Review

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Autoimmune host–microbiota interactions at barrier sites and beyond

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The microbiota is considered to be an important factor influencing the pathogenesis of autoimmunity at both barrier sites and internal organs. Impinging on innate and adaptive immunity, commensals exert protective or detrimental effects on various autoimmune animal models. Human microbiome studies of autoimmunity remain largely descriptive, but suggest a role for dysbiosis in autoimmune disease. Humanized gnotobiotic approaches have advanced our understanding of immune–commensal interactions, but little is known about the mechanisms in autoimmunity. We propose that, similarly to infectious agents, the microbiota mediates autoimmunity via bystander activation, epitope spread, and, particularly under homeostatic conditions, via crossreactivity. This review presents an overview of the current literature concluding with outstanding questions in this field.

Host–microbe interactions: from Pasteur to present

> 'Messieurs, c'est les microbes qui auront le dernier mot.' (Gentlemen, it is the microbes who will have the last word) – Louis Pasteur

Louis Pasteur was right in many ways. All animals are indeed 'walking culture dishes' and will be decomposed and metabolized by the collection of all commensals that colonize us: the microbiota (see Glossary). We are colonized from birth by a large diversity of microbes spanning all three domains of life that form evolutionary ties with the human host. These microorganisms have a range of relationships with their hosts, and exist as mutualists, symbionts, or pathobionts. To date, our understanding of commensal–immune interactions comes principally from studying the gut microbiota. However, the microbiota outside the gut is likely to be equally important, especially with regards to 'barrier diseases' that occur on mucosal or skin surfaces that are colonized with niche-specific microbiota [1].

Far beyond the crucial role of gut bacteria in nutrition and metabolism, it is well established that their influence reaches many host physiologic systems [2,3]. Gnotobiotic animal experiments have shed light on how the microbiota influences the metabolism of drugs, neurological function, immune development and homeostasis, and various chronic diseases of modern societies [3]. Commensals exert profound effects on the development and function of the immune system and therefore likely influence immune-mediated diseases [4]. Indeed, gut bacteria prevent, exacerbate, or induce numerous autoimmune, allergic, or inflammatory diseases and malignancies in animal models [5–26]. A causal role for multifactorial autoimmune diseases in humans is still outstanding, but such a role has been demonstrated in murine models of multiple sclerosis (MS) [7–9], rheumatoid arthritis (RA) [11–13], and type 1 diabetes (T1D) [14–18], and is likely to extend to systemic

Glossary

***Bacteroides fragilis*:** *B. fragilis* is a Gram-negative, human gut commensal with unique immunoregulatory functions. Strains containing polysaccharide A (PSA) induce gut T_{reg} and splenic T_H1 cell responses. Other strains contain toxins implicated in chronic colon inflammation and tumorigenesis that are not covered in this review.
Clostridia: Gram-positive, spore-forming bacteria that include pathogens, soil bacteria, and several commensals with immunomodulatory functions. Strains within *Clostridium* clusters IV, XIVa, and XVIII are able to induce T_{regs}.
Gnotobiotic: a state describing germ-free animals or animals colonized with a defined microbiota, usually with a small community (e.g., altered Schaedler flora – ASF) or single species (monocolonization).
Microbiome: the genetic material within a microbiota, typically defined by 16S rDNA sequencing. Often used synonymously with microbiota although strictly speaking not identical. The full gene content and composition of microbiomes is termed the metagenome, which can be assessed by whole-genome shotgun sequencing.
Microbiota: the sum of all commensals within a niche (gut, skin, mouth, lung, vagina, etc.). Preferentially used compared to outdated terms such as flora or microflora that describe the same.
Molecular mimicry: a longstanding theory that T and B cells specific for pathogen-derived antigens can crossreact with a self-antigen, leading to the development of autoimmunity.
Pathobiont: a commensal that promotes non-infectious disease and is detrimental to health and homeostasis under particular circumstances (e.g., genetic predisposition to over-react to a commensal, inflammation and translocation due to barrier disruption, suppression of beneficial commensals within the same niche). A pathobiont should be differentiated from a pathogen or opportunistic pathogen that causes an infectious disease.
Segmented filamentous bacteria (SFB): until recently non-culturable commensal bacteria that can act as pathobionts or symbionts in autoimmunity depending on the host or model. SFB are capable of inducing IgA, intraepithelial lymphocytes, and antigen-specific T_H17 cell responses in the small intestine, which is their physiologic niche in mice.
Short-chain fatty acids (SCFAs): acetate, butyrate, and propionate are produced by the gut microbiota, particularly after a high-fiber diet. SCFAs act via multiple mechanisms including histone modification and G protein-coupled receptor signaling. SCFAs have multiple biological effects including the induction and recruitment of T_{regs}.
Symbiont: a commensal beneficial for the host. Both the commensal and the host need each other, and thus are living in symbiosis by providing factors that improve fitness.

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Box 1. Genetics of autoimmunity

Autoimmunity is grossly broken down into monogenic and polygenic multifactorial diseases. Monogenic autoimmune diseases represent rare and highly devastating diseases, typically arising from defects in either central or peripheral tolerance pathways such as *FOXP3* and *AIRE* [119]. Polygenic multifactorial autoimmune diseases represent complex diseases that are caused by a combination of genetic and environmental factors. Diseases such as T1D, RA, MS, and SLE are multifactorial autoimmune diseases. Genetic influence over these diseases is becoming clearer in large part through advances in bioinformatic approaches and sharing of patient genomic data through large consortia [120,121]. How genetics influences the microbiota and thus autoimmunity is not well understood. Numerous genetically modified mouse models display an altered microbiota, although these findings could be biased by non-genetic factors [122].

Genome-wide association studies (GWAS) led to the identification of SNPs associated with disease such as those in genes within the HLA locus or cytokine receptor genes (e.g., *IL2RA*, *IL7RA)* implicated in T_{reg} homeostasis and function [28,120]. Each SNP confers a small risk, but taken together the risk is thought to be additive [29]. Consistent with a shared autoimmune pathogenesis, particular SNPs impact upon immune pathways across autoimmune diseases such as HLA loci, co-stimulatory, and proinflammatory pathways [29]. Despite the wealth of information gained from GWAS, this approach has been criticized for lacking the power to identify causal variants. Only a few functional SNPs have been characterized to date, such as those in *PTPN22* and *TNFAIP3* [111,123]. Groups using novel bioinformatic techniques are addressing this critique [124]. Clearly, combining environmental/commensal factors with genetics will lead to a more complete understanding of autoimmunity.

Box 2. Commensals by the numbers

It is estimated that human bacteria alone outnumber human cells by a factor of 10 with roughly 100 trillion bacteria colonizing an individual at any point in time [104]. The number of bacterial genes constituting the microbiome is even larger with recent reference catalogs of over 9.8 million non-redundant genes [105].

Improvements in 16S rDNA sequencing methods and dramatic decreases in sequencing costs are allowing a more complete and individualized picture of the microbial communities colonizing each person over time. It is believed that around 1000 species of gut bacteria are present across the human population, with each person harboring 100–160 species at a given moment in time [125,126]. Gut bacterial communities, in the absence of medical interventions (such as antibiotic use) or pathogen infection, tend to be stable over time [126,127]. It should, however, also be noted that the gut microbiota encodes 100-fold more proteins than the human genome, including over 2 million proteins that are found in less than 20% of individuals [128]. This immense number provides a major source for antigenic variation that could contribute to immune stimulation and crossreactivity in autoimmunity.

lupus erythematosus (SLE) [27]. Many autoimmune diseases have a female sex bias, and animal studies have provided insight into gut commensal effects on hormonal status and gender bias in autoimmunity [17,18]. The purpose of this review is to provide a basic overview of autoimmunity in the context of commensals, and to explore recent advances in the understanding of the complex interactions between commensal bacteria, the immune system, and the effects these interactions have on autoimmune disease.

Genetics, environment, and commensals: pieces of the autoimmunity puzzle

Genetic polymorphisms, in particular at the human leukocyte antigen (HLA) loci, play a key role in the predisposition to autoimmunity (Box 1) [28,29], but genetics alone cannot fully explain multifactorial autoimmunity. In addition to genetic factors, several environmental and dietary factors have been identified in epidemiologic studies and have been mechanistically linked to autoimmunity *in vitro* and *in vivo*. Exactly how these dietary or environmental factors influence autoimmunity in patients, however, is still largely unknown [30].

Because any dietary and environmental trigger needs to enter the host through one of the mucosal or skin barriers, its effects on autoimmunity are likely modulated by the colonizing microbiota, which is itself shaped by the environment and diet [31]. Diet and antibiotic use, for instance, are well established to profoundly alter the gut microbiota, leading to long-lasting changes in metabolic profiles [32,33]. These metabolites, such as short-chain fatty acids (SCFAs) or retinoic acid, can either dampen or ignite inflammation depending on their effects on host adaptive immune cells, in particular on regulatory T cells (T_{regs}) and T helper 17 (T_H17) cells [34–37]. Environmental triggers of autoimmunity may thus enhance commensal-mediated inflammatory processes and thereby influence autoimmunity, but these links have yet to be demonstrated. However, independently of environmental factors, the gut microbiota is emerging as a key player in the development of autoimmunity.

The abundance and diversity of organisms colonizing the host (Box 2) represent an enormous challenge to the innate and adaptive immune system. On one hand, the immune system needs to recognize any potentially detrimental non-self antigen that enters the host, while on the other hand it cannot eliminate the commensal communities at barrier organs because it would negatively affects host fitness [38]. This conundrum was aptly termed 'learning tolerance while fighting ignorance' [39]. The interplay between pathogen and commensal recognition by the immune system is a delicate balance which, when altered, may lead to unintended consequences such as chronic inflammatory and autoimmune diseases.

Dysbiosis: intestinal homeostasis and inflammatory bowel disease

Dietary changes, antibiotic use, and excessive hygiene disrupt commensal homeostasis leading to dysbiotic states that drive chronic inflammation (Figure 1). Chronic inflammation in the gut is the hallmark of inflammatory bowel disease (IBD), which encompasses a spectrum of diseases from ulcerative colitis (UC) to Crohn's disease (CD). The gut microbiota is known to play an especially important role in IBD, which is not surprising given that the gut is a barrier organ that carries the largest microbiota, and an abnormal innate and adaptive immune response to multiple members of the gut microbiota is thought to drive inflammation [10,23,24,40].

16S ribosomal DNA (rDNA) profiling studies revealed a decrease in the diversity of microbial communities in IBD compared to controls [41]. Despite efforts to understand these diseases, reports of the specific microbial communities associated with dysbiosis in IBD are variable