Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

T cell subsets and environmental factors in *Citrobacter rodentium* infection

Brigitta Stockinger

Infection with *Citrobacter rodentium* constitutes an attack on the intestinal barrier and results in concerted action by innate and adaptive immune responses to limit bacterial translocation and destroy those bacteria that have breached the intestinal barrier. Among the many immune cell types that are involved in the defence against this infection, Th17 cells as the major producers of the barrier protective cytokine IL-22 during the adaptive phase of the response are most numerous. Their extensive plasticity furthermore results in the production of additional cytokines that previously were ascribed to Th1 cells, such as IFNγ. The timely and coordinated repair of damaged epithelium requires input from environmental factors derived from diet and microbiota metabolism of tryptophan which are transmitted through the aryl hydrocarbon receptor (AHR). Thus, the combination of a robust immune response, coupled with intestinal stem cell differentiation guided by environmental factors, ensures resistance to barrier destruction by intestinal infection.

Address
The Francis Crick Institute, London, UK

Corresponding author:
Stockinger, Brigitta (BriGitta.Stockinger@crick.ac.uk)

Current Opinion in Microbiology 2021, **65**:92–97

This review comes from a themed issue on **Host–microbe interactions: bacteria**

Edited by **Vanessa Sperandio** and **Gad M Frankel**

https://doi.org/10.1016/j.mib.2021.06.006

Introduction

Infection with *Citrobacter rodentium* is a mouse model of human infection with pathogenic *Escherichia coli* and has been widely studied for its impact on the mucosal immune response in the gut. The importance of T and B cell responses as well as cytokines such as IL-12 or IFNγ in the defence against this pathogen are well established [1,2]. Because of the complexity of intestinal immune cell types involved and their roles at different stages of the infection there is ongoing research trying to define many of the molecular mechanisms underlying *Citrobacter*-induced changes in mucosal immunity. In addition to more direct effects of *Citrobacter* on immune cells, there are also indirect effects on the immune system via perturbations of the microbiota and the involvement of environmental factors transmitted via the ligand dependent transcription factor aryl hydrocarbon receptor (AHR). The activation of a particular CD4 T cell subset, Th17, by *C. rodentium* was first demonstrated by Torchinsky *et al.* [3] who showed that this infection creates the cytokine conditions that promote the *in vivo* differentiation of this T cell subset via phagocytosis of infected apoptotic cells by dendritic cells. However, the specific role of Th17 cells in *Citrobacter* infection was only addressed in later years and will be discussed in this review. Likewise, the role of the aryl hydrocarbon receptor (AHR) in immune cells was first defined in the Th17 subset [4]. The AHR is a ligand dependent transcription factor from a family of transcription factors which are environmental sensors. AHR had previously been studied mainly in the toxicology field for its role in promoting the toxic effects of man-made pollutants, such as dioxin [5]. However, in the last 10–12 years it has emerged that AHR has important physiological functions particularly in barrier organs, such as the skin, the lung and the gut [6]. It is known that AHR is highly expressed in Th17 cells, where its activation promotes the expression of IL-22, a crucial cytokine in *Citrobacter* infection. Moreover, in the last few years the AHR has also been shown to have prominent additional roles in intestinal homeostasis and infection which will be addressed in more detail here.

This review compiles findings over the past 3–5 years and focuses on the role of CD4 T cells as orchestrators of inflammatory as well as regulatory responses while briefly mentioning other cell types that participate in the defense against this infection. Furthermore, in light of environmental influences on gut physiology via dietary and microbiota metabolites, the impact of the environmental sensor aryl hydrocarbon receptor (AHR) on infection with *C. rodentium* is discussed.

T cell responses and their consequences in *C. rodentium* infection

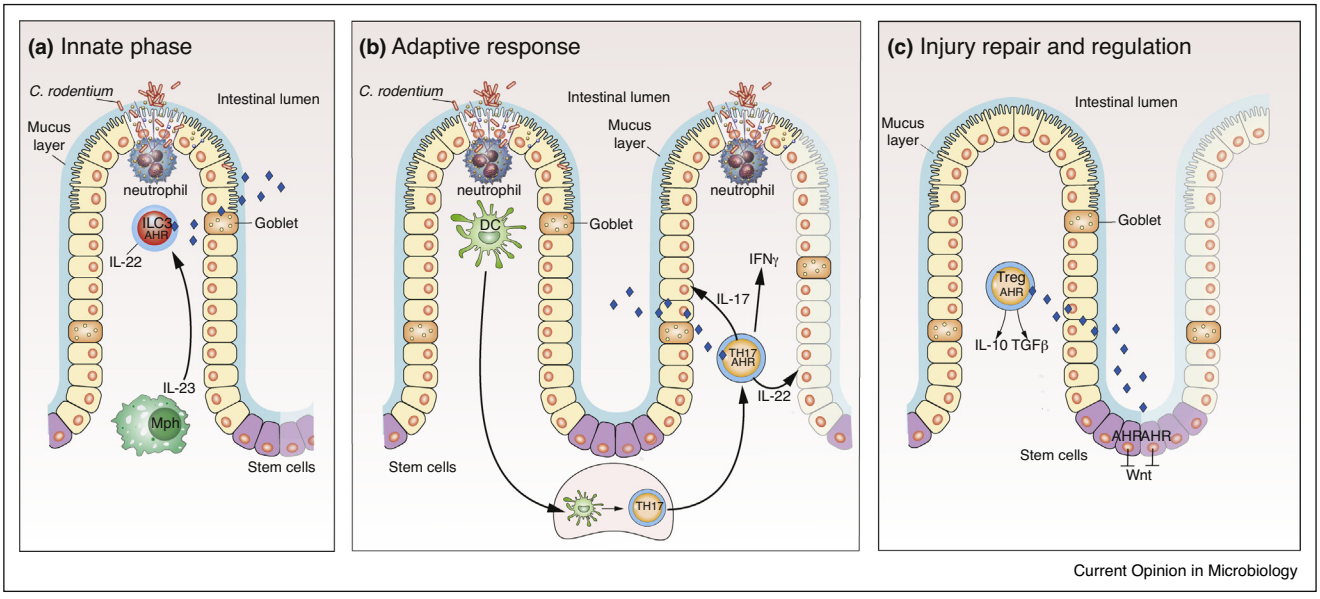
This attaching and effacing pathogen elicits strong immune response in keeping with the necessity to protect the intestinal barrier from the destruction wreake attaching and effacing pathogen d by this bacterium.

Early studies using knockout mouse models established that both CD4 T cell responses and antibody responses are needed to defend the body, whereas CD8 T cells

seem dispensable [1]. However, there is a period of about 5–7 days before the activation of adaptive T cell responses which is bridged by innate immune cell responses, notably innate lymphoid cells (ILC), particularly the ILC3 subset as well as other innate immune cells such as macrophages, dendritic cells and neutrophils (Figure 1 left panel) [7]. A critical cytokine secreted by ILC3 is IL-22, which contributes to barrier defence via the induction of anti-microbial peptides from intestinal epithelial cells, wound healing, fucosylation of mucins and induction of complement [7,8]. IL-22 production by the NCR+ subset of ILC3 is dependent on the provision of IL-23 by dendritic cells and macrophages [9], which itself is induced by IL-1 family members such as IL-36γ [10]. Furthermore, short chain fatty acids derived from microbiota promote IL-22 [11]. ILC3-derived IL-22 is particularly important in the early stage of infection to protect against damage of the cecum, which is the first site of *C. rodentium* colonisation [12]. Beyond day 5, however, the adaptive CD4 T cell response takes over and overshadows the contribution of cytokines by ILC3 (Figure 1 middle panel). In addition to their cytokine-secreting functions, the adaptive CD4 T cell response includes the induction of B cell responses by T follicular helper cells (Tfh) in draining lymph nodes to activate the crucial humoral response to *Citrobacter* infection [13•]. In particular, antibodies of the IgG isotype are critical for the elimination of virulent bacteria in the gut with the help of neutrophils that eliminate IgG-coated intraluminal bacteria [14].

Early studies on cytokine deficient mice suggested that *Citrobacter* infection elicits type I skewed responses as deletion of IL-12 and IFNγ, cytokines required or typical for type I responses, caused increased death from infection [2]. The interest in the field subsequently shifted to the Th17 subset of CD4 T cells, which are characterised by secretion of the cytokines IL-17 and IL-22. Th17 cell differentiation is facilitated by *Citrobacter*-induced epithelial cell death leading to the release of IL-6 and TGFβ by antigen presenting cells, such as dendritic cells [3]. While these are two important mediators favouring the differentiation of Th17 cells, additional factors such as caspase-1 expression in pathogen elicited Th17 cells function in optimal priming of Th17 responses [15] and the expression of resistin like molecule α (RELMα) in epithelial cells promotes their cytokine response [16]. While older classification of T cell responses has emphasised the existence of different CD4 T cell subsets, such as Th1, Th2, Th17 and Treg, it is now clear that there is substantial plasticity in T cells which can blur such boundaries. This has been demonstrated clearly in Th17 cells with the generation of cytokine fate reporters that allow detection of Th17 cells, based on induction of a

Figure 1



Phases of the defence again *C. rodentium* include an innate phase (left panel) where neutrophils phagocytose *C. rodentium* and debris from damaged epithelium, ILC3 are activated by IL-23 from antigen presenting cells such as macrophages and kept alive by AHR signaling induced by tryptophan metabolites from microbiota and the diet (blue lozenges). The middle panel depicts the adaptive phase where dendritic cells present fragments of apoptotic infected neutrophils to induce Th17 cells in draining lymph nodes. Th17 cells migrate to the colonic lamina propria and secrete IL-17, as well as IFNγ and upon stimulation by AHR ligands (blue lozenges) IL-22. The right panel depicts the repair and restoration phase where *Citrobacter* is cleared, regulatory T cells have controlled the inflammatory response via secretion of IL-10 and TGFβ and AHR activation in intestinal stem cells regulates differentiation via coordinated control of the stem cell response to Wnt ligands to replace damaged epithelium.

permanent fluorescent marker when they activated IL-17 [17]. Importantly, the fluorescent marker makes it possible to follow such cells even if they no longer produce IL-17. This established that Th17 cells in the context of an inflammatory milieu rapidly expand their repertoire of pro-inflammatory cytokines and often switch off production of their hallmark cytokine IL-17 [17], thus disguising their identity as Th17 cells if they are classified solely in the basis of IL-17 expression. The IL-17 fate reporter indicated that Th17 cells are the main source of inflammatory cytokines such as IFNγ and GM-CSF during infection with *C. rodentium*. An IL-*22* fate reporter [18•] that allowed determination of the origin of IL-*22* in the intestinal milieu confirmed that the initial source of IL-*22* in the first 5 days after infection was ILC3, whereas thereafter CD4 T cells were the main source [18•].

It is debated that there may be a Th*22* subset [9], but the fate reporter data suggest that such cells may be the product of Th17 cell plasticity. A recent study attempted to determine the state of effector T cells in the intestinal environment following infection with *Salmonella* or *Citrobacter* by single cell transcriptomics. Similar to what was found with fate reporters they report that the expression of cytokines, which traditionally would have been linked to T cell subsets, did not match transcriptomes of the individual cells, but rather indicated that the infecting agent plays a role in determining T cell heterogeneity with polarisation towards particular cytokines irrespective of subset defining transcriptomes [19]. Th17 cells activation by *C. rodentium* infection is initiated in the mesenteric lymph node (mLN) and the cells subsequently migrate to the intestinal lamina propria [20]. They have a distinct transcriptional and metabolic profile from the indigenous Th17 population in the small intestine which is induced by contact with segmented filamentous bacteria (SFB) [21]. *C. rodentium*-elicited Th17 cells show a typical effector cell profile and strong plasticity towards IFNγ expression with a metabolic profile typical of inflammatory effector cells with high oxidative phosphorylation as well as glycolysis, whereas SFB elicited Th17 cells have the metabolic profile of resting memory cells and little plasticity towards inflammatory cytokines [22•]. Effector T cell generation in mesenteric lymph nodes and their expansion in the colon depend on the expression of mTOR as mTOR deficiency in T cells impairs the response against *C. rodentium* infection [23]. Another feature of the T cell response in *C. rodentium* infection highlights the connection between the nervous and immune systems. T cells recruited in this infection express choline acetyl transferase (ChAT), the enzyme required for biosynthesis of acetylcholine (ACh). T cell specific deletion of ACh caused increased inflammation with heightened levels of IL-6, IL-1β and TNF and higher bacterial burden [24]. The latter was attributed to reduced induction of NOS2 expression in epithelial cells from such mice.

Controlling tissue damage: regulatory T cells and environmental triggers via AHR

While the inflammatory response to *C. rodentium* infection is important for the defence against this pathogen, it also represents a threat to tissue homeostasis. Defence against a pathogen needs to be balanced with regulation and the need for tissue restoration (Figure 1 **right panel**). Regulatory T cells expressing Foxp3 are an integral part of the mucosal immune system and in the intestine many of them co-express RORγt, the master regulator of the Th17 cell lineage which may facilitate their regulation of excessive Th17 responses [25]. Plasmacytoid dendritic cells in colon draining lymph nodes were shown to be involved in induction of Foxp3+ Treg and their deficiency led to increased accumulation of inflammatory markers [26•]. A substantial amount of literature has focused on the cytokine IL-10 which can be produced by macrophages, dendritic cells, ILC2 [27], as well as T cells and act on a range of cell types [28]. IL-10 deficiency in CD4 T cells was reported to exacerbate IFNγ and IL-17 responses during infection with *C. rodentium* [29], albeit another study described attenuation of intestinal inflammation during infection in IL-10 deficient mice, attributing a role in downregulation of inflammatory pathways to induction of IL-*27* [30]. Given the multiple sources for this cytokine, different outcomes might be expected depending on whether IL-10 was absent in all cells or deleted in specific subsets.

The intestinal environment is also a site rich in expression of the aryl hydrocarbon receptor (AHR), a ligand dependent transcription factor, which shapes immune and epithelial cell responses at mucosal sites such as the gut (reviewed in Ref. [31]). AHR is activated by ligands in the intestinal environment derived from indoles and tryptophan metabolites generated by the microbiota or formed from dietary pro-ligands that undergo transformation through non-enzymatic condensation reactions [32]. In general, physiological AHR activation by these natural components serves to maintain barrier homeostasis by direct effects on immune cell types as well as epithelial cells, which generally ameliorates inflammation. Some immune cell types in the intestine depend on AHR signals for their survival, such as type 3 innate lymphoid cells (ILC3) [33–35] or subpopulations of intraepithelial lymphocytes (IEL) [36]. As ILC3 are the major producers of the barrier protective cytokine IL-*22* under steady state conditions, AHR deficiency which results in loss of ILC3 will also deplete the intestine of IL-*22*. Under infection conditions the initial protection by ILC3 is normally taken over by the adaptive Th17 cell response that is the major producer of IL-*22* from day 5 after infection with *C. rodentium*. However, AHR is essential for the production of IL-*22* in Th17 cells [4], while not affecting their lifespan to the same degree as that of ILC3. Thus, AHR deficient mice are extremely vulnerable to infection with *C. rodentium* and succumb to widespread bacterial

dissemination about 10 days after infection [37]. Absence of AHR also affects regulatory T cells in the gut environment, reducing their expression of gut homing molecules and increasing the expression of inflammatory cytokines such as IFNγ [38], making them more likely to contribute to inflammation rather than curb it.

AHR ligands are normally rapidly metabolised by cytochrome P4501 enzymes such as Cyp1a1 which are induced upon AHR activation and constitute a feedback mechanism that curtails the duration of AHR signaling [39]. Constitutive expression of Cyp1a1 results in excessive metabolic clearance of AHR signaling and a phenocopy of an AHR deficient state [37]. Such mice succumb to infection with *C. rodentium* as rapidly as AHR knockout mice. In contrast to AHR KO mice, however, mice with overactive Cyp1a1 can be rescued by feeding with a diet supplemented with indole-3-carbinol(I3C) [37], an AHR pro-ligand that is transformed in the acidic compartment of the stomach into a high affinity AHR ligand, indolo[3,2-b]carbazole (ICZ) [40]. Dietary supplementation with I3C attenuates the intestinal inflammation in *C. rodentium* infection and is suggested to reduce the risk of developing colorectal cancer [41].

I3C supplementation restored the IL-22 response and prevented systemic bacterial dissemination [37]. Nevertheless, it became clear that the reconstitution of the immune protective arm is not able to fully restore the defects to the epithelial barrier that occurs in *C. rodentium* infection. This is due to the fact that AHR is an important component facilitating the repair of damaged epithelium by differentiation of new epithelial subsets such as mucus producing goblet cells. AHR restrains the activity of the Wnt pathway by increasing the expression of the negative Wnt regulators *Znrf3* and *Rn43* [42]. A previously identified genetic determinant for susceptibility to *C. rodentium* infection, Rspo2, a member of the Rspondin family which potentiate the canonical Wnt pathway and function as stem cell growth factors [43], is also associated with impaired epithelial differentiation [44•]. Rspo2 expression on the other hand is reduced by exposure to AHR ligands [45], emphasizing the regulatory role of AHR in IEC differentiation.

Thus, both regulatory T cells as well as environmental factors from diet and microbiota serve to counteract the inflammatory consequences of *C. rodentium* infection. While they do not directly constitute an anti-pathogen response, their functioning is nevertheless crucial for the recovery from the cytopathic effect and consequential inflammatory immune response elicited by this infection.

Conclusions

A concerted interaction between innate immune cells in the early stage after infection with *C. rodentium* and induction of adaptive T and B cell responses is required to induce immune protection against this pathogen. Cytokines such as IL-17, IFNγ and IL-22 play prominent roles in the defense, but cannot be simply attributed to defined T cell subsets as the adaptive CD4 T cell response is highly plastic and contains intermediate stages that defy subset categorisation. In addition, the counterbalance for inflammatory T cell responses by regulatory T cells is an essential feature for a successful outcome from infection. Last, but not least the pathogen inflicted damage to epithelium needs to be repaired to prevent barrier disruption and here the influence of environmental factors such as microbial or dietary tryptophan metabolites that activate the AHR is important to safeguard efficient restoration of the barrier by regulated differentiation of intestinal epithelial stem cells.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK, The UK Medical Research Council, and the Wellcome Trust (FC001159).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

1. Simmons CP, Clare S, Ghaem-Maghami M, Uren TK, Rankin J, Huett A, Goldin R, Lewis DJ, MacDonald TT, Strugnell RA *et al.*: **Central role for B lymphocytes and CD4+ T cells in immunity to infection by the attaching and effacing pathogen *Citrobacter rodentium***. *Infect Immun* 2003, **71**:5077-5086.

2. Simmons CP, Goncalves NS, Ghaem-Maghami M, Bajaj-Elliott M, Clare S, Neves B, Frankel G, Dougan G, MacDonald TT: **Impaired resistance and enhanced pathology during infection with a noninvasive, attaching-effacing enteric bacterial pathogen, *Citrobacter rodentium*, in mice lacking IL-12 or IFN-gamma**. *J Immunol* 2002, **168**:1804-1812.

3. Torchinsky MB, Garaude J, Martin AP, Blander JM: **Innate immune recognition of infected apoptotic cells directs T(H)17 cell differentiation**. *Nature* 2009, **458**:78-82.

4. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B: **The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins**. *Nature* 2008, **453**:106-109.

5. Schmidt JV, Bradfield CA: **Ah receptor signaling pathways**. *Annu Rev Cell Dev Biol* 1996, **12**:55-89.

6. Stockinger B, Di Meglio P, Gialitakis M, Duarte JH: **The aryl hydrocarbon receptor: multitasking in the immune system**. *Annu Rev Immunol* 2014, **32**:403-432.

7. Silberger DJ, Zindl CL, Weaver CT: ***Citrobacter rodentium*: a model enteropathogen for understanding the interplay of innate and adaptive components of type 3 immunity**. *Mucosal Immunol* 2017, **10**:1108-1117.

8. Zenewicz LA: **IL-22: there is a gap in our knowledge**. *Immunohorizons* 2018, **2**:198-207.

9. Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, Hatton RD, Weaver CT: **Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria**. *Immunity* 2012, **37**:1061-1075.

10. Ngo VL, Abo H, Maxim E, Harusato A, Geem D, Medina-Contreras O, Merlin D, Gewirtz AT, Nusrat A, Denning TL: **A**

cytokine network involving IL-36gamma, IL-23, and IL-22 promotes antimicrobial defense and recovery from intestinal barrier damage. *Proc Natl Acad Sci U S A* 2018, **115**:E5076-E5085.

11. Yang W, Yu T, Huang X, Bilotta AJ, Xu L, Lu Y, Sun J, Pan F, Zhou J, Zhang W *et al.*: **Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity**. *Nat Commun* 2020, **11**:4457.

12. Rankin LC, Girard-Madoux MJ, Seillet C, Mielke LA, Kerdiles Y, Fenis A, Wieduwild E, Putoczki T, Mondot S, Lantz O *et al.*: **Complementarity and redundancy of IL-22-producing innate lymphoid cells**. *Nat Immunol* 2016, **17**:179-186.

13. • Bai X, Chi X, Qiao Q, Xie S, Wan S, Ni L, Wang P, Jin W, Dong C: **T follicular helper cells regulate humoral response for host protection against intestinal *Citrobacter rodentium* isnfection**. *J Immunol* 2020, **204**:2754-2761

This paper demonstrates the importance of Tfh cells in driving protective IgG1 antibody responses to *Citrobacter* in the later adaptive phase.

14. Kamada N, Sakamoto K, Seo SU, Zeng MY, Kim YG, Cascalho M, Vallance BA, Puente JL, Nunez G: **Humoral immunity in the gut selectively targets phenotypically virulent attaching-and-effacing bacteria for intraluminal elimination**. *Cell Host Microbe* 2015, **17**:617-627.

15. Gao Y, Deason K, Jain A, Irizarry-Caro RA, Dozmorov I, Coughlin LA, Rauch I, Evers BM, Koh AY, Wakeland EK *et al.*: **Transcriptional profiling identifies caspase-1 as a T cell-intrinsic regulator of Th17 differentiation**. *J Exp Med* 2020, **217**.

16. Chen G, Chan AJ, Chung JI, Jang JC, Osborne LC, Nair MG: **Polarizing the T helper 17 response in *Citrobacter rodentium* infection via expression of resistin-like molecule alpha**. *Gut Microbes* 2014, **5**:363-368.

17. Hirota K, Duarte JH, Veldhoen M, Hornsby E, Li Y, Cua DJ, Ahlfors H, Wilhelm C, Tolaini M, Menzel U *et al.*: **Fate mapping of IL-17-producing T cells in inflammatory responses**. *Nat Immunol* 2011, **12**:255-263.

18. • Ahlfors H, Morrison PJ, Duarte JH, Li Y, Biro J, Tolaini M, Di Meglio P, Potocnik AJ, Stockinger B: **IL-22 fate reporter reveals origin and control of IL-22 production in homeostasis and infection**. *J Immunol* 2014, **193**:4602-4613

This paper describes the source of IL-22 in *Citrobacter* infection using an IL-22 fate reporter mouse model.

19. Kiner E, Willie E, Vijaykumar B, Chowdhary K, Schmutz H, Chandler J, Schnell A, Thakore PI, LeGros G, Mostafavi S *et al.*: **Gut CD4(+) T cell phenotypes are a continuum molded by microbes, not by TH archetypes**. *Nat Immunol* 2021, **22**:216-228.

20. Schreiber HA, Loschko J, Karssemeijer RA, Escolano A, Meredith MM, Mucida D, Guermonprez P, Nussenzweig MC: **Intestinal monocytes and macrophages are required for T cell polarization in response to *Citrobacter rodentium***. *J Exp Med* 2013, **210**:2025-2039.

21. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV *et al.*: **Induction of intestinal Th17 cells by segmented filamentous bacteria**. *Cell* 2009, **139**:485-498.

22. • Omenetti S, Bussi C, Metidji A, Iseppon A, Lee S, Tolaini M, Li Y, Kelly G, Chakravarty P, Shoaie S *et al.*: **The intestine harbors functionally distinct homeostatic tissue-resident and inflammatory Th17 cells**. *Immunity* 2019, **51**:77-89 e76

This paper shows that Th17 cells elicited by *Citrobacter* infection are highly plastic and metabolically active effector cells in contrast to resident SFB derived Th17 cells.

23. Lin X, Yang J, Wang J, Huang H, Wang HX, Chen P, Wang S, Pan Y, Qiu YR, Taylor GA *et al.*: **mTOR is critical for intestinal T-cell homeostasis and resistance to *Citrobacter rodentium***. *Sci Rep* 2016, **6**:34939.

24. Ramirez VT, Godinez DR, Brust-Mascher I, Nonnecke EB, Castillo PA, Gardner MB, Tu D, Sladek JA, Miller EN, Lebrilla CB *et al.*: **T-cell derived acetylcholine aids host defenses during enteric bacterial infection with *Citrobacter rodentium***. *PLoS Pathog* 2019, **15**:e1007719.

25. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY *et al.*: **TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function**. *Nature* 2008, **453**:236-240.

26. • Poysti S, Silojarvi S, Toivonen R, Hanninen A: **Plasmacytoid dendritic cells regulate host immune response to *Citrobacter rodentium* induced colitis in colon-draining lymph nodes**. *Eur J Immunol* 2021, **51**:620-625

This paper demonstrates the importance of pDC in inducing Foxp3+ Treg to control intestinal inflammation in *Citrobacter* infection.

27. Bando JK, Gilfillan S, Di Luccia B, Fachi JL, Secca C, Cella M, Colonna M: **ILC2s are the predominant source of intestinal ILC-derived IL-10**. *J Exp Med* 2020, **217**.

28. Kole A, Maloy KJ: **Control of intestinal inflammation by interleukin-10**. *Curr Top Microbiol Immunol* 2014, **380**:19-38.

29. Seiffart V, Zoeller J, Klopfleisch R, Wadwa M, Hansen W, Buer J, Riedel C, Westendorf AM: **IL10-deficiency in CD4(+) T cells exacerbates the IFNgamma and IL17 response during bacteria induced colitis**. *Cell Physiol Biochem* 2015, **36**:1259-1273.

30. Dann SM, Le C, Choudhury BK, Liu H, Saldarriaga O, Hanson EM, Cong Y, Eckmann L: **Attenuation of intestinal inflammation in interleukin-10-deficient mice infected with *Citrobacter rodentium***. *Infect Immun* 2014, **82**:1949-1958.

31. Stockinger B, Shah K, Wincent E: **AHR in the intestinal microenvironment: safeguarding barrier function**. *Nat Rev Gastroenterol Hepatol* 2021 http://dx.doi.org/10.1038/s41575-021-00430-8.

32. Hubbard TD, Murray IA, Perdew GH: **Indole and tryptophan metabolism: endogenous and dietary routes to Ah receptor activation**. *Drug Metab Dispos* 2015, **43**:1522-1535.

33. Lee JS, Cella M, McDonald KG, Garlanda C, Kennedy GD, Nukaya M, Mantovani A, Kopan R, Bradfield CA, Newberry RD *et al.*: **AHR drives the development of gut ILC22 cells and postnatal lymphoid tissues via pathways dependent on and independent of notch**. *Nat Immunol* 2012, **13**:144-151.

34. Qiu J, Heller JJ, Guo X, Chen ZM, Fish K, Fu YX, Zhou L: **The aryl hydrocarbon receptor regulates gut immunity through modulation of innate lymphoid cells**. *Immunity* 2012, **36**:92-104.

35. Kiss EA, Vonarbourg C, Kopfmann S, Hobeika E, Finke D, Esser C, Diefenbach A: **Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles**. *Science* 2011, **334**:1561-1565.

36. Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, Veldhoen M: **Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation**. *Cell* 2011, **147**:629-640.

37. Schiering C, Wincent E, Metidji A, Iseppon A, Li Y, Potocnik AJ, Omenetti S, Henderson CJ, Wolf CR, Nebert DW *et al.*: **Feedback control of AHR signalling regulates intestinal immunity**. *Nature* 2017, **542**:242-245.

38. Ye J, Qiu J, Bostick JW, Ueda A, Schjerven H, Li S, Jobin C, Chen ZE, Zhou L: **The aryl hydrocarbon receptor preferentially marks and promotes gut regulatory T cells**. *Cell Rep* 2017, **21**:2277-2290.

39. Bergander L, Wincent E, Rannug A, Foroozesh M, Alworth W, Rannug U: **Metabolic fate of the Ah receptor ligand 6-formylindolo[3,2-b]carbazole**. *Chem Biol Interact* 2004, **149**:151-164.

40. Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, Bradfield CA: **Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin**. *Proc Natl Acad Sci U S A* 1991, **88**:9543-9547.

41. Wu Y, He Q, Yu L, Pham Q, Cheung L, Kim YS, Wang TTY, Smith AD: **Indole-3-carbinol inhibits *Citrobacter rodentium* infection through multiple pathways including reduction of bacterial adhesion and enhancement of cytotoxic T cell activity**. *Nutrients* 2020, **12**.

42. Metidji A, Omenetti S, Crotta S, Li Y, Nye E, Ross E, Li V, Maradana MR, Schiering C, Stockinger B: **The environmental sensor AHR protects from inflammatory damage by maintaining intestinal stem cell homeostasis and barrier integrity**. *Immunity* 2018, **49**:353-362 e355.

43. Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, Avello M, Lei H, Mickanin C, Liu D, Ruffner H *et al.*: **ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner**. *Nature* 2012, **485**:195-200.

44. • Kang E, Zhou G, Yousefi M, Cayrol R, Xia J, Gruenheid S: **Loss of disease tolerance during *Citrobacter rodentium* infection is associated with impaired epithelial differentiation and hyperactivation of T cell responses**. *Sci Rep* 2018, **8**:847

This paper shows that Rspo2, a genetic determinant of the outcome of *Citrobacter rodentium*, is linked with a transcriptional signature of increased Wnt signaling, loss of epithelial differentiation and exaggerated T cell responses that underlie intestinal dysfunction in susceptible strains.

45. Zhang H, Yao Y, Chen Y, Yue C, Chen J, Tong J, Jiang Y, Chen T: **Crosstalk between AhR and wnt/beta-catenin signal pathways in the cardiac developmental toxicity of PM2.5 in zebrafish embryos**. *Toxicology* 2016, **355-356**:31-38.