The Impact of Nutrition on Autoimmune Disease

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Abstract

Autoimmune diseases are increasingly prevalent in Western society and carry a substantial burden in terms of health and economic costs. The cause of autoimmunity was long thought to be primarily genetic, but recent research has unveiled potential mechanisms by which lifestyle factors, including diet, can influence autoimmune pathology. The presence of lymphocytes that recognize self antigen in the mucosal membrane of the intestines and in obese adipose tissue suggest potential sources for the activated, self-reactive cells that drive the attack on self tissue characteristic of autoimmunity. While there is scant research directly addressing the impacts of nutrition on autoimmunity, the influence of dietary factors on the homeostasis of the mucosal immune system, the gut flora, and the metabolic regulation of immunity present routes by which diet can promote the inflammation necessary for the activation of self-reactive lymphocytes, and subsequent autoimmune pathology.

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Introduction

Autoimmunity is the response of the immune system to self-antigens, and is increasingly prevalent in industrialized countries, affecting roughly 50 million people in the United States (The Cost Burden of Autoimmune Disease: The Latest Front in the War on Healthcare Spending, 2011). It is marked by a loss of tolerance to self tissue and subsequent tissue destruction, indicating a breakdown in central or peripheral tolerance that leads to an autoimmune attack. Loss of central tolerance is rare because of its powerfully destructive effects, and leads to multi-organ autoimmunity and high levels of systemic inflammation, as seen in mouse models with mutations in the autoimmune regulator (AIRE). Despite the checks and balances in place in lymphocyte development that prevent the escape of autoreactive cells, a system of peripheral tolerance is necessary to prevent activation of self-reactive lymphocytes outside of the primary lymphoid organs. This system makes heavy use of T regulatory cells (Tregs) that suppress the activation of other lymphocytes and antigen-presenting cells, and many Treg cells recognize self antigen but display an inactivated and regulatory phenotype. In general, self-reactive lymphocytes become anergic in response to antigenic stimulation without costimulation or the presence of inflammatory signaling, but this leaves open the possibility of stimulation in the presence of inflammation and costimulation, which could potentially lead to the activation of autoreactive B and T cells. Notably, autoimmune diseases are more likely to manifest following infection, and have high comorbidity with a number of other inflammatory diseases including obesity, the metabolic syndrome, and allergic disease.

It is increasingly evident that lifestyle factors such as sleep status, stress, and nutrition can influence immune function, though this body of evidence is emerging and many questions remain unanswered. The food we eat is highly variable and contains a nearly unlimited scope of potentially bioactive compounds, making the influence of nutrition on immunity an intriguing line of research. The effects of micronutrient deficiencies on immune function are fairly well established, but the effects of factors such as carbohydrate content, fatty acid composition (e.g.
saturated fats, n-3/n-6 ratio, etc.), fiber, and potentially immunogenic plant compounds have less well-established evidence bases. It is an exciting time in this field of research, as diverse lines of inquiry are beginning to converge to paint an intricate and complex picture of the influences of nutrition on immunity. The development of techniques for the rapid profiling of microbial communities allows us insight into the impacts of the microbiota on our physiology, while the elucidation of the metabolic pathways governing leukocyte activation provides a mechanistic link between systemic energy balance and the maintenance of tolerance. Clues from celiac disease, one of the most well-researched autoimmune conditions, shine light on potential mechanisms behind a loss of tolerance in the mucosal immune system, which lies as the primary interface between our immune systems and the environment.

Because there is scant research on the direct impacts of nutrition on autoimmunity, any attempt to draw causal connections between these two must, at this point, be mostly indirect. Given that autoimmunity is precipitated by a loss of tolerance and the presence of chronic inflammation, this paper will attempt to draw connections between the impact of nutrition on inflammation and tolerance, and then connect these to the development of autoimmunity. Autoimmunity is well known to be an inflammatory disease, and the necessity of a loss of tolerance is not controversial, so the main focus will be on nutrition and the development of an inflammatory, non-tolerogenic environment. I propose that nutrition can set the stage for autoimmunity through a disruption in the homeostasis of mucosal immunity and energy balance, both of which may activate autoreactive lymphocytes via inflammatory signaling.
Mucosal Immune System

• Overview
• Intraepithelial lymphocytes
  o Natural vs. induced IELs
  o Tδ T cells
  o TCRαβ T cells
  o Induced IELs
• Leukocytes in the LP
  o General characteristics
  o Tregs
  o Th17 cells
  o DCs
The mucosal immune system is the largest interface between our internal and external environments – the small intestine alone has an approximate surface area of 400 m² – and is responsible for the elimination of a diversity of pathogens while maintaining tolerance to large populations of commensal bacteria in the large intestine and the continuous flow of food-derived particles that pass through the digestive system every day (Murphy, Travers, & Walport, 2011). While this system includes everything from the epithelia of the urogenital tract, the respiratory tract, and the mammary glands to that of the digestive tract, the mucosal immune system of the intestines is of primary importance when considering the impact of nutrition on immunity. The presence of a variety of self-reactive lymphocytes poised for activation makes it an intelligent starting point in the hunt for a potential trigger, and source of, autoimmune pathology.

In brief summary of its main anatomical features, beneath the intestinal epithelia lies a web of connective tissue called the lamina propria (LP), which contains local secondary lymphoid organs called Peyer’s patches and isolated lymphoid follicles that drain into mesenteric lymph nodes; together, these are referred to as the gut associated lymphoid tissue (GALT). Specialized epithelial cells called microfold (M) cells are scattered throughout the epithelia, and serve to transport luminal contents into the LP, where they are sampled by local dendritic cells that generally promote tolerance to food particles and constituents of commensal bacteria (Murphy et al., 2011). The intestinal mucosa is home to a wide variety of leukocytes, including specialized populations of lymphocytes that are site-specific. Together, these cells coordinate a fine balance between tolerance and reactivity, and in healthy mucosa inflammation is largely absent, demonstrating the effectiveness of local homeostatic control given the large numbers and volume of potential pathogens passing through the intestines every day, and the presence of $10^{12}$ bacterial cells/ml in the colon contents (Murphy et al., 2011).
**Intraepithelial lymphocytes**

Cells residing in between epithelial cells, called intraepithelial lymphocytes (IELs), are the first line of cellular defense against pathogens, and are present in large numbers (approximately 10-15 for every 100 epithelial cells; Murphy et al., 2011). This population of cells generally comprises mature TCR-γδ and TCR-αβ CD8 T lymphocytes that can be further divided into “natural” and “induced” IELs, and each subset exerts unique effects on the local environment (about 90% of IELs are CD8+; Cheroutre, Lambolez, & Mucida, 2011; Murphy et al., 2011). Natural IELs are TCR-γδ or TCR-αβ cells that do not express CD4 or CD8αβ, but may express the CD8αα homodimer, a CD8 configuration that is repressive instead of costimulatory; these cells are present in relatively large numbers in immature epithelia but decrease with age (Cheroutre et al., 2011). Cells bearing TCR-γδ or TCR-αβ and the CD8αα homodimer are also referred to as type b IELs, and these cells can express high levels of NKG2D, which binds the non-classical MHC molecules MIC-A and MIC-B expressed in epithelial cells in response to injury or stress; type a IELs are conventional TCRαβ CD8αβ T cells, also referred to as “induced” IELs (Cheroutre et al., 2011; Murphy et al., 2011). Natural IELs undergo alternative selection in the thymus, and migrate to the epithelium as CD4- CD8- TCRαβ+/γδ+ naïve T cells; CD8αα expression is induced locally in the intestinal epithelium (Cheroutre et al., 2011).

TCRγδ T cells are implicated in the regulation and repair of epithelial structure in response to inflammatory damage via the secretion of keratinocyte growth factor (KGF) and TGF-β, which promotes epithelial integrity and is generally tolerogenic (Cheroutre et al., 2011). These cells can express NKG2A, an inhibitory receptor that increases TGF-β in the context of TCR stimulation, and can suppress expression of NKG2D in CD8αβ TCRαβ induced IELs (Cheroutre et al., 2011). They maintain cytotoxic activity, show a restricted TCR repertoire, and may be capable of binding self and non-self antigens, potentially without MHC presentation (Atarashi, Umesaki, & Honda, 2011; Cheroutre et al., 2011). A subset of γδ T cells produce IL-
17 and express RORγt and IL-23R in response to infection, complimenting the activity of Th17 cells and may be implicated in EAE; γδ T cells have been demonstrated to promote Th17 cell expansion and limit Treg responses in the CNS of mice with EAE (Atarashi et al., 2011). γδ T cells also have some innate characteristics, such as the expression of TLR1 and TLR2, and are sometimes viewed as a bridge between the innate and adaptive immune systems (Murphy et al., 2011).

TCRαβ natural IELs are also implicated in the maintenance of epithelial integrity. These cells are often self-reactive and display an antigen-experienced and activated phenotype, expressing high levels of granzyme, Fas ligand (CD95 ligand), CD69, and CTLA-4, but are immunologically quiescent, and bind thymus leukemia antigen (TLA) on epithelial cells via CD8αα (Cheroutre et al., 2011; Honda & Littman, 2012). Lymphopenic mice receiving TCRαβ natural IEL transfers show resistance to experimentally-induced colitis, providing indirect evidence for their role in maintaining epithelial integrity, though direct evidence is lacking (Honda & Littman, 2012). The presence of self-reactive TCRs in these natural IELs is not typically problematic, but their high barrier of activation can be overwhelmed with excessive inflammatory signaling and the production of IL-15, suggesting a possible source of activated, self-reactive T cells in autoimmune disease and highlighting the importance of maintaining mucosal homeostasis (Cheroutre et al., 2011).

Induced IELs (TCRαβ CD8+/CD4+) undergo traditional positive and negative selection in the thymus and populate the epithelium in increasing numbers with age. After leaving the thymus and encountering their antigen in epithelial tissues, they reenter the circulation and return to the epithelium via ligation of CD103 (αE integrin) and β7 integrin with E-cadherin on the basolateral surface of enterocytes; the expression of αEβ7 integrin is imprinted by mucosa-specific CD103+ CCR7+ dendritic cells (Cheroutre et al., 2011; Murphy et al., 2011). CD8+ induced IELs have traditional cytotoxic effector function and are important for protection against viral infection, but have different costimulatory requirements, requiring CD40L ligation and indicating a second level of regulation in the mucosal environment that is not necessary in other peripheral tissues (Cheroutre et al., 2011). CD8 induced IELs,
however, are also implicated in disease, and are responsible for tissue damage in celiac disease following ligation of MIC on epithelial cells via NKG2D, as discussed below. CD4+ induced IELs also accumulate with age and are assumed to have a regulatory role due to their production of IL-10 and their ability to suppress Th1 responses (Honda & Littman, 2012). In some cases CD4 induced IELs can upregulate CD8α and adopt a “cytotoxic yet regulatory” IEL phenotype, suggesting another layer of tolerogenic regulation that diverts differentiation away from inflammatory T helper responses (Cheroutre et al., 2011).

Lamina propria leukocytes

The lamina propria, in contrast to the epithelium, contains an approximate 3:1 ratio of CD4 to CD8 T cells, and these cells localize via the expression of α4β7 integrin (Murphy et al., 2011). CD4 T cells are present in their usual effector subsets, i.e. Th1, Th2, Th17 and Treg, as appropriate for a tissue that is tasked with defense from many varieties of pathogens. The CD4 T cells in the LP are predominantly antigen-experienced effector cells displaying cell-surface activation markers such as CD45RO and the CCL5 receptor, but respond poorly to antigen stimulation and secrete large amounts of the cytokines IFN-γ, IL-5, IL-17, and IL-10, even in the absence of inflammation (Murphy et al., 2011). The activated nature of these cells reflects continual stimulation by commensal bacteria, especially in the colon, where the large majority of Th17 cells reside, and reflects a careful balance between bacterial defense and an anti-inflammatory environment. The sheer volume of effector T cells in the intestine would suggest the presence of inflammation, but the activity of Th1, Th2 and Th17 cells is balanced by the presence of large numbers of Treg cells that serve to maintain oral tolerance and an appropriate response to commensal bacteria (Murphy et al., 2011).

Treg cells display multiple phenotypes in the intestinal LP, and deserve special attention because of their important role in mucosal homeostasis. As with IELs, Treg cells are present in “natural” and “induced” subsets, reflecting the location of their maturation. Natural Tregs migrate to the LP via the α4β7 integrin
after maturation in the thymus, while induced Tregs enter the intestines as naïve T cells and differentiate in response to TGF-β and retinoic acid (RA) produced by dendritic and epithelial cells (Marc Veldhoen & Brucklacher-Waldert, 2012). The transcriptional signature of Tregs varies in response to the type of infection: Th1 cytokines upregulate Tbet, allowing Tregs to localize to sites of Th1-based inflammation via CXCR3; Treg expression of interferon regulatory factor 4 (IRF4) allows the expression of ICOS and CCR8 and the suppression of Th2 responses; and STAT3 activation allows Treg homing to tissues undergoing Th17 responses via, for example, CCR6 (Atarashi et al., 2011; Honda & Littman, 2012). This variety in effector responses suggests a functional plasticity in Treg cells that allows appropriate responses to different inflammatory environments in real time. A key role of LP Tregs is the expression of IL-10, which has generally tolerogenic effects on effector cells, though not all FoxP3+ T cells in the intestines produce IL-10. IL-10 suppresses γδ T cell proliferation, prevents unnecessary activation of STAT6 in myeloid cells, and acts in a feed-forward loop to further activate Treg cells via IL-10R binding and subsequent STAT3 activation; the loss of STAT3 or IL-10R in mice interferes with their ability to suppress Th17 responses (Honda & Littman, 2012). A population of IL-10-producing FoxP3+ Treg cells, called T\(_r\)1 cells, is also present in the LP, and differentiate in response to IL-10 and IL-27, which upregulates the aryl hydrocarbon receptor (AhR), a key regulator of Treg responses (discussed below; Atarashi et al., 2011). Together, Treg cells localize to sites of inflammation and act as a negative regulator of inflammatory responses. Loss of Treg function and IL-10 secretion is associated with spontaneous colitis in mice in the presence of intestinal bacteria, underlining the importance of Treg function in mucosal homeostasis (Honda & Littman, 2012).

Th17 function is also critically important for intestinal health, and Th17-mediated inflammation is implicated in a variety of inflammatory diseases, including EAE, colitis, rheumatoid arthritis, Crohn’s disease, and psoriasis (Honda & Littman, 2012). The protective functions of Th17 cells include the induction of antimicrobial peptides from epithelial cells via IL-22 production, neutrophil recruitment via IL-17, and B cell class switching and germinal center formation, among others (Honda &
Th17 differentiation is driven by TGF-β and RA, but, unlike Treg cells, requires the presence of IL-6, hinting at their role in inflammatory responses. A subset of “natural” Th17 cells that matures in the thymus has also been discovered, and these cells display a restricted TCR repertoire that is potentially self-reactive, speaking to the suggested role of Th17 cells in inflammatory disease (Honda & Littman, 2012). In extraintestinal tissues Th17 cells are present at low numbers (<1% of CD4 T cells), but are enriched in the intestinal mucosa, where ~20% of CD4 T cells in the small intestine and 10% in the large intestine are IL-17+, in agreement with the well-documented role of intestinal flora in Th17 differentiation (Atarashi et al., 2011; Honda & Littman, 2012); the details of the impact of the microbiota on T cell differentiation are discussed below.

Dendritic cells in the mucosa are unique in that they are tolerance-promoting in the absence of infection and are present in several different subsets. DCs expressing CD11b (αM-integrin) and CCR6 that are CD8α- reside in Peyer’s patches and produce IL-10 in response to the uptake of antigens from M cells; these cells reside directly under the epithelium and can become pro-inflammatory in the presence of infection (Murphy et al., 2011). CD103+ (the receptor for E-cadherin) DCs secrete RA constitutively and drive Treg induction via RA and TGF-β, and suppress Th17 activity via RA, thus acting redundantly to suppress Th17 (and Th1) function via RA and Treg promotion (Murphy et al., 2011; M Rescigno & Sabatino, 2009). Suppression of inflammatory responses is also driven by epithelial-derived signals such as thymic stromal lymphopoietin (TSLP), which prevents IL-12 production in and subsequent Th1-promotion by LP DCs, and TGF-β and RA, which drives conversion of CD103- DCs into CD103+ tolerogenic DCs (M Rescigno & Sabatino, 2009). Th17 responses are promoted by another subset of DCs that are CD11b*TLR5*, a class of DCs that are unusual in their expression of TLR5 given the low levels of TLR expression in most intestinal DCs; these synergize with CD70*CXC3R1* DCs that express ATP receptors and strongly drive Th17 differentiation in response to treatment with ATP in a TLR-independent manner (M Rescigno & Sabatino, 2009). The interactions between DCs and CD4 T cells in the
The mucosal epithelium is a complex and highly regulated compartment of the immune system that, under physiological conditions, maintains a tight balance between pathogen elimination and tolerance to food particles and commensal bacteria; while there are multiple layers of regulation in place to prevent pathogenesis, centering around Treg cell induction, suppression of inflammatory T cell development via IL-10, TGF-β and retinoic acid, and DC-induced non-reactivity, a breach in the tolerogenic environment could lead to pathology. The presence of self-reactive T cells that rely on suppressive cytokine signaling to maintain quiescence suggests that inflammatory events could lead to activation of these cells and, potentially, autoimmunity, as seen with the involvement of Th17 cells in the
inflammatory diseases discussed above. Evidence that CD4 T cells have functional plasticity in their effector function and type (e.g. Treg to Th17 and Th17 to Th1 differentiation; Hooper & Macpherson, 2010) indicates that inflammation may also divert regulatory cells to inflammatory lineages, contributing to a loss of tolerance. The inflammatory bowel diseases (Crohn’s disease and ulcerative colitis) and CD are examples of the potential consequences of a breakdown in mucosal barrier function, and provide insight into the potential mechanistic underpinnings of autoimmune disease. While an overview of each of these conditions is beyond the scope of this paper, Celiac disease affords an example of a disease specifically driven by a food constituent (gluten) that potently affects immune function in the mucosal environment, and is covered in the following section.
Celiac Disease

- Introduction
- Epidemiology
  - Prevalence
  - Haplotype
- Pathogenesis
  - Gliadin, the environmental trigger of CD
  - Zonulin and Tight Junctions
  - Initiation of the immune response
  - Development of adaptive immunity
  - Perpetuation of inflammation
- Implications for other autoimmune conditions
- Conclusion
Celiac disease (CD) is an inflammatory disease of the small intestine that affects 1 in 133 people in the US and is characterized by a severe reactivity to gluten that manifests itself in autoimmune destruction of the villi of the small intestine (Fasano et al., 2003). CD was first “discovered” by the British physician Samuel Gee in 1877, who described it as a kind of “chronic indigestion” that affected mainly children between the ages of one and five; he also surmised that “errors in diet may perhaps be the cause.” (Fasano, 2009) We know now that CD is initiated by the ingestion of gliadin - part of the protein gluten that is found in wheat, rye, and barley - and that gliadin can have a potent effect on the integrity of the small intestinal epithelium, leading to increased immune reactivity, inflammation, and eventual autoimmune pathology targeting the tissue transglutaminase (tTG; a deaminating enzyme) found in small intestinal epithelial cells. Multiple genetic risk markers for Celiac have been identified, the most prominent of which are the human leukocyte antigen (HLA) class II alleles HLA-DQ2 and HLA-DQ8. These molecules appear to have enhanced binding affinity to certain gliadin-derived peptides, allowing for increased responsiveness of lymphocytes after presentation by antigen-presenting cells (APCs). The presence of HLA-DQ2 or HLA-DQ8, however, is not enough to ensure the development of CD, and research has yet to identify the other factors necessary for the disease to progress.

Research seeking to identify the mechanistic basis of CD has uncovered previously unknown regulatory pathways of the tight junctions (TJs) that hold epithelial cells together in the small intestine. The protein zonulin, identified first from *Vibrio cholerae*, the pathogen responsible for cholera, is a potent modulator of TJ integrity and shows greatly enhanced expression in CD and other autoimmune conditions. Zonulin release is triggered by the binding of a gliadin peptide to CXCR3 – a chemokine receptor previously though to be present only on cells of the immune system – on small intestinal epithelial cells. After TJs are loosened via zonulin signaling, gliadin peptides penetrate the epithelial wall into the lamina propria, where the mucosal immune system lies in a state of alert quiescence. APC processing of gliadin and gliadin/tTG complexes leads to the maturation of effector
lymphocytes targeting them, and autoimmunity results from the production of antibodies targeting tTG, which is a hallmark of the disease. The end result of this process is an intensely inflamed small intestine, villous atrophy, and intestinal malabsorption. A number of other conditions are also more common in CD patients, including type 1 diabetes and other autoimmune diseases, suggesting that steps in the pathogenesis of CD may overlap with those of other conditions.

Epidemiology

The prevalence of CD remained debatable through the late 1990s, when new plasma tests for serum anti-gliadin antibodies (AGA), anti-endomysial antibodies (EMA), and anti-tissue transglutaminase antibodies (anti-tTG) became available for widespread use (Fasano et al., 2003). An international effort to establish the true incidence of CD culminated in a 2003 publication by Fasano, et al., using the newly available screening technologies on a large sample of U.S. patients in 32 states. This study recruited first- and second-degree relatives of CD patients, patients showing symptoms of CD, and those with related complications (the “at-risk” group; 9,019 subjects), and used blood donors, schoolchildren and patients from outpatient clinics in for routine check-ups (“not-at-risk” group; 4,126 subjects) as a control.

The diagnostic criteria for CD in this study required either EMA-positive serological finding with a CD-positive intestinal biopsy or EMA-positive sera with an HLA haplotype consistent with CD. With these criteria, the authors established the prevalence of CD as 4.55% in first-degree relatives of CD patients (95% CI: 3.90-5.20%), 2.59% in second-degree relatives of CD patients (95% CI: 1.80-3.60%), 1.47% in symptomatic adults (95% CI: 0.97-2.10%), 4.00% in symptomatic children (95% CI: 2.99-5.20%), and 0.75% in not-at-risk subjects (95% CI: 0.50-1.10%). This level of reported prevalence was unprecedented, but is now generally accepted as accurate. Previous diagnostic criteria included a biopsy to confirm villous crypt atrophy in the small intestine, as well as signs and symptoms associated with the disease, and the success of a gluten-free diet (Fasano, 2009). These diagnostic criteria had led to an estimated CD prevalence as low as 1 in 10,000 in the general population.
CD is known to be strongly correlated to a patient’s HLA haplotype, with 95% of CD patients possessing the HLA-DQ2 allele, and remaining patients usually possessing the HLA-DQ8 allele (both are variants of the HLA type II gene; Sapone et al., 2012). The prevalence of HLA-DQ2 in Caucasian individuals, however, is approximately 30%, and the risk effect of the HLA-DQ2/8 genes is between 36 and 53% percent, implying that they are necessary but not sufficient for the development of CD (Anna Sapone et al., 2012). This genotypic reliance is supported by the observation that specific alterations in the HLA molecules encoded by the HLA-DQ2/8 genes change the binding of gliadin fragments in the intracellular and extracellular space, effecting the immune response to these epitopes, as discussed below.

**Gliadin, the environmental trigger of CD**

It is commonly accepted that the development of autoimmune disease requires both a genetic predisposition (in all likelihood the HLA-DQ2/8 alleles in CD) and an environmental trigger. In the case of CD, one environmental trigger is gliadin, a protein that complexes with the glutenin found in wheat, rye, and barley to form gluten (glutenin is also toxic for CD patients; Fasano, 2011). Gluten belongs to a family of proteins called “prolamines” that are notable for the unusually high content of the amino acids glutamine and proline (Fasano, 2011). The high glutamine content of gluten makes it a good substrate for tissue transglutaminase (tTG), an enzyme found in epithelial cells of the small intestine that converts positively charged glutamine residues into negatively charged glutamate (Nikulina, Habich, Flohé, Scott, & Kolb, 2004). While most food proteins are substantially digested by proteases present in the small intestine (the brush border enzymes), it is more difficult for these proteases to cleave after proline residues present in prolaminres, generating unusually long, undigested peptide sequences (Fasano, 2011; Schuppan, Junker, & Barisani, 2009). There are at least 50 bioactive epitopes in gluten peptides that exert a range of physiological effects, including immunomodulatory, cytotoxic, and gut-permeability-enhancing actions, as shown below (Fasano, 2011). Specific fragments of α-gliadin that have physiological effects,
known by their order in the primary structure of the protein, include: peptide 31-43 (cytotoxic effects); peptide 57-89 (immunomodulatory); peptides 113-130 and 151-170 (bind CXCR3; discussed below), and peptide 261-277 (IL-8-releasing) (Fasano, 2011). Peptide 57-89, a 33-mer, contains 6 overlapping immunogenic epitopes that can bind HLA-DQ2 after deamidation by tTG, and is considered a “superantigen” in celiac pathogenesis (Schuppan et al., 2009).

The peptides 113-130 and 151-170, marked in blue in the figure, are particularly important in the pathogenesis of CD because they have been shown to bind the chemokine receptor CXCR3 on small intestinal epithelial cells; the discovery of this interaction in 2008 was the first time CXCR3 expression had been observed in the intestinal epithelium (Lammers et al., 2008). CXCR3 (also known as CD183) is also present on plasmacytoid dendritic cells and is bound by the chemokines CXCL9, CXCL10, and CXCL11, which are released by T cells; on natural killer cells; on malignant B cells in chronic lymphoproliferative disorders; and on activated T cells (Murphy et al., 2011). In activated T cells, CXCR3 is associated with the recruitment of the Th1 subset and subsequent immune-mediated damage in viral and bacterial infections and autoimmune disease (Lammers et al., 2008).
CXCR3 is also present on some TCRαβ, CD8αβ, CD103+ T cells that home specifically to the small intestine (Lammers et al., 2008). Lammers, et al. demonstrated in 2008 that the binding of gliadin epitopes to CXCR3 induced the release of the signaling protein zonulin via a MyD88-dependent mechanism.

Zonulin and Tight Junctions

Zonulin is a ~47 kDa protein that participates in primate innate immunity by regulating the permeability of intestinal epithelia to luminal contents, and is overexpressed in CD, type 1 diabetes, and some other autoimmune conditions (Tripathi et al., 2009). Drago, et al. demonstrated in 2006 that zonulin reduces the transepithelial electrical resistance (TER; a measure of permeability) in celiac and non-celiac small intestinal epithelium after exposure to gliadin peptides. A mechanism proposed by Fasano (2011), begins with gliadin binding to CXCR3; subsequently, via MyD88-dependent signaling, zonulin is released from epithelial cells and binds to protease-activated receptor 2 (PAR2), which transactivates endothelial growth factor receptor 2 (EGFR2), triggering the production of inositol triphosphate and diacylglycerol by phospholipase C. Protein kinase C, after activation by DAG or IP3-mediated Ca2+ release, phosphorylates proteins involved in the formation of tight junctions, leading to their disassembly. This pathway is summarized in the figure below (Fasano, 2012).
The permeability of mucosal epithelia to luminal contents is tightly regulated, mainly via the modification of tight junctions. In the small intestine, molecules can cross the epithelium via transcellular (through cells) or paracellular (between cells) pathways; the transcellular pathway is mediated by transcytosis and transporter or pore proteins and is important for the absorption of sugars, peptides, lipids, vitamins and minerals (Suzuki, 2012). The paracellular pathway allows the passive diffusion of some solutes, ions, and water, and the magnitude of solute movement via the paracellular pathway is known as permeability and is measured by TER or the uptake of certain dietary macromolecules (Suzuki, 2012). The TJs between cells that largely determine paracellular permeability are made up of protein complexes that include claudins, occludin, junctional adhesion molecule (JAM) and tricellulin, all of which have transmembrane domains. The intracellular domains of these proteins interact with structural proteins including zonula occludens proteins (ZO) that anchor them to the perijunctional actomyosin ring, as illustrated in the figure above.
Initiation of the immune response

While the primary route of gliadin penetration into the epithelium remains controversial, there are three possible mechanisms discussed in the literature. In the first, epithelial transcytosis allows the translocation of gliadin peptides from the apical to basal pole of the epithelium; in the second, TJ disregulation initiated by CXCR3 binding and zonulin signaling allows gliadin peptides to diffuse across a compromised epithelial barrier; and in the third, DCs extend processes between epithelial cells and are exposed to gliadin peptides in the lumen (Fasano, 2011; Schuppan et al., 2009). The third route is purely hypothetical, as DCs have been observed extending processes through the epithelium but the endocytosis of gliadin in this environment is unconfirmed; the other routes of entry have more direct evidence in support (Maria Rescigno & Sabatino, 2009; Schuppan et al., 2009).

Each of these routes initiates a complementary sequence of events. In the case of transcytosis, gliadin peptides stimulate the release of IL-15 from epithelial cells as well as the upregulation of MIC-A, a non-classical MHC molecule that does not bind TCRs (Murphy et al., 2011; Schuppan et al., 2009). IL-15 activates DCs in the lamina propria and activates NKG2D on intraepithelial lymphocytes (IELs), allowing interaction with its ligand, MIC-A; at the same time, the gliadin peptide 31-49 binds TLR4 on epithelial cells, leading to HLA-E expression (Murphy et al., 2011; Schuppan et al., 2009). Gliadin peptides taking the paracellular route also interact directly with intestinal DCs and macrophages via TLR4 and other MyD88-dependent mechanisms, leading to IL-15 secretion that complements that of epithelial cells; APCs have also been shown to increase expression of IL-1β, TNF-α, IL-8, and MCP-1 in response to gliadin (Nikulina et al., 2004; Schuppan et al., 2009).

The MHC molecules play a crucial role in the activation of effector T cells in CD. The MIC-A expressed on epithelial cells binds its ligand, NKG2D, on IELs, leading to direct cytolytic effects that are independent of TCR specificity in CD8 T cells (Murphy et al., 2011; Schuppan et al., 2009). HLA-E binds its ligand NKG2C on IELs, stimulating proliferation and cytokine secretion that is synergistic with the effects...
induced by NKG2D/MIC-A interactions (Schuppan et al., 2009). A summary of innate immune responses in CD is shown in the figure below from Schuppan et al. (2009).

There are a few mechanisms in place that negatively regulate this generally inflammatory process. The NKG2A expressed by a subset of γδ T cells can initiate suppressive signaling after binding HLA-E, releasing TGF-β; in line with this, γδ IELs have been shown to control the activation of cytotoxic CD8αβ TCRαβ IELs by reducing transcription of NKG2D in these cells (Cheroutre et al., 2011). NKG2A expression, however, is depressed in CD patients despite the increased number of γδ T cells, suggesting a loss of regulatory control (Meresse, Malamut, & Cerf-Bensussan, 2012). Evidence from duodenal biopsies suggests that the presence of these cells is reduced in patients with active CD (Schuppan et al., 2009). Both CD4+ and CD8+ FoxP3+ T regulatory cells also play a role in mitigating the inflammatory process. Following maturation induced by DC-secreted TGF-β and retinoic acid, CD4+ Treg cells recirculate and return to the lamina propria where they down-
regulate immune responses (Schuppan et al., 2009; M Veldhoen & Brucklacher-Waldert, 2012). CD8αα TCRαβ cells also contribute to the regulation of the inflammatory process, though mechanistic details are unclear (Schuppan et al., 2009).

The tissue remodeling seen in CD is driven mainly by the activation of matrix metalloproteinases (MMPs) that are released by macrophages in response to IFN-γ; MMPs are also released by intestinal myofibroblasts in response to TNF-α and IFN-γ signaling (Schuppan et al., 2009). MMPs act on the extracellular matrix that provides structure in the lamina propria and epithelium, leading to the villous atrophy seen in CD histology (Murphy et al., 2011; Schuppan et al., 2009). The cytolytic activity of CD8 T cells in response to MIC-A and HLA-E binding is also a major contributor to crypt hyperplasia and loss of mature epithelial cells, contributing to malabsorption (Murphy et al., 2011).

**Development of adaptive immunity**

The onset of inflammation and structural damage is followed by the development of an adaptive immune response that targets tTG and gliadin. Anti-tTG antibodies are a hallmark of CD and the major marker for diagnosis, though the route of development of these antibodies in unclear, as no tTG-specific T cells have been identified (Murphy et al., 2011). tTG has a high affinity for gliadin peptides because of their glutamine content, and the transformation of these residues into glutamate makes them bind strongly to HLA-DQ2 due to the high affinity of the binding pocket for negatively charged residues; this process is illustrated in the
figure above (Abadie, Solld, Barreiro, & Jabri, 2011). This affinity is strong enough to allow extracellular binding of gliadin to HLA-DQ2 molecules, and overall allows the development of a strong CD4 T cell response to gliadin peptides that is Th1-polarized (Murphy et al., 2011; Nikulina et al., 2004). Given the lack of tTG-specific T cells, the development of anti-tTG antibodies is controversial. One possible mechanism involves the endocytosis of gliadin/tTG complexes by tTG-specific B cells followed by cytosolic processing and presentation of gliadin peptides to gliadin-specific T cells; after recognizing their antigen, these T cells can prime the B cells for anti-tTG antibody production (Murphy et al., 2011).

While the development of these auto-antibodies is a hallmark of CD (and a defining characteristic of the autoimmune nature of the disease), IgA does not generally promote inflammation, and has not been shown to contribute to tissue destruction in CD (Meresse et al., 2012). The production of anti-tTG remains robust, however, with 10% of IgA plasma cells from the lamina propria of untreated CD patients producing anti-tTG IgA; at the same time, patients with latent CD retain anti-tTG IgA in their mucosa, suggesting that its presence is not sufficient for intestinal damage (Meresse et al., 2012). Interestingly, tTG is a multifunctional enzyme, with roles in the assembly and repair of extracellular matrix, proliferation, cell signaling, and endocytosis, and anti-tTG titers drop off concomitantly with the reversal of structural damage upon treatment with a gluten-free diet (Fasano, 2012; Meresse et al., 2012). This suggests a potential role for IgA antibodies to tTG as preventing the normal function of extracellular tTG in the mucosa.

Perpetuation of inflammation

Once established, intestinal inflammation can initiate a positive feedback loop in which inflammatory cytokines further enhance intestinal permeability and tissue damage, leading to continued entry of luminal antigens and the perpetuation of inflammation. The ability of inflammatory cytokines - including TNF-α, IFN-γ, and various interleukins and growth factors – to regulate the integrity of TJs is well documented, and their effects are often synergistic (the combined effects of TNF-α and IFN-γ are notable examples; Capaldo & Nusrat, 2009; Suzuki, 2012). An
overview of these effects is beyond the scope of this section, but in general terms, the pro-inflammatory cytokine milieu contributes to TJ disassembly. Outside of allowing the paracellular passage of gliadin peptides into the lamina propria, other luminal pathogens, including bacteria and other food constituents, can gain entry via the same mechanism (Fasano, 2011). LPS from luminal microbiota is an obvious candidate for further contribution to an inflammatory mucosal environment, and various plant lectins may contribute as well (Cordain, 1999; Freed, 1999; Lindeberg, 2010).

The effects of inflammatory cytokines on TJ integrity are not reliant on the presence of gliadin-initiated enteropathy, and the Western lifestyle provides a number of pro-inflammatory influences, including inadequate sleep, high levels of stress, a generally sedentary lifestyle, and an abundance of nutrient-poor food (Gaesser, Angadi, Ryan, & Johnston, 2011). Given that the presence of HLA-DQ2/8 and a gluten-containing diet is necessary but not sufficient for the development of CD, the presence of chronic inflammation – promoted by a detrimental lifestyle – may be a necessary piece in the puzzle of celiac disease pathogenesis.

Implications for other autoimmune conditions

The effects of a loss of tight junction integrity, subsequent loss of tolerance and recruitment of autoreactive T cells, and development of chronic inflammation seen in CD offer a potential template for the development of other autoimmune diseases. Before discussing these connections, however, it is worth noting that the effects of gliadin on epithelial integrity are not limited to celiac tissue. Research using models of the intestinal epithelial barrier have confirmed the ability of gliadin fragments to induce TJ disassembly via zonulin release, although these effects are not as severe as those seen in tissue from CD patients (Clemente, Virgiliis, & Kang, 2003; Drago et al., 2006; Lammers et al., 2008). Clemente et al. (2003) found a transient increase in zonulin production and transepithelial intestinal resistance in rabbit intestinal epithelium following treatment with gliadin, a process that was shown to be PKC-dependent in rat IEC-6 cells treated with gliadin digests. Drago et
al. (2006) investigated the impact of gliadin digests on IEC-6 and Caco2 cells (designed to model the human intestinal epithelium), observing rearrangement of the cytoskeleton and loss of TJ protein interactions following zonulin release. Furthermore, intestinal biopsies from CD patients showed greatly increased permeability and zonulin release in response to gliadin that was ameliorated by application of a zonulin inhibitor, while biopsies from non-CD patients demonstrated a significant decrease in transepithelial electrical resistance (TEER) 60 minutes after exposure to gliadin. Finally, Lammers et al. (2008) demonstrated a significant increase in zonulin release and decrease in TEER in wild-type mouse epithelium in response to gliadin digests that was CXCR3-dependent. These results indicate that while decreased in comparison to CD patients, normal epithelial cell lines experience increased intestinal permeability in response to gliadin treatment that is zonulin-dependent.

Type 1 diabetes shares high comorbidity with CD, with the prevalence of CD in T1D patients estimated to be 20 times higher than in the general population (Barbeau, 2012). T1D shares many features with CD, including a predisposition for development accompanying certain HLA II alleles, gastrointestinal symptoms, and increases in intestinal permeability, though the environmental trigger is still uncertain (Vaarala, 2012). Increases in intestinal permeability precede the onset of diabetes in BioBreeding diabetes-prone (BBDP) rats by at least a month (Fasano, 2011), and increases in zonulin production and permeability were shown to precede development of diabetes by 2-3 weeks in the same mouse model, while treatment with a zonulin inhibitor blocked the increase in permeability and formation of antibodies (Watts et al., 2005). This suggests that zonulin secretion and subsequent increases in intestinal permeability are key in the development of T1D, and human studies have demonstrated an increase in serum zonulin levels in T1D patients that correlated to increased intestinal permeability (A. Sapone, 2006).

In line with these findings, gluten has been implicated in the pathogenesis of T1D. Gliadin treatment of BBDP rats leads to increased zonulin release and
intestinal permeability, while gluten-free hydrolyzed-casein diets delay the onset and severity of T1D in these animals; furthermore, treatment with the zonulin inhibitor AT1001 prevented a loss of intestinal barrier function and the appearance of antibodies in BBDP rats, strengthening the hypothesis that zonulin-induced permeability increases are necessary for T1D development (Watts et al., 2005). Evidence from humans shows an increase in IFN-γ and TNF-α production in the jejunal biopsies of T1D patients, while biopsies from children with T1D demonstrate increases in intraepithelial CD3+ cells and LP CD25+ mononuclear cells; treatment of these specimens with gliadin increased α4β7- and ICAM-expressing LP cells, as well as CD25+ and CD80+ cells (Auricchio et al., 2004). Further implicating gliadin in T1D is work demonstrating an increase in anti-tTG antibody deposits in the small intestines of children with T1D (Maglio, Florian, & Vecchiet, 2009). T cells isolated from T1D patients also show significantly enhanced reactivity to a variety of wheat antigens compared to healthy controls, and culture of peripheral blood mononuclear cells from T1D patients with gliadin leads to a significant increase in the production of TNF-α, IFN-γ, and IL-6 (Barbeau, 2012). This evidence supports the notion that gliadin can be a trigger in T1D via increases in zonulin-mediated intestinal permeability and a successive loss of tolerance, though the timeline is unclear.

Conclusion

While the roles of gliadin and zonulin in CD and, to a lesser extent, T1D, is apparent, there is a dearth of research on the impacts of zonulin signaling in other autoimmune conditions. The presence of zonulin as a biomarker of disease is gaining traction, however, and preliminary connections between serum zonulin levels and a number of conditions have emerged. These conditions include ankylosing spondylitis, Crohn’s disease, rheumatoid arthritis, and systemic lupus erythematosus, and some cancers and nervous system diseases, as summarized below (Fasano, 2012).
The hypothesis that a loss of intestinal barrier integrity could lead to autoimmune disease is a tempting one given the nature of the mucosal immune system as described above. The presence of relatively large numbers autoreactive lymphocytes and other immune cell populations separated from massive amounts of potentially antigenic molecules - whether from food, bacteria, or viruses – by a single layer of epithelial cells paints a picture of a system on the edge of activation. The potential for the a loss of oral tolerance (as seen in CD) or a break of self tolerance via the activation of normally quiescent, autoreactive cells seems not only possible, but likely, given the number of inflammatory influences present in our modern environment and the strong impact of inflammation on mucosal barrier integrity. Implicating a single dietary compound like gluten in the pathogenesis of a wide range of autoimmune diseases is likely a stretch, despite its demonstrated role in zonulin signaling, but the possibility that other foods could contribute to a compromised intestinal epithelium via the promotion of inflammation may be a stronger hypothesis. Thus, there may be a mechanism by which food can impact autoimmune disease in the context of the mucosal immune system, even if the impact is indirect.

One strong candidate for a role in the regulation of intestinal barrier integrity and self-tolerance is the large population of intestinal bacteria present in all mammals, termed the microbiota. The microbiota have a demonstrated impact on the development and maintenance of intestinal homeostasis, and disregulation of bacterial populations is present in a number of inflammatory diseases. This disregulation is driven by inflammation itself but also potentially by alterations in diet that promote the expansion of pathogenic and the contraction of beneficial species. Recent advancements in this field of research provide a number of clues that support the hypothesis that nutrition can have an impact on autoimmunity.

Table 1. Major Diseases Associated With Zonulin (Pre-HP2) Biomarker

1. Autoimmune diseases
   - Ankylosing spondylitis
   - Celiac disease
   - Inflammatory bowel disease (Crohn’s disease)
   - Rheumatoid arthritis
   - Systemic lupus erythematosus
   - Type 1 diabetes
2. Cancers
   - Brain cancers (gliomas)
   - Breast cancer
   - Lung adenocarcinoma
   - Ovarian cancer
   - Pancreatic cancer
3. Diseases of the nervous system
   - Chronic inflammatory demyelinating polyneuropathy
   - Multiple sclerosis
   - Schizophrenia
The Microbiota

- Introduction
- Homeostasis: interactions with the mucosal barrier
- Mechanisms of immune regulation
  - Th17 cells
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  - TLR signaling
- Microbiota and disease
- Nutrition and the microbiota
The existence of large numbers of bacteria in our large intestines, and, to a lesser extent, our small intestines, has long been recognized, but the impact of the microbiota (colloquially, gut flora) on human health has only recently been targeted as a major area of research. The development of new culture-independent techniques, such as high-throughput sequencing of 16s ribosomal RNA, has allowed researchers to rapidly quantify the composition of the microbiota and compare profiles between diseased and healthy populations, as well as track the development of the microbiota with age (Hooper & Macpherson, 2010; Sellitto, Bai, Serena, & Fricke, 2012). Investigations into the impact of the microbiota on immune function complement this work, and contribute to a developing, though still nascent, picture of the functional relationship between bacteria and their hosts in homeostasis and disease, and research has identified potential roles for microbiota in a growing number of conditions, including inflammatory bowel disease, asthma and allergic diseases, rheumatoid arthritis, EAE, and type 1 diabetes (Honda & Littman, 2012; Kranich, Maslowski, & Mackay, 2011; Vaarala, 2012). Many of these associations may be driven by the influence of the microbiota on Th17 and Treg cells, both of which appear important in the pathogenesis of autoimmunity (Littman & Rudensky, 2010).

The composition of the microbiota is highly variable between individuals and within populations, but there are some taxonomic commonalities. Major phyla in the human intestine include Bacteroidetes and Firmicutes, while Proteobacteria and other phyla are represented at lower levels (Murphy et al., 2011). Within the gram-positive Firmicutes phyla, the Clostridia class dominates, but members of the Enterococcaceae and Lactobaccillaceae families and Lactococcus species are also present; within gram-negative Bacteroidetes, Bacteroides species including Bacteroides thetaiotaomicron, Bacteroides fragilis, and Bacteroides ovatus predominate (Hooper & Macpherson, 2010). These account for about 90% of the bacteria in the human gut, while the remaining 10% is composed of the Proteobacteria, Fusobacteria, Actinobacteria, Veruccomicrobia, and Spirochaetes phyla (Hooper & Macpherson, 2010).
These species maintain a complex homeostasis with the host that is generally mutualistic. Humans lack the capacity to digest a number of plant polysaccharides, but bacteria have a wide variety of saccharolytic enzymes that are capable of processing most of the polysaccharides present in our diets, and change in composition based on the intake of these sugars; the microbiota also produce vitamins that are essential for human health, including biotin, folate, and vitamin K, that are absorbed in the GI tract. (Brenchley & Douek, 2012; Hooper & Macpherson, 2010; Maslowski & Mackay, 2011). Beyond nutrient transformations, commensal bacteria are critical for the health of the mucosal epithelium; germ-free mice have under-developed and dysfunctional GI tracts, are more susceptible to infection, and have a suppressed mucosal immune system, while these effects can be reversed with colonization by a single bacterial species (Brenchley & Douek, 2012). While bacteria can be beneficial, penetration into the subepithelial space can be inflammatory and is undesirable, and three main mechanisms have developed to prevent this: the secretion of mucus, antimicrobial proteins, and IgA (summarized in the figure above; Hooper & Macpherson, 2010). Mucus is secreted by goblet cells and forms a protective barrier on the apical pole of the epithelium, and GF mice have fewer, and smaller, goblet cells, and a thinner mucus layer, suggesting that the presence of microbiota is important for goblet cell function and development (Kandori, Hirayama, Takeda, & Doi, 1996). Antimicrobial proteins are secreted by a number of cells in response to TLR stimulation and cytokine signaling, and are contained almost exclusively in the mucus layer in a gradient, concentrated mostly towards the epithelial surface.
IgA plays a special role in intestinal homeostasis, and deserves particular attention.

IgA produced in the intestines is known as secretory IgA (sIgA) and is secreted as a dimer by intestinal plasma cells in response to class-switching induced by TGF-β; there are over 75,000 sIgA-secreting plasma cells in the human intestine, and these cells produce 3-4g of sIgA per day (Murphy et al., 2011). sIgA binds commensal and pathogenic bacteria and food particles in the gut lumen, and GF mice have impaired secretion of sIgA, hinting at the importance of bacterial induction of B cell class switching, differentiation and expansion (Pabst, 2012). This process is driven by DC sampling of the luminal contents, either via extension of a process through the epithelium or transcytosis by M cells, after which they migrate to the mesenteric lymph nodes to activate and induce homing molecule expression on B cells (Hooper & Macpherson, 2010). This process involves TLR signaling, either in B cells, where TLR signals can substitute for CD40L and act with TGF-β initiate class switching, or in DCs, where TLR activation leads to the induction of inducible nitric oxide synthase and subsequent stimulation of a proliferation-inducing ligand (ARPIL) and B cell activating factor (BAFF), both of which promote IgA class-switching (Honda & Littman, 2012; Murphy et al., 2011). The induction of IgA class-switching is crucial for the maintenance of the epithelial barrier, and a loss of IgA production could potentially contribute to a loss of mucosal barrier integrity; notably, IgA deficiency is associated with malabsorption, lactose intolerance, CD, and ulcerative colitis, among other diseases, due to the damaging immune responses that can develop once pathogens gain access to submucosal tissue (Brenchley & Douek, 2012).
Mechanisms of immune regulation

Th17 cell development is strongly influenced by the microbiota, and is of particular interest given the potential role of Th17-mediated responses in the pathogenesis of autoimmunity (e.g. in EAE, as discussed above). GF mice have greatly reduced Th17 populations and expanded Treg populations, likely reflecting the need for inflammatory signaling in Th17 development (Kranich et al., 2011). Though Th17 differentiation is commonly accepted to be driven by IL-6 and TGF-β, recent work demonstrated that microbiota-induced IL-1β signaling, and not IL-6 signaling, is required for the induction of steady-state Th17 cells in the LP, suggesting that there are different requirements for Th17 induction with or without infection (Shaw, Kamada, Kim, & Núñez, 2012). IL-6 has been shown to activate STAT3, a suppressor of FoxP3, thereby promoting Th17 over Treg differentiation in the presence of TGF-β, which promotes FoxP3 expression and its inhibition of Rorγt (Atarashi et al., 2011). These interactions are illustrated in the figure above (Littman & Rudensky, 2010). Th17 cells secrete IL-21 during differentiation and expansion, which acts to upregulate their expression of IL-23R via STAT3 activation, leading to a full inflammatory response upon IL-23 stimulation (Honda & Littman, 2012). A T cell transfer model of EAE suggested that Th17 cells only expanded and became pathogenic in the presence of IL-23, speaking to the importance of this signaling pathway (McGeachy et al., 2007). IL-1β secretion by activated Th17 cells also generates an autocrine loop, via the promotion of Rorγt expression (Chung et al., 2009). There are multiple mechanisms implicated in microbiota-induced Th17 differentiation, including TLR-9 signaling; flagellin-activated TLR-5 signaling; and the production of IL-6 by DCs stimulated by TLR ligands on apoptotic intestinal epithelial cells in the presence of bacterial infection.
(Honda & Littman, 2012). There is little research on the impact of particular bacterial strains on Th17 cells development, and though segmented filamentous bacteria (SFB) has been shown to be necessary for Th17 induction in mice, the relevance of SFB in humans is unverified at this point; however, the necessity of microbial signaling in normal Th17 development is not controversial.

Treg cells are of primary importance in the regulation of potentially pathogenic inflammatory responses, and their effector functions are generally in contrast to those of Th17 cells. Treg cells are induced by TGF-β and retinoic acid, as described above, and this redundancy in signaling requirements with Th17 cell development serves to prevent excessive inflammation in response to infection. Evidence suggests that Treg cells can be induced by the microbiota, potentially via the polysaccharide A carbohydrate (PSA) and by SFB, though the presence of normal Treg populations in the small intestines of GF mice suggests that these interactions may not be necessary for Treg development; on the other hand, the colons of GF mice have depleted relative and absolute numbers of Treg cells (Cong, Weaver, Lazenby, & Elson, 2002; Honda & Littman, 2012; Hooper & Macpherson, 2010). The bacterium B. fragilis, a human commensal, positively modulates IL-10 production in the colon of mice via PSA binding of TLR-2 on CD4 T cells, leading to suppression of Th17 responses (Round & Mazmanian, 2010). Indigenous murine commensal bacteria, specifically Clostridium clusters XIVa and IV, have been shown to potently induce Treg cells; this interaction is mediated by the production of MMPs from intestinal epithelial cells that cleave inactive TGF-β, generating the active form, as well as the expression of indoleamine 2,3-dioxygenase, which is implicated in Treg induction (Honda & Littman, 2012). Mice with increased frequency of Clostridium strains are protected against intestinal inflammation and allergy, indicating an important role for these bacteria in intestinal homeostasis (Microorganisms et al., 2011). Recent evidence shows that Treg cells mature in the colon in response to antigens from commensal bacteria, and that Treg responses are dependent on this recognition, while commensal-specific TCRs on conventional T cells are capable of inducing colitis, further speaking to the importance of microbial signaling in Treg development and the restraint of inflammatory responses (Lathrop et al., 2011).
TLR signaling via the microbiota is necessary for mucosal homeostasis, as evidenced by the development of DSS-induced colitis in antibiotic-treated mice and the rescue of colitis by administration of LPS, which activates TLR4, or lipoteichoic acid, which activates TLR2 (Rakoff-Nahoum, Paglino, Eslami-Varzaneh, Edberg, & Medzhitov, 2004). TLR2 signaling has been shown to be important for maintenance of TJ integrity, and mice lacking TLR show mucosal ulceration and destruction of epithelial crypts, while TLR2 stimulation induced IL-10 production in CD4 T cells, as discussed above (Cario, Gerken, & Podolsky, 2007). In this vein, administration of *Lactobacillus plantarum* has been shown to promote TJ integrity via increased expression of ZO-1 and other TJ proteins, and observations in epithelial cell cultures confirmed this finding (Vaarala, 2012). Because of the potential inflammatory consequences of excessive TLR signaling, negative regulation of their signaling pathways is crucial. Notably, IL-10 signaling suppresses the activity of TLR4, though the mechanism is unclear; other examples include the production of the protein A20 in response to TLR signaling, which terminates TLR responses, and the single-immunoglobulin IL-1 receptor-related molecule (SIGGIR), which is released by intestinal epithelial cells and DCs and sequesters signaling components in the IL-1 and TLR signaling pathways (Honda & Littman, 2012). Because TLR activation induces inflammatory cytokine production that can lead to a breakdown of mucosal barrier integrity, chronic stimulation of TLRs by penetrant bacteria may play an important role in intestinal inflammation.

*The microbiota and disease*

The potential role of the microbiota in the maintenance of systemic homeostasis is suggested by the presence of altered microbial communities in diseases such as obesity, inflammatory bowel disease, allergies, asthma, rheumatoid arthritis, and EAE (Maslowski & Mackay, 2011; Wu & Wu, 2012). Type 1 diabetes is fairly well researched in this regard compared to most other conditions. Intestinal populations of Treg cells are reduced in T1D, suggesting the presence of dysbiosis, and GF non-obese diabetic (NOD) mice show increased disease severity compared
to colonized controls (Vaarala, 2012; Wu & Wu, 2012). NOD mice lacking MyD88 were protected from T1D development while GF MyD88−/− NOD mice showed accelerated development, suggesting that the presence of microbiota was necessary to obtain the protective effect; this indicates that MyD88-independent signaling was required and allowed the expansion of beneficial bacteria that would have been suppressed with MyD88 present, and speaks to the role of the immune system in shaping microbial populations (Wen et al., 2008). This may have been due to the activity of Bacteroidetes strains, increased numbers of which were found in SPF NOD mice lacking MyD88 (Wen et al., 2008). The bacterial strain *Lactobacillus johnsonii*, when isolated from diabetes-resistant BioBreeding rats and administered to diabetes prone rats, conferred protection against the development of T1D, possibly via enhanced IL-17-induced production of antimicrobial proteins; *L. johnsonii* was shown to activate Th17 cells in a TCR-dependent fashion, suggesting a source of the IL-17 and potentially complicating our understanding of the role of Th17 cells in autoimmunity (Vaarala, 2012).

Inflammatory bowel disease (IBD) is also marked by changes in the composition of the microbiota, including, generally, a decrease in bifidobacteria and lactobacilli and increased numbers of *Escherichia coli* (Atarashi et al., 2011); decreases in the *Clostridia* subgroup of Firmicutes and Bacteroidetes have also been observed in IBD patients, along with increases in Actinobacteria and Proteobacteria (Maslowski & Mackay, 2011). Genomic analysis has revealed that patients with chronic inflammation show less microbial diversity than healthy patients, and patients with IBD have 25% fewer bacterial genes than those without IBD (Qin et al., 2010); the decreased presence of Clostridia clusters IV and XIVa may be particularly important given their role to induce Treg differentiation (see above). IBD is also associated with increased numbers of bacteria adhering to the intestinal epithelium, suggesting impaired mucosal barrier defense function; this is supported by evidence that defects in the NOD2 gene in humans lead to decreased production of α-defensin by Paneth cells, and that polymorphic NOD2 genes confer increased risk for Crohn's disease (Hooper & Macpherson, 2010).
It has been proposed that intestinal inflammation drives dysbiosis in IBD instead of the other way around. T-bet−/− Rag−/− ulcerative colitis (TRUC) mice show overproduction of TNF-α by intestinal DCs and transfer of TRUC microbiota into healthy mice induces colitis, while inflammasome-signaling-deficient (NLRP−/−) mice are also prone to colitis and microbial transfer to healthy mice also leads to colitis (Elinav et al., 2011; Garrett et al., 2007). The ability to induce IBD in healthy mice via microbial transfer indicates that the presence of pathogenic bacteria in the context of impaired mucosal immune function may be the driver of IBD, rather than a result. Bacterial treatments for patients with IBD are in development, and a recent pilot study using fecal transplantation in children with ulcerative colitis showed promising results, with 78% of children showing clinical improvement within 1 week of treatment (Kunde et al., 2013). This provides further support for the hypothesis that dysbiosis drives IBD, and that its correction can have therapeutic effect.

There is further evidence for dysbiosis in rheumatoid arthritis (RA). The removal of anaerobic bacteria (i.e. Bacteroidetes and Firmicutes) in a mouse model of inflammatory arthritis intensifies disease, and GF mice show reduced severity of disease; this was shown to be caused by microbiota-induced Th17 cell differentiation and subsequent IL-17 production in secondary lymphoid tissue that drives B cell activation and the production of autoantibodies (Wu et al., 2010). Introduction of SFB into GF mice was sufficient to drive the development of arthritis, via induction of Th17 cells; an illustration of this pathway is provided in the figure below (Wu & Wu, 2012). In humans, early RA patients not on medication showed decreased Bifidobacteria, Bacteroides and Eubacterium compared to patients with fibromyalgia, while early RA patients also showed a decrease in anaerobic bacteria compared to healthy controls (Kranich et al., 2011).
Finally, a role for the microbiota in the development of EAE has also been established. EAE is known to involve Th17-driven inflammation, and a contribution of TCRγδ T cells via IL-17 production has also recognized, where they promote Th17 function and suppress Treg responses in the CNS (Atarashi et al., 2011). Given the role of Th17 cells in EAE, it is unsurprising that SFB can promote EAE in GF mice, and that antibiotic administration can dampen disease severity (Wu & Wu, 2012). A number of beneficial bacterial strains have been shown to ameliorate EAE, including *B. fragilis* and three *Lactobacillus* strains (*L. paracasei* DSM 13434, and *L. plantarum* DSM 15312 and DSM 15313), where Treg produced IL-10 was implicated in the beneficial activity of *Lactobacillus* (Wu & Wu, 2012). Taken together, the evidence from T1D, IBD, RA, and EAE suggest an important role for the maintenance of a healthy microbial community for the prevention of disease. Mouse models of RA in particular provide a possible mechanism for the extraintestinal development of autoimmunity that is driven by intestinal effects via Th17 cell-mediated inflammation. The contribution of γδ T cells in EAE is also unsurprising, given the presence of potentially self-reactive TCRs in these cells (discussed above), and may represent an activation of normally quiescent mucosal lymphocytes that drive a loss of tolerance.
The intestinal microbiota produce a number of different substances that have effects on our physiology, including vitamins (e.g. biotin, folate, and vitamin K), short-chain fatty acids, polysaccharide A (PSA) and peptidoglycan (PTGN) (Kranich et al., 2011). Short-chain fatty acids (SCFAs) are produced primarily by Bacteroidetes via the fermentation of plant polysaccharides, and are defined as fatty acids with 4 or fewer carbons, i.e. acetate, propionate, and butyrate (15) (Kranich et al., 2011). SCFAs produced by the microbiota are absorbed by enterocytes of the large intestine and are important for the health of enterocytes, who use butyrate as a primary metabolic substrate (Kranich et al., 2011). SCFAs exert anti-inflammatory effects through the binding of G-protein-coupled receptors, including GPR43, which is present mainly on cells of the innate immune system; mice lacking GPR43 show increased inflammation in models of arthritis and colitis, and neutrophils lacking GPR43 are hyper-reactive (Maslowski & Mackay, 2011). The administration of butyrate either topically or by enema is effective for patients with ulcerative colitis, who show impaired butyrate metabolism (Honda & Littman, 2012). GPR109A suppresses NF-κB signaling and inflammatory cytokine production in intestinal epithelial cells, and is activated by butyrate, while SCFAs ligation of GPR41 on intestinal endocrine cells in the epithelium promotes peptide YY secretion (a gastrointestinal regulatory hormone) (Samuel et al., 2008; Thangaraju et al., 2009). These effects suggest a potent immunoregulatory role for SCFAs.

PSA is also produced by the microbiota, specifically B. fragilis, following metabolism of plant polysaccharides, and increases the production of IL-10 in Treg cells via TLR2 binding, as mentioned above; feeding mice lacking B. fragilis PSA is sufficient to induce the normal immunomodulatory effects of B. fragilis (Honda & Littman, 2012). The enhanced function of Treg cells from PSA treatment led to suppression of Th17 responses and allowed colonization of the colon by B. fragilis, while mice colonized with B. fragilis that lacked PSA showed enhanced Th17 activity (Wu & Wu, 2012). PSA treatment is also independently capable of restoring Th1/Th2 balance in GF mice, which show a Th2 bias (Kranich et al., 2011). Finally,
the bacterial product PTGN is able to cross the mucosal barrier, enter systemic circulation and bind NOD1 on neutrophils, promoting appropriate neutrophil responses to pathogenic bacteria (Kranich et al., 2011).

The production of these beneficial microbial products are dependent on the health of the microbiota, and dietary changes have been shown to alter the composition of the microbiota in mice, where the switch to a diet high in fat and sugar from a diet with substantial amounts of plant polysaccharides leads to an increase in Firmicutes, a decrease in Bacteroidetes, and altered gene expression and metabolism in resident bacteria (Maslowski & Mackay, 2011). Further evidence for the impact of diet on the composition of the microbiota is epidemiological and beyond the scope of this paper, but it has been widely acknowledged that populations eating adequate amounts of dietary fiber have decreased incidence of inflammatory disease (Slavin, 2003). More research is needed to tease out the relationships between diet and microbial populations, and the initiation of large-scale projects such as the American Gut Project (www.americangut.org) are a step towards identifying the patterns that drive health and disease.

Probiotics

The potential health benefits of orally ingested, transient bacteria, termed “probiotics,” have been recognized since the early 20th century, when Eli Metchnikoff proposed that the fermented milk products consumed by Bulgarian peasants played a part in promoting their long and healthy lives (Grangette, 2010). The immunomodulatory effects of probiotics, either from fermented products or commercially available supplements, have been the subject of increasing research interest, but information on their effects is still limited. Probiotic bacteria have a limited ability to colonize the colon, and likely exert most of their effects through the small intestinal epithelium during gastrointestinal transit via bacterial products such as peptidoglycan, as mentioned above, and lipotechoic acid, as well as various peptides (Klaenhammer, Kleerebezem, Kopp, & Rescigno, 2012). Unfortunately, conflicting evidence from many trials makes it difficult to ascribe particular
functions to many strains of probiotic bacteria; while probiotic supplementation has been suggested to benefit allergy, for example, 15 clinical trials investigating the effects of probiotic on allergy have shown no effect on allergic rhinitis, bronchial asthma, and allergic sensitization (Klaenhammer et al., 2012). The ability of probiotic strains to promote wellness in healthy populations is also questionable, and is difficult to research, while the mechanisms at play in models of disease may not translate to healthy individuals; there is some evidence to support the use of probiotics in IBD, but success in these trials was limited (Gareau, Sherman, & Walker, 2010). At this point in time it is hard to pinpoint direct effects of probiotics beyond those discussed earlier in this section (e.g. for the lactobacillus species), but advances in the field may provide further evidence for the potential benefit of probiotics on inflammation and autoimmunity.

Conclusions

Evidence from the study of the microbiota and human health provide another link between diet and immunity. The ingestion of adequate amounts of plant polysaccharides is likely crucial for the maintenance of a symbiotic microbiota, while diets that promote the growth of pathogenic bacteria may lead to dysbiosis and inflammatory disease. The effects of bacteria and their metabolites on Treg/Th17 balance is of particular importance, as suppression of Treg responses is associated with the development of autoimmune disease, and Th17 cells have been implicated in their pathogenesis. In consideration of the profound effects of the microbiota on intestinal homeostasis and our knowledge of the mucosal immune system, we can hypothesize that a shift in diet could lay the foundation for aberrant activation of self-reactive T cells and a suppression of the regulatory mechanisms that normally contain them, leading to a loss of tolerance both locally and systemically. This may be complimented by the enhanced epithelial permeability seen in the context of inflammation and gliadin ingestion, which allows the passage of macromolecules into the mucosa and their potential activation of inflammatory responses. Together, dysbiosis and a diet that promotes inflammation and
permeability may lead to a feed-forward cycle of inflammation that promotes further intestinal permeability, loss of regulatory function, and chronic immune activation.

The presence of dysbiosis and increased intestinal permeability in obesity is notable given recent insights into the inflammatory, and possibly autoimmune, nature of the disease. Chronic inflammation driven by the accumulation of fat and activated lymphocytes in adipose tissue is a likely contributor to intestinal inflammation and its negative effects on bacterial populations. The ability of microbiota from obese mice to induce obesity in healthy mice underlines the interrelation of obesity and dysbiosis, and suggests that diet-induced weight gain and alterations in the microbiota may be complementary in the development of autoimmune disease (Blaut & Klaus, 2012). The role of obesity and metabolic dysfunction in inflammation and the loss of tolerance is discussed in the next section.
Immunometabolism and Tolerance

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• Conclusion
The ever-progressing obesity epidemic is one of the greatest public health challenges of 21st century, and there is strong evidence that is it primarily diet- and lifestyle-induced (Lindeberg, 2010). Obesity shares comorbidity with a number of other chronic diseases, and is most strongly associated with the metabolic syndrome, which is comprised of central obesity and two of the following: high plasma triglycerides, reduced HDL cholesterol, hypertension, pathological insulin resistance, or diabetes (Nunn, Bell, & Guy, 2009). Metabolic dysfunction is the unifying factor in these various conditions, and is accompanied by increased sympathetic nervous system and hypothalamic-pituitary-adrenal axis activation, and elevated cortisol, but the high comorbidity of the metabolic syndrome with chronic inflammatory diseases suggests that the pathogenesis of metabolic disease may have an immune component (Nunn et al., 2009). Evidence from mice strongly suggests that calorie restriction and starvation ameliorate experimental autoimmune disease (e.g. experimental autoimmune encephalomyelitis, EAE), and these conditions are generally immunosuppressive (i.e. anti-inflammatory) and marked by increased Treg and decreased Th17 and Th1 cell activation (Fadini, Ceolotto, Pagnin, De Kreutzzenberg, & Avogaro, 2011; Galgani et al., 2010). Despite the promising results seen in animal models, there have been no clinical trials investigating the impact of calorie restriction in autoimmunity (Fadini et al., 2011), but emerging evidence concerning the interplay between metabolism and the immune system may assist in the instigation of this line of research.

While connections between metabolism and immunity have been recognized since Otto Warburg’s observation in 1958 that activated leukocytes are primarily glycolytic, only recently has a push been made to connect these disciplines more completely (Rathmell, 2012). That the development and proliferation of leukocytes requires immense resources in order to be successful is not surprising, and the immunosuppressive effect of calorie restriction speaks to the likely sacrifice of immune function in favor of more immediate metabolic needs during times of famine (Rathmell, 2012). The new field of “immunometabolism” seeks to elucidate...
the mechanisms behind these and other observations, and recent developments offer us fascinating insights into the potential impacts of over- and under-nutrition on inflammatory disease.

Observations from sepsis provide some insight into potential connections between immunity and metabolism. Sepsis is marked by massive, global inflammation, and elicits metabolic effects almost identical to those seen in type II diabetes (T2D) and obesity. During septic events, blood concentrations of amino acids, free fatty acids (FFA), glucose, and lactate skyrocket alongside increases in plasma insulin, cortisol, glucagon, and catecholamines (Andersen, 2004). Release of inflammatory cytokines triggers systemic insulin resistance and the release of substrate from metabolic tissues, marking an overwhelming shift towards catabolism, as illustrated in the figure below (Andersen, 2004). These observations are notable because they demonstrate a situation in which the presence of inflammation triggers metabolic derangement, instead of the other way around. The cytokine leptin, popularized primarily as a nutrient-sensing hormone, also plays a crucial role in the immune response to sepsis, and is now known to modulate the activation of the mucosal immune system and to play a role in the maintenance of tolerance, as well (Mackey-Lawrence & Petri, 2012; Matarese, Procaccini, & De Rosa, 2012; Tschöp et al., 2010). This begs the question: is metabolic derangement driving chronic inflammation in obesity and the metabolic syndrome or vice versa?

![Cytokine Signaling in Obesity](image)

**Fig. 1.** The sepsis induced changes in circulating hormones and metabolites. The bold arrows across the endothelial barrier indicate the direction of net metabolite flux. VLDL, Very low-density lipoprotein.
The hormone leptin was discovered in the early 1990s as a primary regulator of systemic energy homeostasis and satiety, and was found to originate in adipose tissue; its antagonistic counterpart, adiponectin, was discovered soon thereafter, providing a partial illumination of the primary regulatory pathways involved in overweight and obesity. Leptin secretion by adipocytes correlates positively and directly to fat mass, while adiponectin correlates negatively, and both were initially thought to act primarily on the arcuate nucleus, the paraventricular and dorsomedial nuclei and the lateral hypothalamus to induce anorexigenic or orexigenic behavioral responses, respectively (Duntas & Biondi, 2012). While these effects are important pieces of the puzzle in the pathological energy imbalance seen in obesity, the discovery of immunomodulatory effects of both leptin and adiponectin have allowed a more complete picture of their importance to emerge.

Lean adipose tissue displays a number of differences from overweight or obese adipose tissue. In healthy (lean) individuals, adipose tissue is home to a high percentage of T regulatory cells, low numbers of CD8 T cells, and low numbers of macrophages; this is accompanied by a cytokine milieu dominated by tolerogenic IL-10 and adiponectin and low levels of pro-inflammatory cytokines (e.g. TNF-α, IL-6) and leptin (Feuerer et al., 2009). Obese adipose tissue, on the other hand, is marked by high levels of leukocyte infiltration and activated CD4 and CD8 T cells, and expression of proinflammatory genes and cytokines, and greatly reduced levels of adiponectin, IL-10, and Tregs (Deng et al., 2013; Feuerer et al., 2009); notably, macrophages can constitute up to 50% of total cells in obese adipose tissue, compared to ~10% in healthy adipose (Odegaard & Chawla, 2013).

*Leptin, adiponectin, mTOR, and AMPK*

Leptin plays a crucial role in the accumulation of leukocytes in adipose tissue and the generation of a proinflammatory environment. The leptin receptor acts via the janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway and has generally proinflammatory effects in leukocytes (Moraes-vieira,
Olsen, & Câmara, 2010). Mice lacking the leptin gene or the leptin receptor (ob/ob or db/db mice) demonstrate lymphopenia, impaired CD4/CD8 T cell responses, thymic atrophy, and have lower T cell IFN-γ and adipose Th1 cell infiltration than wild-type mice, but are resistant to experimental autoimmune diseases such as EAE (Lumeng, Maillard, & Saltiel, 2009; G Matarese et al., 2001). Leptin directly participates in macrophage and monocyte activation by favoring the production of leukotriene B4, the cyclooxygenase enzymes, nitric oxide, TNF-α and IL-6, and increases production of TNF-α and IFN-γ in T cells (Giuseppe Matarese et al., 2012; Moraes-vieira et al., 2010). Leptin appears, via induction of the signaling molecules described above, to promote Th1 and Th17 cell differentiation over Treg and Th2 differentiation, and the Th1/Th17 cell deficiency seen in ob/ob mice can be reversed by leptin administration (Galgani et al., 2010). In contrast to leptin, adiponectin signaling promotes an opposing immune profile: the differentiation of Treg cells, suppression of NF-κB, the production of IL-10 in dendritic cells and macrophages, the downregulation of costimulatory molecules in dendritic cells, and suppression of IL-6 and TNF-α production in macrophages (Procaccini & Matarese, 2012; Tilg & Moschen, 2006). Given these effects, leptin can generally be viewed as proinflammatory (though essential for normal function), and adiponectin as anti-inflammatory.

Recent evidence suggests that many of these effects are mediated by the mammalian target of rapamycin (mTOR), a serine/threonine kinase that is partly responsible for the regulation of cellular metabolism, growth and development (Procaccini & Matarese, 2012; Waickman & Powell, 2012). mTOR plays a crucial role in metabolic regulation in most cells and insights into its actions have provided strong evidence for the close interrelation of metabolism and immunity. mTOR is activated by phosphatidylinositide-3 kinase (PI3K) and Akt in response to nutrient influx and is inhibited by AMP-activated protein kinase (AMPK) in cases of nutrient deficiency marked by a low intracellular ATP:AMP ratio (Fadini et al., 2011; Procaccini & Matarese, 2012). AMPK is the target of metformin, a common treatment for insulin resistance, and its activation reduces the severity of EAE via
increased production of IL-10 and a reduction in proinflammatory cytokines (Procaccini, Galgani, De Rosa, & Matarese, 2012).

There is evidence that there are two major metabolic programs associated with mTOR and AMPK activity: one biased towards glycolysis and one towards respiration. Rapidly proliferating cells require large amounts of metabolic substrate for their expansion, and glycolysis is the process best equipped to meet these demands. Accordingly, activated T cells are observed to have upregulated GLUT1 expression and have increased rates of glutamine and leucine influx, both of which serve to activate mTOR (Waickman & Powell, 2012). Activated T cells also display increased use of the pentose phosphate pathway, an alternative to glycolysis that utilizes glucose-6-phosphate and leaves the cell with metabolic intermediates that can be used to meet the substantial substrate demands of proliferating cells (Düvel et al., 2010; Tozzi, Camici, Mascia, Sgarrella, & Ipata, 2006). Memory T cells and Treg cells, on the other hand, utilize primarily oxidative metabolism and have markedly lower levels of mTOR activation, and mTOR-deficient T cells preferentially differentiate into Tregs (Delgoffe et al., 2009; Procaccini et al., 2010).

mTOR has a number of downstream targets, many of which are transcriptional regulators, including HIF-1α (critical for cellular adaptation to hypoxic conditions), Myc (an oncogenic transcription factor), PPARα (peroxisome proliferator-activated receptor α; a nuclear hormone receptor involved in fatty acid metabolism regulation), PPARγ (homolog of PPARα; regulates lipid metabolism and glucose homeostasis), and SREBPs (sterol regulatory element binding proteins; regulators of lipogenesis and cholesterol synthesis) (Waickman & Powell, 2012). These regulatory elements are summarized in the figure below from Waickman and Powel (2012). The general outcome of mTOR activation of these transcription factors is the promotion of glycolytic metabolism (and the accompanying bias towards T cell activation) and prevention of oxidative metabolism (and suppression of Treg development), though the action of PPARγ appears to play a counter-regulatory role.
PPARγ is involved in the regulation of lipid storage and glucose metabolism in metabolic tissues, and promotes non-pathological adipogenesis and lipid accumulation (Nunn et al., 2009). PPARγ activity is associated with the ability of ob/ob mice with over-expressed adiponectin to store massive amounts of fat without pathological consequence, and appears to play an important role in the suppression of inflammation in metabolic tissue and leukocytes (Nunn et al., 2009). Agonists of PPARγ suppress IL-6, TNF-α, and leptin secretion in adipose tissue, and inhibit the development of Th1 and Th17 cells via the modulation of dendritic cells; the ligation of PPARγ has also been shown to directly suppress Th17 development by inhibiting RORγt transcription (Cheroutre et al., 2011; Nunn et al., 2009). This highlights the interrelation of metabolic and immunoregulatory pathways, as PPARγ plays a protective role against the accumulation of pathological amounts of intracellular lipids by suppressing inflammation and promoting fatty acid metabolism.
Given the importance of Treg cells in the regulation of inflammation and disease, the effects of mTOR and AMPK on Treg cells is of particular interest. mTOR activity is known to suppress Treg development, and inhibition of mTOR with rapamycin promotes Treg differentiation even in the presence of supraphysiological levels of IL-2 (Procaccini et al., 2010). In mice with EAE, treatment with rapamycin increased rodent survival with concomitant suppression of inflammatory signaling, Th1 and Th17 responses, and glucose and amino acid absorption, while promoting Treg responses (Galgani et al., 2010). A summary of the effects of mTOR activity is shown below (figure from Waickman and Powell, 2012).

The mTOR pathway and its control of T cell activity is strongly regulated by leptin and adiponectin. Ob/ob mice show impairment of the Akt/mTOR pathway in autoreactive T cells, improving survival in an adoptive transfer model of EAE; this effect was reversed with leptin administration, and the same protective effects were seen with calorie restriction, which also suppresses leptin secretion (Galgani et al., 2010). LepR is expressed in T cells and macrophages in the central nervous system of mice with EAE, and its expression is reduced during remission (Sanna & Giacomo, 2003); inflammatory lesions in multiple sclerosis patients also show increased leptin expression (Matarese et al., 2012). Treg cells have high levels of LepR and produce leptin themselves, and neutralization of leptin can induce proliferation by reducing mTOR activity, suggesting a negative feedback loop (Procaccini et al., 2010). Th1 and Th17 cells, on the other hand, are promoted by leptin via enhanced
secreton of IFN-γ and TNF-α, and secrete leptin themselves in an autocrine loop (Matarese et al., 2012).

Adiponectin acts via the adiponectin receptor (ADIPOR), which in T cells is stored in vesicles co-localized with the inhibitory protein CTLA-4, and is expressed in response to T cell stimulation (Procaccini & Matarese, 2012); ADIPOR signaling also leads to the activation of AMPK, PPARα, and PPARγ, directly opposing the effects of leptin signaling (Kadowaki, Yamauchi, & Kubota, 2008). The suppressive effects of adiponectin on T cells may also be due to alterations in dendritic cell activation, as adiponectin-treated DCs show impaired expression of CD80, CD86, and MHC II molecules, and induce Treg differentiation in naïve T cells (Tsang et al., 2011). In summary, leptin acts to increase mTOR activity in contrast to adiponectin signaling, which is inhibitory. mTOR and AMPK signaling are largely in control of T cell fate decisions via induction of glycolytic or oxidative metabolism, representing a direct connection between nutritional status and the degree of systemic immune activation.

Adipose tissue, metabolic disregulation, and inflammation

Inflammation in adipose tissue is a hallmark of obesity and stands in stark contrast to healthy adipose tissue, which shows no signs of inflammatory activity. The source of this inflammation is now considered to be largely metabolic, and comes as a result of chronic macronutrient overload in the context of over-nutrition (Gregor & Hotamisligil, 2011). Storage of excess nutrients in adipose tissue serves as the primary defense against metabolic overload in other tissues, but once the nutrient buffering capacity of adipose tissue has been exceeded, multiple intracellular and extracellular consequences arise, marked by exposure to supraphysiological levels of glucose and fatty acids. In the extracellular compartment, ectopic lipid deposition and protein modification due to high circulating glucose levels (e.g. the production of advanced glycation end products) are the main outcomes (Odegaard & Chawla, 2013). Inside the cell, the presence of excess lipids leads to saturated fatty acid and ceramide accumulation and increased
flux through the electron transport chain, as well as activation of TLR4 (Procaccini et al., 2012). The increased mitochondrial workload demanded by excess substrate results in the accumulation of reactive oxygen species, and a decrease in the ATP:ROS ratio, a key indicator of cellular metabolic health (Nunn et al., 2009); the resultant oxidative stress directly insulin resistance, and appears to be the metabolic trigger of inflammation (Hoehn et al., 2010).

Hyperlipidemia and hyperglycemia activate a number of stress response pathways, including activation of the inhibitor of nuclear factor κB kinase β (IKKβ), mTOR, Jun N-terminal kinases (JNK), endoplasmic reticulum-to-nucleus signaling-1 (IRE-1), extracellular signaling-related kinases (ERK), protein kinase Cθ (PKCθ), suppressor of cytokine signaling (SOCS) proteins, and RNA-activated protein kinase (PKR) (Odegaard & Chawla, 2013). These pathways converge to induce insulin resistance (likely a protective mechanism against the further accumulation of glucose, fatty acids, and their metabolites) via insulin receptor substrate (IRS) protein phosphorylation, as well as inflammation via the JNK and IKKβ pathways, both of which work to activate the proinflammatory transcription factors AP-1 and NF-κB (summarized in the figure below from Odegaard and Chawla, 2013).
The effects of pharmaceuticals on inflammation provide support for the idea that metabolic dysfunction can drive inflammation. The drug metformin, an activator of AMPK, reduces gluconeogenesis in the liver, lowering blood glucose concentrations and glucose influx into - and subsequent ROS production in - metabolic tissues (Hundal et al., 2000). Given the effects of AMPK as a counter-regulator of mTOR, we could expect that metformin would have effects on T cell lineage commitment, and this appears to be the case. Metformin treatment inhibits the secretion of Th1 and Th17 cytokines and induces Treg differentiation via increased IL-10 secretion, and prevents EAE induction by preventing inflammatory cytokine, chemokine, and adhesion molecule production (Matarese et al., 2012; Procaccini & Matarese, 2012).

The accumulation of activated lymphocytes in obese adipose tissue has been noted for some time, and a July 2009 edition of Nature Medicine included three often-cited reports that shed considerable light on the nature of this accumulation. Feuerer et al. provided evidence that lean adipose tissue is home to a considerable number of Treg cells (greater than 50% of CD4 T lymphocytes), and that these cells promote insulin sensitivity in adipocytes. Furthermore, adipose tissue Treg cells were found to have a restricted TCR repertoire that was significantly different from lymph node Treg cells and adipose tissue conventional T cells (T_{conv.}), while the receptor sequences were similar between mice, suggesting antigen-specific selection in the local environment. Adipose tissue Treg cells also demonstrated a 136-fold increase in IL-10 production compared to splenic Treg cells, and elevated transcription of genes downstream of the IL-10 receptor, suggesting that adipose Treg cells not only secrete IL-10 but respond to it as well. Production of molecules affecting leukocyte migration were also upregulated compared to Treg cells from other sites, including CCR1, CCR2, CCR9, CCL6, integrin αv, activated leukocyte cell adhesion molecule, and CXCL10. Alongside the evidence of a restricted and site-specific TCR repertoire and the ability of adiponectin to drive Treg differentiation, it appears that adipose tissue Treg cells may be a unique subset of FoxP3^+ CD4 T cells that recognize a cognate antigen in the context of a supportive cytokine milieu, leading to their accumulation in lean adipose tissue.
Winer et al. (2009) presented evidence that adipose-resident T cells are responsible for the regulation of metabolic activity in the local environment, and that there are lineage-specific effects on the insulin resistance observed in diet-induced obesity. The expansion of Th1 cell populations in the visceral adipose tissue of DIO-mice was accompanied by a body mass-dependent progressive decrease in Treg cells, and this general pattern was confirmed in human adipose tissue, where obese adipose tissue showed a 12:1 ratio of Th1:Treg cells compared to a 6:1 ratio in lean adipose tissue. Adipose-resident CD4 T cells also demonstrated a restricted TCR repertoire, reiterating the findings of Feuerer et al. and suggesting localized antigen-specific expansion of the T cell population; the authors noted that tissue-driven TCR bias is common among autoimmune disorders, but has not been associated with obesity or type II diabetes up to this point. To investigate the effects of different T cell lineages on metabolic parameters, the authors reconstituted Rag-1 deficient mice fed a high fat diet (HFD) with either CD4 or CD8 T cells, and found that reconstitution with CD4, but not CD8, T cells led to improvement in insulin sensitivity and glucose tolerance. Using adoptive transfer of ovalbumin-specific T cells into HFD Rag-1 deficient mice, they observed no improvement in metabolic parameters and a lack of TCR rearrangements in splenic and adipose T cells, suggesting an antigen-specific role of T cells in metabolic control. Finally, the authors demonstrated a positive effect on metabolic parameters from the promotion of Th2 and Treg populations. Taken together, these results suggest that the composition of T cell populations in adipose tissue play a role in regulating local metabolism, and that this regulation is antigen-dependent.

The report from Nishimura et al. (2009) provided key information regarding the order of immunological events in the development of obesity. The accumulation of proinflammatory macrophages is an important facet of obese pathology, but it was previously unknown whether their recruitment to adipose tissue preceded or followed the recruitment and expansion of T cell populations. To examine this, the researchers sampled adipose tissue from mice fed a HFD or normal chow between 4 and 30 weeks of age and determined the percentage of macrophages, CD8 T cells, CD4 T cells, and Treg cells in the sample at different time points. CD8 T cell
populations increased significantly after 30 weeks, beginning at 6 weeks, while CD4 T cell populations decreased beginning at 10 weeks, and Treg cell depletion arose at 15 weeks. Macrophage populations, however, increased significantly beginning at 10 weeks, demonstrating that CD8 T cell accumulation precedes macrophage recruitment and the depletion of Treg cells. Curious about the role of CD8 T cells in adipose tissue inflammation, the authors determined that depletion of CD8 T cells via CD8-specific antibodies was sufficient to ameliorate pre-existing adipose tissue inflammation, and that treatment with anti-CD8 antibodies prevented the initiation of inflammation in mice fed a HFD, suggesting a key role of cytotoxic T cells in adipose tissue inflammation.

The role of macrophages in obesity is significant, despite their late involvement in the development of the disease. Many tissues have specific populations of macrophages that play a role in homeostasis and regulation of the local environment, and adipose tissue is no different, demonstrating the presence of two distinct types of macrophages, named M1 and M2. M1 macrophages are predominantly pro-inflammatory and are distinguished by their CD11c$^{hi}$Nos2$^{+}$TNF-$\alpha^{+}$ phenotype and high levels of secretion of IL-6, TNF-$\alpha$, IL-1$\beta$, Th1-type cytokines (e.g. IL-12) and reactive nitrogen species; their generation is, in turn, promoted by Th1 and classically inflammatory cytokines (Odegaard & Chawla, 2013; Ouchi, Parker, Lugus, & Walsh, 2011). M2, or “alternative,” macrophages are largely anti-inflammatory, are distinguished by a CD206$^{+}$CD301$^{+}$Arg1$^{+}$ phenotype, and secrete IL-10 and TGF-$$\beta$$, promoting Treg populations and a tolerogenic environment; M2 macrophages are promoted by Th2-type cytokines (e.g. IL-4 and IL-13) and adiponectin from adipocytes, and show activation of the transcription factors STAT6 (also implicated in Th2 activation) and PPAR-$$\gamma$$ and –$$\delta$$ (Ouchi et al., 2011; Shapiro, Lutaty, & Ariel, 2011). The actions of M1 and M2 macrophages are summarized below in the figure from Odegaard and Chawla (2013).
Consistent with its effects in T cells, adiponectin promotes M2 polarization at the expense of M1 cells via the activation of PPARγ and AMPK. PPARγ mediates many of the positive effects of M2 cells, and its activation with thiazolidinediones attenuates insulin resistance, while inhibition compromises M2 activation and maturation* (Shapiro et al., 2011). PPARγ can also inhibit M1 activation via inhibition of the transcription factor NF-κB* (Shapiro et al., 2011). The activation of PPARγ and AMPK is accompanied by a bias towards respiration over glycolysis, similar to the effects discussed above in Treg and memory T cells, while M1 macrophages, predictably, display a glycolytic bias similar to activated Th1/Th17 cells, further highlighting the interrelation between metabolic regulation and inflammation (Shapiro et al., 2011).

The interplay between adipocytes, Tregs, activated T cells, and macrophages in obesity is complex and often reciprocal, and the collective evidence strongly
implicates a role for self-reactive T cells in the pathogenesis of the disease. A report published in the March 5th (2013) edition of Cell by Deng, et al. provides a fascinating addition to this evidence base by demonstrating the expression of MHC II molecules in obese adipocytes, a finding that illuminates a likely route of activation for adipose-resident activated T cells independent of macrophages, which, as discussed above, are late arrivals to inflamed, obese adipose tissue. In an attempt to identify adipocyte changes in obesity that could affect immune function, the researchers micro-array profiled adipocytes from obese women and, to their surprise, found that the expression of a number of MHC II-associated genes, including the class II transactivator (CIITA), a master regulator of the MHC II pathway, were significantly increased. This finding was confirmed in mice fed a HFD, in which expression of MHC II genes were increased after only 2 weeks; after 3 months of HFD, MHC II mRNA expression in murine adipocytes approached levels seen in activated macrophages isolated from HFD- or chow-fed mice. Seeking to uncover a stimulus for increased MHC II expression, the authors observed that supernatant from leptin-stimulated Th1 cells was able to induce MHC II expression in adipocytes but was attenuated by IFN-γ-specific antibodies, suggesting the action of IFN-γ, the secretion of which is a known consequence of leptin stimulation of Th1 cells. Furthermore, using ovalbumin-specific T cells in co-culture with adipocytes, they demonstrated that CD4 T cell activation is antigen- and MHC II-dependent, established adipocytes as legitimate antigen-presenting cells. The authors also confirmed the delayed activation and M1 polarization of adipose tissue macrophages in the development of obesity. The order of events, as proposed by the authors, is illustrated to the right.
Conclusion

These findings offer significant insight into the mechanisms implicated in the development of obesity and further blur the line between metabolic and immune regulation. We have seen that nutrient sensing pathways typified by leptin/adiponectin and mTOR/AMPK are active in the regulation of metabolic tissue as well as T cell lineage commitment and follow predictable patterns. Activation of mTOR via leptin signaling induces proliferative and proinflammatory Th1 responses in macrophages in T cells and induces a glycolytic metabolic bias, while adiponectin activation of AMPK leads to the promotion of Treg responses and a respiratory metabolic bias. In lean individuals these systems act in opposition to each other in order to maintain homeostasis and tolerance, but in the context of overabundance the activation of cellular stress pathways, increased leptin and proinflammatory cytokine secretion, and suppression of tolerogenic Treg and M2 cell populations serve to promote the accumulation of activated T cells and M1 macrophages.

Given the expression of MHC II in adipocytes, the presence of restricted TCR repertoires and evidence for the macrophage-independent activation of T cells, we can now say with near certainty that the T cell accumulation seen in the development of obesity is antigen-specific, and likely autoimmune in nature; the identification of the self-antigen must be the next major objective in the field. Evidence from Galgani et al. (2010) that autoreactive T cells are non-reactive in ob/ob mouse models of EAE, and that leptin repletion recovers reactivity, provides us with a possible mechanistic link between the pathology of obesity and other autoimmune diseases. Leptin may act systemically to promote the activation of self-reactive T cells that have escaped negative selection, allowing the initiation of an autoimmune attack on various tissues, directly linking overnutrition to chronic, inflammatory disease. We may soon achieve an understanding that the metabolic syndrome and obesity do not have effects on inflammatory disease because they are inflammatory diseases themselves, and share many mechanisms in common with the development of these conditions.
Conclusions and Perspectives

This discussion has considered evidence from two main lines of inquiry: that of the impact of the homeostasis of the mucosal immune system on the development of autoimmunity, and that of immunometabolism and obesity. Both of these facets are clearly impacted by nutrition, though many of the details remain to be elucidated; mucosal immunity is impacted strongly by gliadin, and potentially as-yet-undiscovered immunogenic plant compounds, and by the health of the microbiota, whose composition is influenced by the polysaccharide content of our diets, while obesity and metabolic derangement are driven primarily by unfavorable dietary habits and caloric excess. Recent evidence suggests that circulating zonulin is elevated in patients with obesity-associated insulin resistance, and an emerging concept of “metabolic endotoxemia” – an increased translocation of LPS-containing bacteria across the mucosal membrane in obesity that may contribute to metabolic dysfunction – provide evidence for the impact of a compromised intestinal barrier on obesity-associated metabolic derangement (Blaut & Klaus, 2012; Moreno-Navarrete, Sabater, Ortega, Ricart, & Fernández-Real, 2012). The acknowledged influence of obesity-associated inflammation on intestinal permeability, on the other hand, suggests that this is a two-way road, while the ability of dysbiosis to drive inflammation and obesity and vice-versa complicates the picture greatly.

In all likelihood, however, the progression of intestinal barrier dysfunction, dysbiosis, and obesity-associated metabolic derangement are complementary to each other, and there may be different initiating factors in different situations. In one context, chronic caloric excess may lead to weight gain that drives intestinal dysfunction, while in another, a low-fiber diet may promote dysbiosis, the penetration of harmful bacteria into the mucosa, and subsequent metabolic derangement. In both cases, however, the terminal impact is the promotion of an inflammatory, non-tolerogenic environment that may lead to a break in self tolerance. Increased leptin secretion in the context of weight gain almost certainly influences the metabolism and tolerogenic properties of not just local but peripheral
lymphocyte populations, given its systemic actions, suggesting that the metabolism-driven loss of lymphocyte quiescence seen in obese adipose tissue may also be at play in the intestinal mucosa, and providing a mechanistic link between metabolic syndrome/obesity and mucosal immune dysfunction.

The fact that intestinal permeability, dysbiosis, and metabolic derangement present a web of comorbidity paints a complex picture of autoimmune pathogenesis whose details have yet to discerned, and the ability of inflammation to drive intestinal permeability, metabolic dysfunction, and dysbiosis by itself makes it difficult, if not impossible at this point in time, to pinpoint the initiating factor in autoimmune pathology. The unifying factor, however, is diet-driven inflammation, and evidence for the potential impact of diet on the development of autoimmunity suggests that non-pharmaceutical interventions may be possible and effective if they can slowly dial back inflammatory stimuli and encourage the reclamation of tolerance. Research into the direct impacts of diet on autoimmunity is sorely needed, as are studies investigating the chronology and mechanistic commonalities of the pathogenesis of different autoimmune diseases. The evolution and emergence of immunometabolism, intestinal microbiota, and mucosal immune system research make this an exciting time in the field, and we await a unifying picture of the interrelations of these fields and their impacts on disease.


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Introduction


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Appendix:
Non-technical summary of concepts
Introduction

The immune system lies as our primary defense against infection and disease, serving to protect us from the constant bombardment of potential pathogens that we are exposed to on a daily basis. It is necessarily complex, given the almost unlimited variety of molecules it must recognize and either ignore, or eliminate. The cells of the immune system, called white blood cells or leukocytes, originate from a common progenitor in the bone marrow and differentiate into various mature cell types in the bone marrow, the thymus (located on the midline, near the heart), the blood, or peripheral tissues. These mature cells (and the immune system in general) can be divided into two branches: those of innate immune system, and the adaptive immune system. The innate immune system is responsible for recognizing and eliminating 99.9% of the potential pathogens that we encounter, and it does this in large part through the recognition of molecular patterns that many pathogens share. The receptors that recognize these patterns (pattern recognition receptors) are numerous but invariant, and do not change in response to infection; once a pathogen is identified through these receptors, it is destroyed through a variety of mechanisms. The adaptive immune system, on the other hand, works to recognize most of the possible patterns present on pathogens through the production of highly variable receptors that are specific to individual parts of individual pathogens. This is accomplished, amazingly, through the shuffling and recombination of DNA, and generates essentially random mutations to these receptors, accounting for the huge variability in antigen binding capacity found in these cells (antigens are parts of pathogens that generate immune responses).

The adaptive immune system is made up of two cells types, collectively termed lymphocytes: B cells, which develop in the bone marrow, and T cells, which develop in the thymus. B cells are responsible for the production of antibodies that bind antigens in the extracellular space, permitting their clearance by cells of the innate immune system, which can recognize antigen/antibody complexes. T cells come in two varieties: those responsible for the elimination of virally infected cells (termed cytotoxic lymphocytes), and those that assist other cells in developing the
appropriate response to other types of infection (termed *T helper cells*). All of these are important in the production of an appropriate immune response. During an infection, the recognition of a pathogen by the innate immune system induces *inflammation*, which is marked by the recruitment of more immune cells to the site of infection, and the production of *cytokines*, signaling molecules of the immune system that activate other leukocytes. Leukocyte recruitment is accompanied by swelling and occlusion of the site of infection due to the activation of clotting factors.

The inflammatory response is essential to the successful elimination of an infection, but when allowed to persist unchecked, can have a variety of consequences. The term *chronic inflammation* describes this self-perpetuating response, and is widely used today to describe the conditions behind many modern ailments, from asthma and allergy to heart disease and obesity. The simplest way to describe chronic inflammation is a perpetual, non-lethal low-grade activation of the immune system on a systemic level. While chronic inflammation does not lead to acute mortality, it can cause significant, and in some cases serious, health issues. The incidence of inflammatory disease is rising rapidly in industrialized countries, and there are no easy solutions to this increasingly damaging situation.

A particularly intense manifestation of inflammatory disease are the *autoimmune diseases*, a wide variety of conditions that develop when the immune system attacks our own tissues instead of pathogens. Normally, checks and balances during the development of lymphocytes eliminate self-reactive cells and prevent an attack on self-tissue, but some self-reactive cells escape into the circulation. These cells are kept in check through the promotion of *tolerance* (non-response), primarily via *regulatory T cells* (Tregs) that produce suppressive cytokines when activated. Autoimmune diseases are universally marked by a reduction in the presence and effectiveness of Treg cells, and a generally inflammatory environment, suggesting that they develop following a breakdown in tolerance. Recent estimates put the incidence of autoimmune disease (AD) at 16% in the United States, while ADs are the second-highest cause of chronic illness. The absence of AD in non-westernized cultures, however, and the increasing incidence in Western society, suggest that a difference between these may be behind the surge in disease. Because genetic
evolution is a slow, generation-by-generation process, it is extremely unlikely that
genetic changes are of principle importance, and more likely that a change in
environment has left us vulnerable to a loss of tolerance and AD.

Nutrition is one of many environmental pressures that may contribute to the
development of AD, but one that we have control over, and the differences in
nutritional environment between industrialized and non-industrialized cultures are
widely acknowledged. There is scant evidence, however, linking nutrition and
autoimmune and inflammatory diseases specifically, so we must look to evidence for
the potential impact of nutrition on inflammation, and the impact of inflammation
on autoimmunity, to suggest a connection. There are two main mechanisms that
appear to play a large part in the development of inflammation and loss of tolerance
that leads to AD: a breakdown in the intestinal barrier that allows the activation of
potentially self-reactive lymphocytes, and metabolic disregulation in lymphocytes
themselves that may also drive activation. We will address these in order.

The Intestinal Barrier: Where the Rubber Meets the Road

The mucosal immune system\(^1\) is the largest interface between our internal
and external environments – the small intestine alone has an approximate surface
area of 400 m\(^2\) – and is responsible for the elimination of a diversity of pathogens
while maintaining tolerance to large populations of resident bacteria and the
continuous flow of food-derived particles that pass through the digestive system
every day. While this system includes everything from the epithelia of the urogenital
tract, the respiratory tract, and the mammary glands to that of the digestive
tract, the mucosal immune system of the intestines is of primary importance when
considering the impact of nutrition on immunity. The intestinal mucosa is home to a
wide variety of leukocytes, including specialized populations of lymphocytes that
are site-specific. Together, these cells coordinate a fine balance between tolerance
and reactivity, and in healthy mucosa inflammation is largely absent, demonstrating

\(^{1}\) The “mucosal epithelia” refers to those epithelial barriers lining the body's internal cavities (e.g. the
digestive tract) that secrete a protective layer of mucus. The mucosal immune system is a distinct
compartment of the immune system associated with the mucosal epithelia.
the effectiveness of local homeostatic control given the large numbers and volume of potential pathogens passing through the intestines every day, and the presence of $10^{12}$ bacterial cells/mL in the colon contents.

*Celiac disease, barrier disruption, and inflammation*

Disruption of this sensitive intestinal barrier is associated with a number of diseases, most notable Celiac disease (CD) and the inflammatory bowel diseases (IBDs: Crohn’s disease and ulcerative colitis). CD is an increasingly prevalent autoimmune/allergic disease that is triggered in genetically susceptible individuals by the ingestion of the gliadin fraction of gluten, a protein found in wheat, rye, oats, and barely, and similar proteins found in other cereal grains. The prevalence of CD in the US was recently established as 1 in 133, with a higher incidence in close relatives of CD patients. CD shares comorbidity with a number of other autoimmune diseases, most notably type 1 diabetes (an autoimmune disease of the pancreas), and provides an example of the potential impact of a bioactive food protein on the homeostasis of the intestinal mucosa. In CD, digestion of gliadin into peptide fragments allows them to bind to receptors on intestinal epithelial cells, beginning an intracellular signaling cascade that loosens the structural connections between epithelial cells, called *tight junctions* (TJs). TJs normally function to prevent the passage of the luminal contents into the connective tissue lying underneath the epithelium, and the disruption of TJ integrity can allow them access to leukocytes that are normally isolated from the luminal contents.

In CD, gliadin fragments are taken up by cells of the innate immune system and presented to T cells, generating an adaptive response to gliadin and the production of inflammatory cytokines by innate cells. This is mediated by specialized molecules of the innate immune system that “present” peptide fragments to T cells, dubbed *MHC molecules* after the gene that encodes them, the *major histocompatibility complex*. When gliadin makes its way into the epithelium, it is modified by the enzyme *tissue transglutaminase* (tTG), converting the amino acid glutamine into glutamate and allowing it to bind more effectively to MHC molecules. Certain alleles of these molecules constitute the genetic risk factors for celiac, as
their high affinity for gliadin fragments allows for enhanced recognition by T cells. Once activated, these T cells promote inflammation that recruits additional leukocytes to the small intestine, and promotes the production of antibodies that target gliadin and self-proteins in the local environment. T cells that are activated in this context can target epithelial cells for destruction, leading to the intestinal damage found in biopsies of CD patients.

Inflammatory cytokines can themselves alter TJ function, worsening the barrier dysfunction initiated by gliadin signaling, and generating a feed-forward loop of TJ disruption and further inflammation. The mucosae possess a variety of self-reactive lymphocytes, and these may escape into the circulation, allowing them access to tissues that they can target for destruction. Under physiological conditions this is prevented by Treg cells and other tolerance-promoting mechanisms, but the presence of inflammation can provide an activating signal that is normally absent. This mechanism of activation, and the potential to generate an autoimmune attack on tissues beyond the intestines, is hypothetical at this point, but the presence of barrier disruption in a number of autoimmune diseases provides support for the concept.

**Gut flora and regulation of the mucosal immune system**

The existence of large numbers of bacteria in our large intestines, and, to a lesser extent, our small intestines, has long been recognized, but the impact of the microbiota (colloquially, *gut flora*) on human health has only recently been targeted as a major area of research. The development of new culture-independent techniques has allowed researchers to rapidly quantify the composition of the microbiota and compare profiles between diseased and healthy populations, as well as track the development of the microbiota with age. Investigations into the impact of the microbiota on immune function complement this work, and contribute to a developing, though still nascent, picture of the functional relationship between bacteria and their hosts in homeostasis and disease. Research has identified potential roles for microbiota in a growing number of conditions, including
inflammatory bowel disease, asthma and allergic diseases, rheumatoid arthritis, mouse models of multiple sclerosis, and type 1 diabetes.

The gut flora, under physiological conditions, are symbiotic and promote the proper development of the mucosal immune system and its proper function once established. Cells of the innate immune system regularly sample luminal contents in the intestines, and the recognition of bacteria by these cells provides activating signals that generate a baseline level of inflammation (physiological inflammation) that is crucial in maintaining a proper mucosal barrier. The importance of these interactions are evident in germ-free mice raised without bacterial exposure that demonstrate altered immune responses and reduced numbers of Treg cells in the large intestine. Furthermore, bacteria produce a number of metabolites that promote mucosal homeostasis, including short-chain fatty acids that are the primary fuel source of colonic epithelial cells. The gut flora rely on nutrients that we ingest in order to survive, and changes in diet are accompanied by changes in our bacterial communities. Bacteria possess a number of enzymes that can metabolize plant polysaccharides that we are incapable of digesting, and a diet low in plant material fails to support these potentially important bacterial species. A key role of commensal bacteria is to prevent colonization by pathogenic bacteria, and dysbiosis generated by a low-polysaccharide diet may allow the infiltration of harmful bacterial species, the promotion of inflammation, and the subsequent activation of self-reactive lymphocytes, as discussed above.

The concept of *dysbiosis* – a pathological imbalance of intestinal bacterial populations – as a possible mechanism of disease is gaining traction, and evidence that dysbiosis can impact TJ integrity is a key observation. While continual stimulation by the gut flora is necessary for proper immune function, inflammation beyond physiological levels may hamper the negative feedback mechanisms that are in place to prevent penetration of bacteria into the epithelium. Excessive exposure to luminal bacteria may, once again, generate a feed-forward inflammatory loop that serves to further damage the integrity of the mucosal barrier.

An important detail to keep in mind in this discussion is the ability of systemic inflammation from other sources to alter TJ integrity and damage mucosal
barrier integrity and to change the composition of bacterial communities independently. Dysbiosis may be a driver of disease, but can also be a consequence of inflammation and its effects on the mucosa. While it is tempting to pin the source of inflammation (and subsequent activation of self-reactive cells) on bioactive plant proteins like gliadin and a disrupted microbial community, other sources of inflammation may precede changes in the mucosal immune system. One such source of inflammation is obesity, the prevalence of which in industrialized society makes it a likely contributor to the epidemic of autoimmune disease we see today.

**Metabolism and Immunity**

The obesity epidemic is widely recognized, and while the precise causes are not entirely clear, the presence of excessive nutrient intake and subsequent systemic metabolic dysfunction is not controversial. Obesity is frequently accompanied by a variety of conditions that are collectively referred to as the metabolic syndrome, including high plasma glucose, high plasma fatty acids/triglycerides, hypertension, and insulin resistance; immunological alterations in obesity are increasingly recognized as well, and the presence of chronic inflammation is a hallmark of the condition. These immunological alterations are quite pronounced, and include the accumulation of activated lymphocytes and innate cells in obese fat tissue, and a depletion of Treg cells, as well as mucosal barrier disruption and dysbiosis. Recent work has begun to unravel the interrelation of immunity and metabolism, and provides some intriguing evidence for our developing understanding of autoimmune disease.

Like every other cell, leukocytes require energy and metabolic substrate to maintain normal cellular function, and to divide and proliferate. The metabolic requirements of dividing lymphocytes are particularly pronounced, and their activation is accompanied by a switch to almost exclusively glycolytic metabolism, which allows for the production of metabolic intermediates that are required to generate amino acids, lipids and nucleic acids for the generation of new cells during an immune response. On the other hand, non-activated lymphocytes and Treg cells rely primarily on oxidation, and are non-proliferative. Lymphocytes rely on
intracellular and extracellular energy-sensing pathways in order to determine the proper response to a given stimulus, and fat cells provide signals to this effect via the hormones leptin and adiponectin, which signal energy sufficiency/excess and insufficiency/deficit, respectively. Leptin secretion increases in proportion to fat mass, while adiponectin is secreted in inverse proportion, and both act strongly on lymphocytes to elicit specific responses. In lean fat tissue, adiponectin secretion far outweighs leptin output, and in response, leukocytes in fat tissue are primarily tolerogenic Treg cells and non-activated leukocytes that rely on oxidative metabolism. In obese fat tissue, on the other hand, leptin production predominates, and the presence of glycolytic, activated, inflammatory lymphocytes is dramatically increased.

The presence of activated lymphocytes in fat tissue is surprising, given that activation is dependent on recognition of an antigen and subsequent stimulation. In recent years, the hypothesis that these cells recognize self-proteins in obese fat tissue has gained credibility, and the very recent discovery that fat cells can participate in immunity as members of the innate immune system by activating T cells makes a strong case that obesity is, in fact, an autoimmune disease. In the presence of excess energy (i.e. the high circulating levels of macronutrients found in obesity) and permissive signaling through energy-sensing pathways (i.e. leptin), inactive, self-reactive T cells can make the switch to glycolytic metabolism and proliferation, allowing the generation of an autoimmune response; the depletion of Treg cells and a loss of adiponectin secretion compounds and permits this aberrant activation, and could potentially be a key mechanism in the generation of autoimmune disease in the rest of the body. In this way, there is a clear line of evidence connecting chronic over-nutrition and the development of autoimmune disease.
Conclusion and Perspectives

We have seen how multiple pathways may contribute to the development of autoimmune disease, and that these act, potentially, through the disruption of energy homeostasis and mucosal immune function. A lingering issue in the discussion of dietary influences on autoimmunity is the order of events; inflammation can drive metabolic dysfunction, alterations in intestinal permeability, and dysbiosis, so causality could lie in any of these directions, or originate from some other source entirely. The unifying factor in the discussion is a loss of tolerance to self tissues via the generation of inflammation, and multiple roles of diet in driving the development of an inflammatory environment. Evidence for the metabolic effects of immunity on the loss of tolerance suggest that over-nutrition may be a crucial component of this process, and could effect the mucosal barrier, as evidenced by the presence of dysbiosis and intestinal permeability in obese patients. Bioactive compounds in cereal grains, like gliadin, provide a source for the initiation of intestinal inflammation, while a lack of fiber and other potentially disruptive nutritional behaviors can drive dysbiosis. Taken together, this evidence suggests that the modern nutritional environment can promote inflammation and the initiation of autoimmune disease, and increasing research interest in the direct impacts of nutrition on autoimmunity may soon provide dearly-needed evidence to support a true mechanistic understanding of the causal relationships at play.