Review

The Unique Lifestyle of Crohn's Disease-Associated Adherent-Invasive *Escherichia coli*

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Abstract

Escherichia coli is one of the most genetically and phenotypically diverse species of bacteria. This remarkable diversity produces a plethora of clinical outcomes following infection and has informed much of what we currently know about host–pathogen interactions for a wide range of bacteria–host relationships. In studying the role of microbes in disease, adherent-invasive *E. coli* (AIEC) has emerged as having a strong association with Crohn's disease (CD). Thus, there has been an equally strong effort to uncover the root origins of AIEC, to appreciate how AIEC differs from other well-known pathogenic *E. coli* variants, and to understand its connection to disease. Emerging from a growing body of research on AIEC is the understanding that AIEC itself is remarkably diverse, both in phylogenetic origins, genetic makeup, and behavior in the host setting. Here, we describe the unique lifestyle of CD-associated AIEC and review recent research that is uncovering the inextricable link between AIEC and its host in the context of CD.

Introduction

Digestion and nutrient acquisition, part of the homeostatic functions of the intestine, pose a unique challenge for the host immune system. On one hand, our immune system is continually exposed to a diverse array of dietary antigens, commensal microbes and their products, and self-antigens, all of which require the maintenance of immune tolerance. On the other hand, the intestinal mucosa is occasionally exposed to pathogenic microorganisms that require a rapid and decisive host response to minimize damage. The ability to maintain this fine balance between tolerant and protective immune responses in the gut is central to the maintenance of gut health. However, common microbes that can adopt dualistic relationships with the host, behaving at times as both commensal and pathogen, can destabilize this delicate balance.

Crohn's disease (CD) is one type of inflammatory bowel disease in which the fine balance operating in the gut breaks down, leading to immune activation and chronic inflammation. It is generally accepted that CD is a multifactorial condition with an etiology influenced by genetic, environmental, and immunological factors [1]. While genetic predisposition clearly plays a central role in CD [2–4], there is low penetrance for any one genetic risk allele, suggesting that a multitude of genetic and environmental factors can converge on disease expression [5–7]. Indeed, external risk factors including antibiotic use, diet, prior episodes of acute infectious gastroenteritis, and the acquisition of disease-associated microbes have received attention due to their epidemiological connections to CD [5,8–10]. While no single risk factor explains CD etiology, the combination of risk factors that have been established seems to

converge on the unifying feature of loss of intestinal homeostasis.

Maintenance of Gut Homeostasis

Mammals maintain gut integrity and prevent colonization by invading pathogens using a variety of immunological and physical barriers. While generally tolerant to commensal gut microbes in unperturbed states, the mucosal immune system can be uniquely and rapidly deployed in response to invading pathogens [11–15]. The epithelial lining of the intestine creates a physical interface between "inside" and "outside" the host. Alone, this interface forms an important barrier separating sterile body sites from the trillions of microbes in the gut lumen. Protecting the epithelium is a layer of mucus, a highly regenerative glycoprotein complex secreted by intestinal goblet cells. Mucus itself is an important intestinal compartment that creates a physical and chemical barrier that restricts the type of bacteria that can come in contact with the epithelial surface [16]. The release by specialized gut epithelial cells of antimicrobial peptides into the mucus creates an additional physicochemical barrier to restrict bacterial contact. Although the inner mucus layer is considered to be sterile [17], the outer layer houses a distinct bacterial community that requires specialized metabolic capacities and likely mechanisms to resist the action of innate antimicrobial peptides [18,19].

In addition to the direct antimicrobial strategies employed by the host, commensal microbes are another robust means to protect the gut from colonization by pathogenic bacteria. The human body harbors trillions of non-pathogenic commensal bacteria, which are non-uniformly distributed along the longitudinal gut axis [20,21]. Indeed, this resident microbiota provides a high level of "colonization resistance" through the physical occupation of colonization sites, as well as competing for and utilizing essential nutrients [22,23]. Whereas in states of health, the resident microbiota is capable of providing robust colonization resistance, this effect is rapidly diminished or lost following exposure to certain antibiotics [24]. In addition to providing direct competition, resident commensal bacteria contribute to the maintenance of an educated mucosal immune system. For example, sampling of the intestinal lumen by intestinal immune cells results in exposure to the antigens derived from commensal organisms, providing the necessary stimulation required for the maturation of gut-resident immune cells [11,25–30]. Animal studies under axenic conditions have demonstrated that the mucosal immune system is severely underdeveloped in germ-free mice, leading to multiple immunological deficiencies, including impaired T-cell maturation [31], differential distribution of innate lymphoid cells [32], and impaired development of regulatory mechanisms [33].

Commensal, Pathogen, and Pathobiont: The Many Faces of *Escherichia coli*

E. coli is a ubiquitous, albeit typically low-abundance, member of the human microbiome. The versatility of this species is showcased by its varied relationships with hosts, ranging from benign commensals to pathogenic variants [34]. For example, whereas cattle can carry enterohemorrhagic *E. coli* (EHEC) asymptomatically at ranges of 200–10,000 colony-forming units per gram of feces [35], as few as 10–100 of these same bacteria in humans can give rise to the potentially deadly hemolytic uremic syndrome [36]. Different pathotypes of *E. coli* show remarkable genetic diversity, which affects their clinical presentation and tissue tropism [37,38]. It is this exceptional level of genomic diversity that has made *E. coli* a fascinating case study in microbial evolution and adaptation. Pathogenic variants of *E. coli* have evolved multiple mechanisms to ensure their survival and persistence. For example, in addition to expressing flagella, EHEC and enteropathogenic *E. coli* (EPEC) use a type III secretion system (T3SS) to adhere to and manipulate the gut epithelium, allowing them to persist by forming microcolonies at attaching and effacing lesions (A/E lesions) [39]. A/E lesions represent a pathogenic hallmark formed at the location of bacterial adhesion to the surface of gut epithelium [39]. This direct contact with host cells gives access to an intestinal niche that typically restricts most other microbes. While this niche may provide a competitive advantage, it also places bacteria in close proximity to sites of heightened immune surveillance, specifically the intestinal epithelium and underlying immune cells residing in the lamina propria [39]. Commensal *E. coli* may occasionally come in contact with the intestinal epithelium and can be sampled through microfold cells and dendritic cells, yet these interactions are likely transient and the bacteria lack evolved strategies to persist in this locale [40]. In addition, immune regulatory mechanisms employed by the gut prevent these transient encounters from inducing inflammation, likely because they generate little to no damage to the host. Pathogenic variants of *E. coli,* on the other hand, can have prolonged contact with the epithelium, inducing a sustained and pronounced engagement with the immune system [39]. Moreover, the T3SS itself can be detected by certain immunological sensors of the immune system, leading to activation of host inflammasomes and bacterial recognition [41].

Adherent-Invasive *E. coli*

While many strains of *E. coli* can be distinctly classified as either overtly pathogenic or commensal based on genetics and phylogeny, defining the pathogenic potential of *E. coli* is somewhat of a moving target. A relatively recently recognized *E. coli* pathotype (discovered in the late 1990's), adherent-invasive *E. coli* (AIEC) falls within this shifting spectrum. AIEC is distinct from the six major diarrheagenic *E. coli* pathotypes (enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and enterotoxigenic *E. coli* (ETEC)), in that it lacks common virulence factors found in these pathogenic relatives (for an excellent comprehensive review on *E. coli* pathotypes, see Ref. [38]. AIEC are most notable for being implicated in the pathogenesis of CD [42]. Owing to this association, which has been established and validated through clinical, experimental, and epidemiological studies [43–46], there has been much interest in a definitive genetic signature of the AIEC pathotype. So far, this has remained elusive [43–46]. AIEC strains can also be found, albeit much less frequently, in healthy individuals [9,10,44], suggesting that AIEC might work in concert with other CD risk factors.

Although the gut microbiome is a resilient ecosystem, it can be restructured through diet, xenobiotics, enteric infection, and host genetic factors [12]. Compositional shifts in the microbial community, known as dysbiosis, can exert pathophysiological effects on the host [47]. What are the drivers of host pathology during dysbiotic imbalances? There are numerous microbial, metabolic, and immunological explanations; however, in the context of this discussion, the outgrowth of so-called pathobionts has received attention for their ability to exert a pathologic effect on the host [48]. Pathobionts can be benign members of the indigenous microbial community under normal physiological conditions; however, they can act as disease modifiers following perturbations to the gut environment, bringing about alternative bacterial phenotypes. AIEC have been found to asymptomatically colonize healthy individuals yet are more commonly isolated from CD patients. Given their ability to exacerbate gut inflammation, there is growing appreciation that AIEC are a likely pathobiont in the pathophysiology of CD [47] (Fig. 1). A common feature of CD-associated dysbiosis is the decline in microbial diversity, specifically a decrease in the abundance of Firmicutes and Bacteroidetes, and the expansion of Proteobacteria, of which *E. coli* is a member [49–51]. In line with these observations, high titers of *E. coli* antibodies have been detected in the sera of CD patients [52,53], suggesting a pathogenic role for *E. coli* during disease progression. Evidence from experimental animal models suggests that AIEC strains too can modify the gut microbiota in genetically susceptible hosts, leading to the expansion of additional pro-inflammatory microbial species [54]. Therefore, AIEC may be both a cause and a consequence of CD manifestations.

Features Distinguishing AIEC from Commensal *E. coli*

To date, only a handful of *E. coli* strains have been fully characterized as having the AIEC phenotype. Despite their genetic diversity, these strains share a number of common features, most notably their ability to adhere to, and invade, epithelial cells. Below we discuss some of the central features most commonly seen with AIEC strains.

Phylogeny

Genetically, AIEC are most closely related to extraintestinal pathogenic *E. coli* that cause disease outside of the gut, including uropathogenic *E. coli* and strains associated with neonatal meningitis [55]. Although there is a strong line of evidence supporting the role of AIEC in promoting gut inflammation and exacerbating CD pathology, the genetic determinants that discriminate AIEC from commensals have remained elusive. In the absence of a definitive genetic signature, the identification of AIEC strains relies on *in vitro* phenotypic assays that monitor the ability of *E. coli* to adhere to and invade epithelial cells, and to survive in macrophages where they can elicit a proinflammatory response [8]. The ability of AIEC to replicate extensively in macrophages was originally reported [56]; however, this attribute may not be the most reliable marker of the AIEC phenotype, as many strains do not share this character [57,58]. Given this, survival or persistence, rather than replication within the macrophage, may be a more reliable AIEC indicator. Comparative genomic studies have revealed that the majority of AIEC isolates are distributed across the B2 and D phylogenetic groups of *E. coli*. Furthermore, the distribution of AIEC strains across the *E. coli* phylogenetic space suggests that convergent evolution is the dominant force shaping this pathotype [43–46], making it likely that several different host environments can select for the AIEC phenotype.

Biogeography

Similar to commensal *E. coli*, AIEC uses type I pili as an adherence structure to attach to surfaces, with FimH being the terminal adhesin. However, genetic polymorphisms have been described in FimH from the LF82 strain of AIEC that allow it to bind more efficiently to the CEACAM6 glycoprotein expressed

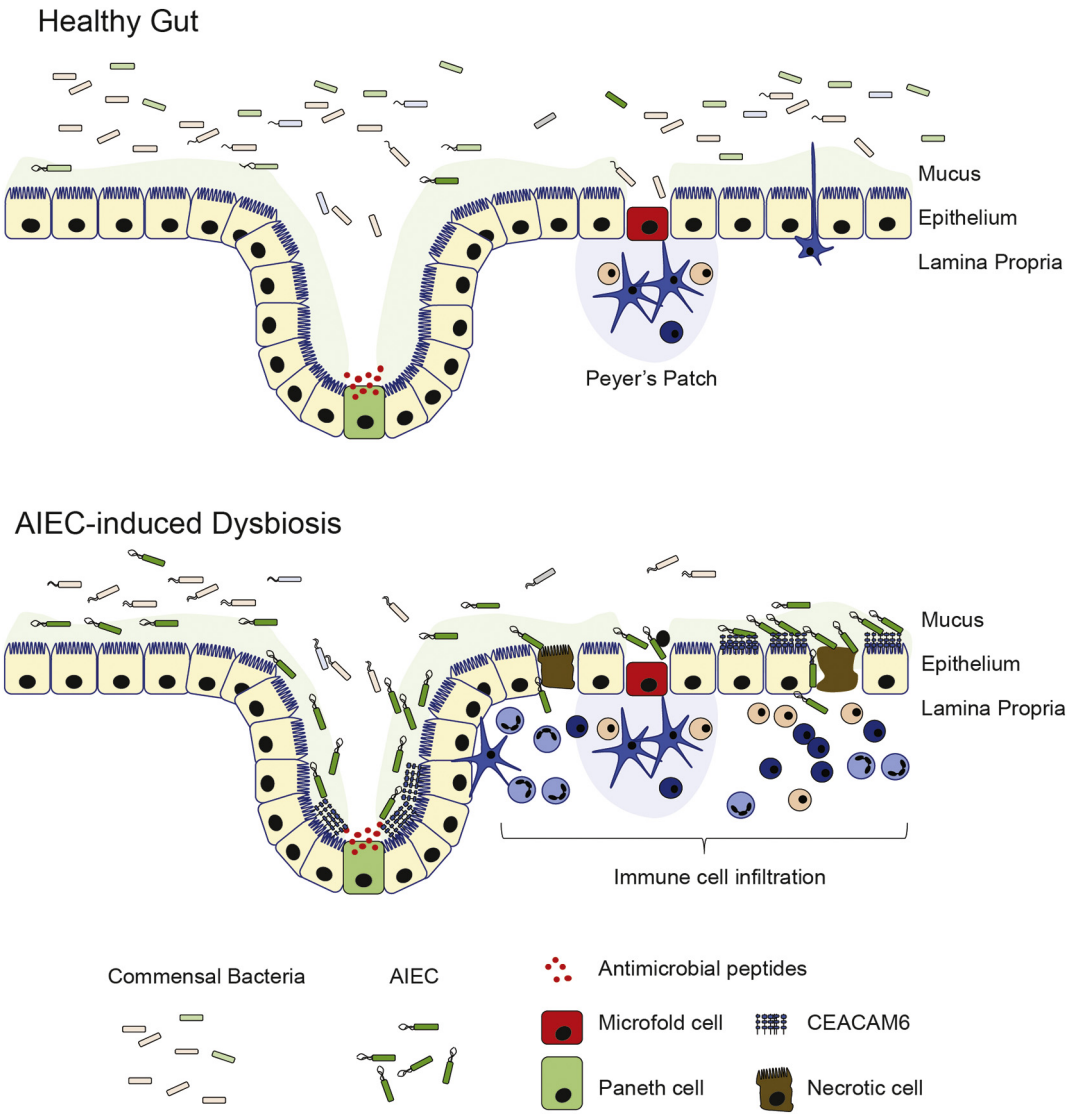

Fig. 1. The healthy gut is characterized by a single epithelial layer covered in mucus that functions to restrict bacterial access to the lamina propria. The small intestine uses finger-like extensions, termed villi, to absorb nutrients. These villus invaginations contain the stem cells used to populate the various types of epithelial cells comprising the small intestine. Paneth cells reside at the base of these crypt formations and are responsible for secreting a series of antimicrobial peptides that protect the crypt from microbial invasion, as well producing epithelial growth factors responsible for regulating epithelial cell differentiation and growth. These systems maintain gut homeostasis by restricting access of commensal bacteria to the intestinal epithelium and sampling local antigens. AIEC have been shown to exploit inflammation-derived metabolites following antibiotic treatment to expand their metabolic niche and outcompete resident commensals. AIEC-induced dysbiosis induces the upregulation of CEACAM6 on the intestinal epithelium, resulting in bacterial infiltration deep within the crypts. AIEC strains of *E. coli,* unlike the commensal strains, undergo prolonged contact with the epithelium, inducing a sustained and pronounced engagement of pattern recognition receptors, which activates inflammatory responses and results in distinct immune cell recruitment.

on the surface of human gut epithelial cells, compared to the orthologous FimH expressed in commensal *E. coli* [59]. CEACAM6 expression was found to be elevated in patients with ileal CD, potentially giving AIEC a competitive advantage over other *E. coli* strains in the CD gut [60]. Moreover, unlike in commensals, flagellar motility was shown to be upregulated in AIEC upon exposure to mucin [61]. Accordingly, AIEC strains are better able to penetrate the intestinal mucosa compared to *E. coli* symbionts [61,62]. Similarly, the endoplasmic reticulum stress response glycoprotein, Gp96, was found to be abundant in intestinal biopsies obtained from CD patients [63]. AIEC can bind to Gp96 using outer-membrane protein A, which might aid in targeting AIEC to sites of active disease.

In addition to their enhanced adhesive traits, AIEC display higher levels of cell invasion compared to non-pathogenic *E. coli*. Unlike many Gram-negative bacteria that employ a T3SS to invade host cells, AIEC do not use this invasion mechanism and appear to use surface invasins instead (e.g., IbeA) to trigger their entry into intestinal epithelial cells [64]. For example, the IbeA-encoding locus was more frequently detected in the genomes of extraintestinal *E. coli* and AIEC strains compared to commensal *E. coli*, suggesting a global role for this invasin in AIEC pathogenesis [64,65].

Community lifestyle

Biofilms are bacterial aggregates embedded in a matrix of complex exopolysaccharides, DNA, and proteins. The ability to produce cellulose, a matrix component associated with biofilm formation, is important for AIEC pathogenesis in mice, as well as their ability to promote gut inflammation [66]. In addition to maintaining a stable bacterial community, biofilms can confer protection against both antibiotics and sentinel immune cells [67]. Martinez-Medina *et al.* [68] demonstrated that biofilm formation is another pathogenic trait that distinguishes AIEC from non-pathogenic *E. coli*, with the majority of AIEC strains producing robust biofilms. Although AIEC biofilms have not been formally demonstrated *in vivo*, their biogenesis has been found to be modulated by host cues, including iron and mucin. For example, iron was shown to induce the aggregation of the AIEC strain, NC101 [66], whereas mucin-derived *N*-acetyl-glucosamine reduced biofilm formation in the prototypical AIEC strain, LF82 [69], suggesting that biofilm production may be spatially regulated depending on the localization of cues within the host environment.

Immune evasion

The human gut contains an abundance of antimicrobial peptides (AMP) known to regulate immune function and kill microbes via a variety of mechanisms [70]. CD is associated with increased mucosal secretion of certain AMP classes (e.g., human neutrophil proteins and human β-defensin-3) [71]. Due to their proximity to the gut mucosa, AIEC strains are likely exposed to elevated concentrations of AMPs during inflammation. Thus, it is conceivable that the host environment would select for AMP-resistant AIEC strains. In agreement with this, McPhee *et al.* [72] demonstrated that the AIEC strain NRG857c is equipped with multiple genes that confer AMP resistance, including an outer-membrane protease, revealing an additional mechanism by which AIEC can persist in the CD environment. Further evidence supporting the role of AMP resistance in AIEC pathogenesis comes from murine models of polymicrobial gastroenteritis. In these models, exposing AIEC-colonized mice to the enteric pathogen, *Salmonella* Typhimurium, resulted in the expansion of the AIEC gut population and higher levels of inflammation relative to the mono-infected groups [73]. Unlike the wild-type strain, an AMP-sensitive AIEC mutant was unable to expand in the gut of *Salmonella*-infected mice, a finding consistent with the important role of AMP resistance in promoting AIEC expansion during inflammation.

"Inflammophilic" traits

In addition to creating a hostile environment for invading pathogens, inflammation can contribute to gut dysbiosis, with the expansion of facultative anaerobes being one hallmark [74]. Several studies have shown that inflammation can function as an ecological switch that promotes the bloom of Proteobacteria through modulating the metabolic landscape in the gut [51,75–77]. For example, antibiotics can induce mild levels of inflammation that trigger an increase in epithelial oxygenation and nitrate species, thereby conferring a fitness advantage to *E. coli in vivo* [76]. Furthermore, the induction of nitric oxide synthase during colitis was shown to generate nitrate, which can serve as a terminal electron acceptor for *E. coli*, and to promote the formation of oxidized sugars (e.g., glucarate and galactarate) that can be preferentially used by invading pathogens [77]. In a similar manner, AIEC have been shown to exploit inflammation-derived metabolites following antibiotic treatment, leading to their expansion at mucosal-associated sites in the gut [51]. In these studies, AIEC mutants deficient in glucarate and galactarate utilization, or nitrate reduction, are severely attenuated in mouse models following antibiotic treatment. Several AIEC strains carry the propanediol utilization locus [45,78] used by *Salmonella enterica* in the respiration of 1,2-propanediol during inflammation [79]. However, the role of propanediol utilization in AIEC colonization remains undetermined. In summary, while AIEC strains often share a number of similar traits, including their ability to adhere and invade epithelial cells, to date, no clear unifying molecular definition of AIEC has emerged. Future genetic studies may be capable of elucidating a central "signature" trait common to all AIEC strains, however thus far, a strict genetic signature has remained elusive.

AIEC in States of Health And Disease

In animal models, AIEC strains are associated with heightened inflammation and pathogenic potential in genetically susceptible hosts or in hosts exposed to secondary stimuli leading to loss of homeostatic

balance in the gut [45,51,59,64,66,73,80–82]. However, AIEC is also found in healthy individuals, suggesting that the behavior of AIEC in these settings might be fundamentally different than in states of disease. Whether AIEC strains remain asymptomatic because the host actively constrains them or because the strains themselves are genetically distinct is an open question. In one recent study, certain adherent strains of bacteria, including AIEC LF82, were required for maintaining anti-inflammatory effects in the gut [28]. Specifically, AIEC were able to interact with CX3CR1+ mononuclear phagocytes and induce the production of the regulatory cytokine, IL-10, to limit inflammatory T-cell responses [28], possibly as a mechanism of immune evasion. In contrast, IL-10 has also been previously shown to play a central role in intestinal homeostasis, whereby mice deficient in IL-10 develop spontaneous colitis [83]. Another recent paper showed that IL-10$^{-/-}$ mice mono-associated with murine AIEC developed chronic inflammation with an attendant increase in IL-12/23p40 production in the small and large intestines [81]. An additional immunological outcome in this model was elevated production of IFN-γ and IL-17 by CD4 T-cells, making IL-10$^{-/-}$ mice potentially useful to untangle the immunological response to pathobionts in states of health and disease. Together, these findings make it difficult to assess the role of IL-10 in CD models. Specifically, while AIEC might drive IL-10 production potentially as a way to mediate immune evasion, IL-10 also seems required to prevent the spontaneous development of colitis in mice.

One of the original mouse models of AIEC infection was developed in CEABAC10 transgenic mice that express various human CEACAM receptors, including CEACAM6, which are lacking in mice [84,85]. When infected with AIEC strain LF82 in conjunction with low-doses of dextran sulfate sodium (DSS), a chemical inducer of colitis, CEABAC10 mice exhibit dramatically enhanced intestinal inflammation, weight loss, and lethal outcomes, generally over a short period of time (~1 week) and to a greater magnitude then DSS alone controls. Moreover, specific genetic associations with CD, such as NOD2, have been studied in the context of AIEC infection. Impaired expression of NOD2 has been shown to facilitate enhanced intramacrophage survival of AIEC strains, including LF82 [86,87], possibly through an impairment in autophagy-mediated control of intracellular bacteria [86]. The enhanced intracellular survival of AIEC was also shown to induce elevated pro-inflammatory cytokine production, providing a direct link between intracellular AIEC and a pathophysiological response by the host [86]. Thus, the enhanced survival and proinflammatory effect of AIEC in NOD2$^{-/-}$ macrophages provides a possible mechanism to account for the greater inflammatory potential of AIEC in genetically susceptible hosts. A similar enhanced survival and pro-inflammatory response is observed when primary monocytes isolated from CD patients are exposed to AIEC [87], suggesting that at least one genetic subset of CD patients may have a primary impairment in macrophage-mediated control of AIEC.

In *tlr5*$^{-/-}$ mice, LF82 exacerbates chronic colitis in the absence of CEACAM6 [88,89]. The development of chronic colitis in *tlr5*$^{-/-}$ mice persisted after host clearance of LF82, possibly through elevated levels of LPS and flagellin, as well as a lack of low-level signaling through TLR5. Continuous low-level stimulation through TLR5 has been shown to induce IL-22, a cytokine associated with maintenance of gut integrity [88,89]. IL-22 is increased during active CD, as well as following DSS-induced colitis [90], suggesting that the host may induce IL-22 to help reconstitute the gut following injury. Of note, the IL-22 receptor shares a common subunit with the IL-10 receptor, IL-10R2; polymorphisms in both IL-10R1 and IL-10R2 have been associated with early-onset colitis in children [91,92], indicating that IL-10 and/or IL-22 may be a key player in regulating disease development in children.

Genetic models such as those described above provide useful tools to study how host genetics might create selective gut environments that predispose to disease states when pathobionts are present. Additional models are beginning to explore other combinatorial risk factors that might work in concert with AIEC. For example, Oberc *et al.* [51] reported that treatment with several classes of antibiotics dramatically compromises intrinsic colonization resistance to AIEC. In this study, multiple antibiotic classes strongly potentiated AIEC expansion in chronically infected mice. AIEC outgrowth was not correlated with a stereotypical shift in the gut bacterial community but was linked to reduced diversity and a divergence from the pre-antibiotic state. AIEC expansion was due, in part, to the generation of oxidized sugars that AIEC has the capacity to metabolize more effectively than commensal *E. coli* variants. Looking through a public health lens, another use for this xenobiotic model might be in studying initial AIEC colonization of naïve hosts, as almost nothing is known about infectious doses and natural routes of transmission of AIEC in humans.

It is possible that long-term colonization by AIEC can shift the gut equilibrium to a state of hyper-responsiveness, making colonized individuals more susceptible when exposed to secondary insults linked to CD. This idea might be relevant to the enigmatic finding that CD is more common in individuals exposed to acute infectious gastroenteritis caused by *Salmonella* and other enteric pathogens, sometimes with onset times years after the infectious episode [6,93,94]. The mechanistic basis for this long-term risk association following an acute

event is unresolved; however, one possibility is that resident gut microbes, like AIEC, could perpetuate inflammatory reactions in the post-infectious period. In mouse models, AIEC-colonized animals exposed to acute infectious gastroenteritis exhibit mortality and disrupted gut barrier function, whereas in AIEC-naïve animals, the same enteric infection is non-lethal and self-limiting [73]. In these studies, co-infection with AIEC and *Salmonella* resulted in enhanced T-cell and myeloid cell recruitment into the cecal tissue when compared with *Salmonella* infection alone, indicating that AIEC is an active disease modifier in this model. These immunological changes were accompanied by enhanced cytokine (e.g., TNF-α and IL-17A) and chemokine (e.g., IP-10, MIG, MIP1α, and MIP1β) production in infected ceca, with attendant increases in immunopathology. The source of enteric inflammation on AIEC infection outcome was not specific to *Salmonella*, as similar results were seen in a co-infection model with AIEC and *Citrobacter rodentium*.

The ability of AIEC to drive inflammation has been partially linked to the expression of hypoxia-inducible factor (HIF)1α within inflamed tissues. Heightened expression of HIF1α is found in the ileum and colon of CD patients during active stages of disease, with greater than 80% of ileal and colonic CD biopsies staining positive for HIF1α [95]. This number contrasts with the ~35% of remission-stage biopsies that are positive for HIF1α and ~20% in healthy individuals. Importantly, in a CEABAC10 mouse model, HIF1α mRNA was upregulated in the colonic tissue of mice infected with LF82 but not when infected with the non-pathogenic *E. coli* strain, K12 [95]. As a sensor of oxygen saturation, HIF1α is degraded under normal tissue oxygenation and stabilized under hypoxic conditions leading to downstream signaling events. Growing evidence suggests that HIF1α may also be capable of sensing certain inflammatory products (i.e., nitric oxide) [96]. This is relevant because during infection, nitric oxide is a potent antimicrobial product produced through the activation of nitric oxide synthase (NOS) 2. Intriguingly, *E. coli* colonization is enhanced in mice treated with streptomycin through the production of NOS2-dependent nitrate that provides alternative electron acceptors for anaerobic respiration in *E. coli* [97,98]. Moreover, elevated production of nitrate accompanies the enhanced epithelial oxygenation previous shown to augment AIEC growth *in vivo* [76]. These studies highlight a potential axis between AIEC, HIF1α, and nitric oxide in the development of inflammatory pathology. However, this relationship might be more complicated. A recent paper showed that hypoxia and HIF1α activation may dampen inflammation during colitis and in CD patients [99], suggesting that HIF1α observed in CD patients may be in response to, rather than the cause, of inflammation.

Adaptive Evolution of AIEC

Comparative genomics suggest that AIEC strains have varied ancestries, suggesting that the adaptive traits acquired by these pathobionts in independent host environments can lead to the manifestation of the adherent-invasive phenotype. One plausible explanation for the genetic heterogeneity and phylogenetic diversity of AIEC is that AIEC might be inherently more adaptable to the gut environment compared to commensal *E. coli.* However, very little is known about the pace and specificity of the adaptations that AIEC employs to colonize the gut. To study the adaptive behavior of AIEC *in vivo*, we recently leveraged a previous CD infection model first developed to study the impact of long-term AIEC colonization in mice [82], and combined it with natural host-to-host transmission to understand the genetic changes that occur in the AIEC population over chronic timescales in a host [62]. Starting with a single clonal isolate of AIEC that was originally isolated from a CD patient, the host environment quickly selected for multiple diversified lineages of AIEC capable of infecting new hosts. We found a common propensity for AIEC to evolve hypermotility and to undergo metabolic rewiring to better consume host- and microbe-derived short-chain fatty acids; phenotypes that were confirmed in a large collection of *E. coli* isolates from human CD patients. Strikingly, these phenotypes were absent in *E. coli* isolated from healthy individuals, suggesting the presence of common selection signatures for CD-associated pathobionts. The evolved hypermotile AIEC lineage was more pro-inflammatory *in vivo* relative to less motile strains, which is consistent with the observation that flagellin is an immunodominant antigen in CD patients [100]. Given the anti-inflammatory properties of short-chain fatty acids, the increased consumption of these metabolites by AIEC may accelerate the perturbations leading to dysbiosis and provides a plausible link between AIEC outgrowth and disease development [14]. Within-host evolution experiments revealed that the commensal *E. coli* strain HS did not acquire the motility or metabolic traits that were commonly seen in the evolved AIEC lineages, despite being capable of rapid acquisition of adaptive mutations *in vivo* [101–103]. The implication of this might be that the evolutionary landscapes of AIEC and commensal *E. coli* are fundamentally different, possibly resulting from unique interactions between each of the two groups and the immune system in different biogeographic locations. A recent study tracked the compositional fluctuations in the microbiome over time in a single CD patient, revealing a shift in the abundance of specific *E. coli* strains that appeared to correlate with the patient's disease state [104]. During peaks of inflammation, *E. coli* strain ST1 significantly expanded in the gut of the CD patient, suggesting a possible

role in driving disease. Comparative genomics revealed that strain ST1 genetically resembles the well-studied AIEC strains, LF82 and NC101. Using standard *in vitro* assays, the authors were able to confirm that strain ST1 belongs to the AIEC pathotype. The authors used a bioinformatics approach to reconstruct the metabolic networks of several AIEC strains and the commensal *E. coli* K-12 strain MG1655. In doing so, they were able to detect distinct metabolic traits between AIEC and commensals [104]. Together, these findings strongly highlight the power of using evolutionary models to delineate the different adaptations acquired by AIEC and commensals within the host.

Concluding Remarks

As a bacterial species, *E. coli* takes on many faces and forms, ranging from a benign commensal that is ubiquitous in the human microbiome to some of the most highly evolved pathogens capable of causing a range of clinical manifestations. AIEC appear to be neither commensal nor a true pathogenic variant of *E. coli*, but rather a pathobiont capable of rapid expansion in the host where it can exert unique pathogenic effects. New models that track within-host evolution of AIEC provide evidence that AIEC are remarkably adaptable and most likely stratify themselves into discrete populations *in vivo,* separated spatially, and possibly exerting different subversive activities on the host immune system. A fascinating area of new study will be to understand the degree to which the host "personalizes" AIEC genomic evolution and how adaptable these diversified lineages are when transmitted to genetically divergent hosts that exert different selective pressures. A deeper dive into the population genomics of AIEC from individual patients is also needed. The first study reporting such an accounting was published recently, revealing considerable flux in the dominant AIEC strains found in one Crohn's patient over a 3-year period [104]. Correlating the waxing and waning of different strains with clinical presentation during remission and flares was possible, bringing us closer to a more nuanced view of AIEC in health and disease. In this vein, AIEC might be uniquely susceptible to intervention strategies that target the genetic mechanisms underlying bacterial adaptation [105,106]. It seems that the clinical enigma that is CD has presented us with an equally compelling microbiology challenge for the future.

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AIEC, adherent-invasive *E. coli*; AMP, antimicrobial peptides; A/E Lesions, attaching and effacing lesions; CEACAM6, carcinoembryonic antigen-related cell adhesion molecule 6; CD, Crohn's disease; CX3CR1, CX3C-chemokine receptor 1; DAEC, diffusely adherent *E. coli*; DSS, dextran sulfate sodium; EAEC, enteroaggregative *E. coli*; EHEC, enterohemorrhagic; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic; ETEC, enterotoxigenic *E. coli*; HIF, hypoxia-inducible factor; IP-10, IFN-γ-inducible protein 10; IL, interleukin; LP, lamina propria; LPS, lipopolysaccharides; MIP, macrophage inflammatory protein; MIG, monokine induced by gamma interferon; NOS, nitric oxide synthase; STEC, Shiga toxin-producing *E. coli*; TLRs, Toll-like receptors; Tg, transgenic; TNF, tumor necrosis factor; T3SS, type III secretion system.

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