Physiology and Immunology of Digestion

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Just over an inch under the abdominal skin, even of the most comely human being, lies the malodorous, heaving mass of bowels without which none of us could survive. The gut serves two simultaneous functions, which are mutually antagonistic. On the one hand its 400 square meters of surface has the job of absorbing nutrients from the food - approximately one ton per year in adults. On the other hand most of our food (being entirely composed of foreign proteins etc) is antigenic, and the gut must simultaneously prevent antigens and other undesirable dietary products from gaining access to the body tissues. The key is good digestion - in the same step, dangerous molecules are both made safe and converted into useful currency for the body's economy.

Microbial contamination of food is not normally a major issue in the modern Western world, and what germs remain after cooking are usually killed by the stomach acid, so the healthy small bowel has few if any microbial residents. (This is not true in less-developed countries where cooking fuel is scarce, and the small bowel is frequently colonised by bacteria, protozoa and parasites, even in apparently healthy people.) The *large* bowel is host to approximately 100,000,000,000,000 bacteria - more cells than in the whole of the rest of the body. The composition of the large bowel flora is somewhat mysterious as many of the species are ultra-strict anaerobes and some cannot be grown at all on known bacteriological media, being known only by their DNA fingerprints. Most of the bulk of normal faeces is indeed made up of bacteria, followed by desquamated cells from the gut, and only a minor proportion of the stool is undigested food because anything not absorbed by the small bowel is usually consumed by the voracious bacteria in the large. This "normal flora" is essential for many normal functions of the gut - germ-free animals have abnormal villi, subnormal nutrition and cannot be orally tolerised (see below).

The absorptive surface of the small bowel is in a constant state of turnover, with the absorptive cells (enterocytes) sloughing from the tips of the villi and being replaced by mitosis from the crypts. This tremendous daily wastage is due to the toxicity of the intestinal luminal contents, one of the most poisonous environments in the world. Food, perhaps surprisingly, contains numerous toxins (especially the portion derived from the Plant Kingdom), and we survive the daily assault only because of efficient defence systems, one of which is our use of a disposable gut mucosa. Defaecation can be virtually abolished by adopting the kind of high-protein diet used by early astronauts, in spite of the alleged difficulty of digesting meat, because mammalian meats, with few exceptions, are relatively non-toxic. (They can however be horrendously *allergenic* - please distinguish). Small wonder that the intestine is the largest lymphoid organ in the body.

This immunological factory is perpetually busy, day and night, sorting out the useful moieties of the diet for absorption and nutrition, and rejecting the rest. 40% of all body lymphocytes at any one time are resident in the gut, where they comprise a discrete immune system operating somewhat independently from that of the rest of the body. So immunologically-activated is the gut immune system that the healthy gut (including the tonsils) has been described as being in a permanent state of physiological inflammation (although this is nothing in comparison to the degree of inflammation that can be achieved in Crohn's disease, or even gastroenteritis).

GUT-ASSOCIATED LYMPHOID TISSUE (GALT)

This begins at the throat, where the tonsilloadenoidal ring is strategically placed to sample everything swallowed. There are lymphoid nodules scattered within the mucosa throughout the alimentary tract but these are particularly concentrated in the small bowel into Peyer's patches, which serve no direct nutritional role but form the front line of the gut's immune defences. The average human small bowel contains 45 Peyer's patches at 24 weeks' gestation, 80-120 at birth (reflecting the swallowing of maternal dietary antigen from the amniotic fluid), 250 in the mid-teens as the dietary range reaches maximum variety, then declining to about 100 at age 90. The appendix, situated at the junction between the sterile small bowel and the septic large bowel, again samples everything that passes the ileocaecal sphincter.

The main sites of *antigen* absorption are the M (microfold) cells of the Peyer's patches, which take up whole protein molecules and pass them to the dense meshwork of dendritic cells that lie immediately beneath. These process the antigens and present their antigenic determinants (epitopes) to T lymphocytes in the Peyer's patch and in the local mesenteric lymph nodes, which in turn prime associated B cells. These lymphocytes enter the bloodstream via the thoracic duct, rapidly homing to the lamina propria of the intestine and other mucosal surfaces (lungs, nose, mammary glands, lacrimal and urogenital mucosa) where they mature into effector cells. All types of immune effector cell are found in the lamina propria but the largest contingent are plasma cells producing secretory IgA (sIgA) antibodies. 80% of all the body's plasma cells (antibody factories) are normally resident in the gut lamina propria, and of those 90% are sIgA secretors. Relatively small amounts of other immunoglobulin classes are also found in the gut. Interestingly, isolated secretory IgA deficiency is a fairly common condition even though the *serum* IgA levels of these individuals are usually normal. SIgA deficiency can sometimes be compensated by secretion of IgM instead.

The physiological uptake of antigens by the Peyer's patches leads simultaneously both to immune sensitisation of the gut *and* systemic tolerance. That is, there is normally plentiful anti-food sIgA antibody bathing the gut mucous membranes, but little or no corresponding antibody in the bloodstream. This is called **oral tolerance** although the term is somewhat misleading as only the inner organs are tolerised while the gut itself is actively immunised. Enough antigen is absorbed via the absorptive mucosa (see below) to stimulate normal amounts of sIgA in the gut,

but the Peyer's patch route is needed for systemic tolerance. Interestingly, a "normal" gut flora is also needed for oral tolerisation.

SECRETED ANTIBODIES

SIgA antibodies are secreted between the tight-packed enterocytes that comprise the absorptive surface of the small bowel and pass into (a) the lumen, but also and more importantly into (b) the thin unstirred layer of mucus (glycocalyx) that coats and lies between the microvilli of the enterocytes. The gut secretes sIgA antibodies against *all* antigens present in the gut, both dietary and microbial. Most gut-mucosal sIgA is found in the crypt cells, with diminishing amounts as the cells migrate up the villus. The same repertoire of sIgA antibodies bathes all of the other mucosal surfaces as well, providing an "antiseptic paint".

The lungs and nose also have antigen-sampling cells that elicit the production of appropriate sIgA antibodies, and the activated lymphocytes from these also pass to each of the other mucosal surfaces, so that each of these surfaces is protected against antigens belonging to all of the other organs. There are therefore sIgA antibodies against dusts and pollens to be found in the gut secretions, and - most important - sIgA antibodies against *dietary* antigens in breast milk.

The gut immune system excludes most dietary antigens from entry to the portal circulation (**immune exclusion**), though a small percentage of ingested antigen does sometimes reach the sytemic bloodstream, to be mopped up into circulating immune complexes (CIC) which are then cleared via the reticuloendothelial system (no doubt making their own modest contribution to the overall nutrition of the body). Inflammation of the gut mucosa causes the tight junctions between adjacent enterocytes to open, allowing greater antigen permeability. Small quantities of CIC containing diet-derived antigens are found in many healthy individuals after meals but significant levels, by and large, are most often found in food allergic disorders and in individuals with sIgA deficiency. Indeed, failure of immune exclusion in the gut often leads to food allergy affecting non-alimentary organs. The majority of healthy people do not have significant circulating levels of anti-food antibodies (they are *systemically* relatively tolerant while simultaneously being fully immune at the mucosal surfaces).

It is not uncommon for a bout of gastroenteritis to be followed by a transient episode of lactase deficiency and/or food intolerance, presumably because the tight junctions open during the acute episode of inflammation, allowing access of dietary antigens to the circulation and thus systemic sensitization. As the gut inflammation recedes, immune exclusion and oral tolerance are (usually) re-established with suppression or deletion of diet-reactive clones. Some unlucky patients do not recover their oral tolerance and remain food allergic or intolerant. Interestingly, Peyer's-patch-deficient mice produce normal sIgA levels in the intestine but cannot develop oral tolerance. Neither can germ-free animals.

TESTS

Unfortunately, circulating anti-dietary antibodies are not a reliable marker of food intolerance or allergy; they merely demonstrate that at some time within the lifespan of the patient's memory-lymphocytes (possibly many years ago) there was some immunologic recognition of that food epitope. The same is true of tests of cellular immunity against food antigens. RAST and similar tests measure only IgE antibodies, and not always very well. And if the illness is mediated by toxicology mechanisms and not immune mechanisms, tests of immune reactivity are totally irrelevant. Theoretically some form of cytotoxic test should be more relevant for those, but in practice none of the current versions has proved reliable.

The whole field of "allergy testing" is a dismal mess. There is no test, orthodox or heterodox, that reliably gives the correct answer every time. That includes the classic withdrawal-and-challenge technique, originally introduced to this country by our own Professor Ronnie Finn and now used, ironically, by our conventional colleagues as a stick to beat us with . Withdrawal-and-challenge is conclusive (within statistical and methodological limits) *only when positive* - a negative challenge series proves (and disproves) nothing. In my view challenge studies have too many drawbacks, both practical and theoretical, for routine use in the clinic [1].

That is not, however, to say that allergy tests are totally useless. Whether or not they give the correct diagnosis, following the indicated diet often succeeds in converting ill people into well people, and that, after all, is what we're all in business for. Speaking for myself, I hardly ever use diagnostic tests since I can get at least 60-70% of probable food-intolerant patients better merely by applying informed guesswork, and that is the same success rate that any diagnostic test can claim. If you get *all* patients merely to give up wheat and milk, giving appropriate advice to optimise their nutrition, probably half will automatically get better. If you feel the need to use "diagnostic tests", just be honest with the patients and tell them that although the test may give useful clues, it is not expected to be entirely accurate. There are numerous allergy tests on the market and if I were forced to make a cautious recommendation, it would currently (2003) be the Alcat (which is not strictly an allergy test but a test of toxicity).

Elimination diets carry dangers, one of which is the recruitment of new allergies/intolerances with time.This can be avoided by using one of the modern forms of low-dose desensitisation, which bypass both the need for accurate diagnosis and that for extensive elimination diets.

NORMAL PROTEIN DIGESTION

Since the antigens that cause food allergy/intolerance are largely proteins, we allergists are mainly interested in the fate of dietary proteins. The digestion and absorption of carbohydrates, fats and other dietary moieties follow quite different pathways through the gut wall.

As every schoolboy knows, proteins entering the gut are progressively digested by enzymes in the gastric and intestinal juices so that by the time they arrive at the surface of the absorptive cells of the mucosa - the enterocytes - they are in the form of free amino acids. Unfortunately this is **not entirely true**. It would take about 200 hours for the unaided action of the gut enzymes fully to degrade all ingested protein, and although there is some absorption of isolated amino acids, most of the absorption of protein is in the form of **small peptides** (oligopeptides) consisting of 2-3 amino acids. Oligopeptides of three or more amino acids are too small on their own to be *immunogenic* but are fully capable of being *antigenic* epitopes, as will be recalled from the immunology section of the first Training Session. That is, although they cannot on their own evoke the production of new antibodies, they can be recognised and bound by pre-existing antibodies of the appropriate specificity.

The absorption of oligopeptides is considerably more efficient than that of single amino acids, and is indeed the only route of protein absorption available in coeliac disease and Hartnup disease, in which free amino acids cannot be absorbed. Oligopeptides are first bound by specific receptors at the enterocyte surface. Since there are 8000 possible tripeptides, this implies a corresponding degree of variation in the receptors. These receptors bind to the appropriate oligopeptides and immobilise them, initially in the glycocalyx where further enzymic digestion occurs, then drawing them into vesicles within the cytoplasm of the phagocytic enterocyte. Final enzymatic breakdown occurs at one of these two sites, that is, either in the glycocalyx or inside the body of the enterocyte. The resultant free amino acids then pass out of the enterocytes at the basolateral cell membranes to enter the portal circulation. Oral pre-immunization (that is, normal feeding with that protein) results in increased adsorption of oligopeptides to the microvilli and also increased endocytosis of oligopeptides and intracellular breakdown.

At least some of these intracellular digestive vesicles contain sIgA as well as oligopeptide, that is, this sIgA is being carried back in through the enterocyte, presumably still bound to their antigen. By contrast, non-absorptive epithelia such as gall-bladder and female genital tract have sIgA *between* the epithelial cells but not within them. In a series of classic experiments at Harvard in the 1970's, Allan Walker and others showed that secretory antibodies and pancreatic enzymes co-operate in the digestion of antigens, *both* being required for optimal efficiency [2-3]. Probably the antibodies' function is to immobilise the oligopeptides while the enzymes degrade them. Since most of our food is antigenic, this co-operation between enzymes and antibodies would appear to be the normal physiological state of affairs.

True, most of us incorporate novel foods in moderate doses into our diets from time to time without notable malabsorption, even without the benefit of pre-existing gut antibodies. In the healthy gut there is plenty of spare digestive capacity and adequate nutrition will normally proceed even when there is suboptimal surface area, antibodies or enzymes. Nevertheless severe damage to any of these factors may compromise gut function, and Frank Green and I noted in 1977-78 that failure of the "antibody-facilitated digestion" (AFD) pathway would lead

to a number of predictable consequences [4], which started me out on my lifelong fascination with food intolerance. Note, sIgA production (quantity and/or quality) can be compromised by a number of environmental insults including certain common infections.

Miller reported in 1983 a series of bottle-fed infants with protracted diarrhoea of infancy syndrome. These unfortunates lacked sIgA, failed to digest their feeds and often died of malnutrition, unless given human milk [5]. "Holiday diarrhoea", usually attributed to exotic infection (in *Paris?*), could equally well be due to ingesting unaccustomed protein antigens in the food and water - not necessarily microbial in origin.

Failure of gut immunity simultaneously explains gut dysbiosis, some "leaky guts", digestive enzyme deficiency and food allergy/intolerance, and could partly explain Crohn's and coeliac diseases. If AFD is a pathway of major nutritional importance it could also help to explain the malabsorption of AIDS and other immunodeficient states. Theoretically the ideal treatment for all these conditions would be to replace the missing antibodies, not add in extra enzymes.

"LEAKY GUT"

Since failure of antigen exclusion in the gut leads to food allergy, several workers have conceived the obvious and attractive hypothesis that excessive gut permeability is a necessary factor in the pathogenesis of this condition. Such hyperpermeability could be caused by gut infection, gut trauma (including chemical and surgical trauma), or impaired production of mucus, digestive enzymes or antibodies. A thriving albeit somewhat arcane industry has grown up in interested laboratories measuring intestinal permeability to intact molecules. Note however that most current tests of "gut leakiness" offer only a very crude assessment since they measure uptake of inert *sugars*, not proteins, and are not necessarily relevant to food allergy because sugars use different absorption pathways. Considerable "leakiness" to intact proteins could have occurred long before these tests became positive.

Suggested reading

General

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Specific References

1) Freed DLJ. False-negative food challenges. *Lancet* (2002), **359**: 980-1.

2) Walker WA, Wu M, Isselbacher KJ, Bloch KJ. Intestinal uptake of macromolecules II. (1975) *Gastroenterology* **69**: 1223

3) Walker WA, Wu M, Isselbacher KJ, Bloch KJ, Intestinal uptake of macromolecules III (1975) *J Immunol* **115**: 854-61.

4) Green FHY, Freed DLJ, Antibody-facilitated digestion and the consequences of its failure. in (ed) Hemmings WA, *Antigen Absorption by the Gut*, MTP Press, Lancaster, 1978.

5) Miller V. Difficulties and values of breast milk for atopic babies. *in* Freed DLJ (ed), *Health Hazards of Milk* (1984) Bailliere Tindall, Eastbourne, 112-118.