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Commentary

Island biogeography effects on microbial evolution may contribute to Crohn's disease

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ABSTRACT

Inflammatory bowel diseases (IBDs), such as Crohn's disease (CD), involve a poorly understood and complex immune response to both the biota of the human gut and the gut itself. The role of the gut biota in human health has been ill defined and attitudes toward the intestinal flora have ranged from judging them largely irrelevant to declaring them a human organ system. A better way to view the intestinal flora is as a group of evolutionarily self-interested species that form large, potentially interbreeding populations that utilize human beings as a series of semi-isolated habitats, like islands in an archipelago. Here we propose that the imposition of modern sanitation and hygiene standards has drastically attenuated the connection between the "islands" inhabited by the gut flora, and that existing work drawn from evolutionary biology studies of island ecosystems, rather than medicine, predicts that the evolution of gut flora should now be pushed toward limited-dispersion forms of intestinal microorganisms – a proposition borne out by the discovery of so-called "adherent invasive *Escherichia coli*." This pathogenic variant of the gut bacterium *E. coli* clings to and invades the intestinal epithelium and has been implicated in CD. Gut flora and diseases of the gut should arguably be studied as ecology as much as medicine, and treated within this context.

1. Introduction

Dispersion into novel suitable environments is a common characteristic of all organisms. Mobile organisms, such as most animals, not only migrate into new productive habitats incidentally, but also often engage in migrations specifically to discover and colonize new territories amenable to their lifestyle. Juvenile grey squirrels (*Sciurus carolinensus*), for example, seek out unoccupied woodland habitat in which to establish themselves as adults, often traveling significant distances in the process [1]. Other animals, ranging from mammals to insects, also travel regularly to distant suitable environments, sometimes crossing large stretches of unsuitable habitat to do so. The ability and inclination of a species to spread to non-contiguous but equivalent environments carries obvious and large benefits under the rules of natural selection, as large populations share greater combinatorial diversity and are statistically more resistant to extinction.

The evolutionary advantages of being able to travel to and colonize spatially separate but ecologically similar habitats is so great that even those organisms that lack significant mobility in their major or mature stage have usually evolved a life stage especially suited to dispersion. Sedentary corals and other aquatic invertebrates usually have a juvenile form capable of drifting great distances in coastal or pelagic currents to reach distant but appropriate habitats. Fungi that inhabit soils, trees, rocks and other solid substrates in which mobility is restricted, likewise often have waterborne or airborne spores capable of reaching similar niches at great distance. Plants also engage in such purposeful dispersion of their seeds. Windborne seeds are evolutionarily quite common, as are seeds capable of being dispersed by animal assistance, water movement, and other means – ranging from expulsion under plant-generated hydraulic pressure to simple maximization of entropic dispersal through structural features such as a smooth, round shape and light weight [2].

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; *E. coli*, *Escherichia coli*; AIEC, adherent, invasive *E. coli*; UC, ulcerative colitis; RA, rheumatoid arthritis; IL-12/23, IL-17, interleukin 12/23, interleukin 17; TNF-α, tumor necrosis factor-alpha; Ig, immunoglobulin; Fim, fimbrial family proteins [FM]; ELISA, enzyme linked immunosorbent assay; NMR, nuclear magnetic ratio; UPEC, uropathogenic *E. coli*; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; ZO-2, zona occludens protein 2; PKCζ, protein kinase C zeta; VacA, vacuolating cytotoxin gene A; GWAS, genome wide association studies; NOD2, nucleotide-binding oligomerization domain-containing 2; ATG16L1, autophagy-related 16-like 1; IRGM, immunity-related GTPase family M.

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2. Isolation selects for limited dispersion

Arrival of mobile organisms into isolated environments such as distant islands, however, presents an evolutionary paradox. Although mobility allowed the initial colonization into the remote location, further mobility is unlikely to result in arrival at another suitable location. Populations of organisms in such circumstances often evolve reduced mobility and limited dispersion as a successful strategy. For example, plants with windborne seeds (such as the common dandelion, *Taraxacum offininale*) sometimes reach quite small islands many miles distant from the mainland from which they originated. Arrival on these specks of isolated habitat is statistically improbable for any given seed, but occurs occasionally due to the large number of such seeds blown out to sea. Once a population is established on such an island though, windborne dispersion becomes nearly suicidal. The overwhelming majority of a plant's seeds will blow free of the island and perish in the water. Under these circumstances, mutant forms of heavy or pappus-free seeds that lodge and germinate near the parent plant are highly advantageous and indeed numerous instances have been documented of plant populations on distant islands evolving reduced mobility and increased adherence [2–4], often quite rapidly [5]. Examples of island existence selecting for reduced dispersion include not only windborne plants, but also waterborne plants such as the well known "coco de mer", a palm of the genus *Lodoicea* that is endemic to the extremely isolated Seychelles islands and that, despite having ancestors that likely reached the islands via conventional waterborne coconuts, has evolved a unique double coconut that is resistant to rolling toward the waterline and is intolerant of long immersion in salt water. The coco de mer is adapted to disperse only within a small range around the base of the parent tree [6]. Islands are replete with other examples of reduced dispersion forms, including flightless birds and insects [3]. It is clear that extreme isolation often reduces or abrogates the typical evolutionary pressure to widely disperse progeny and results in the selection of altered populations of numerous creatures.

3. The changing "Biogeography" of the gut microbe: public hygiene as habitat fragmentation

Multicellular organisms, including human beings, serve as habitat for numerous mutualistic, commensal and parasitic microorganisms, such as the large and diverse biota that typically inhabits the gut. These organisms have evolved in close association with their multicellular hosts and often spread from the gut of one individual organism to that of another, so that a population of any given species of multicellular organism effectively shares a common gut biota. Indeed, from the evolutionary perspective of the gut-dwelling microorganisms, a population of a multicellular host species constitutes a multitude of highly similar habitats separated by small stretches of inhospitable habitat – that is, the outside world. The separation of these host-habitats is usually quite incomplete, especially in gregarious or social organisms that often live in close physical proximity to one another and engage in unintentional sharing of fecal fomites. Indeed, many animals also engage in intentional coprophagy, presumably to actively transfer gut biota, which is often beneficial to the host.

Under such circumstances, if gut microorganisms are subject to the same evolutionary pressure to disperse to new habitats as are other organisms, it is in the long-term interest of the gut-dwelling organism to leave the gut of the individual in which it currently exists and spread to the guts of others. Given the mortality of all individual hosts, this is in fact essential. One means of facilitating spread is for the intestinal flora to contribute to the overall health and well being of the host, perhaps explaining why most gut biota is commensal or mutualistic in habits. Until very recently, human beings doubtlessly constituted a single archipelago of linked habitats for gut microorganisms, in which hundreds of species passed freely from one human to other associated humans by means of simple fecal fomites. Evolutionary fitness favored embrace of dispersion by the human gut biota.

A small fraction of the potential inhabitants of the human gut is harmful, however, including pathogenic bacteria such as certain strains of *Escherichia coli* and *Salmonella* spp. as well as numerous parasites such as helminthes, flatworms, roundworms and diverse viruses. The need to avoid the transfer of this minority of harmful gut biota has motivated modern humans to undertake elaborate and highly efficacious precautions against facilitating the transfer of any fecal fomites from one person to another. These include hygienic food preparation practices, hand washing, modern sewerage and wastewater treatment, the provision of sterile drinking water and robust use of detergents, disinfectants and antibiotics in the human environment. While these measures are aimed at inhibiting the spread of pathogenic gut biota, they have their isolating effects on all gut biota.

In modern, wealthy nations, dispersion is no longer an evolutionarily advantageous strategy for gut-dwelling bacteria and other intestinal flora, as the overwhelming majority of the creatures that leave the gut via defecation is rapidly sequestered and killed.

4. Adherent invasive *E. coli*: a reduced dispersion mutant implicated in Crohn's disease

Under these conditions, evolution is predicted to select for reduced dispersion forms of the species of microorganism residing within the human gut. It is interesting to note then, the relatively recent description of so-called "adherent invasive *E. coli*" in the medical literature [7,8]. As its name suggests, this strain of bacterium is capable of both adhering to the wall of the intestine and invading the intestinal epithelial cell itself, existing within vesicles in the cells in a manner roughly analogous to mycobacterium pathogens. Adherent invasive *E. coli* (AIEC) have been implicated in CD and are hypothesized by some to be involved in the primary etiology of this gut disorder that is closely linked to increased standards of public hygiene [9–11]. Numerous other autoimmune disorders have been hypothesized as well to originate with or be exacerbated by changes in the gut biota [12] and may be possibly relieved by the consumption of so-called "probiotic" organisms, although the correct identification of which organisms are appropriately probiotic is currently under active investigation.

5. John Donne refuted: modern man as an island – the gut microbes' perspective and the epidemiology of Crohn's disease

We propose here that modern hygiene practices have resulted in the rapid and recent imposition of island-like evolutionary pressures on the ecosystem of organisms that have evolved to reside within the human gut. Similar to the effects of urban habitat fragmentation on wind-dispersed plants [13], these pressures select against attempted dispersion as a successful evolutionary strategy for such intestinal organisms and favor the retention of bacteria within the gut. As a result, the long-standing evolutionary steady state that has existed between human host and (principally commensal) gut biota has been fundamentally disrupted. This disruption may be the root cause of some inflammatory gut disorders – as typified by the association of CD and novel strains of adherent invasive *E. coli*.

An increasing insularity of the human gut biota would also be predicted to lead to the emergence of other island population effects as well. One of the most well noted effects of island

ecosystems is that the number of species that an island or archipelago can support is directly proportional to the area of the island and inversely proportional to the distance from the island to other landmasses [14]. According to these rules, division of once linked ecosystems (i.e., human populations before modern hygiene) into small well-separated individual units (the current state in the developed world) is predicted to result in a reduction of species biodiversity in the gut biota. It is intriguing to note then that a reduction in species diversity has been reported in the gut biota of Crohn's patients [15] (relative to matched healthy controls) and that the gut biota of children from a rural village in Burkina Faso was found to be significantly more diverse than the gut biota of age-matched children from urban Florence, Italy [16, see Suppl Table 6].

Other correlates of CD also support a connection to functional insularity of microbiota. Crohn's is more prevalent among the urban than the rural [17]. Caring for livestock or owning a pet is protective [18,19]. It is more common among those of higher socioeconomic class than those of lower socioeconomic class [17], and suggestively, it is less common in those from larger families than in those from small families [18–20] with "only children" being at greater risk than those with siblings [18]. It is less common among those who were breast-fed than those who were fed sterile formula as infants [21], and less common in those who drank unpasteurized milk as children [18]. It is of interest to note that although the gastrointestinal tract in humans is sterile at birth, breast-fed infants develop a more diverse bifidobacteria population compared to formula-fed infants [22]. Gut microflora dominated by Bifidobacterium species have been found to be associated with many beneficial effects to the host including preventing growth of pathogenic microorganisms [23].

The striking temporal and geographic association of CD with economic development and installation of effective public hygiene has been known for some time [9–11]. The mechanism by which stricter hygiene induces disease is unclear, however. Seen in light of the evolutionary self-interest of the gut biota, the appearance of AIEC, associated with Crohn's, may be a normal response to habitat fragmentation, explainable by rules of ecology already well worked out through the study of insular ecosystems.

6. Pharm-ecology – a new field of drug research?

If accurate, this theory (as well as other hypotheses implicating an altered ecology of endogenous microbes in disease) has interesting possible implications for drug discovery efforts. Approaching CD through such an ecosystem prism would lead to more of a focus on pathogen phenotypes, associated with the underlying pathophysiology, as compared to the current pursuits of identifying candidate proinflammatory factors or pathways. It could be argued that this altered approach is indeed warranted for CD based soley on existing clinical pharmacology data that appear to distinguish CD from other autoimmune diseases with which it is typically grouped such as rheumatoid arthritis (RA) and psoriasis. For example, targeting the proinflammatory cytokines IL-12/23 or IL-17 in clinical trials for CD has had some success [24] but overall has been disappointing, compared to the very promising efficacy in RA and psoriasis [24–26]. Moreover, neutralization of TNFα, the archtypical proinflammatory cytokine impicated in autoimmune diseases [27], results in a different clinical pharmacology spectrum in CD as compared to RA and psoriasis. Etanercept, the TNF-α receptor-Ig fusion biologic is not effective in CD whereas the biologics engineered to bind circulating and cell-bound TNF-α are quite effective in these patients, having made an important impact on both induction and maintenance of remission [28]. However, all the anti-TNF-α biologics, including etanercept, are effective in RA and psoriasis [29]. Island theory would posit that enteropathic bacteria which evolve mechanisms for limited dispersion would need to evade the general anti-pathogen inflammatory armamentarium of host immune cells. A likely corollary would thus be the development of a distinct clinical pharmacology of CD as compared to prototypic autoimmune diseases.

Effecting change in a complex and evolving ecosystem will certainly require specificity and selectivity. It is not apparent that AIEC are distinct phylogenetically, however the strains isolated from IBD patients do exhibit clonal clustering with respect to allelic profiles [30]. Such patterns provide some optimism for a rational design to eradicate the reduced dispersion microbes while not affecting beneficial bacterial species. Enteropathogenic strains of *E. coli* have clearly evolved, via horizontal gene transfer techniques, the ability to adhere and colonize intestinal epithelial cells [30]. Therapeutic approaches worth considering would be to target the processes utilized by the AIEC in their ecosystem adaptation of reduced dispersion.

6.1. AIEC adhesins

Bacteria utilize their surface appendages, type 1 pili and flagella, as virulence factors which mediate adhesion to host epithelial cells [31,32]. The FimX family of chaperone, usher and adhesin proteins assemble as repeating subunits in the rodlike type 1 pili projections and bind to either glycoproteins or non-glycosylated epitopes on host cells [31,33]. The family 'adhesin' member FimH has been implicated for orchestrating adhesion diversity among *E. coli* strains [33] and is instrumental in the binding and subsequent invasion of pathogenic *E. coli* to the gut wall [34,35]. Phylogenic analysis of FimH sequences showed a clustering of pathogenic strains including AIEC and uro-pathogenic *E. coli* [36]. Thus both intestinal and extraintestinal *E. coli* strains may have evolved similar mechanisms for adhesion to host cells, as a consequence of habitat fragmentation, which pose possible targets for drug discovery. Larsson et al. [36] used statistical molecular design to construct an optimized peptidic scaffold for inhibiting the binding of FimH with its cytoplasmic chaperone protein FimC. The nonamer library contained non-native peptides which blocked (at 10–200 μM) the binding of soluble FimH to immobilized FimC using an ELISA. The data provide insights into key areas of FimH critical for pilus assembly. Svensson et al. [37] designed 'pilicides' which were either amino acid or pyridinone analogs of a peptide ligand of FimC, and found that these molecules blocked the association of FimC with FimH. NMR analysis revealed up to 3 distinct binding sites on FimC which could be exploited by pilicide scaffolds [38].

Uropathogenic *E. coli* (UPEC) bind to and invade bladder epithelium via an adhesion event between FimH and mannose residues on its epithelium receptor uroplakin-1a [39]. Inhibitors of FimH adhesion, based on the structure of α-D-mannose, effectively (low micromolar) blocked the adherence and the invasion of a UPEC to uroepithelial cells in vitro [40]. Focusing on the carbohydrate-recognizing domain of FimH, Klein et al. [41] developed low molecular weight α-D-mannosides with low, single digit nanomolar activity in a competitive binding assay (compound 16b IC_{50} = 4.8 nM). In a mouse model of urinary tract infection using the UPEC strain UT189, oral administration (50 mg/kg) of the FimH mannoside antagonists reduced bacterial colony forming units by 2 and 4 orders of magnitude, respectively, in the urine and bladder. Whether these attractive FimH antagonists also affect the binding of AIEC to gut epithelium has not been reported.

6.2. Adhesin receptors

FimH contains an N-terminal carbohydrate binding pocket which is involved in the binding of this adhesin to glycosylated

residues on receptor sites. Barnich et al. [42] identified the adhesive glycoprotein carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) as the likely adhesive receptors for AIEC. Selective amino acid substitutions in FimH appear instrumental for directing the binding of AIEC to various CEACAMs and suggest an approach for developing selective adhesion antagonists [43]. In vivo support for this binding interaction was found by Carvalho et al. [35]. Mice expressing human CEACAMs, but not wild type mice, developed a severe form of colitis following infection with the AIEC strain LF82. Administration of an anti-CEACAM antibody was effective in limiting disease development. CEACAMs have been shown to be abnormally upregulated on primary ileal enterocytes from CD patients [42], consistent with the isolation of AIEC from the ileal mucosa of Crohn's patients [44]. Interestingly, CEACAMs can be upregulated on human intestinal epithelial cells following incubation with AIEC strains [42]. Thus, seemingly consistent with island theory, the AIEC have evolved the ability of inducing mechanisms promoting its own adhesion for colonization.

CEACAMs are known to be deregulated in various cancers [45]. Targeting CEACAMs, using an antibody-drug conjugate [46] or a humanized single chain variable fragment [47] has been pursued for pancreatic cancer but not, to date, for blocking colonization of AIEC.

6.3. Probiotics

Although numerous bacteria have been touted as putative probiotic enhancers of health by everyone from scientists to yogurt spokes-models, our ignorance of what components of the human commensal biota are truly beneficial is nearly perfect. As noted above, the dysbiosis characteristic of IBD and in particular CD patents is consistent with the reduction of species diversity predicted to occur in models of island ecosystems. Thus the introduction of live microorganisms for improving health, i.e., probiotics, is a logical approach for improving the ratio of beneficial versus aggressive/invasive gut flora. Probiotic preparations have shown limited success in UC trials but clear efficacy in CD patients has not yet been established [48]. However, in vitro studies have shown that one such probiotic strain, *E. coli* Nissle 1917, prevented the loss of transepithelial resistance in T84 cells (human colonic adenocarcinoma cells) induced by the enteropathogenic *E. coli* strain E2348/69 [49]. This protective effect of *E. coli* Nissle 1917 was associated with a redistribution of tight junction proteins (e.g., ZO-2) to the cellular membrane, an event dependent on a decreased activity and cellular distribution of PKCζ. It is of interest to note that activation of PKCζ has been linked to the uptake of the Helicobacter pylori virulence factor, VacA, into human T lymphocytes [50]. PKCζ is known to have an essential role in several cellular signaling events, including glucose transport [51], but may be part of a host signaling pathway confiscated by rogue bacterial strains undergoing reduced dispersion. Even though probiotics have yet to exhibit promising results in CD patients, unmasking the mechanisms underlying their protective effects in cell-based assays should lead to discrete targets to prosecute for possible blocking colonization of these rogue strains.

Gut biota diversity is largely controlled by predatory bacteriophages which can also influence survival and colonization of probiotic bacteria [52]. However, bactericidal bacteriophages selective for a pathogenic strain (O157:H7) of *E. coli* have been isolated from bovine or ovine fecal samples [53]. Selectivity was associated with bacterial composition of lipopolysaccharide. Phenotypic screening for AIEC-specific bacteriophages from a fecal phage library could lead to a promising new probiotic treatment for CD.

6.4. Biomarkers

In parallel to discerning mechanisms underlying host colonization by limited-dispersion species, biomarkers of the ecology of the gut should be incorporated into clinical trials for the treatment of the associated disease states. The comprehensive species composition of the gut biota should be known at baseline and following treatment. This will allow not only the monitoring of changes in population ecology affected by treatment, but also the possible stratification of patients into different subpopulations of responders and non-responders. The ecology of the gut is a potentially rich source of information in drug trials, contained in an easily and non-invasively collected sample material. With probiotics, microbes could even be engineered to contain reporters that transmit specific information about the state of the gut ecology and physiology into the feces. These probiotic strains would constitute a new sort of "cell-based therapy" – one for which the manufacturing technology is practical, economical and validated, as the engineering and mass culture of defined strains of bacteria is among the mainstays of biotechnology.

Clearly the host environment will influence endogenous ecosystem evolution. Genome-wide association studies (GWAS) have identified host susceptibility genes for IBD although the pathological consequences of these mutations in CD are still being evaluated (e.g., [54,55]). However, these genetic mutations may prove to be valuable biomarkers for enriching clinical trials with patients harboring AIEC. Single nucleotide polymorphisms in the gene encoding for nucleotide-binding oligomerization domain containing 2 (NOD2) protein are associated with AIEC colonization in CD [54]. Defects in NOD2 function would hamper the ability of the host innate immune response to protect against the invasion of an aggressive pathogen such as AIEC [56]. Consistent with the emerging pattern of limited-dispersion AIEC colonization, the polymorphisms in NOD2 are more prevalent in CD patients with ileal versus colonic involvement or compared to patients with UC [54]. GWAS analyses have also linked genetic defects in the autophagy genes *ATG16L1* (Autophagy-related 16-like 1) and *IRGM* (Immunity-related GTPase family M) to CD [55]. Lapaquette et al. [57] have demonstrated that cellular deficiencies in these proteins leads to enhanced intracellular replication of AIEC but not other enteropathogenic strains of *E. coli*. Beyond serving as valuable biomarkers, these gene mutations may predispose regions of the gut wall as safe havens for initiating a process of colonization aimed at forming a limited dispersion ecosystem.

7. Conclusions

In 1624, the poet John Donne famously wrote that "No Man is an Island." From a microbial ecology perspective, this is quite possibly incorrect. While it is only natural to look at human health and disease from the point of view of the human physiological system, it must be kept in mind that a single human being is a natural habitat for trillions of microorganisms, all of which contain their own genomes and are constantly being selected according to their strict evolutionary self-interest. While they may sustain human health when it benefits them to do so, they are under no obligation to remain mutualistic. They do not exist to serve man. If human behavior changes, intentionally or incidentally, the evolutionary landscape in which these organisms dwell, they will adapt – or else perish. The rules governing this evolution of the microbiota are the same as those that have been worked out in other ecosystems, such as islands. Greater efforts to incorporate the lessons of evolutionary biology and ecology into the understanding of

inflammatory gut pathologies could yield rich rewards. Such efforts will likely tax conventional wisdom regarding pathophysiology and open new vistas for pharmacological interventions resulting in translational medicines.

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