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Minireview

Microbiota and Autoimmune Disease: The Hosted Self

Diane Mathis[1,*] and Christophe Benoist[1,*]
[1]Division of Immunology, Department of Microbiology and Immunobiology, Harvard Medical School, Boston, MA 02115, USA
*Correspondence: cbdm@hms.harvard.edu (D.M. and C.B.)
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The trillions of microbial symbionts normally hosted by mammals have important influences on the development and function of the immune system. We highlight recently discovered cellular and molecular mechanisms by which they impact autoimmune diseases—in particular, gut-distal disorders. Besides provoking a reconsideration of the definition of immunological "self" and "nonself," these new findings evoke exciting possibilities for the discovery of a whole new class of immunomodulatory molecules.

Introduction

The principal function of the immune system is to defend the body from pathogenic invasion—by microbes from without and tumors from within. Both the rapidity of the innate and diversity of the adaptive immune systems are mobilized to this end. An unavoidable byproduct of generation of the needed diversity is that T and B lymphocytes capable of recognizing self-constituents occasionally arise in the primary lymphoid organs—the thymus and bone marrow, respectively. A complex network of immunological tolerance mechanisms has evolved to cull these self-reactive specificities from the emerging lymphocyte repertoire or to keep them in check if they somehow manage to exit to the periphery. But occasionally one or more of these mechanisms goes awry, resulting in a state of autoimmunity, which sometimes progresses to a pathological condition, autoimmune disease.

Immunological tolerance, autoimmunity, and autoimmune disease have been elements of immunologists' vocabulary for decades—and hundreds of experiments and debates have been aimed at their elucidation. Nonetheless, recent advances in our understanding of the composition and activities of microbial populations that colonize diverse body sites as commensals, mutualists, or parasites prompt a reconsideration, or at least extension, of some basic concepts. Here we will touch on how postmodern appreciation of the universe of microbes hosted by mammals modifies our definition of self:nonself, how symbiont microbiota impact development of the immune system, how they can influence the initiation or outcome of autoimmunity, and how we might translate emerging knowledge on microbiota and microbiomes to the human context.

Nonself or Self?

Mammals are sterile at birth, the neonate acquiring its microbiota during and shortly after naissance. In adults, symbiont communities can fluctuate with alterations in host diet or physiology; however, they are stable enough over time that kinship relations can be discerned (Ley et al., 2008; Turnbaugh et al., 2009). The relative importance of host environment versus genetics in shaping the composition remains under debate. Certainly, phylogenetic influences are discernable (Ley et al., 2008), and a given species appears to have a "core microbiome" at the gene level (Turnbaugh et al., 2009; Benson et al., 2010). Yet monozygotic and dizygotic twin pairs have a similar degree of variability in gut microbial communities (Turnbaugh et al., 2009). In any case, the composition of the microbiota reflects eons of host symbiont coevolution—with fine tuning of both host and microbe genomes—and variability may be an evolutionary advantage in and of itself.

These observations put a new slant on issues related to immunological tolerance and autoimmunity. If an adult mammal harbors over ten times more bacterial cells in the intestinal tract than there are somatic and germ cells in its entire body, where does "self" end and "nonself" begin? Is the microbiome to be considered an environmental factor (because it responds to surrounding conditions) or an epigenetic factor (because it passes from generation to generation)? It seems appropriate to encompass the core microbiota in the definition of a mammal's "self," and to consider that tolerance mechanisms that evolved to eschew attack on the tissues will be shared with those employed to maintain a balance with the universe of symbionts.

It Takes Two to Tango …

One of the major impacts of the mammalian microbiota is its effect on the development and function of the immune system. In fact, communities of bacterial and immune cells are closely linked, especially those residing in the intestinal tract, each influencing and being influenced by the other (reviewed in Lee and Mazmanian [2010] and Littman and Pamer [this issue, pp. 311–323]). While the means by which the immune system deals with microbes is an old and ongoing preoccupation of immunologists, just how the symbiont microbiota shapes immunity has become amenable to precise mechanistic dissection only relatively recently, reflecting advances both in high-throughput sequencing methods and in our knowledge of lymphocyte subpopulations.

In general terms, the incomplete state of the immune system in adult germ-free (GF) and neonatal individuals argues that microbes drive its maturation. Reported defects include both gut-associated and systemic abnormalities: defective T, B, and innate cell compartments in mucosal tissue, fewer $CD4^+$ T lymphocytes in all peripheral lymphoid organs, a systemic tilt to the T-helper (Th) 2 phenotype, and reduced complements of IgG and IgA antibodies (Abs). All of these aberrancies are reversed within weeks after microbial colonization.

In more specific terms, gut-resident microbes—sometimes even a single species—can have a striking influence on the emergence and/or stability of particular $CD4^+$ T cell subsets. For example, segmented filamentous bacteria (SFB), a gut-resident

Table 1. Mouse Models OF Autoimmune Disease

Human disease	Mouse Model	Reference	Primary Immunological Mechanism(s)	Effect of Introducing Microbiota
Inflammatory arthritis	*Il1rn*$^{-/-}$ (knockout mouse line)	(Abdollahi-Roodsaz et al, 2008)	Emphasizes innate immune system: cytokines, Toll-like receptors.	Full complement (GF vs. SPF): enhanced disease. *lactobacillus bifidus*: enhanced disease
	K/BxN (T cell receptor transgenic mouse line)	(Wu et al, 2010)	T, B, and innate immune cells important. Highlights role of autoAbs.	Full complement (GF vs. SPF): enhanced disease. SFB: enhanced disease
	Collagen-induced arthritis (rat)	(Breban et al, 1993)	T, B, and innate immune cells important. Adjuvant-induced.	Full complement (GF vs. SPF): dampened disease
	SKG (mouse line with a point mutation in *Zap70*)	(Yoshitomi et al, 2005; Hashimoto et al, 2010)	T-cell-mediated. Defective central tolerance of T cells.	Full complement (SPF vs. C): enhanced disease. Fungal β-glucans: enhanced disease
Multiple Sclerosis	Experimental autoimmune encephalomyelitis (EAE) (mouse)	(Lee et al, 2010)	T-cell-mediated, though multiple other cell-types play a role. Adjuvant-induced.	Full complement (GF vs. SPF): enhanced disease. SFB: enhanced disease
Autoimmune polyglandular syndrome	*Aire*$^{-/-}$ (knockout mouse line)	(Gray et al, 2007)	T-cell-mediated. Defective central tolerance of T cells.	Full complement (GF vs. SPF): no effect
Type-1 diabetes	Nonobese diabetic (NOD) (genetically selected inbred mouse strain)	(King and Sarvetnick, 2011; Kriegel et al, 2011)	T-cell-mediated, though multiple other immune cells impact. Multigenic.	Full complement (GF vs SPF): varies in different colonies. SFB: protects females

Only those studies referred to in the text are presented. GF = germ-free; SPF = specific-pathogen-free; C = conventionally housed.

Gram-positive, spore-forming, obligate anaerobe most closely related to *Clostridia* (Kuwahara et al., 2011, Sczesnak et al., 2011, Prakash et al., 2011), promotes the development of a robust Th17 population in the small-intestinal (SI) lamina propria (LP) of mice (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009); a defined mix of *Clostridia* strains induces a population of Foxp3^{+}CD4^{+} regulatory T (Treg) cells in the murine large-intestinal (LI) LP (Atarashi et al., 2011); and the human gut symbiont *Bacteroides fragilis* exerts multiple effects on CD4^{+} effector and regulatory T cell populations when it colonizes mice (Mazmanian et al., 2005; Mazmanian et al., 2008; Round and Mazmanian, 2010; Round et al., 2011). The pathways from microbe colonization to immune cell modulation are so far only poorly defined but have been suggested to include the following: an action of serum amyloid A (SAA) on dendritic cells (DCs) (Ivanov et al., 2009), ATP-mediated activation of DCs (Atarashi et al., 2008), the induction of TGF-β expression by gut epithelial cells (Atarashi et al., 2011), an effect of *B. fragilis* polysaccharide A (PSA) on DCs (Mazmanian et al., 2005), and an interaction between *B. fragilis* PSA and Treg TLR-2 (Round et al., 2011). Whether or not all of these proposed mechanisms will survive close scrutiny, the perception is that we have only scratched the surface so far and that microbes have likely evolved many means to manipulate mammalian immune systems.

Transcending the Neighborhood …

Given the multifaceted interplay between the mammalian microbiota and immune system, it is not surprising that alterations in symbiont microbe communities were long ago linked to immune pathologies, notably allergic and autoimmune disorders (Strachan, 1989; Wills-Karp et al., 2001). Ties to inflammatory bowel diseases are easy to envisage; as these are being reviewed by Littman and Pamer (in this issue), we will not deal with them here. A number of associations between the microbiota (or defined elements of it) and particular gut-distal autoimmune disorders have been reported over the years, but it is only quite recently that techniques that permit one to probe the cellular and molecular underpinnings of such correlations became available.

Comparisons of disease parameters in different autoimmune models (detailed in Table 1) housed under GF versus specific-pathogen-free (SPF) or conventional conditions have shown the full gamut of responses to loss of the microbiota: disease amelioration (e.g., Abdollahi-Roodsaz et al., 2008; Wu et al., 2010; Lee et al., 2011), no significant effect (Gray et al., 2007), or disease exacerbation (Breban et al., 1993). It may seem perplexing that the microbiota can have opposing impacts on the development of autoimmune disease—more so when there are divergent outcomes with models of purportedly the same disorder, e.g., arthritis (Abdollahi-Roodsaz et al., 2008; Wu et al., 2010; Breban et al., 1993), or even with the same model in the hands of different investigators, e.g., NOD mice (Kriegel et al., 2011; King and Sarvetnick, 2011). These "discrepancies" are likely to clear up with more precise knowledge of colonizing microbiota at different animal facilities and more profound appreciation for the heterogeneity of the pathogenic mechanisms underlying the various models.

Recent findings on the impact of SFB on autoimmune manifestations in different mouse models serve to illustrate this last point. Mice kept under GF conditions have few Th17 cells, notably in the major site of their accumulation, the SI-LP; recolonization of GF mice with intestinal microbiota induces a robust Th17 compartment within days (Ivanov et al., 2008). Strikingly, monocolonization with SFB, a filamentous bacterium intimately associated with the intestinal epithelium, can produce the same result (Gaboriau-Routhiau et al., 2009; Ivanov et al., 2009). The implication of Th17 cells and/or IL-17 in a number

of autoimmune manifestations prompted explorations of the influence of SFB in rodent disease models. Arthritis was greatly attenuated in the K/BxN model (Table 1) when housed GF (vis-à-vis SPF); robust disease was restored 10–14 days after introduction of GF mice into an SPF facility, and within days of SFB monocolonization (Wu et al., 2010). The sequence of events was documented to be as so: SFB colonization → development of an SI-LP Th17 compartment → appearance of Th17 cells in the spleen, likely via migration from the gut → generation of arthritogenic B cells and autoAbs in the spleen, promoted by a direct impact of IL-17A on B cells → autoAb deposition in the joints, ultimately provoking arthritis by well-established mechanisms entailing the mobilization of inflammatory cells and cytokines. This scenario is consistent with the fact that treatment of K/BxN mice with anti-IL-17 mAb blocked the production of autoAbs and the consequent development of arthritis (Wu et al., 2010). Next, parallel results on an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) were reported: EAE was attenuated in GF mice, associated with a reduction in Th17 cells (also fewer Th1 and more Treg cells); monocolonization with SFB induced EAE, subtended by an increased Th17 compartment in both the gut and spinal cord (Lee et al., 2011). The consistency in the results on the arthritis and EAE models served to increase the surprise when it was found that SFB was associated with disease protection in NOD mice, a spontaneous model of type-1 diabetes (T1D) (Kriegel et al., 2011). Individuals from the same NOD colony differed in their SFB status, which was reflected in their relative susceptibility to disease: while almost all females that were free of this microbe developed T1D, only about 15% that harbored it got diabetes. Proof of SFB's role in disease protection, in particular whether it is a direct one or mediated through cosymbionts, must await monocolonization or cohousing experiments. In the meantime, the only difference found in the immune systems of SFB^+ and SFB^- female NOD mice was a greatly reduced SI-LP Th17 population in the latter.

How might SFB promote autoimmune disease in one context and dampen it in another? It is important to keep in mind that not all autoimmune disorders have the same mechanisms of initiation, propagation, and regulation. Just as it was too naive in the 1990s to think that autoimmune manifestations universally reflect a Th1/Th2 imbalance, it is an oversimplification today to expect that they always signal an upset in the Th17/Treg balance. Indeed, considering the models discussed above, most murine arthritis models have a strong Th17 dependency; there is still active debate over the relative importance of Th1 and Th17 cells in EAE; and there is little, and contradictory, support for a critical role for Th17 (over Th1) cells in NOD diabetes (discussed in Kriegel et al. [2011]). Th subsets are known to crossinhibit, so it follows that a Th17-inducing microbe (like SFB) can potentially inhibit a Th1-dependent disease (like NOD diabetes). An alternative possibility is that another SFB activity might have differential impact in different autoimmune contexts. For example, SFB induces IL-22 expression, as well (Ivanov et al., 2009; Kriegel et al., 2011), and this cytokine's ability to repair intestinal epithelium might counter breaches of the intestinal barrier thought to promote T1D (Lee et al., 2010; Turley et al., 2005) but not known to impact arthritis or EAE.

Such pleiotropic effects are also characteristic of the human intestinal symbiont, *B. fragilis*. This microbe and its product, PSA, dampened mouse models of colitis and EAE, mobilizing mechanisms ranging from inhibition of Th1 cells, to induction of IL-10-producing $CD4^+$ cells, to reduction of the Th-17 compartment, to enhancement of Treg activity (Mazmanian et al., 2005, 2008; Round and Mazmanian, 2010; Round et al., 2011). What ties these mechanisms together? One possibility is that different processes come into play according to the modality (*B. fragilis* versus PSA), route, or dose of administration, or in different contexts of autoimmunity. A perhaps more satisfying explanation is that Tregs are the lead players, secondarily dampening Th17 or Th1 or even Ab effector responses, depending on the context. This interpretation would be consistent with the recent suggestion that PSA might signal Tregs directly through TLR-2, which in turn restrain Th17 cells (Round et al., 2011). However, this scenario would need to accommodate the current concept that distinct subsets of Tregs have evolved to regulate different Th subsets (Campbell and Koch, 2011).

Lastly, additional complexity derives from the fact that different microbes can impact the same immune system compartments, by similar or dissimilar mechanisms. For example, arthritis in the SKG mouse model (Table 1) is more severe in a colony housed under conventional than under SPF conditions; disease exacerbation in the dirtier facility was attributed to fungal colonization, which, through a β-glucan/Dectin-1 interaction, induced arthritogenic Th17 cells in a complement-dependent manner (Hashimoto et al., 2010; Yoshitomi et al., 2005). And arthritis in the *il-1rn*$^{-/-}$ model can be provoked by monocolonization of GF mice with *Lactobacillus bifidus*, through a TLR-4/Il-1/Th17 axis (Abdollahi-Roodsaz et al., 2008).

All in all, then, this seems like a very fruitful, but exceptionally complicated, area of investigation, reflecting the stunning complexity of both the microbiota and the immune system, and the myriad planes of interaction between them. Almost certainly, systems approaches will be helpful in elucidating important principles that govern host:symbiont interplay as it impacts autoimmune disease. Just as certainly, reductionist strategies, such as examination of monocolonized and gene-manipulated mice, will continue to unravel key processes, pathways, and players. Another line of investigation in its early days is genetic dissection. Genome-wide analysis of a cross between C57Bl/6 mice and an ICR-derived outbred line revealed loci that were associated with individual microbial species, others linked to groups of related taxa, and still others with pleiotropic impacts on groups of distinctly related organisms (Benson et al., 2010). Of even greater interest in the context of autoimmune disease is a report that the MHC/HLA-like molecule Cd1d could regulate the composition of mouse intestinal communities (Nieuwenhuis et al., 2009). Might long-recognized but little-understood MHC/HLA associations with a variety of autoimmune disorders at least in part reflect influences on symbiont microbe colonization? On a related note, it might be worthwhile to extend the concept of molecular mimicry, as a trigger of autoimmunity, to the symbiont microbiome. Indeed, a recent study identified a microbial peptide, common to multiple classes of symbionts, that had weak sequence homology with myelin basic protein and could induce disease in a humanized mouse model of multiple sclerosis (Harkiolaki et al., 2009).

And Human Autoimmune Diseases?

Of course, the end goal is to translate this new knowledge, mostly derived from rodent models, to a better understanding of autoimmune diseases in humans. Most such disorders show a 30%–70% discordance rate in identical twins, leaving plenty of room for environmental, epigenetic, and stochastic elements to play a role. Certainly, genetics cannot explain the disconcerting increase in a number of immune maladies over the past several decades, notably T1D, MS, and asthma (particularly in so-called "developed" nations). Hence the proposal and later modification of the "hygiene hypothesis"—changing diets, improved sanitary conditions, increased use of antibiotics, etc. prevent the immune system from being adequately "primed" during its maturation, resulting in Th subset imbalances, Treg cell deficiencies, and other faults that predispose to immune diseases. It is easy to envisage how the microbiota fits into such a scheme—it is modified in response to diet, sanitation, and antibiotics, and its composition instructs immune-system maturation.

Unraveling the microbiome/immune-system/autoimmune-disease axis in humans will be difficult and complex. Microbiome-wide association studies are currently in progress but are likely to be subject to several of the same weaknesses and disappointments as genome-wide association studies (GWAS) are—and then some, given that, while an individual's genome is constant, its microbiome fluctuates over time, with the environment, with drug treatment, etc. Stem-cell technology should aid in the development of culture systems that capture the interactions between microbial, immune-system, and intestinal cells, but these are likely to be challenging endeavors that require maintaining a three-dimensional structure, optimally under anaerobic conditions. No doubt, rodent models, in particular humanized-mouse models, will continue to elucidate critical principles. Murine and human immune systems are much more similar than they are different; the species' microbiota share dominant groupings, but there are many divergences at the lower taxonomy levels; though there may be greater similarity in the microbiomes (Ley et al., 2008). Encouragingly, human-specific commensals like *B. fragilis* can colonize the mouse intestine, impact the immune system, and modulate autoimmune and inflammatory diseases (Mazmanian et al., 2005, 2008).

Regardless of the impediments, studies on the microbiota and microbiome open new vistas on autoimmunity and autoimmune disease. There may or may not prove to be associations between particular symbionts and particular autoimmune disorders. And their identification may or may not yield novel approaches to prevention or treatment – entailing administration of prebiotics, probiotics or drugs. But even independent of such associations, the microbiome promises to be a treasure-trove of novel immunomodulatory molecules. It has coevolved with its host for eons, developing a multitude of strategies to tame the immune system. We should learn, and heed, its lessons.

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