You AhR what you eat?

B Paige Lawrence & David H Sherr

AhR is more than just a receptor for pollutants. Studies have now identified crucial roles for this environment-sensing transcription factor in the development and maintenance of gut-associated lymphoid tissues.

Any basic immunology text teaches that mucosal tissues are the first line of defense against invading microorganisms. However, this presents an overly simplistic view of these vital, complex and multifunctional tissues. Cells in the dermal layers and at the mucosal surfaces must be nimble and able to respond to an ever-changing variety of environmental signals. Cells in these sites not only maintain barriers but also regulate tissue homeostasis, elimination of invading microorganisms while sparing commensal flora, metabolism of nutrients, elimination of toxicants and controlled repair of physical damage. Many diseases, including ulcerative colitis and Crohn's disease, demonstrate the devastating consequences of deregulated immune response mechanisms at mucosal surfaces and have catalyzed research to elucidate the factors that control these important barriers between an organism and its environment.^ However, understanding of the development, regulation and interactions of cells at mucosal barriers remains incomplete. Three recent papers, including one by Lee *et al.* in this issue of *Nature Immunology*[1], have shed new light on an unexpected role for an environmental chemical receptor and transcription factor, the aryl hydrocarbon receptor (AhR), in the development of critical components of the gut-associated lymphoid tissue[2,3].

The integrity of mucosal barriers and host resistance to the sea of microbes just beyond the epithelial cell wall are maintained by multiple components of both the innate and adaptive immune systems. For example, in the gut these include dendritic cells (DCs) that constantly sample the intestinal milieu for pathogens; intraepithelial lymphocytes (IELs) that interdigitate between epithelial cells in the lining of the gut; T cells that express γδ receptors; B cells that accumulate in draining mesenteric lymph nodes and Peyer's patches; and innate interleukin 22 (IL-22)-secreting lymphoid cells (ILC22 cells)^ that concentrate in cryptopatches and isolated lymphoid follicles (ILFs) in the lamina propria (**Fig. 1a**). The presence of ILC22 cells seems to be particularly important, because IL-22, secreted in response to IL-23 production by activated DCs, increases epithelial cell growth, survival and production of peptides that protect the host against pathogens. In the absence of IL-22–secreting cells, the epithelial barrier is compromised; this gives microorganisms access to the lamina propria, which results in robust inflammatory responses that further damage the mucosal surface (**Fig. 1b**). This working model of mucosal immunity provides a framework for these exciting new reports, which demonstrate a central role for AhR in the development and function of IELs and two subpopulations of ILC22 cells, NKp46$^+$ and lymphoid tissue–inducer (LTi)-like cells, which are known for their contribution to prenatal lymphoid organ development. Notably, these new studies link a receptor known historically for its recognition of and response to pollutants to the development of cells whose main job is to monitor and respond to the microbial environment.

The AhR is a fascinating protein. A generation ago it was discovered as the cytosolic receptor for pollutants such as TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). In the 30 years since its discovery, the known repertoire of ligands of AhR has expanded and includes many anthropogenically and naturally derived compounds, such as many common pollutants, indoles and flavonoids derived from cruciferous vegetables, and tryptophan metabolites produced endogenously. It is now also known that AhR is a member of the PAS family of environment-sensing, basic helix-loop-helix transcriptional regulators and has been highly conserved throughout evolution. Such points speak to what would be assumed to be critical, evolutionarily beneficial biological functions of AhR. However, it has only been in the past few years that the 'normal' function of AhR has been suggested.

The function of a protein is often identified by knocking out the gene that encodes it. The examination of various AhR-deficient ($Ahr^{-/-}$) mouse strains initially presented a mixed picture of consequences to the immune system. Initial reports of AhR-deficient mice described some very slight changes in splenic architecture and cellularity[4] but no obvious changes in immunocyte development or deficits in immune function[5]. Still, the devil is in the details. New work is beginning to identify intriguing changes in the development and function of cells of the immune system. The ultimate importance of these changes will probably depend on the nature of the insult, the site of immune challenge and the cellular response pathways needed to cope with it. For example, $Ahr^{-/-}$ mice effectively combat mild infection with influenza A virus[6] but not infection with *Listeria monocytogenes*[7] or *Citrobacter rodentium*[1,3], which suggests that AhR has pathway-specific roles in modulating host defense mechanisms.

As for mucosal immunity in the gut, the startling finding by Lee *et al.* that $Ahr^{-/-}$ mice produce little or no IL-22 and are all but devoid of cryptopatches and ILFs points to a key role for AhR and provides a mechanistic explanation for the lack of IL-22: failure to generate NK46$^+$ or LTi-like cells. The exquisite

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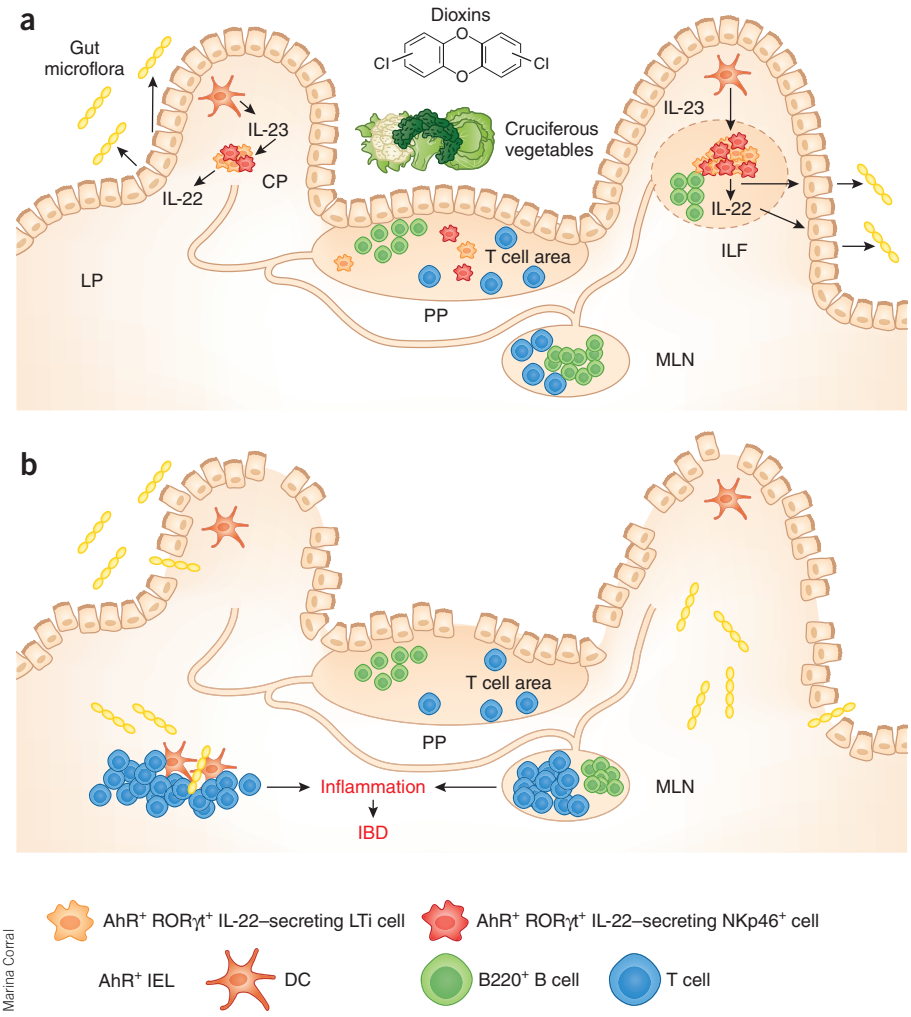

AU3 **Figure 1** ^(**a**) The gut-associated lymphoid tract consists of IELs dispersed among luminal epithelial cells, Peyer's patches (PP), cryptopatches (CP), ILFs and mesenteric lymph nodes (MLN) connected to one another via the lymphatics. ILC22 cells include AhR$^+$RORγt$^+$NKp46$^+$ cells and AhR$^+$RORγt$^+$ LTi cells. The development of ILC22 cells is dependent on AhR expression and, presumably, on the activation of AhR by ligands produced endogenously (such as kynurenine) or absorbed through the diet (such as indole-3-carbinol (I3C) from cruciferous vegetables and/or environmental contaminants in foods). The production of IL-23 by DCs encountering gut bacteria activates NKp46$^+$ and LTi cells to produce IL-22, which increases epithelial cell survival, proliferation and production of antimicrobial peptides. (**b**) In the absence of AhR, both cryptopatches and ILFs fail to develop, and Peyer's patches have fewer NKp46$^+$ or LTi cells. In the absence of these IL-22–secreting cells, the epithelial boundary is compromised; this enables the entry of gut bacteria into the lamina propria, resulting in potentially life-threatening susceptibility to intestinal flora and poorly controlled inflammatory responses, which contribute to inflammatory bowel disease.

sensitivity of these mice to challenge with *C. rodentium* highlights the importance of these ILC22 populations and, therefore, AhR to gut immunity. Furthermore, mucosal hyperplasia and the influx of inflammatory cells into the intestinal lamina propria of *Ahr*$^{-/-}$ mice link AhR to the prevention of inflammatory bowel disease. Similar results for mucosal integrity and susceptibility to *C. rodentium* infection in either *Ahr*$^{-/-}$ mice or mice in which *AhR* is conditionally knocked out in RORγt ILCs have been obtained by Li *et al.* [2] and Kiss *et al.*[3]. Complementing results showing the loss of IL-22–secreting cells reported in the paper by Lee *et al.*[2], data from Li *et al.* demonstrate that IELs from *Ahr*$^{-/-}$ mice fail to accumulate in the gut lining[3]. Furthermore, Lee *et al.* show that AhR regulates Notch activity in intestinal lymphoid cells, which connects AhR to myriad downstream, Notch-regulated biological effects. Thus, at least three of the gut-associated lymphoid cell subsets critical to mucosal immunity—IELs, NK46$^+$ and LTi cells—depend on AhR for development and/or function.

The development of cryptopatches and ILFs, which develop postnatally, but not of Peyer's patches, which develop prenatally, is affected by deletion of AhR[1], which suggests that some postnatal environmental stimulus drives the development of cryptopatches and ILFs. Li *et al.*[3] and Kiss *et al.*[2] suggest that this stimulus is provided by dietary AhR ligands, including indoles produced by cruciferous vegetables. However, the study by Lee *et al.*[1] fails to demonstrate an effect of feeding mice a synthetic diet apparently devoid of natural AhR ligands on development of intestinal lymphocytes. These observations are important because the former suggests that diet may have a considerable effect on mucosal immunity and prevention of inflammatory bowel diseases through exposure to exogenous, naturally derived AhR ligands, whereas the latter suggests an alternative pathway of AhR activation and tissue protection via production of endogenous tryptophan-derived ligands.

From the Lee *et al.* paper discussed here[1], and other studies investigating 'normal' AhR functions in T cells and B cells, it has become evident that AhR is no longer just about environmental pollutants. This remarkable protein is a powerful modulator of the immune system. Moreover, it has broad effects on the development, activation, proliferation and/or differentiation of leukocytes, including considerable effects on DCs[8,9], cells of the T_H17 subset of helper T cells[10,11], regulatory T cells[10,12] and B cells[13,14]. These studies will focus more attention on the role of AhR in the development, regulation and function of the immune system and on the exciting possibility of controlling AhR activity to deliberately modulate immune function. However, it will be important not to overlook the importance of exposure to AhR-binding pollutants as they relate, in the present context, to the increase in diseases at mucosal sites such as the gut and lung. Indeed, in terms of improving human health, research that uses environmentally relevant AhR-binding pollutants (such as dioxins and PAHs) and potentially therapeutically beneficial AhR ligands (such as FICZ and I3C) will form the foundation for future strategies to prevent and treat many diseases. To illustrate this point, the important data provided in the papers discussed here raise the question of whether and how environmental AhR ligands distort the development of AhR-dependent lymphocytes, including NK46$^+$ lymphocytes, LTi-like lymphocytes and IELs. The urgency with which these questions should be considered is underscored by the apparently continual exposure to AhR ligands and their potential adverse effect on health[15].

In short, AhR—previously notorious only for its mediation of the ill effects of environmental pollutants—is a far more important molecule

whose function expands the concept of environmental stimulation from pollutants to dietary components. Present research indicates that AhR is joining the ranks of other regulatory proteins critical for the development and maintenance of crucial components of the immune system, including gut-associated lymphocytes that mediate mucosal immunity.

AU4 **COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.^

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