Mucosal Immunology and Oral Tolerance

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History of adverse food reactions

- First recorded by Hippocrates over 2000 years ago.

- 1921: Prausnitz and Kustner demonstrated that substance responsible for Kustner’s fish ‘allergy’ was present in his blood stream.

- 1950: Loveless reported results of first blinded placebo-controlled food trial.

- 1960: Goldman et al established diagnosis of food allergy with use of elimination diets and subsequent food challenges.
Definitions

- **Adverse food reaction**: any abnormal reaction resulting from the ingestion of food. May be the result of food intolerances, food allergy or food aversions.

- **Oral tolerance**: specific suppression of cellular or humoral immune responses to an antigen, by prior oral administration.

- **Food allergy** represents the failure of development of tolerance or the breakdown in existing tolerance. It must be distinguished from other adverse food reactions which have no immunological basis but may resemble it clinically.
Function of the GIT

- Digestion and absorption of essential nutrients.

- Protective host defence against harmful foreign antigens (food proteins, viruses, bacteria, parasites).

- Intestinal immune system is required to discriminate between harmless and harmful foreign proteins.
GI tract largest immunological organ in the body.

In constant interaction with the external environment.

Must mount a potent and rapid response against potentially harmful foreign substances and pathogenic organisms **BUT** must remain unresponsive to enormous amounts of nutrient antigens and around $10^{14}$ normal flora.
Evolved both innate and adaptive components capable of inhibiting responses to certain antigens and mounting immunoprotective responses against others.

Mucosal system is characterised as a site where antigen is selectively sampled and tolerance develops, to maintain a steady state of controlled and protective inflammation.
Innate mechanisms of defence

- Mucus produced by goblet cells. Composed of a number of glycoproteins that cover the epithelium and limit antigens and microbes from communicating with epithelium.
- Trefoil factors assist with barrier repair and wound healing.
- Antimicrobial proteins (defensins) synthesised by Paneth cells and epithelial cells.
- Secretory IgA (s–IgA) produced by B cells found on mucosal surface.
s-IgA

- Produced along length of MALT by plasma cells in lamina propria.
- Secreted as a dimer bound by ‘J’ chain unlike serum IgA.
- Binds to glycoprotein from epithelial cell ‘Sc’ or the ‘pIgR’.
s–IgA (cont)

- 2 functions of glycoprotein:
  - actively transports s–IgA across epithelium into lumen
  - protects s–IgA from degradation by luminal proteases

- s–IgA binds microbes and toxins in lumen, preventing attachment to epithelial cells and trapping them in mucus layer for excretion in stool.
s–IgA (cont)

- Antigen bound to s–IgA may be presented to dendritic cells which in turn present antigens to lymphocytes.

- s–IgA does not activate complement pathways so actions are non–inflammatory hence ideal for protection of mucosal surfaces.
GALT (Gut–associated lymphoid tissue)

Composed of 4 distinct lymphoid compartments:

1. Peyer’s patches and the appendix
2. Lamina propria lymphocytes and plasma cells
3. Intraepithelial lymphocytes interdigitated between enterocytes
4. Mesenteric lymph nodes
Peyers patches (PP’s)

• Aggregates of lymphoid follicles in intestinal mucosa.

• Located primarily in small distal intestine.

• Composed of large B-cell follicle surrounded by interfollicular T–cell region.
Peyers patches (PP’s)

- Also contains numerous macrophages and dendritic cells (antigen presenting cells or APC’s).

- Epithelial surface overlying PP’s composed of single layer of columnar epithelium, follicle associated epithelium (FAE).

- Within FAE are specialised M cells derived from enterocytes under influence of lymphotoxin, secreted by B lymphocytes.
M Cells

- No microvilli or hydrolytic enzymes
- Express a number of receptors which serve to sample antigens
- Some regional differences in M cells possibly due to changing microflora
- Invaginated subdomain at basolateral membrane forming ‘intraepithelial’ pocket
M Cells

- Main function is transepithelial vesicular transport of antigens from gut lumen to subepithelial lymphoid tissues.

- Antigens include particulate proteins, bacteria, viruses and non-infectious particles.

- Important in development of immune responses and tolerance.
Lymphocytes in the GALT

- Intraepithelial T lymphocytes mainly exhibit a suppressor and cytotoxic phenotype. Protect the mucosa by killing infected cells and attracting other immune cells to combat infection.

- The lamina propria is endowed with B cells and T cells that exhibit a helper and inducer phenotype.
How do antigens enter the bloodstream?

- Food ingested is broken down by gastric acid and digestive enzymes. Decreases the immunogenicity of the proteins.

- Food protein antigens absorbed by specialised cells (DC’s, M cells, epithelial cells) and transported to lymphoid tissue in Peyers Patches.

- Antigens are presented to T lymphocytes of a helper and inducer phenotype, which proliferate and induce B cell response.

- B cells exit PP’s via intestinal lymphatics ➔ mesenteric lymph nodes ➔ thoracic duct ➔ vena cava
Specific antibody–secreting lymphocytes appear in peripheral blood within 48 hrs of antigen exposure and persist in the blood for 2 weeks.

Homing receptors on lymphocytes, which interact with ligands on endothelial cells, target the migration of lymphocytes to other mucosal sites to mediate protection more broadly (‘common mucosal immune system’).

Antigen–specific systemic suppression after oral antigen introduction can be seen after 1–2 days and oral tolerance to systemic challenge becomes established within 5–7 days.
Factors affecting barrier function

- Food allergies could result from epithelial barrier dysfunction resulting in increased intestinal permeability.

- Impaired barrier immunity increases food antigen exposure to the immune system which may result in antigen sensitisation and production of allergic response.

- Animal studies have shown that gut permeability is influenced by numerous factors including epithelial apoptosis, pathogens, cellular inflammatory responses, cytokine release and ingestion of alcohol and NSAID’s.
Changes in barrier function after birth

- An abrupt change in gut barrier function occurs at birth when the gut switches from processing amniotic fluid to digesting milk.

- Food consumption initiates the release of trophic hormones and the activation of secretion, motility, and absorption.

- During postnatal development, further maturational and adaptive events in the gut defense barrier include the appearance of mucosal proteins and digestive enzymes and the development of the intestinal flora.
Food proteins play a critical role in stimulating maturation of the immune system.

Developmental immaturity of these mechanisms in younger children and neonates reduces efficiency of mucosal barrier.

Likely plays a major role in increased prevalence of gastrointestinal infections and food allergy in first few years of life.
Changes in barrier function after birth (cont)

- Basal acid output low in 1st month of life, intestinal proteolytic activity only matures at 2 years old, intestinal microvillus membranes immature (altered antigen binding and transport through mucosal epithelial cells).

- Newborns lack IgA and IgM in exocrine secretions and salivary IgA absent at birth and low in first few months of life.
Oral Tolerance

- Specific suppression of cellular or humoral immune responses to an antigen by prior oral administration.

- Unresponsiveness of T cells to ingested food proteins may be the result of 3 different mechanisms: *T cell anergy, T cell deletion or induction of regulatory T cells (Treg cells).*
T cell anergy

- Characterised by reduced proliferation following stimulation with antigen and APC’s.

- Presentation of antigen in presence of appropriate co-stimulation by APC’s leads to development of active immune response.

- In absence of co-stimulation, effector function does not develop and tolerance ensues.
T cell deletion

- Clonal deletion of antigen–specific T cells by apoptosis demonstrated in animal models fed very large doses of antigen.

- Not thought to play a major role in humans.
Induction of regulatory (Treg) cells

- Result in production of inhibitory cytokines such as $TGF-\beta$, $IL-4$ and $IL-10$.

- $Th3$ and $Tr-1$ cells are potent sources of $TGF-\beta$. Generated in mucosal lymphoid tissue in response to low-dose antigen and mediate ‘bystander tolerance’ by inhibiting activation of surrounding lymphocytes.
Factors influencing induction of oral tolerance

1. Age of the host

2. Nature of the antigen ie. soluble vs particulate; protein vs carbohydrate

3. Dose of antigen administered

4. Nature of antigen presenting cell

5. Genetics of the host
Age of the host

- Neonates are not ‘tolerized’.

- Ingestion of dietary proteins early in life has been associated with food intolerance.

- Due to immature intestinal barrier with increased permeability, which facilitates priming of humoral and cell-mediated immune responses.
Nature of the Antigen

- Best tolerogens demonstrated to be proteins in experimental models.
- Majority of dietary proteins induce tolerance, (comprise bulk of antigens in most diets).
- Soluble antigens more ‘tolerogenic’ than particulate ones.
- Process of digestion degrades many particulate antigens to soluble ones.
Repeated low doses of antigen elicit activation of regulatory T cells.

Whereas single high dose of antigen induces either clonal deletion or anergy.
Nature of the APC

- Intestinal epithelial cells (IEC’s) act as non-professional APC’s but lack appropriate co-stimulatory molecules to prime T cells efficiently.

- DC’s within PP’s express \( IL-10 \) and \( IL-4 \) which favour generation of tolerance.
Role of Bacterial Flora in oral tolerance

- Luminal bacteria aid digestion, produce vitamins and communicate with epithelium to promote growth and differentiation.

- Prevent colonisation by many potential pathogens by creating a hostile environment for them (compete for nutrients, physically block access to the epithelium).

- Probiotics stimulate production of cytokines involved in suppression of inflammation
Complementary food introduction

- Large prospective studies have proven that later introduction of solid foods had no protective effect on the development of asthma or atopic disease.
- Complementary foods should be introduced after 17 weeks and no later than 26 weeks.
- Restriction of maternal diet during pregnancy and breastfeeding NOT recommended.
- Restriction of allergenic foods in infants after 4–6 months of age NOT recommended.
Role of probiotics

- Limited number of studies done to assess efficacy and safety of probiotics in infants.
- Evaluation and interpretation of results further hampered by fact that there is wide variation in probiotic strains and doses used, as well as age of initiation and duration of supplementation.
- More well-controlled clinical trials are needed.
Immunotherapy

- OIT (oral immunotherapy) and SLIT (sublingual immunotherapy).
- Involves regular administration of small amounts of allergen via the oral or sublingual route.
- Aim is to rapidly induce desensitisation and then in time, induce tolerance to the antigen.
**Conclusion**

- Dominant immune response in the intestine is one of suppression resulting in oral tolerance.

- Complex regulation of immune system in the gut.

- Oral tolerance is determined by multiple factors incl. nature and dose of antigen as well as age of host.

- As more becomes known about immune regulation and antigen processing in the gut, hopefully more definitive treatments for food allergies can be discovered.
References

1. *Mucosal immunology, eosinophilic oesophagitis, and other intestinal inflammatory diseases*, Atkins et al, Journal of Allergy and Clinical Immunology, February 2012 (S255–S261).


5. *Pediatric food allergy and mucosal tolerance*, Scurlock et al, Pediatric Allergy and Immunology, Feb 2010, online publication.
