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EDITORIAL

Fanning the Flames of Autoimmunity: The Microbiome in Rheumatic Disease

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Marie Curie once said, "Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less." And so it goes with the microbes that live in and on us, i.e., the commensal microbiota. Historically, microbes were feared by the public because much of microbiology focused on the study of infectious pathogens. But we have since learned that in the nooks and crannies of mucosal and skin surfaces of the human body, a diverse collection of microbes contributes to normal host physiology. Indeed, we rely on our microbiome, which refers to both the microbes and the genes they encode, to extend the capabilities of our own genome. This knowledge comes from studies of germ-free (GF) animals, which lack a commensal microbiota. GF animals are abnormal in multiple respects—they fail to gain weight as quickly, are more susceptible to infections, and have multiple neurologic abnormalities relative to their colonized counterparts. Thus, the commensal microbiota contributes to diverse processes, including metabolism, immune system maturation, and neurologic development.

But commensal microbes may contribute to disease as well. Armed with new technologies, we are discovering the ways in which commensal microbiota contribute to autoimmunity. Physicians, scientists, and patients alike are asking: in genetically prone individuals, do specific commensal microbes trigger disease? Or do microbes exacerbate preexisting disease and, if so, can we target the microbiome to modify disease progression? Can we alleviate autoimmunity if we get rid of disease-aggravating microbes? Or can we target specific bacterial proteins or metabolites that contribute to disease?

Several lines of evidence suggest that the microbiome contributes to rheumatic disease. First, antibiotics have efficacy in the treatment of rheumatic diseases. Examples include the use of minocycline in rheumatoid arthritis (1,2) and sulfasalazine in rheumatoid arthritis and spondyloarthritis (3,4). Second, host-targeted antirheumatic drugs, including methotrexate (5), azathioprine, and leflunomide (6), have off-target effects on gut microbiota, raising the possibility that these drugs work by targeting both host cells and the microbiota to modulate inflammation. Third, therapies specifically targeting the gut microbiota, such as probiotics, have demonstrated success in alleviating autoimmunity, albeit in small patient populations (7). Finally, patients frequently report that dietary interventions alleviate disease, but whether this is due to shifts in the microbiota or direct effects on host immunity remains difficult to dissect in patients.

Animal models of autoimmunity are powerful tools for dissecting the microbial contributions to disease and enable scientists to investigate questions that are impossible to evaluate in patients. A key example is the study of GF animals, or "gnotobiology," in which animals are reared in isolators that enable experimental manipulation of the commensal microbiome. Whereas GF mice are devoid of all microorganisms, specific pathogen–free (SPF) mice are free of a defined set of pathogens that infect mice in the wild; most murine studies performed since the 1960s have been conducted at SPF facilities. GF mice lacking an intact microbiota enable researchers to learn whether specific microbes trigger disease, whether they exacerbate established disease, or whether microbial communities or microbial genes are responsible for variable host phenotypes (e.g., lean versus obese state).

Compelling evidence from animal models suggests that commensal microbes can trigger rheumatic disease in genetically prone animals. HLA–B27–transgenic rats reared in GF isolators were protected against autoimmune arthritis and colitis, suggesting that commensal microbes are required for these manifestations (8). In contrast, dermatitis and inflammatory genital lesions persisted in the GF state, suggesting that microbiota are dispensable for development of these lesions. Similarly, interleukin-1 receptor–knockout mice fail to develop arthritis in the GF state (9). Wu et al demonstrated that arthritis was highly attenuated in

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K/BxN mice (although autoantibody production persisted), and that introduction of just a single bacterial species, segmented filamentous bacteria (SFB), was sufficient to restore the arthritis phenotype in these mice (10). Interestingly, SFB is rarely found in human gut microbial communities, but other human gut commensals have since been shown to accelerate arthritis in K/BxN mice (11), highlighting the importance of studying human-associated species and strains. Finally, SKG mice, which have impaired T cell receptor signaling due to a hypomorphic mutation in ZAP-70, show attenuated arthritis and colitis in the GF state (12), and colonization with RA patient microbiota leads to worse disease than colonization with microbiota from healthy individuals (13). These models suggest that both host genetic mutations and the presence of specific microbes are necessary for the development of disease.

On the other hand, several mouse models of rheumatic disease show that commensal microbes are dispensable for disease onset. GF MRL-*lpr* mice, which harbor a null mutation in the *Fas* gene required for apoptosis, continue to have lupus-like manifestations in a GF environment, including autoantibody production, splenomegaly, lymphadenopathy, and glomerulonephritis (14). In a mouse model of psoriatic arthritis, in which mice have targeted mutations in a negative regulator of NF-κB, GF animals develop arthritis with the same severity as their SPF counterparts (15). Thus, in some models of rheumatic disease driven by single-gene mutations in proteins that are critical for limiting the immune response, host genetics dominates over the commensal microbiota in triggering autoimmunity.

In this issue of *Arthritis & Rheumatology*, Hong et al (16) add to our knowledge of the role of the commensal microbiota in contributing to autoimmunity in a genetically complex model of lupus. BXD2 mice spontaneously develop autoantibodies, splenomegaly, glomerulonephritis, and erosive inflammatory arthritis (17). Disease primarily starts in older mice, with 50% of mice experiencing arthritis and proteinuria by 8 months. Multiple genetic loci contribute to this phenotype. Like humans, BXD2 mice undergo a "preclinical phase" and develop autoantibodies prior to the onset of overt organ manifestations.

To determine the contribution of commensal microbiota to disease onset and progression, Hong et al derived BXD2 mice in GF isolators and studied phenotypes at 6 and 12 months of age (Figure 1). The authors compared GF BXD2 mice to their SPF counterparts as well as to SPF C57BL/6 (B6) mice, which lack manifestations of autoimmunity. At 6 months, GF BXD2 mice and SPF BXD2 mice both developed splenomegaly and had elevated levels of total IgG, IgG anti-DNA, and IgG anti–macrophage receptor with collagenous structure (anti-MARCO). MARCO is a receptor on macrophages needed to clear apoptotic debris, and elevated levels of anti-MARCO are seen in murine models of lupus and in patients (18), suggesting that the commensal microbiota is dispensable for these early manifestations. SPF BXD2 mice, GF BXD2 mice, and B6 mice all showed similar levels of activated

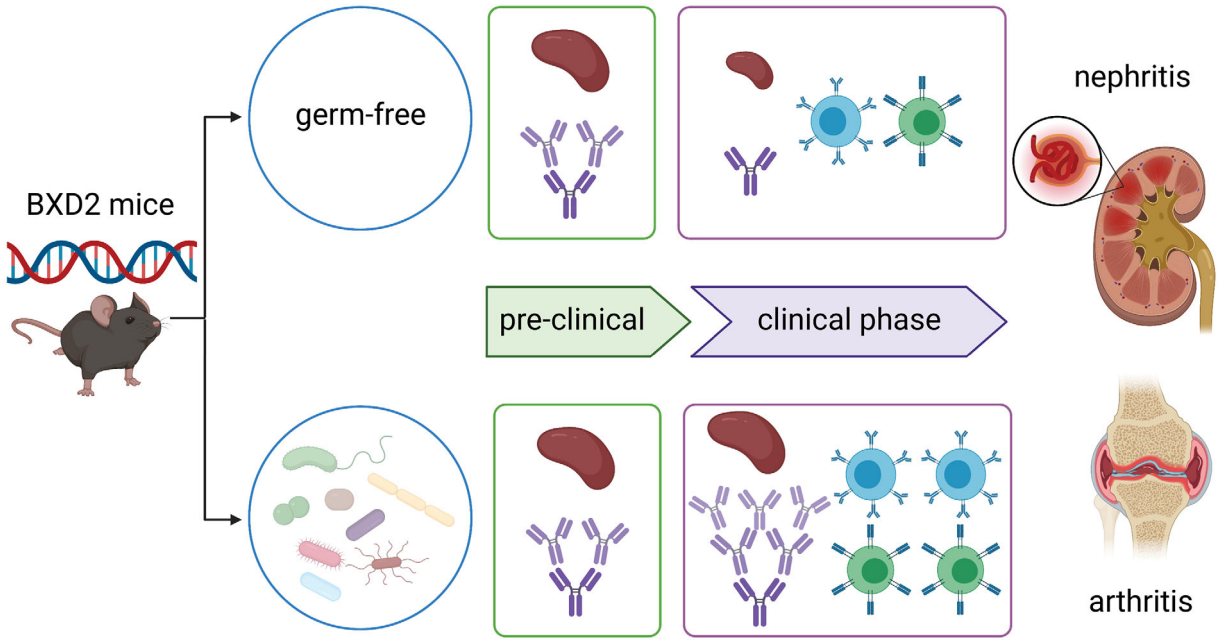

Figure 1. Schematic illustration of the role of the microbiota in a mouse model of lupus in the study by Hong et al (16). Germ-free (GF) BXD2 mice (top) and specific pathogen–free (SPF) BXD2 mice (bottom) were compared during the preclinical phase (at 6 months of age) and clinical phase (at 12 months of age). Both groups had splenomegaly and elevated levels of autoantibodies at 6 months of age. At 12 months of age, GF BXD2 mice had a smaller spleen size, lower autoantibody levels, and lower percentages of activated B cells (blue) and Th17-expressing CD4+ T cells (green) compared to SPF BXD2 mice, suggesting that microbes enable persistence of genetically driven autoimmunity. By 12 months of age, both groups of mice developed glomerulonephritis and inflammatory arthritis of similar severity.

splenic B cell populations, including germinal center and age-associated B cells, and activated splenic T cell populations at 6 months.

In contrast, the commensal microbiota contributed to the persistence and progression of cellular and molecular markers of autoimmunity in older mice. At 12 months, GF mice had less splenomegaly and lower levels of IgG autoantibodies compared to their SPF counterparts, suggesting that the commensal microbiota are needed to enable autoimmunity to persist or progress in BXD2 mice. Additionally, compared to SPF BXD2 mice, GF BXD2 mice showed lower levels of activated B cells and Th17-expressing CD4+ T cells.

Finally, consistent with markers of autoimmunity seen at 6 months, the authors found that commensal microbiota were dispensable for the onset of both glomerulonephritis and inflammatory arthritis in BXD2 mice at 12 months. Histopathologic features of disease were similar between SPF and GF mice.

Overall, in this genetically complex mouse model of lupus, the host genetic program drives the initial manifestation of autoimmune disease. While the BXD2 mouse model has features that are not characteristic of human disease (e.g., lupus patients typically have nonerosive arthritis), and studying other models will be important, the findings of Hong et al suggest that the BXD2 model might be useful for studying pathogenesis and therapy in the absence of an intact commensal microbiota. For example, studies of GF BXD2 mice may reveal whether specific diets and drugs directly impact the host (and not the microbiota) to alleviate disease or whether an intact gut microbiome mediates the beneficial effects of these and other interventions. Furthermore, these findings suggest that in some models of autoimmunity, modifying the microbiome may not be sufficient to prevent disease, which patients often inquire about.

However, these findings leave open the possibility that the microbiome can add fuel to the fire of autoimmunity over the course of a lifetime. As Hong and colleagues note, while they were not able to detect differences in the onset of renal and joint disease, this finding does not preclude the possibility that the microbiota aggravates preexisting disease. Indeed, it is possible that differences in disease severity or mortality might have been detected in older mice. The penetrance of disease seemed to be low at 12 months, with many SPF and GF mice exhibiting low overall renal and arthritis scores. Would allowing more time for the development of full-blown arthritis or glomerulonephritis enable us to discern differences in disease severity between GF BXD2 mice and SPF BXD2 mice? And would transfer of defined microbes from SPF mice to GF mice cause worse disease, suggesting that microbes exacerbate existing disease?

These questions are of clinical relevance because while modifying the human genome is challenging, altering the human microbiome via diet, supplements, probiotics, and medications is more feasible. And while prevention or cure are the ultimate goals, altering modifiable factors in patients with disease could reduce suffering. Knowledge of how microbes fan the flames of autoimmunity could enable the development of microbiome-targeted therapies to alleviate autoimmunity.

In summary, the findings of Hong et al suggest that in BXD2 mice, commensal microbiota are not needed for the onset of autoimmunity, but they do contribute to the persistence and progression of autoimmunity. While it remains a formidable challenge to decipher the multiple mechanisms by which the microbiota modulate host immunity, given the complexity of both the microbiome and the host immune system, it is a worthwhile task for our patients and our field. With advances in science and medicine, now is the time to understand more about microbiota–host interactions in rheumatology.

AUTHOR CONTRIBUTIONS

Dr. Nayak drafted the article, revised it critically for important intellectual content, and approved the final version to be published.

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