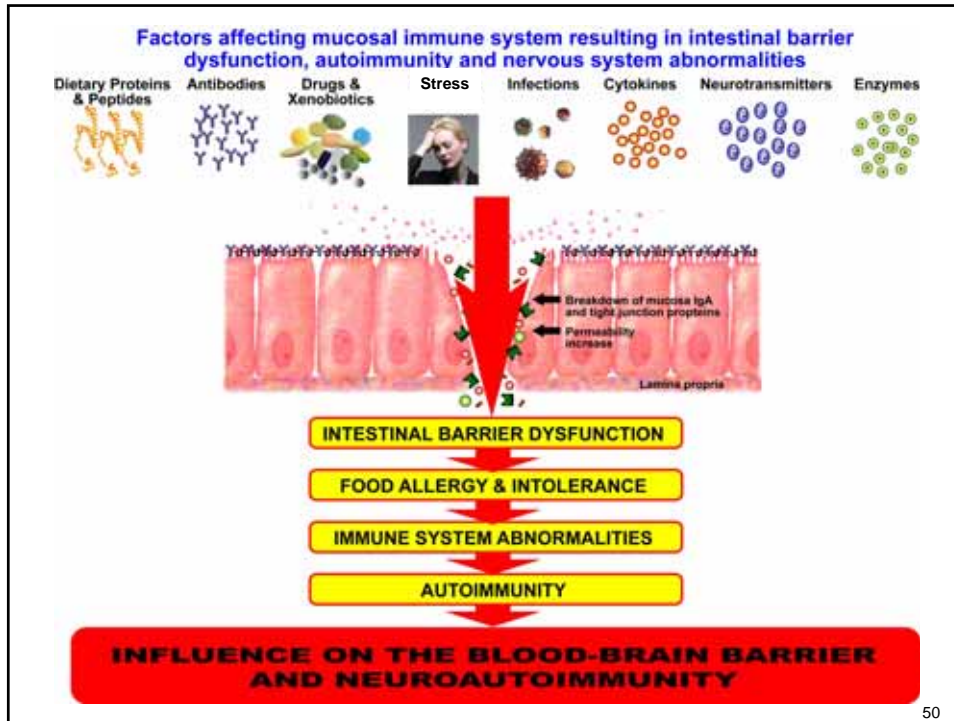


INTESTINAL BARRIER FUNCTION TEST IS RECOMMENDED FOR PATIENTS WHO:

- ◆ Have candidiasis, which appears to be resistant to standard therapy.
- ◆ Are suspected of suffering from disturbances of intestinal permeability and absorption.
- ◆ Complain of food intolerance (including “food allergy”).
- ◆ Complain of chemical hypersensitivity.
- ◆ Present multiple symptom complaints (including Chronic Fatigue Syndrome).
- ◆ Suffer from abnormal immune cell count and function (including auto-immune diseases).
- ◆ May develop post-operative sepsis due to bacterial translocation.

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The gut-brain barrier in major depression:
Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression

Michael MAES¹, Marta KUBERA² and Jean-Claude LEUNIS³ *Neuroendocrinology Letters* Vol. 29 (1) 2008

- **There is now evidence that major depression (MDD) is accompanied by an activation of the inflammatory response system (IRS) and that pro-inflammatory cytokines and lipopolysaccharide (LPS) may induce depressive symptoms.**
- We found that the prevalences and median values for serum IgM and IgA against LPS of enterobacteria are significantly greater in patients with MDD than in normal volunteers.
- It is suggested that the increased LPS translocation may mount an immune response and thus IRS activation in some patients with MDD and may induce specific “sickness behaviour” symptoms.
- **It is suggested that patients with MDD should be checked for leaky gut by means of the IgM and IgA panel used in the present study and accordingly should be treated for leaky gut.**

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Mechanism of IL-1- β Induced Increase in Intestinal Epithelial Tight Junction Permeability

Rana Al-Sadi, Dongmei Ye, Karol Dokladny, and Thomas Y. Ma
The Journal of Immunology, 2008, 180: 5653–5661.

The IL-1- β induced increase in intestinal epithelial tight junction (TJ) permeability has been postulated to be an important mechanism contributing to intestinal inflammation of Crohn's disease and other inflammatory conditions of the gut.

In conclusion, our data indicate that the IL-1 increase in Caco-2 TJ permeability was mediated by an increase in MLCK expression and activity. Our findings also indicate that the IL-1-induced increase in MLCK protein expression and Caco-2 TJ permeability was mediated by an NF- κ B-dependent increase in MLCK gene transcription.

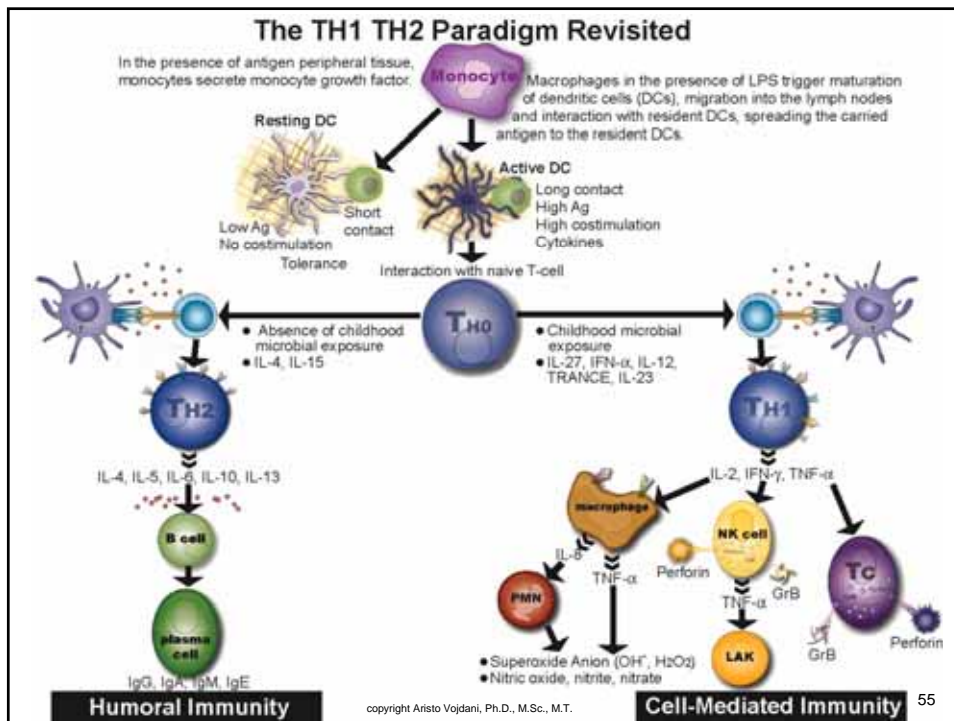
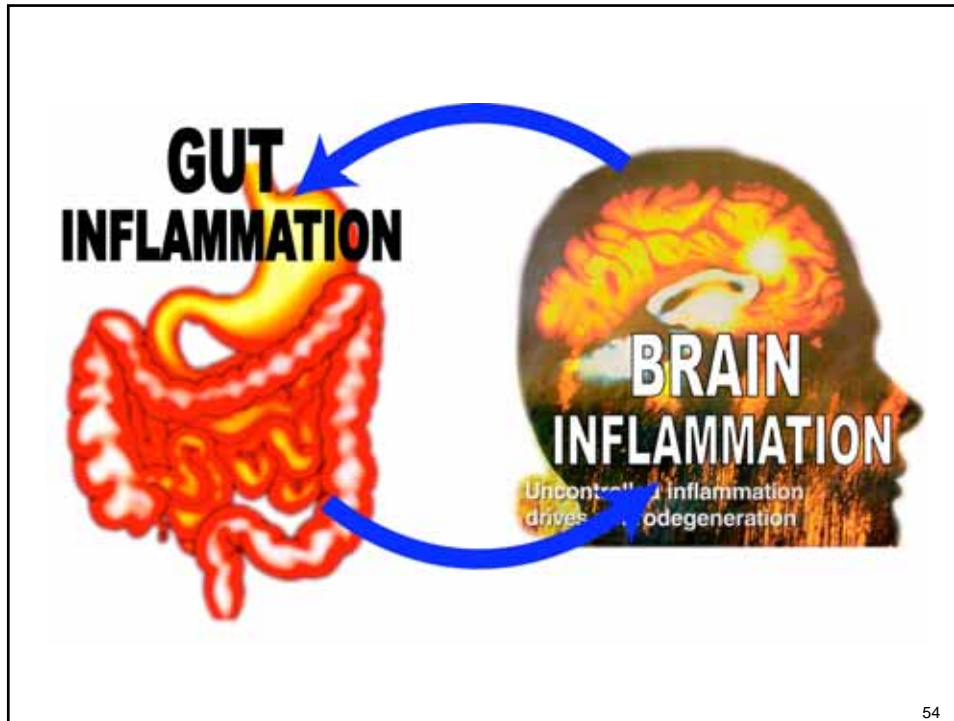
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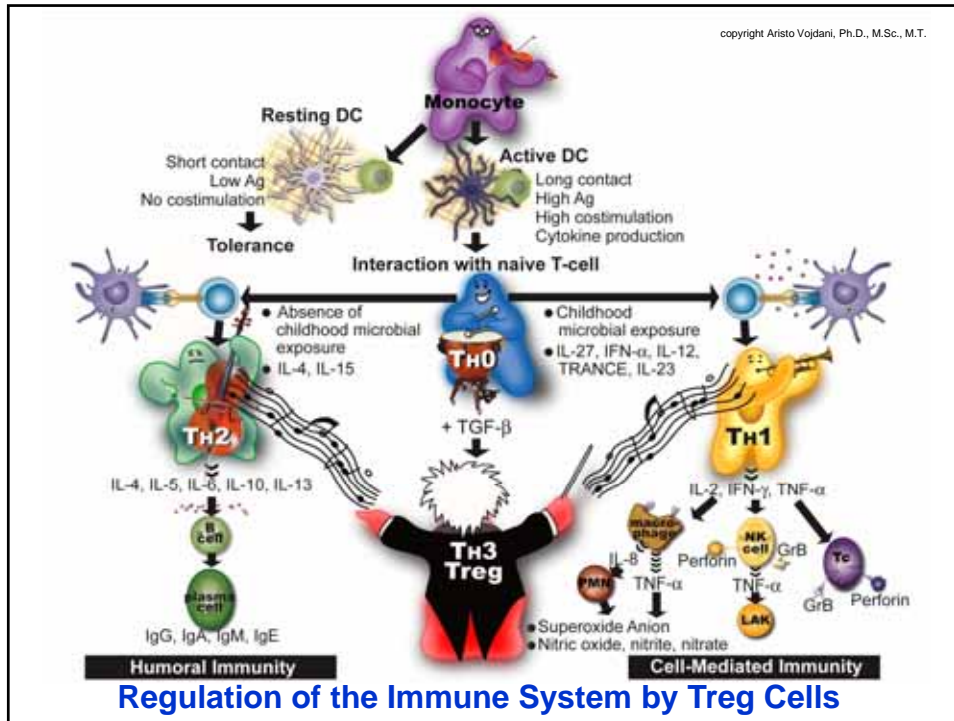
Do Salivary Antibodies Reliably Reflect Both Mucosal and Systemic Immunity?

Brandtzaeg, P., *Ann. N.Y. Acad. Sci.*, 2007, 1098: 288–311

“Two major antibody classes operate in saliva: secretory IgA (SIgA) and IgG. The former is synthesized as dimeric IgA by plasma cells (PCs) in salivary glands and is exported by the polymeric Ig receptor (pIgR). Most IgG in saliva is derived from serum (mainly via gingival crevices), although some is locally produced. In patients with active celiac disease, IgA antibodies to disease-precipitating gliadin are reliably represented in whole saliva but not in parotid secretion. **Saliva remains an interesting biological fluid with great scientific and clinical potentials.**”

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Opinion

Cell
PRESS

The hygiene hypothesis and psychiatric disorders

Graham A.W. Rook¹ and Christopher A. Lowry²

The hygiene hypothesis proposes that several chronic inflammatory disorders (allergies, autoimmunity, inflammatory bowel disease) are increasing in prevalence in developed countries because a changing microbial environment has perturbed immunoregulatory circuits which normally terminate inflammatory responses. Some stress-related psychiatric disorders, particularly depression and anxiety, are associated with markers of ongoing inflammation, even without any accompanying inflammatory disorder. Moreover, proinflammatory cytokines can induce depression, which is commonly seen in patients treated with interleukin-2 or interferon- α . **Therefore, some psychiatric disorders in developed countries might be attributable to failure of immunoregulatory circuits to terminate ongoing inflammatory responses.** This is discussed in relation to the effects of the immune system on a specific group of brain serotonergic neurons involved in the pathophysiology of mood disorders.

Trends in Immunology Vol.29, No.4, 2008

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