Gut microbiota linked to autoimmune uveitis

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Autoimmune uveitis is a group of intraocular inflammatory diseases that arise without known infectious etiology. Uveitis is one of the leading causes of blindness in the developed world and is responsible for up to 15% of severe visual handicap. The disease affects population of working age and has a significant impact on public health. Patients often show detectable immune responses to unique retinal proteins such as retinal arrestin and interphotoreceptor retinoid binding protein (IRBP), which are involved in visual function, and particular HLA haplotypes have been associated with disease, supporting the autoimmune nature (1). Although anecdotal evidence suggested a link between microbial infections and uveitis, our understanding of the etiology of disease, its driving mechanisms and treatment options are still limited. Animal models have been instrumental to advance our understanding on pathogenesis of uveitis. A 'classic' uveitis model in mice is referred as experimental autoimmune uveitis (EAU), involving active immunization with IRBP in complete Freund's adjuvant (CFA) that contains heat-killed *Mycobacterium tuberculosis*. Co-administration of bacterial adjuvants is required to activate innate immune cells and create the proinflammatory milieu to induce adaptive immune responses and to trigger the autoimmune effector pathway (1). However, unlike experimental diseases in the animal models, most cases of human autoimmune uveitis cannot be connected to an exposure of the immune system to ocular antigens that are present behind the blood retinal barrier. These target antigens in uveitis are sequestered inside the eye and are not expressed in the periphery (2), but retina-specific T cells that would recognize them must

be activated to enter the eye to drive pathology in uveitis. This presents a paradox and raises a fundamental question: where and how do autoreactive T cells, that can recognize retinal antigens and trigger uveitis, first become activated?

In recent years, intestinal microbiota emerged as a candidate responsible for providing innate stimuli to prime the immune system, and its contribution to pathogenesis of autoimmune diseases has been intensively studied. Clinical studies support a link between changes in gut commensal microbiota ('dysbiosis') and human autoimmune diseases including rheumatoid arthritis, spondyloarthritis, lupus, diabetes, inflammatory bowel disease and multiple sclerosis (MS) (3-5). The relative abundance of some bacterial taxa has been associated with the diseases in patients and in the corresponding animal models. While studies in uveitis patients are still underway, animal studies on autoimmune uveitis in rodents have unraveled strong associations of gut microbiota with disease. The examples include HLA-B27 transgenic rats, a model of spontaneous spondyloarthritis analogous to human ankylosing spondylitis (a disease known to be associated with uveitis), that exhibited altered microbiota compositions in the cecum compared to healthy controls (6), and the mouse models of spontaneous or induced uveitis, in which disease was strongly attenuated in the absence of gut microbiota (7-9). The latter will be discussed ahead with a particular focus on the recently published paper by Lin and colleagues in the July 2016 issue of IOVS (8).

It would be worth mentioning our preceding findings with a 'spontaneous' mouse model of uveitis first. To

study environmental factors including microbiota that might involve in triggering autoimmune uveitis, our group developed and used a spontaneous uveitis model in R161H mice that express a transgenic for T cell receptor (TCR) specific for IRBP on the disease-susceptible B10.RIII background (10). R161H mice have an expanded population of IRBP-specific CD4+ T cells (approximately 20–30%) in the peripheral lymphoid tissues, and spontaneously develop uveitis with 100% incidence by 2 months of age (10). In these mice, IRBP-specific T cells are activated in the gut by a combination of antigenically cross-reactive and innate bacterial stimuli, whereupon they acquire pathogenicity to trigger disease. These autoreactive T cells receive a signal through their clonotypic TCR in the gut lamina propria and become pathogenic effectors. Depletion of commensal microbiota by oral broad-spectrum antibiotic treatment or by rearing under the germ-free (GF) condition resulted in protection, whereas the exposure of GF R161H mice to the conventional environment by co-housing restored disease development. Proteinaceous components of bacteria-rich intestinal contents activated R161H T cells *in vitro* and made them sufficiently 'pathogenic' to transfer disease in naïve WT recipients (7). These findings strongly support a need for a TCR-driven signal, perhaps from a microbiota-derived antigenic mimicry, but they do not negate a requirement for innate adjuvant effects, which also can come from microbiota. Although the putative antigenic mimic in uveitis has not been identified, a mimic of an antigen responsible for type 1 diabetes was recently reported (11). Thus, antigenic mimicry by commensals may be a more common and frequent trigger of autoimmune diseases than it is currently appreciated.

The paper by Nakamura *et al.* extends the notion on a contributory role of gut microbiota to the development of autoimmune uveitis (8). Using mice that have a normal polyclonal T cell repertoire (i.e., WT mice) on the uveitis-susceptible B10.RIII background, they demonstrated a critical role of gut commensal microbiota in the 'induced' EAU model by active immunization with an uveitogenic IRBP peptide emulsified in CFA. By comparing oral versus intraperitoneal administration of broad-spectrum antibiotics (ampicillin, metronidazole, neomycin and vancomycin), they examined how the severity of EAU could be affected by antibiotic treatments. Not surprisingly, intraperitoneal treatment did not affect the mass of gut microbiota, whereas oral treatment significantly reduced the overall bacterial load in the gut. The severity of uveitis was correlated with the presence of gut microbiota, and

was significantly attenuated by oral antibiotic treatment. The dramatic reduction of the major phyla, *Firmicutes* and *Bacteroidetes*, and the bacterial class, *Alphaproteobacteria*, was confirmed by quantitative PCR of the 16S rRNA gene following oral antibiotic treatment. Increased frequencies of Foxp3+ regulatory T cells (Tregs) were noted both in lymphoid tissues (eye-draining LN and gut-draining LN and lamina propria) and in the eye of EAU-challenged mice that were treated with oral antibiotics. This increase was first detected in the gut 2 weeks after antibiotic treatment (1 week after immunization) before the onset of disease, and later in the extra-intestinal lymphoid tissues and in the eye when disease had already developed. Although fewer T cells infiltrated the retina of antibiotic-treated EAU mice, the proportion of Tregs detected in their retinas was significantly higher than that in untreated EAU mice. By contrast, the frequencies of effector T cells and production of inflammatory cytokines including IFN-γ, IL-17 and TNF-α from eye-draining LN appeared to be lower in the oral antibiotic-treated mice at the end of the priming stage of EAU (1 week post-immunization) compared to those in untreated mice. However, unlike other reports in which elimination of gut microbiota by oral antibiotic treatment decreases inflammatory cytokines in the gut-associated lymphoid tissues, no obvious reduction of IFN-γ or IL-17 was seen in the gut lamina propria, presumably because only the early time point before the onset of disease was examined. Whether these increased Tregs in the eye were recruited from the periphery (including the gut) or were they converted *in situ* within the eye in the antibiotic treated mice remains to be investigated.

Metagenomic analysis for microbial identification by 16S rRNA sequencing revealed that there was segregation in microbiota compositions in EAU-challenged mice from matched non-immunized (naïve) control mice (8). The difference was not detected 1 week after immunization (before the onset of uveitis), but became significant 3 weeks after immunization. Thus, immunization may have resulted in alteration of gut microbiota, and the changes were more dramatic after the onset of disease. Classes of bacteria that were increased in the abundance in EAU-challenged mice included Clostridia, Deltaproteobacteria, Bacilli and Coriobacteria. There was also a trend of segregation in the gut microbiota in EAU mice that exhibited high disease scores versus low scores, although the difference was not statistically significant. These findings suggest that host immune responses may modulate the intestinal microbiome and alter the composition in normal mice. However, it is

also possible that the robust immune reactions by CFA that are given at the time of immunization may have influenced the alteration. A 'blank' immunization of adjuvant(s) alone as a control would be needed to address this possibility. Since some clusters of Clostridium species have been reported to induce Tregs in the gut (12,13), it would be interesting to investigate the correlation between Treg induction in the gut and the amount and/or composition of Clostridium in feces in EAU-challenged mice.

Notably, single treatment of metronidazole or vancomycin was effective in decreasing clinical scores of EAU, the results similar to the quadruple antibiotic treatment, in this study by Nakamura *et al.* (8). The protective effect of antibiotics on disease progression by inducing Tregs in the lamina propria, as well as in the eye, was also supported by single treatment of either metronidazole or vancomycin. It is remarkable that the gut microbiota of mice treated with these uveitis-protective antibiotics clustered together, and that they segregated from those of non-protective antibiotics (ampicillin and neomycin). The effective protection by single antibiotic treatment might allow to narrow down the microbes that are sensitive to the antibiotic but would have a promoting role in uveitis. These results validate an association of the gut microbiota composition with protection of uveitis, and suggest good potential bacterial targets including *Coprococcus*, *Dorea*, *Lactobacillus*, *Adlercreutzia* and *Clostridium* for further investigations. Identification of microorganisms that may support the emergence of Tregs during the course of EAU would be one of the important next steps.

Intriguingly, however, the disease protection demonstrated by Nakamura *et al.* in microbiota-reduced mice immunized for EAU was different from our results. We used the same broad-spectrum antibiotic cocktail and started treatment in drinking water to pregnant dams, and treatment continued indefinitely after weaning until the mice were used for experiments at adult ages (8–16 weeks old). We induced EAU to WT littermates of R161H mice described above to address the possibility that the attenuation of spontaneous uveitis by long-term antibiotic treatment could stem from nonspecific immunosuppressive effects of antibiotics, rather than depletion of the resident gut microbiota. In our hands, these antibiotic-treated WT mice that were immunized for EAU developed full-blown disease undistinguishable to untreated WT mice that received regular water and were immunized as controls, eliminating the concern about immunosuppression (7). Because we did not see treatment effects on the disease

susceptibility, we did not further investigate the presence of Tregs in the tissues and eyes of EAU-challenged mice. However, in the R161H spontaneous model of uveitis, frequencies of Tregs were not increased but rather decreased in the gut lamina propria of antibiotic treated mice (7). Furthermore, unlike the results from Nakamura *et al.* in which individual antibiotic treatment of either metronidazole or vancomycin was effective (8), we did not see the protection from spontaneous disease in R161H mice in any of the single antibiotic treatment groups, which had been treated with individual antibiotics in drinking water from before the birth as above (14), and therefore we did not immunize their WT littermates to examine the susceptibility to EAU. The reasons for the differences between these studies are currently not clear. However, disease protection was reported in an analogous immunization-induced model of experimental autoimmune encephalomyelitis (EAE, a model for MS), as well as in a model of EAU, in mice raised under the GF conditions (9,15), further corroborating a role of gut microbiota in immunization-induced autoimmune diseases. Attenuated disease after a short-term course treatment with antibiotics was also reported in the model of EAE using the same antibiotic combination (16), or in the model of EAU on the C57BL/6J background with a different antibiotic combination (9). It is conceivable that the length and timing of antibiotic treatment that was used in different studies and/or specific microbial environments in various facilities may underlie these differences. Nonetheless, these results support the notion that uveitis is a heterogeneous disease with potentially different environmental, immunologic and genetic influencing factors.

Established therapies for human uveitis are mostly based on nonspecific immunosuppression (corticosteroids, antimetabolites and alkylating agents). Because of the severe side effects of the treatments, it is necessary to develop new approaches based on increased understanding of basic disease mechanisms, so as to intervene more specifically in the pathogenic processes. Although a role for gut microbiota in animal models of uveitis has been strongly supported as described here, it is still not known whether and how gut dysbiosis might affect human uveitic diseases. The study from Nakamura *et al.* indicates that changes in the 'mature' gut microbiota in adulthood by oral antibiotic treatment can rapidly affect the immune system to alter the susceptibility to autoimmune disease at the sites distant from the gut. As the authors propose, it may be plausible to use broad-spectrum antibiotics for a short period of time to

reset or modulate the gut microbiota and then to repopulate the gut with beneficial microorganisms as probiotics (or prebiotics) to mitigate the inflammatory flare of uveitis.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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