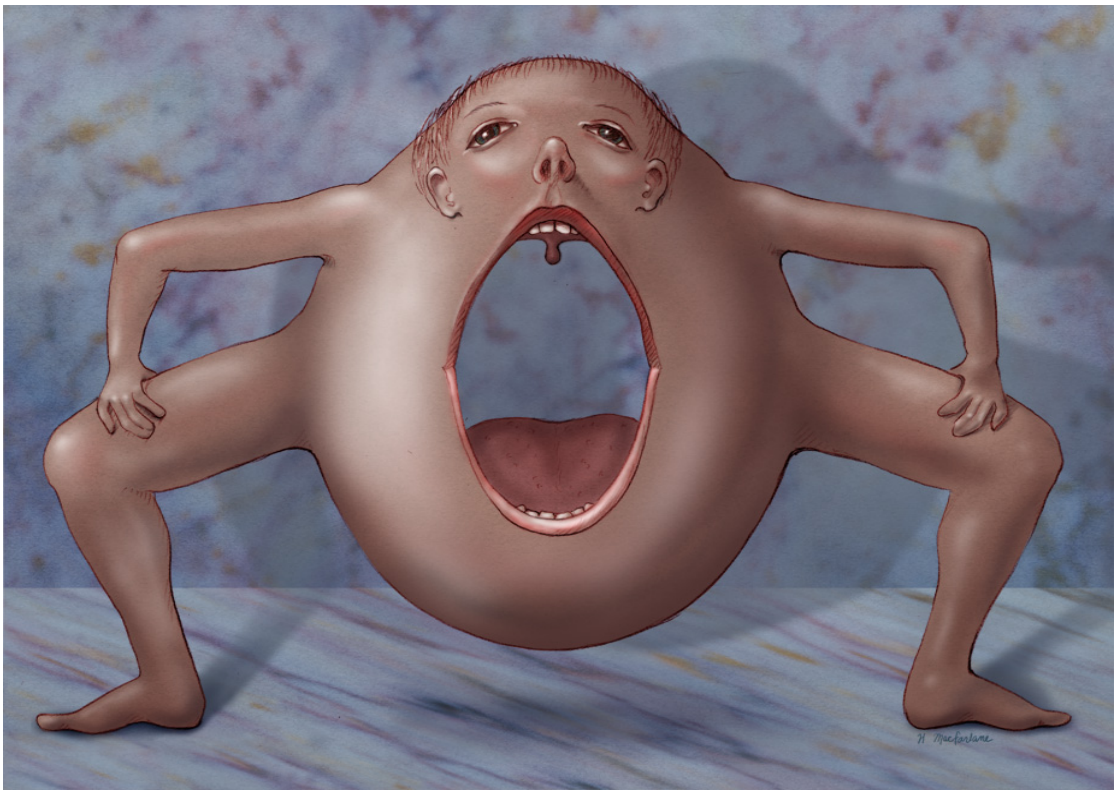
CHRONIC FRUSTRATED IMMUNE RESPONSES & REGULATION

THE IMMUNE SYSTEM'S NEARLY IMPOSSIBLE JOB. The immune system has a difficult enough job: to recognize anything that is foreign, and therefore potentially harmful, and arrange for its destruction. It must do this without recognizing 'self' in a destructive way. But in the gut the job is nearly impossible: it has to let in all sorts of foreign molecules (here called *food*, not *antigen*) without attacking them, and tolerate the immediate adjacency of as many as 10^{12} foreign organisms, bacteria and archaea and their viruses, and fungi, in a single milliliter of gut contents, while at the same time detecting and combatting a minority of dangerous organisms in the same milieu.

Remember that we are, topologically, toruses: our insides are really outside[1]:



As we start to understand the intricacies of the immune system in the gut we are going to find out, I think, how the immune system regulates itself in spite of its daunting job assignment. And that will lead us to entirely new ways of thinking about autoimmune and chronic inflammatory conditions, and maybe—finally—allow us to move from the current 'shotgun' approaches to treatment (i.e., suppressing everything) to a focused, antigen-specific, mechanism-based approach that will be more effective and much less risky.

Now here is the situation in the gut. ►There is normally abundant TGFβ in the submucosal Peyer Patches, and that favors the differentiation of Th0 cells into Treg. The resident dendritic cells here make IL-10, and that also favors Treg development. Thus these sites are rich in Treg cells, which

[1] This is Torusman, © by Helen Macfarlane.

is desirable considering the constant exposure to bacteria- and food-derived, non-pathogenic, potential immunogens coming through the M cells of the gut epithelium. If a peptide comes in unaccompanied by damage or inflammation, you probably don't want to make an immune response to it, so it's good to make Tregs. Also very common in Peyer Patches are Tfh that specifically drive B cells towards making IgA, so that the mucus layer nearest the epithelial cells that line the gut is, surprisingly, almost sterile. More than one group has suggested the Tregs can differentiate easily into such Tfh, and vice versa.

►However, the combination of TGFβ *and IL-6* has been shown to downregulate Treg and upregulate Th1 and Th17 (the CD4+ Th that makes IL-17 and is expanded by IL-23; both these cytokines are also common in areas of inflammation.[2]) IL-6 is produced by epithelial and other cells ►in response to stress or damage. This model links a lot of disparate observations. Normal commensal gut organisms have evolved to live in the lumen and not try to invade; the immune response to them, taking place in an environment dominated by TGFβ, is mostly by Treg at a steady level. When the innate response indicates a threat, it makes stress cytokines, IL-6 and others, and the response switches from Treg production to defensive Th1, Th17, or Th2.

The recognition of normal vs. abnormal organisms is doubtless mostly carried out by innate immunity via PRR that bind various PAMPs. These include the TLRs we discussed early on, and several other PRR systems, including one called NOD2. NOD2 detects muramyl dipeptide, a component of bacterial cell walls, and triggers cytokine production by activating NF-κB. There are also complex PAMP-recognizing assemblies called "inflammasomes." Some loci of innate immunity genes are linked to autoimmune disease risk. For example, the inflammasome NLRP1 is linked to the risk of vitiligo, a condition in which melanocytes are the target of a CTL attack.

CHRONIC FRUSTRATED IMMUNE RESPONSES. Any time the immune system is trying to get rid of a foreign antigen that it can't eliminate or encapsulate, it will remain chronically active and the tissues in which it takes place will become a metaphoric battlefield, as ravaged and scarred as the real thing. We discuss a few major examples here; you may be able to think of others. The CFIR term is not standard, but it is wrong to think of these conditions as autoimmune (though autoantibodies may eventually develop), "autoinflammatory" (which is sort of meaningless), or "autoaggressive" (ditto). At least CFIR is correctly descriptive.

INFLAMMATORY BOWEL DISEASE, IBD. This term includes Crohn disease (CD) and ulcerative colitis (UC). CD affects the small intestine, especially the terminal ileum, which leads into the ascending colon. The colon can also be affected. There are microabcesses in the wall of the intestine, generalized inflammation throughout the wall (so that fistulas can develop between the lumen and the peritoneum), and the disease process is 'patchy' with affected areas interspersed with healthy ones. The abscesses eventually become granulomas. UC is usually more superficial in the large intestine, and can erode the surface leading to bleeding. ►Both are thought to involve dysregulated immune responses, possibly to commensal bacteria. Genome-wide association studies (GWAS) have identified 163 loci associated with significant risk in IBD. 30 are specific for CD, and 23 for UC. A hundred and ten loci are in common between the two conditions. So there is a strong genetic component; but the environment and 'bad luck' also play important roles, since concordance in monozygotic twins is only 30-35% for CD, and 10-15% for UC[3].

[2] Veldhoen M, et al. 2006. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 24:179-89.

[3] Excellent review: B. Kohr, A. Gardet, and RJ Kavier. 2011. Genetics and pathogenesis of inflammatory bowel disease. Nature 474: 307-317.

One interesting model with support from human studies suggests that in some IBD patients, an early (genetic? post-infectious?) event is an increase in gut permeability so that certain secreted defensins, made by gut lining cells, are able to penetrate *back into* the tissues. There, acting as DAMPs, they stimulate macrophages to produce inflammatory cytokines, including IL-6.

Whatever the proximate causes, the outcome is that the patient has activated Th1, Th17, and Th2 against normal commensal organisms as if trying to rid the gut of these creatures; but they never can, so the inflammation goes on and on. This will eventually change the populations of microorganisms in the intestines (the microbiome) and that may further exacerbate the condition. Some workers have gone so far as suggesting 'fecal transplants' to replace the microbiome in IBD patients with one derived from healthy donors.

CELIAC[4] DISEASE. Also called gluten-sensitive enteropathy, this condition affects almost 1% of the world's population. In infants it presents as malabsorption, diarrhea, and failure to thrive; in adults it can be so nonspecific as to defy clinical diagnosis, with a variety of symptoms (osteoporosis, anemia, rash) secondary to malabsorption as the villi in the gut atrophy. The diagnostic gold standard is a small intestinal biopsy. Also useful in diagnosis is autoantibody to the gut endomysium, the lining that supports the smooth muscle layer; the specific antigen is tissue transglutaminase 2 (TG2). This enzyme turns glutamine into glutamic acid by deamidation, and in some people may, if it couples to but can't release digestion-resistant, glutamine-rich gliadin (wheat) peptides, ►inadvertently turn itself into a B-cell autoantigen by the 'foreign + self hybrid antigen' help mechanism (review that in the Type II Unit). Note, though, that ►it is T cell immunity to gliadin peptides that is responsible for the chronic inflammation. Ninety percent of people with this condition are HLA-DQ2, and most of the rest are HLA-DQ8[5]; but most HLA-DQ2 or 8 people don't get celiac disease, implicating other genetic and environmental factors. This is another example of a frustrated immune response: the body has decided that gluten is dangerous and must be destroyed, so the gut becomes the battleground on which this endless battle is waged. However, unlike IBD, there is a fix available: if the patient does not eat gluten (wheat, rye, barley) the symptoms will fade and the gut can revert to normal.

Some patients with poorly-controlled celiac disease will develop a skin condition called dermatitis herpetiformis. Biopsy shows that there is autoantibody in the skin to skin-specific transglutaminase 3 (TG3). There is evidence that *this* antibody actually causes the skin lesions. Perhaps not surprisingly, the antibody is IgA, which probably arose from the anti-TG2 of celiac by somatic hypermutation/epitope spreading.

CHRONIC BERYLLIUM DISEASE. This is a pulmonary inflammatory and fibrotic disease caused by exposure to inhaled beryllium dust. It is seen in miners (the largest mine is in Utah) and machinists, especially in the nuclear industry where Be alloys find many uses. Perhaps a million people have been exposed, and 15% of them are symptomatic. Inhaled Be can become covalently linked to various peptides and it is thought that this creates novel epitopes to which a Th1 (Th17 also?) response is made, and later a scarring Th2 response as well. Since the Be cannot be removed effectively by macrophages, the condition can become established and chronic even after a single inhalation exposure. It is strongly linked to HLA-DP alleles that have a glutamic acid at position 69 of the β chain (DPβE69). This creates a negatively charged pocket which could bind a Be+ coupled peptide, something non- DPβE69 alleles can't do.

[4] From Greek *koiliakos*, of the belly.
[5] The same HLA alleles that are associated with Type 1 diabetes, in which celiac is commonly seen.

PSORIASIS. There is some evidence that this chronic inflammatory condition of skin also involves an inappropriate, unregulated T cell response, in this case to normal skin organisms. It is associated with the allele HLA-Cw*06:02 (a class I gene, which in this case may be in linkage disequilibrium with a pathogenic Class II allele; or CTL may play a key role in the pathogenesis of psoriasis.) Interestingly, this allele is high in African Americans, who are in most studies at greater risk for psoriasis than Caucasians—but in sub-Saharan Africa the allele is high among Black people but prevalence of psoriasis is low, clearly suggesting environmental factors play a role, see below. Genome-wide association studies implicate HLA, a gene that affects skin cell differentiation, and IL-23 (a Th17 cytokine). A 2016 Phase 3 trial of the specific anti-IL-23 monoclonal antibody tildrakizumab showed startling effectiveness.

PERIODONTAL DISEASE. This chronic inflammatory condition is the major cause of tooth loss; prevalence in the US is about 8% in the young, 16% in the elderly. It is strongly associated with several bacterial species, but the association is complex and not well understood. The gingival crevice between the gum and the tooth root is not easily cleared by saliva, and yet cell-mediated immunity cannot reach there because it's outside the body; so it's a great place for bacteria to live, if it's not kept scrupulously clean. The condition has many of the characteristics of inflammatory bowel disease, including a shift from a TGFβ milieu to one with IL-6 and TGFβ. There is an association with rheumatoid arthritis (antibodies to citrullinated peptides are seen in both conditions, and they are both linked to smoking and environmental pollution.) Immunosuppressive therapy improves periodontitis, though it's rarely prescribed for it. Tocilizumab, a blocking monoclonal antibody to the IL-6 receptor (licensed for rheumatoid arthritis), has been reported to be effective in severe periodontal disease, but the side effects were worrisome.

DYSREGULATED T CELLS: WHAT'S GOING ON? There has been, over several decades, a true increase in many countries in the prevalence of both autoimmune and allergic diseases. Bad luck doesn't vary much, probably, and genetics changes but not that fast. So what has changed in the environment? This is a good time to think about this: we no longer live in the world we evolved for. We have been hunter-gatherers right from when we evolved from other apes. Is this our life-style today? Hardly. This disparity between our genetic heritage and our current environment has led to another, very visible, problem: the explosive increase in obesity.

HYGIENE HYPOTHESIS. First proposed by D.P. Strachen in 1989, this was an attempt to explain certain non-uniformities in the world-wide increase in allergy and asthma. Broadly, there has been *less* of an increase in: poor countries as compared to rich ones; equatorial versus northern countries; rural populations as opposed to urban; inner cities as opposed to rich neighborhoods; children of large families as opposed to only children. All of this suggested that exposure to environmental dirt and infections helped the immune system mature normally, while lack of such exposure might leave a child in an infantile state. There is good evidence that newborns start out with a Th2-dominated system which gradually balances out with Th1. So Strachen suggested that this might explain the increase in Th2- (and the Th2-like Tfh that drive B cells to switch to IgE) mechanism diseases. It's an appealing idea, but it ran into some trouble because the same clean rich people who should, by this explanation, be Th2-dominated are also at increased risk of Th1 diseases like ulcerative colitis and Crohn disease, multiple sclerosis and juvenile diabetes. How can the same group be Th2- and Th1-dominated at the same time? **The model was too simple**, not surprisingly since Treg were barely on the scene at that time.

A newer formulation[6] of the hygiene idea is the "**Old Friends Hypothesis.**" It says that certain harmless microorganisms—notably non-tuberculosis *Mycobacteria*, lactobacilli, and helminth worms— have been in humans so long that we rely on their presence to instruct our immune systems not to overreact against commensals or low-grade pathogens. Specifically, ►if you have adequate exposure to these old friends, you develop a balance between activation and regulation, driven by the right number of Treg. ►But if you have been old-friendless most of your life, you may have **too few Treg** and be too ready to make a strong Th1 or Th2 or even Th17 response to some organism that really isn't much of a threat (gut flora) or should be no threat at all (pollen), especially if you already have allelic variants of genes that predispose you to do so.

> **ASK YOURSELF:** Do you find the evidence, though incomplete, persuasive? If so, what do you recommend—more dirt for our kids? Move to the Equator? Feed them yoghurt? *Go eat worms?*

WHIPWORMS: IT'S WHAT'S FOR DINNER. A group of Iowa gastroenterologists decided that in Crohn Disease (CD) and Ulcerative Colitis (UC), Th1 are bad and Th2 might, by opposing Th1, be good. In 2005 it was still thought that the important event was Th1-Th2 "sibling rivalry." (Th1 do, to an extent, suppress Th2 development, and vice versa, as they perhaps compete for limiting growth factors.) How to effect a switch? Well, they reasoned, parasite responses are strongly Th2-dominated. So they recruited a group of quite ill CD patients and fed them some drinks of fresh pig whipworm ova. This was safe because the worms will only live a few days in the human gut. In a short, open-label study, the improvement in their patients' symptom scores was remarkable[7]. Subsequent work has shown that the mechanism of the effect was not Th2 suppressing Th1, but rather an impressive **increase in Treg** in the gut, which can suppress Th1, Th17, and Th2 responses[8]. It is fascinating to think that this could still take place in adults, and we are fortunate that although Treg are stimulated by recognizing their specific epitopes as are any other T cell, ►the effect of their suppression is *not* antigen-specific, so that many nearby activated T cells are down-regulated or do not differentiate into effectors.

The FDA has approved several trials of worm ova recently. This therapy is not as harmless as many non-scientific sites on the Web would have you believe; too strong a Treg response can suppress needed responses to viruses and bacteria. It *is* all about balance.

A new 5-second rule?

If it falls on the floor and is there for any length of time,
but you can tell by examining it for not more than 5 seconds
that it was once food,

your little brother may eat it.

Thanks to a class of astute high school students for devising this version of the rule.

[6] Rook GAW. 2012. Hygiene hypothesis and autoimmune diseases. Clin Rev Allergy Immunol. 42:5-15.
[7] Summers RW, et al. 2005. Trichuris suis therapy in Crohn's disease. Gut 54:87–90.
[8] A 2016 paper has identified a protein released by hookworms that is very immunosuppressive; it works by stimulating Tregs. *Science Translational Medicine* 26 Oct 2016. DOI: 10.1126/scitranslmed.aaf8807

Learning Objectives for
Chronic Frustrated Immune Responses & Regulation

1. Describe the factors that regulate the differentiation of Th0 cells in the Peyer's Patches to Th1, Th2, or Th17 versus into Treg cells.

2. Discuss the relative influence of environment and genetics on the risk for inflammatory bowel disease.

3. Discuss the pathogenesis of celiac disease, and the relative role played by antibody and T cells. Discuss the importance of HLA alleles in this condition.

4. Discuss immunological aspects of celiac disease that are non-autoimmune and autoimmune, and describe the mechanism whereby antibody to tTG2 is made.

5. Outline the Hygiene or Old Friends Hypothesis, and some of the observations that support it.

6. Discuss the idea that it may be possible to switch Th1/Th2/Th17 responses to Treg instead.

7. Discuss the mechanism of chronic beryllium disease.