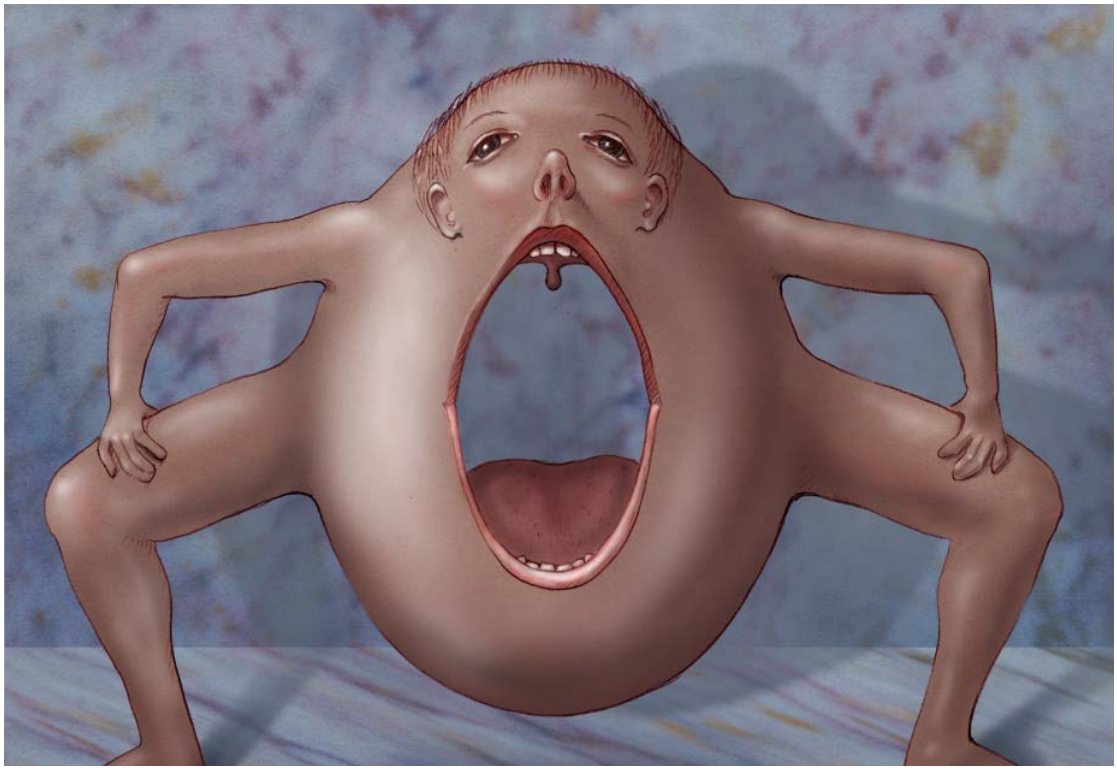
CHRONIC FRUSTRATED IMMUNE RESPONSES & REGULATION

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 their viruses, in a single millilitre 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's 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 is desirable considering the constant bombardment with bacteria- and food-derived, non-

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

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's 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; a nice touch, as they'd both prevent harmful responses and help protective ones.

►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; what Polly Matzinger famously called 'danger.' This is the first model that links a lot of disparate observations. Normal commensal gut organisms have evolved to live in the lumen and not try to invade; the innate response to them, producing mostly TGFβ, would be at a low, steady level to which we have adapted by setting a cut-off, above which we switch from Treg production to the defensive Th1, Th17, or Th2.

The recognition of normal organisms is doubtless mostly carried out by innate immunity via PRR that bind various PAMPs. These include the TLRs we discussed right at the beginning, 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.

ASK YOURSELF: Suppose you didn't make enough Treg in your gut, or in your lung, or skin: *what might happen?*

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 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).

INFLAMMATORY BOWEL DISEASE, IBD. This term includes Crohn's Disease (CD) and ulcerative colitis (UC). Crohn's affects the large and small intestine, especially the terminal ileum. 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. 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, perhaps to commensal bacteria.

Genome-wide association studies (GWAS) have identified 71 loci associated with significant risk in Crohn's, and 47 loci in UC. Twenty-eight loci are in common between the two conditions; ►NOD2 is one of them. Together they predict 23% of the risk in CD and 16% in UC, so there is

[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.

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].

One interesting model with support from human studies suggests that in some IBD patients, an early (genetic?) 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 cytokines, including a lot of 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 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 hallmark is antibody to the gut endomysium, the lining that supports the smooth muscle layer; the specific antigen is tissue transglutaminase 2 (TG2). This enzyme makes protein crosslinks through glutamines, 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 illicit 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; 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 reverts 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). Perhaps not surprisingly, the antibody is IgA, which probably arose from the anti-TG2 of celiac by epitope spreading. There is evidence that this antibody actually causes the skin lesions.

BERYLLIOSIS. Fortunately not common, 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.) 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. Since the Be cannot be removed effectively by macrophages, the condition can become established and chronic even after a single inhalation exposure.

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

PSORIASIS. There is some evidence that this chronic inflammatory condition of skin also involves an inappropriate, unregulated T cell response to normal skin organisms. It is associated with the allele HLA-Cw*06:02 (a class I gene, which may be in linkage disequilibrium with a pathogenic Class II allele.) Interestingly, this allele is high in Blacks, who are in most studies at greater risk for psoriasis than Caucasians—except in sub-Saharan Africa, where the allele is high but 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.

DYSREGULATED T CELLS: WHAT'S GOING ON? There has been, over several decades, a true increase in many countries in 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 genes 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; slums 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-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's 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 had barely made it onto the scene.

A newer formulation[4] 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 were old-friendless at a critical developmental stage, perhaps between 0 and 2 years, 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 is no threat at all (pollen), especially if you have a genetic predisposition to do so.

I find the evidence, though incomplete, persuasive. So what do we do—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's Disease (CD) and Ulcerative Colitis (UC), Th1 are bad and Th2 might, by

[4] Rook GAW, et al. 2004. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin. Immunopathol. 25: 237-55.

opposing Th1, be good. In 2005 it was still thought that the important event was Th1-Th2 "sibling rivalry," as Treg were just being unraveled. How to effect a switch? Well, parasite responses are strongly Th2-dominated, to generate IgE. 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[5]. Subsequent work has shown that the mechanism of the effect was not Th2 suppressing Th1, but rather a remarkable increase in Tregs in the gut, which can suppress both Th1 and Th2 responses. 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.

[5] Summers RW, et al. 2005. Trichuris suis therapy in Crohn's disease. Gut 54:87–90.

CAN WE PUSH T CELL POPULATIONS AROUND? Let's consider a little basic science that reveals a lot. First, in a highly artificial system involving T cell clones, single defined epitopes, and purified antigen-presenting cells, it has been found that, all things being equal, lowering the affinity of the tripartite MHC-peptide-TCR reaction shifts T cell responses from Th1 to Th2. Second, in human rheumatoid arthritis, as well as in several clever animal models, Th1 cells directed against a defined collagen epitope are pathogenic; Th2 cells are not, and in fact, oppose Th1 cell effects.

Here's an example of that: Mice that were transgenic for human HLA-DR-1 (an arthritis-associated allele) were immunized with a peptide from collagen II (see box.) They developed a strong Th1 response and symptoms and histopathology reminiscent of rheumatoid arthritis. ►But if immunized with the analog (an **Altered Peptide Ligand**, APL) in which two of the MHC-binding anchor peptides are altered (F→N, E→D), lowering affinity for MHC a hundred-fold, a Th2 response (IL-4, not IFNγ) was obtained. Immunizing with the analog and the collagen II peptide together *prevented* arthritis[6]. This result has been confirmed in HLA-DR4-transgenics[7].

collagen II
*GEBGIAG***FKGEQGPK***GEBGPA*

analog
*GEBGIAG***NKGDQGPK***GEBGPA*

So in theory, and in mouse practice, and in a lot of labs in Big Pharma, altered peptide ligands (APL) may be the Next Big Thing. Will they work once disease is established? If you have already got tissue damage due to an autoaggressive Th1 response, can you still switch things over to Th2 (or more likely, and the focus of most of the research now, Treg) and alleviate symptoms while preventing disease progression? Time will tell. The very appealing thing about this approach, even when compared to highly effective cytokine antagonists, monoclonals, and immunomodulators, is the focus on a specific antigenic response, rather than global immunosuppression. Will APLs become the Magic Bullets of autoimmunity?

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 child may eat it.

[6] Myers LK, et al. 2004. An Analog Peptide that Suppresses Collagen-Induced Arthritis. Am J Med Sci 2004,327: 212–216.

[7] Boots AM et al. 2007. Identification of an altered peptide ligand based on the endogenously presented, rheumatoid arthritis-associated, human cartilage glycoprotein-39(263–275) epitope: an MHC anchor variant peptide for immune modulation. Arthritis Research & Therapy 2007, **9:**R71.

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.

4. Outline the Hygiene or Old Friends Hypothesis.

5. Discuss the idea that switching Th1 to Th2 responses may be a way to treat certain autoimmune diseases.

6. Define altered peptide ligands, and comment on their possible uses in the future.