Chapter 4: Tolerance in the intestine

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Objectives:

- Special features of the mucosal immune system
- Define the mechanisms and relative roles of innate versus adaptive immunity
- Identify the cell populations that contribute to immunity and tolerance in the gut and their locations
- Tolerance to commensal bacteria
- Low- and high dose tolerance
- Tolerance to dietary antigens
- Immunotherapies by inducing tolerance
Immune tolerance is the process by which the immune system does not attack an antigen.

A. Central tolerance:
   • lymphocyte development and operates in the thymus and bone marrow. T and B lymphocytes that
   • against self antigens: T and B lymphocytes deleted before they develop into fully immunocompetent cells.

B. Peripheral tolerance is developed after T and B cells mature and enter the periphery. Some T- and B-cells which still react against self-antigens will escape the negative selection in the thymus, and will be controlled by
   • Activation-induced death leading to apoptosis
   • Anergy: hyporesponsiveness in lymphocytes
   • Cytokines like IL10 and TGFbeta.

C. Acquired/Induced tolerance:
   • to external antigens
   • always antigen-specific
Oral tolerance is the most rigorously investigated form of tolerance
Intricate balance between host defense and immunoregulation

- Large antigenic burden of dietary antigens and commensal bacteria.
- Barrier for pathogens, but permeable to water and other nutrients

Normal immune response in the gut is unresponsiveness.
Mucosal immune response

1. luminal digestion of potential antigens
2. strong physical barrier
3. production of antibodies (sIgA)
4. selective antigen sampling sites
5. unique T cell subpopulations that effect suppression

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Digestion of Antigens

**Proteolytic enzymes**

Stomach: pepsin, papain  
Small bowel: trypsin, chymotrypsin  
Pancreatic proteases, lipases,  
Enzymes that break down carbohydrates, etc

**Function:**

Digestion into di- and tripeptides for  
• absorption of nutrients  
• to render nonimmunogenic peptides  
(< 8-10 aa are poor immunogens)

**Bile salt**

emulsification

**pH**

**Bacterial flora**

$10^{12}$ to $10^{14}$ bacteria/g colonic tissue: aid in digestion
The physical and innate barrier: Goblet and Paneth cells

Goblet cells
- **Glycocalyx**, a semipermeable barrier
- Reservoir for sIgA: binds to bacteria, viruses and prevents epithelial attachment
- **Trefoil factors**

Without mucin products or trefoil factors the host is more susceptible to inflammation and less capable of repairing breeches.

Paneth cells
- **Antimicrobial peptides**: lyse bacteria
  - defensins $\alpha$ and $\beta$

The physical barrier: epithelial junctions

Epithelial junctional and tight junctions regulate gut permeability to bacteria & dietary proteins:

- Permeable only for ions

Increased permeability exist:

a) During inflammation: IBD
b) In the perinatal period
c) In food allergic individuals

tight junctions are permeable for macromolecules

The permeability of tight junctions is regulated by:

Exogenous factors: alcohol, non-steroidal anti-inflammatory drugs, pathogen exposure

Immunologic influences: cytokines, immune cells, apoptotic pathways
Intestinal epithelial cells – sentinels in the gut

- Defective sensing of PAMP except for crypt cells
- Recruitment of neutrophils by secretion of IL8
- Can act as nonprofessional APC
- Constitutively express MHC class I and class II, MHC class Ib (CD1d, HLA-E), MICA/B, gp180 (ligand for CD8 cells), LFA-3 (CD2 ligand), CD23
- CD86, ICAM-1 can be induced

sIgA

- cannot bind complement
- can agglutinate antigen
- can be compensated by IgM (by IgA deficiency, incidence 1/300-1/600, higher levels of serum antibody to food antigens)
- sIgA system does not fully mature until the age of 4

Secretory component (SC) protects IgA against proteases.
IgM can also bind SC.

IgG, IgE is found in lumen – transport?
IgE heavily glycosilyated, but degrades easily in stomach and small intestine

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**Bacterial flora**  
$10^{12}$ to $10^{14}$ bacteria/g colonic tissue

- aid in digestion,
- promote epithelial cell growth and differentiation,
- shape immune-repertoires. (germ-free animals – immunedeficient)

**Mechanisms for intestinal tolerance to commensal bacteria**

**Commensal bacteria**
- Impaired ability to escape trapping in mucus
- Impaired ability to adhere and invade epithelial barrier
- Low endotoxicity as a result of having pentacylated lipid A

**Mucosal epithelium**
- Defective sensing of PAMP except for crypt cells
- Early warning system (NOD) for detection of invading pathogens
- Permanent induction of active-inflammatory system under pressure of the gut microflora

**Lamina Propria**
- Contains tolerogenic DCs, macrophages and Treg

_Sansonetti PJ. Nat Rev Immunol. 2004 Dec;4(12):953-64._
Inflammatory Bowel Disease (IBD)

- Intestinal flora required: no colitis without bacteria
- Genetic predisposition by gene defect associated with the innate immune response
- Inflammatory response results in continued epithelial injury which causes erosions, ulcerations and a decreased production of defensins. → increased exposure to microbiota and amplification of the inflammatory response
- Hallmark of active IBD: infiltration of innate (neutrophils, macrophages, DCs and NKT cells) and adaptive immune cells (B- and T cells) → TNFα, IL1β, INFγ, IL23 elevated

Crohn's disease: Th1, Th17 associated can affect the entire digestive system

Ulcerative colitis: Th2, Th17 associated, affects only colon

Antigens elicit qualitatively distinct immune responses based on their portal of entry.

<table>
<thead>
<tr>
<th>Route of Antigen</th>
<th>Usual outcome</th>
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<tbody>
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<td>Subcutaneous</td>
<td>Immunization</td>
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<td>Intramuscular</td>
<td>Immunization</td>
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<td>Injury</td>
<td>Immunization</td>
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<tr>
<td>Intravenous</td>
<td>Tolerance</td>
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<td>Mucosal (oral, nasal and respiratory)</td>
<td>Tolerance</td>
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<tr>
<td>Portal vein</td>
<td>Tolerance</td>
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<td>Anterior chamber of the eye</td>
<td>Tolerance</td>
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</table>

Immunization is characterized by local inflammation and specific antibody production. Tolerance is characterized by inhibition of systemic immunity to the specific antigen.
Delicate balance between tolerance to innocuous antigens and immunity to pathogens is histologically recognized as controlled or physiological inflammation.
1. Pathway of tolerance through absorptive epithelial cells

Through absorptive epithelial cells (~200m²):

- can act as non-professional APC (constitutively express MHCII on basolateral membran)
- can activate CD8+ T cells

1. Transcytosis through intestinal epithelial cells
2. Capillaries into portal vein
3. proceeding to the liver
Immune privilege and tolerance in the liver

- Acceptance of allogenic liver transplants in animal models without immunosuppression (in contrast to skin, kidney or heart transplants).
- in humans, liver transplants are better accepted than other vascularized organ grafts (fewer T-cell mediated rejection episodes, requiring less immunosuppressive therapy).
- direct injection of antigen or allogenic cells into the portal vein, resulting in tolerance. When the liver is bypassed as a result of porto-systemic shunting of the blood flow, oral tolerance cannot be achieved dissemination and spread of many cancers (e.g. colon, breast, prostate), and the development of liver metastasis is often associated with the final stages of cancer development.

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Calne RY. Immunol Rev 2000;174:280-282
LSECs, Kupfer cells, NKT cells have been implicated in portal vein tolerance.
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University Clinics of Dermatology, and Institute Of Pathophysiology and Allergy Research

Epithelial cell /portal vein

Source: Barrett KE: Gastrointestinal Physiology: http://www.accessmedicine.com
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2. Pathway through Peyer’s patches

M cells: specialized cells, which is able to transcytose particulated antigens without further processing

Ablation of Peyer’s patches by genetic or pharmacological means does not affect tolerance.
Soluble antigens lead to systemic tolerance, whereas particulated or aggregated antigens generally prime the immune response.

**Pasteurization:**
> 30 min at 63 °C, > 17 sec at 72 °C

Gelfiltration by a Sephadex G-200 column

Pasteurization of β-lactoglobulin leads to aggregation of BLG. This changes the path of uptake away from absorptive enterocytes to solely Peyer’s patches.
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The Pathophysiology of Allergy

Antigen-specific antibody response

![Graph showing IgG1 and IgE levels](image)

Antigen-specific cytokine response from splenocytes

![Graph showing IL5 and IL13 levels](image)

* p<0.05  ** p<0.01

Roth-Walter F et al. Allergy;63(7), 882-90, 2008
The formation of aggregates

- changes the path of antigen uptake (enterocytes to Peyer’s patches)
- Leads to an enhanced immune response
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Antigen

M-cells

Peyer's patch
3. Pathway via Dendritic cells

Dendritic cells intercalate between epithelial cells and sample antigens directly from the lumen.

Expansion of dendritic-cell populations however lead to enhanced induction of oral tolerance.

1. Uptake of Antigens via Dendritic cells
2. Via the lymphatic vessels to Mesenteric lymphnodes

Ablation of Mesenteric lymphnodes by genetic or pharmacological indicate that these structures are essential for oral tolerance tolerance.

Mayer L et al. Nature Reviews Immunology 4, 407-419, 2004
Potential mechanisms of oral tolerance

Oral tolerance occurs after either administration of a single high dose of antigen (>20mg) or repeated exposure to lower doses (100ng-1mg)


Active immune response requires

- ligation of the TCR with the peptide-MHC complexes.
- Co-stimulatory molecules (CD28/CD80: CD86)
- cytokines
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- TCR crosslinking
- Absence of co-stimulation
- concurrently in the presence of inhibitory ligands (CD95 and CD95L or TNFR-family death receptors)

High Dose tolerance leads to anergy and/or deletion.

... leads to activation of regulatory T-cells which suppress immune responses by

- Secretion of IL10 and TGF-beta
- cell-surfaced associated suppressive cytokines
- Inhibitory receptors.
Dendritic cells and their role in oral tolerance

- are abundant in mucosal tissue (PP&LP)
- Propensity to induce Th2 responses in vitro
- Express IL10 and TGFβ?
- DCs from PP promote IgA production in naive B cells
- CD8+ plasmacytoid DCs induce Tr cells
- Process apoptotic enterocytes and sample commensal organisms in steady state

- Adoptive transfer of DCs from LP can induce Ag-specific tolerance in host
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Cytokines required for Th cell differentiation

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<thead>
<tr>
<th>Cytokines</th>
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<th>Th2</th>
<th>Th9</th>
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Transcription factors associated with Th cell subsets

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Cytokines expressed by Th cell subsets

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<tr>
<td>Th1</td>
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<tr>
<td>Th22</td>
<td>Induction of delayed type hypersensitivity</td>
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<tr>
<td>Th2</td>
<td>Autoimmunity</td>
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<td>Th9</td>
<td>Defense against helminth infection, asthma, allergy</td>
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<tr>
<td>Th17</td>
<td>Induce tissue inflammation, promotion of autoimmunity</td>
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<td>Treg</td>
<td>Anti-inflammatory</td>
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<th>Cytokines</th>
<th>Responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>Defense against intracellular pathogens</td>
</tr>
<tr>
<td>Th22</td>
<td>Induction of delayed type hypersensitivity</td>
</tr>
<tr>
<td>Th2</td>
<td>Autoimmunity</td>
</tr>
<tr>
<td>Th9</td>
<td>Defense against helminth infection, asthma, allergy</td>
</tr>
<tr>
<td>Th17</td>
<td>Induce tissue inflammation, promotion of autoimmunity</td>
</tr>
<tr>
<td>Treg</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Tr1</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>Th3</td>
<td>Defense against helminth infection, asthma, allergy</td>
</tr>
<tr>
<td>Tr35</td>
<td>Defense against helminth infection, asthma, allergy</td>
</tr>
</tbody>
</table>

Note:
- Activate macrophage
- Activate mast cells
- CD4+CD25+ FOXP3+
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome

Gene mutation of FOXP3 (Scurfy mice)

Key Clinical Features of IPEX
Enteropathy: Watery diarrhea, usually early onset (< 6 months old)
Endocrinopathy: Type I diabetes or thyroiditis most common
Dermatitis: Eczema most common but may be psoriasiform, pemphigoid, etc.
Other Autoimmunity: Autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP), autoimmune neutropenia, autoimmune hepatitis, nephropathy, etc.
Elevated IgE

Sakaguchi S. Nature Immunology 6, 345 - 352 (2005)
Factors important for oral tolerance induction

- Properties of Antigen: stability to heat and digestion, glycosylation
  - Food processing
  - Food matrix
  - Protein family (e.g., vast majority of aeroallergens from animal sources belong to the lipocalin-family, plant food allergens usually originate from the prolamin and cupin superfamilies and the Bet v 1 family)

- Antigen Dose and composition
- Adjuvants (B-subunit of cholera toxin)

- Route of antigen exposure

- Age and genetic susceptibility of the host
- Host commensal flora

To elicit an immune response antigens must reach the immune system in an immunogenic form. (undigested, with an adjuvant, etc.)
Food allergy

- failure in establishing oral tolerance or a breakdown in existing tolerance
- could result from epithelial barrier dysfunction

Tregs

- increased Tregs have been associated with reduced clinical reactivity
- In children with non-IgE mediated milk allergy, active GI disease is associated with high Th2 and low Treg-associated cytokines
- Children who outgrew milk allergy have higher levels of circulating CD4+CD25+Tregs.

References:
Shreffler WG et al. J Allergy Clin Immunol 2009, 123,43-52
Induction of Tolerance in Allergic Patients

Allergen Immunotherapy:

- SIT: works well with aeroallergen, but food allergen immunotherapy is unsafe due to high rate of severe systemic anaphylactic reactions
- SLIT: liquid concentrate administered under the tongue
- OIT: powdered food proteins administered orally with food

Administering increasing doses of antigen in a controlled setting followed by regular home dosing during a build-up phase to reach a maximum tolerated maintenance dose of antigen.

To determine clinical desensitization/tolerance after treatment an open or masked food challenge with antigen or placebo.
The Pathophysiology of Allergy

Tolerance in the intestine
Dr. Franziska Roth-Walter
University Clinics of Dermatology, and
Institute Of Pathophysiology and Allergy Research

Mucosal immunotherapy
(oral, sublingual)

APC

Naive CD4 T cell

TGF-β
IL-10

Reduced clinical symptoms (OFC)

Foxp3

T-regulatory cell

Desensitization
increased IgG4
Decreased IgE, SPT reactivity
Decreased basophil reactivity

Tolerance
Increased Foxp3+ Tregs
increased IFN-γ, IL-10, TNF-α
OIT appears to be safe, well-tolerated treatment when performed by experienced personnel in highly supervised research settings with appropriate safety precautions in place.
### Oral tolerance in animal models of disease

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Human disease</th>
<th>Induction</th>
<th>Effective oral antigens</th>
<th>Prophylactic or therapeutic</th>
<th>Dose</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental autoimmune encephalomyelitis</td>
<td>Multiple sclerosis</td>
<td>Commonly induced by injection of susceptible animals with myelin proteins plus pertussis toxin as an adjuvant to permeabilize the blood–brain barrier</td>
<td>Whole myelin, myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein, platiramer acetate (copolymer 1)</td>
<td>Prophylactic and therapeutic</td>
<td>High dose: 63, 94, 95, 125</td>
<td>42–44</td>
</tr>
<tr>
<td>Collagen-induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Induced by injection of type II collagen in adjuvant</td>
<td>Collagen types II and IX, HSP65</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>74</td>
</tr>
<tr>
<td>Adjuvant-induced arthritis</td>
<td>Rheumatoid arthritis</td>
<td>Induced by injection of Freund’s adjuvant, bacterial products or HSPs</td>
<td>HSP60, HSP65, type II collagen</td>
<td>Therapeutic</td>
<td>Low dose</td>
<td>114</td>
</tr>
<tr>
<td>Experimental autoimmune uveitis</td>
<td>Autoimmune uveitis</td>
<td>Induced by immunization with sequenced retinal antigens or IRBP</td>
<td>Retin S-antigen, IRBP, HLA-B27 mimotope (HLA-B27PD)</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>78, 79, 97</td>
</tr>
<tr>
<td>Experimental autoimmune myasthenia gravis</td>
<td>Myasthenia gravis</td>
<td>Immunization with acetylcholine receptor</td>
<td>Acetylcholine receptor</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>77</td>
</tr>
<tr>
<td>Non-obese diabetic mice</td>
<td>Type 1 diabetes</td>
<td>Spontaneous destruction of pancreatic islet cells</td>
<td>Insulin</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>90, 96, 99, 121</td>
</tr>
<tr>
<td>Rat insulin promoter LCMV diabetes model</td>
<td>Type 1 diabetes</td>
<td>Transgenic expression of LCMV proteins under the rat insulin promoter, infection with LCMV initiates disease</td>
<td>Insulin</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>101</td>
</tr>
<tr>
<td>Middle cerebral artery occlusion</td>
<td>Stroke</td>
<td>Surgical occlusion of the middle cerebral artery</td>
<td>Myelin basic protein</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>64</td>
</tr>
<tr>
<td>LDL-receptor-deficient mice</td>
<td>Atherosclerosis</td>
<td>Mice lacking the LDL receptor are fed a high-fat diet</td>
<td>HSP65</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>65, 66</td>
</tr>
<tr>
<td>Tissue transplant</td>
<td>Tissue transplant</td>
<td>Surgical transplantation of allogeneic tissue</td>
<td>Donor cells, donor MHC proteins</td>
<td>Prophylactic</td>
<td>Low dose</td>
<td>80, 92</td>
</tr>
</tbody>
</table>

Several common models are used to study oral tolerance. Models are prophylactic if the regimen of oral feeding is begun prior to induction or onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after induction or onset of disease. Low doses correspond to <1 mg per day, whereas >1 mg per day is considered a high dose. This division is based on studies with myelin proteins in experimental autoimmune encephalomyelitis and might not accurately reflect doses with other antigens in other disease models. HSP, heat shock protein; IRBP, interphotoreceptor retinoid binding protein; LCMV, lymphocytic choriomeningitis virus; LDL, low-density lipoprotein.

Caveat: genetic heterogeneity in human disease but not in experimental system. Variations in MHC allele alleles can lead to distinct functional outcomes with the same ligand.
Only limited number of tolerance studies in human, since lack of suitable neo-antigen. Antigens must be non-toxic, capable of being ingested and rarely encountered by the general population.

### Table 3 | Oral tolerance in human diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Oral antigen</th>
<th>Dose</th>
<th>Prophylactic or therapeutic</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food allergy</td>
<td>Allergen</td>
<td>Increasing dose over time</td>
<td>Therapeutic</td>
<td>About 80% of patients are successfully desensitized</td>
<td>130</td>
</tr>
<tr>
<td>Autoimmune uveitis</td>
<td>Sequestered retinal antigens, HLA-B27PD,Retinal S-antigen, soluble retinal antigens</td>
<td>4 mg capsules 3 times a week for 12 weeks</td>
<td>Therapeutic</td>
<td>Marginal clinical benefit. All patients relapsed after cessation of treatment</td>
<td>131,132</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Collagen</td>
<td>0.1 mg bovine type II collagen daily for 1 month, followed by 0.5 mg daily for 6 months, 20, 100, 500 or 2,500 μg chicken type II collagen daily for 24 weeks, 0.05, 0.5 or 5 mg bovine type II collagen daily for 6 months, 0.5 mg bovine type II collagen daily for 3 months, 0.1 mg chicken type II collagen daily for 1 month, followed by 0.5 mg for 2 months</td>
<td>Therapeutic</td>
<td>Clinically significant response at 20 μg dose</td>
<td>135,136</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>Insulin</td>
<td>7.5 mg insulin</td>
<td>Prophylactic</td>
<td>No benefit</td>
<td>137</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Myelin</td>
<td>300 mg bovine myelin</td>
<td>Therapeutic</td>
<td>No clinically significant benefit</td>
<td>140</td>
</tr>
</tbody>
</table>

*For trial results see National Institutes of Health News website in Further Information. In contrast to experimental animal models, most human clinical trials have attempted to induce oral tolerance after the onset of disease (therapeutically). Treatments are prophylactic if the regimen of oral feeding is begun prior to the onset of clinical disease, whereas they are therapeutic if oral tolerance is initiated after the onset of disease. HLA-B27PD, HLA-B27 mimotope.*

Key issue remains: Translation of animal models to human subjects. Dose, Antigen, age, genetic and environmental diversity must be taken into account.
Powerful tool for therapy of

⇒ Allergies
⇒ Autoimmunity
⇒ Chronic inflammatory conditions
⇒ alternative to immunosuppressive medications that have undesirable side-effects (such as steroids)
THANK YOU FOR YOUR ATTENTION!