DNA AND CELL BIOLOGY
Volume 35, Number 9, 2016

Pp. 455–458
DOI: 10.1089/dna.2016.3432

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Regulation of Autoimmunity by the Microbiome

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Intestinal microbes have profound effects on inflammatory autoimmunity in sites distant from the gut. The mechanisms whereby this happens are only now beginning to be understood and may include such diverse effects as innate stimulation of migrating immune cells and effects of circulating bacterial metabolites. Our studies add to this the demonstration that microbiota may provide a source of cross-reactive antigenic material that activates autoreactive lymphocytes within the gut environment. In a spontaneous model of autoimmune uveitis, T lymphocytes specific to a retinal autoantigen are activated through their specific antigen receptor in the gut and acquire the ability to fuel inflammatory autoimmunity in the eye. In view of the huge diversity of commensals, it is conceivable that they may provide surrogate antigens for activation of autoreactive lymphocytes(s) of other tissue specificities, and might therefore be involved in the etiology of autoimmune diseases more frequently than is currently appreciated.

The Role of Intestinal Microbiota in Immune Development and Intestinal Homeostasis

Although underappreciated until the last decade, an increasing number of studies have demonstrated the importance of commensals for the development of human physiology, especially in the immune system. A variety of antigens produced by commensal microbiota, associated with skin and mucosal surfaces, provide a major source for immune stimulation (Hooper *et al.*, 2012). In fact, without this stimulation by commensals, development of the immune system is stunted, which is clearly seen in mice reared under germ-free conditions. To maintain steady state, the host–microbiota interaction must be able to contain the commensals within their appropriate niche without triggering inflammatory responses, while at the same time retain the ability to rapidly attack and eliminate pathogenic microbes (Palm *et al.*, 2015). Gut commensals stimulate secretion of a variety of effector molecules, including antimicrobial factors, antibodies, and cytokines, which in turn limit the commensals to the appropriate anatomical locations. Furthermore, commensals restrict colonization by pathogens by keeping the gut mucosa rich in antimicrobial molecules and by populating the environment and functioning as an extra physical barrier over the epithelia and mucus. Indeed, the effects are so strong that fecal microbiota transplantation from healthy donors is a well-established treatment for patients with recurrent *Clostridium difficile* infections (Kelly *et al.*, 2015). Hence, gut microbiota continually provide appropriate signals to immune cells in the steady state without causing pathogenic responses, and serve as a barrier against pathogens.

As part of this host–commensal relationship, numerous lymphocytes are present in the gastrointestinal (GI) tract. They include effector IL-17-expressing T cells (Th17) as well as regulatory T cells (Treg) that have an activated phenotype and are thought to be crucial to a successful host–microbiota relationship (Hooper *et al.*, 2012; Palm *et al.*, 2015). Specific groups of commensals may fulfill different roles in this relationship: as an example, segmented filamentous bacteria (SFB) and members of the clostridium groups IV and XIV were shown to stimulate the induction of Th17 or Treg cells, respectively (Ivanov *et al.*, 2009; Atarashi *et al.*, 2011). Later, other findings revealed the importance of gut commensals on different types of immune cells from both innate and adaptive arms of immunity (Hill *et al.*, 2012; Chung *et al.*, 2012).

This beneficial balance between host and gut microbiota can be affected by diet and hygiene habits (Brown *et al.*, 2012; Daley, 2014), use of antibiotics (De La Cochetiere *et al.*, 2005), invasion of virulent microbes (Barman *et al.*, 2008), and even long-term stress (De Palma *et al.*, 2015), resulting in a situation known as dysbiosis. In some cases, together with host genetic factors, dysbiosis results in uncontrolled immune responses against resident gut microbes and can lead to chronic and relapsing inflammation in the GI tract known as inflammatory bowel diseases, which include Crohn's disease and ulcerative colitis (Nell *et al.*, 2010).

Microbiota Affect Development of Inflammatory/ Autoimmune Disease in Distant Sites

One of the surprising observations of the past decade is that immune responses in sites distant from the gut can also

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be regulated by gut microbiota. Absence of microbiota or alteration in its composition (dysbiosis) affects expression of autoimmune diseases in distal sites in animal models. Thus, some diseases require the presence of microbiota and are ameliorated or absent in antibiotic-treated or germ-free mice. Examples are experimental arthritis (a model for human rheumatoid arthritis, RA) (Wu *et al.*, 2010), experimental autoimmune encephalomyelitis (a model for multiple sclerosis, MS) (Berer *et al.*, 2011), and autoimmune uveitis (a model for human uveitis) (Horai *et al.*, 2015). In contrast, type 1 diabetes in non-obese diabetic (NOD) mice is ameliorated by the presence of microbiota, and germ-free mice have enhanced disease. Furthermore, its sex dependence is, in part, explained by microbiome differences between males and females and can be ameliorated in females by transfer of male microbiota (Wen *et al.*, 2008; Yurkovetskiy *et al.*, 2013).

In some cases, specific genera of microbes were identified to be positively or negatively associated with a disease. For example, SFB was reported to drive pathogenic Th17 responses in the K/BxN mouse model of RA (Wu *et al.*, 2010). *Proteus mirabilis* and *Klebsiella pneumoniae* were needed for expression of arthritis in the TRUC model (Garrett *et al.*, 2010). *Prevotella copri* was found to be more abundant in feces of patients with RA (Scher *et al.*, 2013). In the model for MS, in which the target organ is an immune-privileged site, monocolonization of germ-free mice with SFB enhanced responses of self-reactive T and B cells against the causative protein myelin (Berer *et al.*, 2011), while oral administration of purified polysaccharide A of *Bacteroides fragilis* dampened disease (Ochoa-Reparaz *et al.*, 2010).

Exactly how microbiota in the gut affect development of disease in distant sites is largely unclear. Activation of autoreactive T cells in the gut would have explained it, however, recent studies show perplexing evidence that Th17 cells in the gut of healthy mice were, in fact, specific for gut microbiota (Yang *et al.*, 2014). Alternatively, the innate stimuli originating from gut microbes could play a critical role in linking commensals to adaptive responses, possibly by activating autoreactive T cells recirculating through the gut via their innate immunity receptors, or indirectly by effects on innate cells of the immune system. In this context, specific types of dendritic cells are pivotal for the induction of Treg cells (Telesford *et al.*, 2015), and for the generation of Th17 cells in the gut (Janelsins *et al.*, 2014; Goto *et al.*, 2014), where the group 3 innate lymphoid cells (ILC3) play immunoregulatory roles (Mortha *et al.*, 2014). However, this does not fully explain how these responses trigger autoimmunity at sites distant from the gut.

Uveitis Study Reveals an Unexpected Connection Between Microbiota and Activation of Autoreactive T Cells in the Gut

Autoimmune uveitis, or uveoretinitis, is a sterile inflammatory disorder affecting the retina and uvea (choroid, iris, and ciliary body). It is responsible for up to 15% of blindness in the Western world. The disease is thought to be driven by self-reactive T cells that can respond to retinal proteins. The healthy eye is an immune-privileged site, which is largely devoid of immune cells. Pathogenic T cells that infiltrate the eye must be previously activated to breach the blood–retinal barrier that separates the eye from the rest of the body and helps maintain its immune-privileged status (Caspi, 2010). This presents a paradox, because the antigens that these T cells recognize are by their nature confined to the eye and are not available in the periphery to activate the T cells.

The R161H uveitis model, a T cell receptor transgenic mouse specific for the interphotoreceptor retinoid-binding protein (IRBP) expressed in the retina, offers an opportunity to study the natural triggers of spontaneous uveitis (Horai *et al.*, 2013). R161H mice start to develop uveitis at 3–4 weeks of age with infiltration of inflammatory cells and destruction of the retina in the next 4–8 weeks reaching 100% incidence (Horai *et al.*, 2013). To address the central question where these autoreactive T cells first become activated, we searched for activated T cells in different organs in R161H mice by flow cytometry and confocal imaging. Surprisingly, we found that activated T cells were present in the gut before disease in the retina was apparent (supplemental movies in Horai *et al.*, 2015). Depletion of the gut microbiota by treatment with broad-spectrum antibiotics in drinking water significantly attenuated disease and reduced activated T cells, suggesting that gut commensals are required for the activation of autoreactive T cells in the lamina propria of R161H mice. The contribution of gut commensals as a trigger of disease was further confirmed in germ-free R161H mice, in which disease was also drastically attenuated.

These findings raised the possibility that a commensal-derived signal was required for the activation of retina-specific autoreactive T cells. The activated phenotype of T cells in the gut was maintained in R161H mice that were made genetically deficient for IRBP, supporting the idea that a commensal-derived signal, and not IRBP, is responsible for activation of the IRBP-specific T cells in the gut. In keeping with this, protein extracts from intestinal contents of normal, but not germ-free R161H mice, stimulated IRBP-specific T cells to upregulate expression of the activation marker CD69 and produce IL-2 in an MHC class II-dependent manner, suggesting the presence of an antigenic substance. This notion was further confirmed by the finding that IRBP-specific T cell activation was not induced by innate stimuli alone (bacterial lipopolysaccharide, *M. tuberculosis* extract) or by known bacterial superantigens. Importantly, R161H T cells activated by intestinal stimuli induced uveitis in naive recipient mice, indicating that these stimuli are sufficient to make them pathogenic (Horai *et al.*, 2015). In the aggregate, these results are compatible with the interpretation that commensal organisms can serve as a source of a cross-reactive antigen to a host that harbors autoreactive T cells specific to retina, and is therefore predisposed to develop autoimmune uveitis.

Broader Implications, Limitations, and Future Directions

Ever since it has been demonstrated that commensal microorganisms can contribute to the development of disease at sites distant from the gut, it was generally felt that the stimuli are likely to be bacteria-associated conserved molecular patterns stimulating innate immunity receptors such as TLRs. Our study indicates that adaptive immune responses to cross-reactive bacterial antigens may have an important role to play, at least in the case of uveitis. Furthermore, taking into account the huge variety of commensal

species in the gut, it is plausible that a similar scenario could apply to other autoimmune pathologies.

The putative cross-reactive molecule(s) that can activate the retina-specific T cells and their source (bacterial, fungal, parasitic, viral) remain to be identified and we are using biological and bioinformatic approaches to screen candidate molecule. Also, we have not excluded the need for innate costimulatory effects of microbial components that might be required as an ''adjuvant,'' although innate stimuli alone appear insufficient to activate autoreactive T cells (Horai *et al.*, 2015). It is possible that the antigen and adjuvant effects come from different commensal organisms, adding to the complexity. Finally, it remains to be demonstrated that the activated retina-specific T cells that we detect in the gut actually do reach the eye, but this is not yet possible using currently available technology.

The question that presents itself is the applicability of these findings to clinical uveitis. Extrapolating from the R161H uveitis model, we should keep in mind that these are inductive events, which occur before the onset of disease. We must therefore be able to identify who are the individuals at risk. Genetic and family history studies could make that possible in the future. Further studies will also hopefully identify the particular microbe(s) involved in triggering disease and technological advances will make it possible to develop ways to target them selectively, while sparing the rest of the commensal microbiome. It is to be expected that, as our understanding of the complex interrelationship between the microbiota and the immune system develops and our ability to manipulate them progresses, these goals may become attainable.

Acknowledgment

Funding was provided by NIH/NEI Intramural funding, Project number EY000184.

Disclosure Statement

No competing financial interests exist.

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Received for publication July 1, 2016; received in revised form July 1, 2016; accepted July 1, 2016.