Body Traffic: Ecology, Genetics, and Immunity in Inflammatory Bowel Disease

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Key Words

ulcerative colitis, Crohn's disease, microbiology, mucosal immunology, immunoregulation, mucosal barrier, mucus, disease biomarkers

Abstract

The abundant bacteria and other microbial residents of the human intestine play important roles in nutrient absorption, energy metabolism, and defense against microbial pathogens. The mutually beneficial relationship of host and commensal microbiota represents an ancient and major coevolution in composition and mutual regulation of the human mucosa and the resident microbial community. Inflammatory bowel disease (IBD) is a set of chronic, relapsing inflammatory intestinal diseases in which rules of normal host-microbial interaction have been violated. This review considers the components of this host-microbial mutualism and the ways in which it is undermined by pathogenic microbial traits and by host immune and epithelial functions that confer to them susceptibility in patients with IBD. Recent advances in understanding the genetics of IBD and the immunology of host-microbial interaction are opening new strategies for treatments that target host susceptibility, candidate microbial pathogens, and intestinal ecology.

INTRODUCTION

IBD: inflammatory bowel disease

Inflammatory bowel disease (IBD) is a set of chronic, relapsing inflammatory diseases of the intestine. IBD consists predominantly of ulcerative colitis (UC) and Crohn's disease (CD), which are clinically distinguished by intestinal localization, local features of inflammation, a profile of complications, and familial aggregation. In the United States and Europe, the prevalence of IBD is estimated to occur in 1.4 and 2.2 million individuals, respectively. The incidence of IBD, particularly CD, has increased approximately tenfold during the past half century in Europe and the United States (1, 2). Such findings, and its emergence as a more common disease in industrialized Asia (3), reflect environmental factors for IBD susceptibility that are associated with an urbanized Western lifestyle.

Resident enteric microorganisms are a unique and important class of environmental factors that are probable targets and indeed may represent etiologic agents in IBD-associated inflammation (4). The host interaction with enteric microorganisms is governed by mucosal epithelial and immune cell types, and aberrant features of this interaction are manifest in the chronic intestinal inflammation that is the hallmark of IBD. Much of what we presently understand about IBD pathogenesis derives from animal models of IBD and their use in defining the induction and regulation of mucosal inflammation. This important area has benefited from recent analytic reviews (5, 6). Mechanistic studies of human IBD have benefited increasingly from a biologic assessment of patients undergoing novel therapies targeting immune function or microbial ecology (4, 7).

Genetic susceptibility is also an important factor in IBD, with a greater contribution to CD than to UC (50% and 10%, respectively). Genetic loci are now known for disease risk and clinical heterogeneity and are emerging for environmental susceptibilities and response to treatment (8). The IBD-associated genes now identified appear to encompass allelic differences affecting immune or epithelial functions. Thus, IBD disease biology is set in a large canvas of the interacting physiology of microbiology, immunology, and epithelial cells, modified toward or away from disease activity by genetics and environmental factors (**Figure 1**). In this review, we seek to convey the major elements of this interacting physiology and how to fulfill the desire to simplify and personalize modes of IBD disease biology toward therapy or prevention.

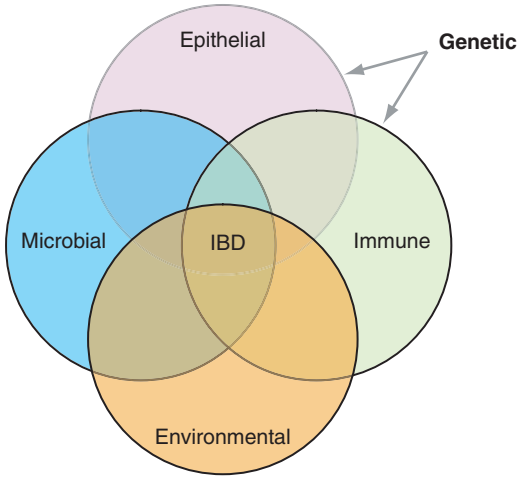

Figure 1

Mucosal inflammation is regulated by the interplay of resident microbiota, intestinal epithelium, and the mucosal immune system, which comprise the native intestinal community. Genetic allelism and environmental factors modify this interplay, driving the mucosal inflammatory state toward or away from clinically significant intestinal inflammation. IBD, inflammatory bowel disease.

RESIDENT INTESTINAL MICROBIOTA

In ancient evolutionary time, we lived in a mixed multitude of diverse species, attached to submarine rocks. In conditions perhaps akin to modern microbial biofilms, the members of these communities coevolved to carry out complementary functions in capture and metabolism of nutrients and in defense of the community from aliens. However, the opportunity for travel beckoned even then, particularly as multicellular organisms formed to

encounter new and potentially more robust locales for their activity. The challenge was how to maintain the diverse microbiologic community that had developed a successful strategy in cooperative metabolism and defense. From this challenge, the intestine was born. The intestine provides a robust but highly controlled environment in which our ancient microbial partners, and ourselves, have continued to evolve over the past five-hundred million years in order to continually refine this ancient biologic partnership.

What is the composition of our intestinal microbial community? For more than a generation, microbiologists have studied the composition of enteric microbiota, in particular its bacterial members. Studies were technologically focused on culturable bacteria in feces, hence reflecting luminal bacteria of the distal large intestine (9). Such work demonstrated more than 100 bacterial species and abundant numbers ($>10^{10}$) of organisms. In general, this microbial composition was stable within each individual over months and years. This microbial mosaic was set in part by dietary habits, but notably by maternal experience of the neonatal or infant period. Although yet to be formally proved, this appears to reflect a strong "founder effect," in which the initial microbial residents (preferentially from the mother's microbial community and shaped by maternal immunoglobulin A, or IgA) fill and retain the intestinal niches of the mother's progeny. Thus, functional diversity related to specific microbial composition may be disproportionately transmitted vertically from mother to child (10, 11).

The insights from these pioneering studies acquired new dimensions with the advent of direct molecular strategies to characterize microbial communities. In most cases, this was accomplished by characterizing and enumerating cloned DNA from intestinal specimens using polymorphic regions of 16S rDNA (**Figure 2**) (12–14). This strategy permits rapid and flexible phylotyping and enumeration of microbial species. Importantly, it does not depend on microbial culture because efficient microbial culture has been established for only certain microbial taxa. Such work revealed that humans typically bear approximately 500 species of the microbial kingdom Eubacteria (pertaining to most bacteria) and a similar number of species derived from Archaebacteria and Fungi (15, 16). Although many phyla and lower taxa of these kingdoms can be detected in the human intestine, the relative representation of microbial species spans a range of many orders of magnitude. In the colon, members of *Bacteroidetes* and *Firmicutes* predominate, although even their relative abundance appears to be a stable trait that distinguishes individuals (**Figure 3**) (17, 18).

The resident microbial community is not organized like a classical biofilm, but its composition is stabilized by intermicrobial competition, metabolic interdependence, and regulation (19). Thus, the intestinal microbiota provides an important element of host defense to alien microbial intruders. Note that the intestine is also the residence for other classes of microorganisms with biologic significance (e.g., viruses, protists, and helminthes). The composition and abundance of such taxa have not yet been subjected to systematic assessment using culture-independent methods.

The abundance of this microbial load is staggering. Intestinal bacteria number 10^{14}–10^{15} and comprise 50% of fecal solids (20). Resident microbiota form a very large community within the large intestine, with distinct composition and abundance in different segments of the cecum and colon. The microbial community is almost entirely restricted to the luminal content, separated from the mucosa by the secreted mucus layer. However, a compositionally distinctive microbial community can be detected in the mucus layer or even in apparent association with epithelium, particularly in the proximal colon (21, 22). Although comprising a very small proportion of intestinal microbiota, this compartment has gained attention for its potentially disproportionate roles in epithelial and immune function. In

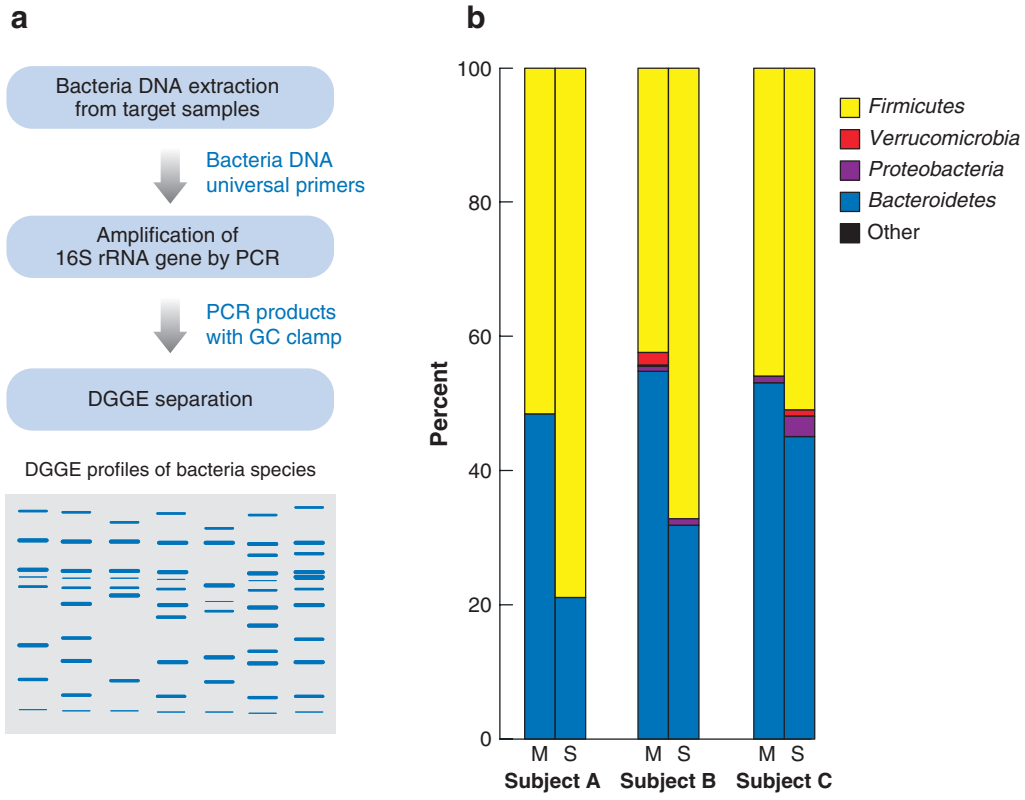

Figure 2

Molecular identification of enteric bacteria. (*a*) Microbial DNA extracted from intestinal samples is amplified by polymerase chain reaction (PCR) using universal primers annealing to conserved regions in the microbial 16S gene bearing terminal guanosine-cytosine (GC) sequences (GC clamp). Mixtures of amplified gene fragments from different samples are then separated from each other by denaturing gradient gel electrophoresis (DGGE). Internal variable regions of microbial 16S sequences from different bacterial species diverge in stability to denaturation and are often distinguishable by DGGE. Microbial communities are categorized by DGGE fingerprint. Adapted from Reference 13. (*b*) High-throughput sequence analysis. PCR-amplified microbial 16S rDNA is cloned and sequenced directly by high-throughput methods and categorized and enumerated for molecular phylotypes according to phylum. Differences between subjects, and between mucosal (M) and stool (S) samples, are tabulated. Adapted from Reference 13a. (*c*) Oligonucleotide fingerprinting of rRNA genes (OFRG). PCR-amplified microbial 16S DNA is cloned and arrayed. Hybridizations with single DNA probes create fingerprint clusters and are statistically categorized using known phylotype glossary. UPGMA, unweighted pair group method with arithmetic mean. Adapted from Reference 13b. (*d*) Summary data for mouse intestinal microbiota analyzed by ORFG. Molecular phylotypes were identified by OFRG in genetically identical mice derived from different breeding colonies (RF, restricted flora; SPF, specific pathogen-free). Significant differences for incidences of enteric bacteria and fungus taxa were identified in the two breeding colonies. Adapted from Reference 15.

the intestine itself, human cells on a per-cell basis represent only a minute proportion of the resident enteric cells. Thus, from the perspective of cell numbers, humans are less than 10% human.

What functional roles can be ascribed to this resident microbial community? In ruminant animals highly dependent on nutrient fermentation, the resident microbiota may contribute more than 50% of energy

recovery capacity for dietary intake. Although the scope of metabolic contribution is less prominent in humans, it has been known for decades that intestinal microbiota contribute substantially to complex carbohydrate and divergent amino acid nutrients (23, 24). Two examples illuminate the resultant host-microbial interdependence. First, microbial metabolic production of buryrate is a significant source of this fermentative metabolite, which represents the preferred energy source of the intestinal epithelium. Second, the osmotic stress and reduced nutrient absorption sometimes observed in patients undergoing systemic antibiotic treatment can reflect the burden of nonmetabolized nutrients when the pertinent microbial taxa are depleted.

However, integration and regulation of resident microbial metabolism in human physiology remains an important area for research. Recent work by Gordon and colleagues has approached this question in a novel way by comprehensive assessment of the genomic metabolic repertoire of the resident microbiota (25). Compared with the human genome, the resident microbiota is significantly enriched for metabolism of amino acids, glycans, xenobiotics, methaneogenesis, and biosynthesis of vitamins and isoprenoids. Conversely, the human genome is enriched for transport and metabolism of inorganic ions and secondary metabolites (nonribosomal peptides, antibiotics, and organic pigments). Note also that the expressed repertoire is distinct from the kingdom-wide genomic representation of this metabolic repertoire. This indicates that the resident microbiota are specially evolved or regulated to express these metabolic functions in the human intestine.

This line of investigation reveals that the metabolic repertoire of host and microbiota are divergent and complementary. Moreover, significant differences were observed in the expressed repertoire between the microbial communities of different human individuals. Indeed, experimental studies in mice indicate that individual variation in enteric microbial composition affects net energy balance and may contribute to important disease phenotypes such as susceptibility to obesity (17).

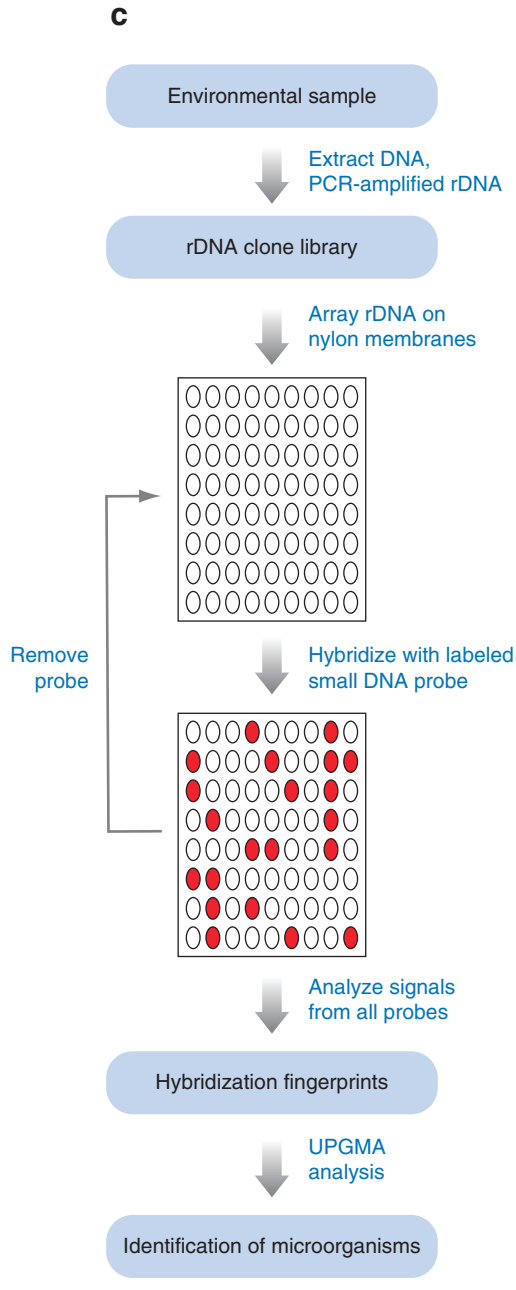

Figure 2

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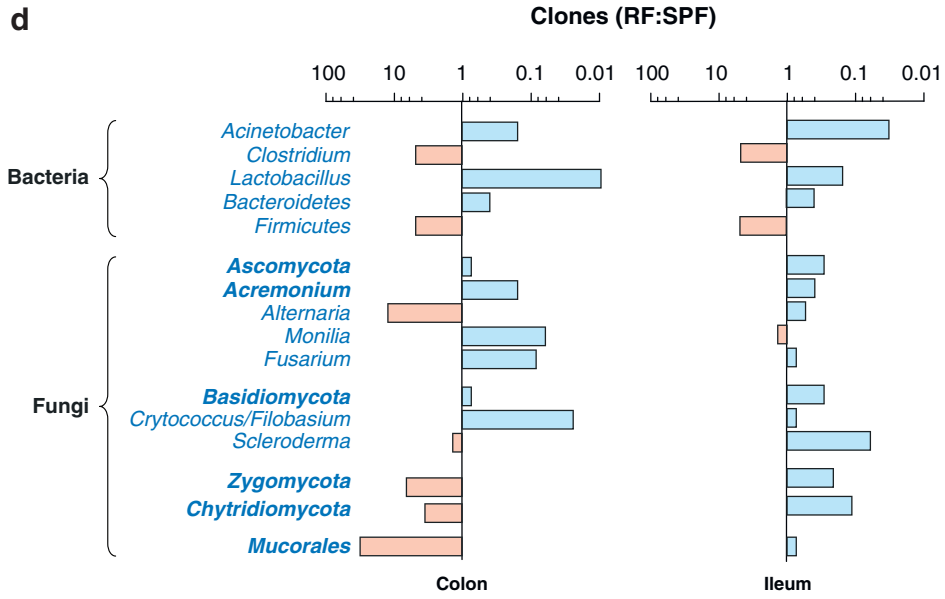

Figure 2

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HOST CONTROL OF THE RESIDENT MICROBIOTA

With such a large microbial population residing in the intestine, it is important to understand what controls its composition and activity and what restricts microorganisms and their products from entry into the host mucosa (**Table 1**). As noted above, this need is met partly by the substantial self-regulation of composition and activity within the microbial community itself. Two categories of molecules play a major role from the host side. First, epithelial cells constantly produce and replenish a barrier mucus layer overlaying the apical epithelium. The intestinal mucus is composed predominantly of a mixture of isoforms of the mucin protein family. These are small serine- and threonine-rich proteins, highly glycosylated at these sites with complex O-linked oligosaccharides comprising 80% of the mucin mass (26). This unusual composition permits interaction with bacterial polyssacharides and motile elements such as flagella. Compounded with a thickness of hundreds of microns, it provides both a physical barrier and an unfavorable molecular substrate for microbial adherence and penetration. It also provides a matrix for accumulation of autocrine epithelial growth and survival factors, such as intestinal trefoil factor (27). Finally, the epithelial cell layer itself represents an important barrier, comprised of a specialized cell surface resistant by several mechanisms to microbial adhesion and invasion and tight junctional complexes constraining intercellular penetration (**Figure 4**).

The abundance and isoform composition of epithelial mucin production is regulated by a variety of sensing systems for microbial encounter and inflammatory products (26). There is also evidence for allelic variation in mucin expression, which may qualitatively or quantitatively affect this mode of barrier defense among individuals (28). Among mucin isoforms, MUC2 (the predominant mucin isoform of the intestine) is particularly modulated by local inflammatory activity, suggesting that it may be an important constituent of mucin function in the intestine. In support of this idea, knockout mice deficient in MUC2

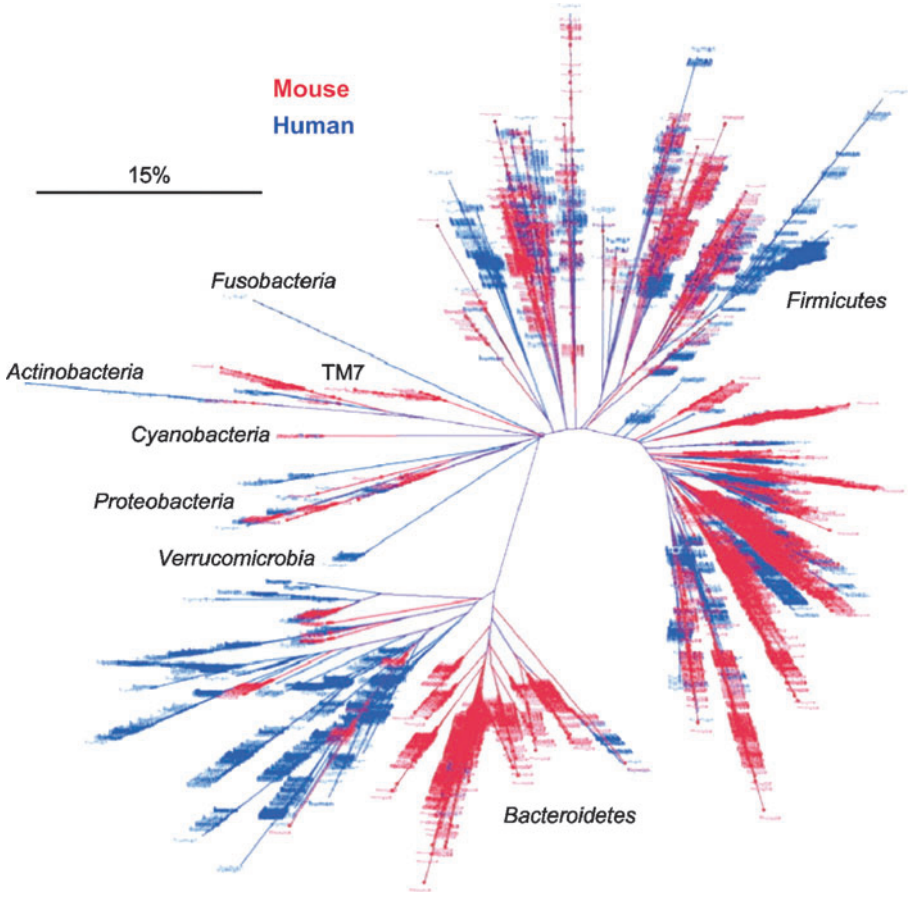

Figure 3

Bacterial diversity in the cecum of a human (*blue*) and C57BL/6 mouse (*red*). Bar indicates 15% sequence divergence. TM7 is a member of the division of *Eubacteria* designated for the German peat bog from which the first sequence was obtained (Torf, mittlere Schicht, or peat, middle layer). Adapted from Reference 17.

are highly susceptible to spontaneous and induced colitis (27). Functionally significant allelisms of MUC3 have also been associated with IBD susceptibility in population-based human studies (29). Validation that the gene-specific allelism is causally related to IBD in such individuals, and that the specific changes in microbial interaction are related to such disease susceptibility, is an area for further investigation.

A second important factor in host control of intestinal microbiota is secretory immunoglobulins. These immunoglobulins are the well-known product of B lymphocytes matured in the mucosal immune system and often represent selection for microbial antigenic products of the intestinal luminal environment. Owing to the characteristics of the

Table 1 Microbial and host traits conferring inflammatory quiescence to resident microbiota

Commensal bacteria
• Deficient escape mechanisms for mucus trapping
• Deficient traits for epithelial adherence and invasion
• Low endotoxicity (pentacylated lipid A in Gram-negative bacteria)
Mucosal epithelium
• Defective sensing of molecular pathogen-associated molecular patterns
• Rapid sensing for invasive microorganisms
• Strong antimicrobial crypt functions
• Attenuation of NF-κB signaling by products of resident microbiota
Lamina propria
• Contains tolerogenic dendritic cells, macrophages, and regulatory T cells producing anti-inflammatory cytokines (e.g., IL-10 and TGF-β) in response to commensal bacteria

Table adapted from Reference 84.

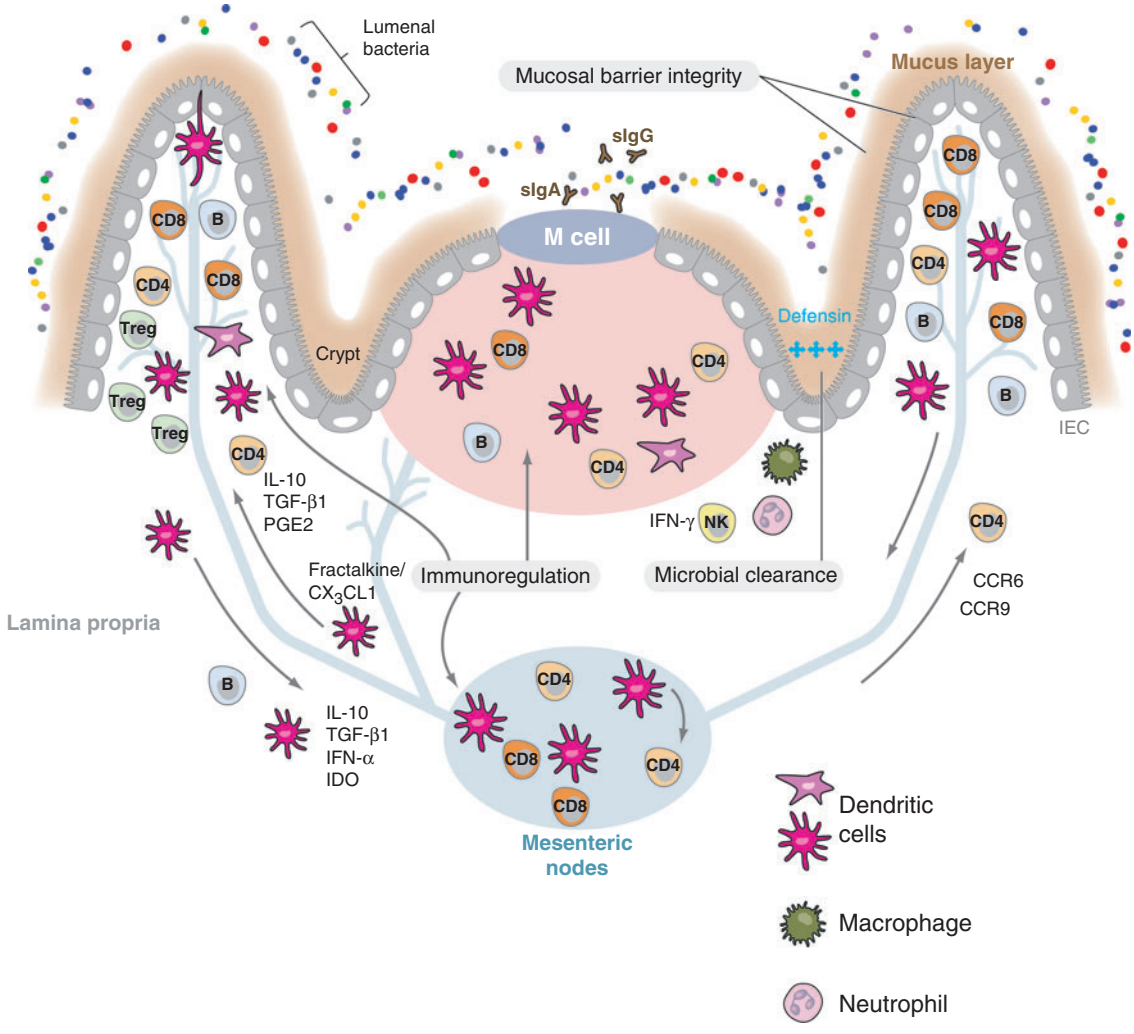

Figure 4

Control of intestinal microbiota and the host response at mucosal interface. Physical barriers to luminal microbiota and their products include interepithelial tight junctions and the epithelium-derived mucus layer. Mucus also provides a matrix for the accumulation of epithelial growth and survival factors for resistance to microbial adhesion and invasion. Secretory immunoglobulin A (IgA) and IgG comprise an important biologic barrier, through neutralization and sequestration of luminal microbiota, food, and microbial products. Innate and adaptive microbial-sensing systems in epithelial cells and immune cell types adjust the levels of local microbial clearance activity. Limits to mucosal inflammation are set by levels and activity of local immunoregulatory cell types (e.g., regulatory $CD4^+$ and $CD8^+$ T cells, plasmacytoid dendritic cells). TGF, transforming growth factor; IL-10, interleukin-10; PGE2, prostaglandin E2; IFN, interferon; IDO, indoleamine oxidase; Treg, regulatory $CD4^+$ T cells; B, B cell; M cell, mucosal follicule-associated epithelial cell; sIgA, secretory immunoglobulin A; CCR, CC-type chemokine receptor; IEC, intestinal epithelial cell.

various immunoglobulin isotypes and the relative absence of luminal inflammatory cells, their host defense function predominantly involves direct effects due to binding of microbial organisms and their molecular products (30). Genetic manipulation of secretory immunoglobulin levels or quality profoundly changes the abundance and composition of the normal resident microbiota (31, 32). This strongly suggests that the host shapes the composition of the normal microbial community. IgA deficiency, a relatively common trait in humans, is not clearly associated with IBD susceptibility (33). Although greatly enriched for the IgA isotype, the IgG isotype is also a major component of secretory immunoglobulins, and each isotype benefits from specialized transport systems for secretion and reuptake for sampling and response by the mucosal immune system (34). The contribution of IgG to the functional secretory immunoglobulin pool may account for the lack of disease susceptibility in individuals lacking IgA.

Microbial sensing by epithelial and immune cell types is a prerequisite for the initiation of inflammation. Because such inflammation toward resident microbiota is minimal in the normal host, it would be likely that such microbial sensing is physiologically attenuated. During the past decade, the toll-like receptor (TLR) family has emerged as one major class of receptors for microbial sensing and programming of inflammation (35, 36). Elegantly, several mechanisms attenuate TLR function at the mucosal surface. Intestinal epithelial cells express a variety of TLR family members, so global down-expression of this recognition system does not account for attenuation. However, colonic epithelium is selectively reduced for expression of TLR, and accessory proteins for TLR2, TLR4, membrane domain 2, and cluster designation 14 (CD14) (37, 38). Their typical ligands, peptidoglycan and lipopolysaccharide, are molecules expressed by common resident enteric bacteria. Moreover, these bacteria on a community level frequently express pentacylated lipid A, which greatly reduces TLR4 agonist activity (39). TLR5, specific for the abundant family of bacterial flagellins, is restricted to basolateral expression, thereby impairing recognition of luminal flagellin not associated with microbial invasion. TLR9 is expressed, but may be signaling deficient in human intestinal epithelium (40).

TLR: toll-like receptor

IκBα: inhibitor of nuclear factor, alpha subunit

Some resident microbiota express traits for inactivation of effector pathways elicited by TLRs. Nuclear factor kappa B (NF-κB) signaling by TLRs and certain inflammatory cytokines [e.g., interleukin (IL)-1 and tumor necrosis factor (TNF) family members] require degradation of IκBα (inhibitor of nuclear factor, alpha subunit) by E3 ubiqutin ligase and proteosome degradation. Nonpathogenic *Salmonella* species, perhaps representative of certain resident bacteria, inhibit the function of the E3 ligase targeting IκBα. Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor with potent anti-inflammatory action through a number of transcriptional targets (41). The classic colonic commensal, *Bacteroides thetaiotamicron*, negatively regulates epithelial NF-κB signaling by inducing and activating PPARγ, a trait also observed for other nonpathogenic Gram-negative bacteria. One surprising mechanism is its downstream action on NF-κB signaling, by forming a reticuloendotheliosis virus homologue B (RelB)/PPARγ complex that redirects RelB to a biologically inactive cytoplasmic compartment (42). Reduced PPARγ epithelial expression is a feature of UC and CD, and may be linked genetically in the latter case (43). Accordingly, PPARγ agonists and gene therapy have proven effective in acute and immune murine colitis (44), although reports of efficacy in human clinical trials have not emerged.

Paradoxically, an important anti-inflammatory role of epithelium is early and definitive antimicrobial function. By preventing elaboration of invasive microbiota and their products, immune activation and mucosal inflammation can be averted. One mechanism for this early response is intestinal

NOD: nucleotide-binding oligomerization domain

CARD: Caspase recruitment domain

DC: dendritic cell

epithelial expression of nucleotide-binding oligomerization domain (NOD) receptors, including NOD1/Caspase recruitment domain (CARD)4, NOD2/CARD15, and NALP (NACHT, LRR, and PYD domain protein). These receptors bind to agonist microbial products (e.g., muramyl dipeptide) present in the cytoplasm, hence representing intracellular microbial encounters. The intestinal crypts and glands are specialized for robust microbial sensing and antimicrobial effector function. In particular, Paneth cells of the small intestine express high levels of various alpha- and beta-defensins—in addition to other microbial-cytotoxic peptides—whose production and secretion are induced through ligation involving a full spectrum of functional TLRs, NODs, and inflammatory cytokine receptors (45, 46).

Although neutrophils are uncommon residents of the intestinal lamina propria, they are efficiently recruited to the mucosa in the context of even subclinical inflammation. Activation of epithelium by microbial encounter produces a wide variety of cytokines and chemokines that serve as neutrophil chemoattractants to the basolateral epithelial border, and lipoxin A4, which drives apical neutrophil migration (47). Such neutrophils are recruited out of the mucosal vasculature through inflammation-related changes in microvascular flow, endothelial adhesion, and transmigration (48). Several lines of evidence link neutrophil activity to innate resistance to microbial mucosal influx, and to curtailing microbially induced inflammation at an incipient subclinical stage (49). Mucosal natural killer (NK) cells physiologically comprise ~10% of intestinal lamina propria leucocytes (50, 51). As in other tissues, resident mucosal NK cells respond to microbial encounter or epithelial injury secondary to IL-15 production by activated epithelium, endothelium, and macrophages, and by expression of activating major histocompatibility complex (MHC) class Ib molecules (52). Such activated NK cells efficiently amplify local antimicrobial effector function (53), thereby further curtailing incipient microbial invasion and resultant inflammation.

Table 2 Distinguishing features of mucosal dendritic cells

Homing	**α4β7 integrin**
	CCL20/CCR6 and CCL9/CCR1 (Peyer's patch) CCR9 (ileum)
Functions	**Immature phenotype**
	Trans-junctional luminal sampling (CD103/E-cadherin interaction) Transport of luminal products through mesenteric node migration IL-23-dependent neutrophil and NK activation (especially ileum) IL-10, TGF-β, IFN-α, Serrate, indoleamine 2,3 dioxygenase Induction of lymphocyte mucosal homing (retinoic acid)

MUCOSAL DENDRITIC CELLS

Dendritic cells (DCs) are common residents of the intestinal mucosa and its attendant lymphoid compartments and are complex with respect to differentiation, state of maturity, and functional capabilities (**Table 2**) (54). At inductive mucosal lymphoid sites such as the Peyer's patch, the unique luminal sampling characteristics of the overlying M-type epithelium facilitate their ongoing encounter with antigenic and microbial products (55, 56), localized to epithelial subjacency via CC-type chemokine receptor 6 (CCR6) and CC-type chemokine ligand 9 (CCL9) chemoattraction (57, 58). In nonspecialized lamina propria sites, DC are drawn to epithelial subjacency through fractalkine/CX3CR1 interaction (56, 59, 60). Moreover, their specific expression of CD103 (α_e integrin) permits the insinuation of dendritic processes across the tight junctional barrier and direct DC sampling of the lumen (59, 61). This intimate interaction with intestinal epithelium also plays a role in cell-cell induction of mucosa-specific DC differentiation. In the human, constitutive production of thymic stromal lymphopoietin and other mediators by intestinal epithelial cells directs mucosal DCs to a noninflammatory state. That is, upon

microbial challenge, such DCs produce IL-10 and IL-6 but not IL-12 and attenuate polarization of $CD4^+$ T cells to the T helper type 1 (TH1) phenotype (62). Transforming growth factor (TGF)-β1, retinoic acid, prostaglandin E2, and endocannabinoids are also prominent products of the mucosal microenvironment and may be both products of and conditioning stimuli of mucosal DCs that attenuate their pro-inflammatory program (5, 20, 63–66).

As key antigen-presenting cells, DCs regulate T cell activation and differentiation, and this may be true both in the lamina propria and after migration to mesenteric lymph nodes (54). Following microbial and host programming in the lamina propria, DCs migrate to the draining mesenteric nodes bearing microbial products and other luminal sampling (32, 57, 63, 67, 68). In the mesenteric node under physiologic conditions, DCs are notable for preferential expression of traits that attenuate inflammatory T cell fates, including IL-10, TGF-β, IFN-α, the Notch ligand Serrate, and indoleamine 2,3 dioxygenase (69–74).

Recently, elegant evidence has further related migrant mucosal DCs to mesenteric immunoregulation. Mice deficient in CD103 are deficient in colonic negative regulation by $CD4^+CD25^+$ regulatory T cells. Through transfer experiments, this requirement was attributed to recipient non–T cells. This was attributed to CD103-dependent differentiation of $CD11c^+$ MHC class II^{hi} DCs and their unique capacity (through retinoic acid production) to promote expression of the gut-homing receptor CCR9 on T cells (65, 75). There is also evidence for functional contribution of DCs to adaptive immunity in the local lamina propria. In the colon, $Foxp3^+$ $CD4^+CD25^+$ T cells form clusters with lamina propria DCs in the setting of protective immunoregulation, and local antigen presentation appears to be required for their optimal activity (75).

From a physiologic standpoint, one should consider the mechanisms by which DCs contribute to the control of microorganisms that may cross the mucosal barrier. Mucosal DCs may condition local lamina propria neutrophils and NK cells through production of IL-23 (76), which, in the context of other bioactive molecules, contributes to their recruitment and activation (77, 78). In ileal DCs, the common IL-12 and IL-23 subunit, p40, is constitutively expressed, whereas there is selective production of IL-23p19, which is further augmented in response to bacterial flora. In contrast, IL-12p35 expression is extinguished in these cells under physiologic conditions and is instead the product of the uncommon $CD11c^+CD8\alpha^-CD11b^-$ DC subset. This suggests that the impact of mucosal DCs on local inflammatory capabilities favors a self-limited innate response. A distinct but functionally analogous biology is reported in the colon, where lamina propria DCs physiologically produce comparable levels of IL-12 and IL-23, but also, prominently, IL-10 (79). The mechanism of such differential segmental programming in the intestine is not yet resolved, but could involve differences in input from the local epithelial cells, resident regulatory lymphocyte populations, or local microbial community and their products (67, 80).

MUCOSAL LYMPHOCYTES

Many excellent reviews have addressed the features of B and T lymphocyte populations in inductive mucosal sites such as the Peyer's patch and their trafficking and fate as they migrate to mesenteric node and intestinal sites (81–83). In important respects, the functional distinctions of this compartment may be secondary to the innate immune sensing of enteric microbial products, its impact on epithelial/dendritic cell interaction and resultant programming of DC functional traits, and the polarization of T cell differentiation by these mucosal DCs locally and after migration to regional lymph nodes. Here, we highlight functional distinctions of mucosal lymphocytes that may be pertinent to disease susceptibility or resistance in IBD.

A major and unusual mucosal lymphocyte population is intimately interspersed within the epithelial layer (intraepithelial lymphocytes). These cells are mainly CD8αβ T cells and express CD103, permitting formation of cell-cell interactions at the epithelial tight junction. Intraepithelial T cells are limited in proliferative capacity and antigen responsiveness. Instead, their effector function is geared for recognition and response to epithelial stress, either through cytolytic activity or production of survival factors such as fibroblast growth factor (FGF)-8. Reciprocally, through expression of IL-15R and the homodimeric CD8αα, these cells are exquisitely responsive to cognate epithelial-derived ligands [IL-15 and the murine epithelial MHC class 1b molecule TL (84)], which promote intraepithelial cell activation and survival (85, 86). Intraepithelial $CD8^+$ T cells share with NK cells the capacity to express various NK cell receptors, including activating isoforms (e.g., NKG2C-E) (52). The cognate ligands for these receptors include a variety of MHC class Ib family members up-expressed in the epithelial cell stress response. In celiac disease, Jabri and colleagues demonstrated that this system may represent the surprising effector of the antigliadin $CD4^+$ T cell response. Thus, $CD4^+$ T cell cytokine-induced epithelial up-expression of NKG2D ligands such as MIC and NKG2D coactivation of TCR signaling lead to epithelial targeting by intraepithelial lymphocytes (52).

Other MHC class 1b–restricted T cells are also notable in the intestinal lamina propria and mesenteric node: Qa-1 or H2-M3 bearing human or bacterial heat-shock peptides (TCR or NKG2C receptors) (87, 88), CD1d bearing endogenous or microbial glycolipids (invariant TCR) (89–91), and MR1 (invariant TCR) (92, 93). MR1 is intriguing because its cognate T cell population is highly restricted to the intestinal lamina propria and is deficient in germ-free or B cell–depleted mice. The common themes of invariant TCR recognition and microbial antigen indicate that such populations provide an innate-like immune function that may regulate immune recognition or effector profile of the mucosal response. Indeed, CD1-restricted invariant NK T cells clearly modify the colitis susceptibility in animal disease models. Somewhat paradoxically, invariant NKT cells in mouse models attenuate mucosal inflammation induced by TH1 responses, but augment inflammation induced by TH2 responses (94–97). These findings suggest that the regulatory roles of invariant NK T cells involve alternate mechanisms and cellular targets that will require further delineation.

An important and controversial issue is whether the limited adaptive immunity to luminal antigens reflects immune ignorance or tolerance. Conceptually, attenuation of such immunity is important to reduce the potentially destructive consequences of immune responses to food antigens and normal resident microbiota. In the normal lamina propria, $CD4^+$ T cells are distinguished from nodal T cells by attenuated TCR-inducible proliferative capacity, but could be overcome by other cell-cell interactions, such as CD2 ligation (98–100). More generally, mucosal antigen encounter can result in systemic immunologic tolerance, and much has been done to identify the different cell types and locales for tolerance induction (54, 101). With respect to the mucosa and constitutive luminal antigens, Duchmann and colleagues find evidence for specific tolerance (102). However, a more abundant body of evidence suggests that such nonresponsiveness is due to immunologic ignorance, as mucosal immunity can readily be elicited when luminal antigens with appropriate innate activation are elicited across the mucosal barrier (6).

Natively, such energy could be programmed by mucosa-conditioned DCs and various regulatory T cell populations. Indeed, the various types of $CD4^+$ and $CD8^+$ regulatory T cells are readily demonstrable by phenotype and function in the Peyer's patch, lamina propria, and mesenteric node

compartments (6, 51, 56, 103). For Foxp3$^+$ CD4$^+$ T cells, one prominent mucosal factor directing their regulatory differentiation is TGF-β. However, recent studies indicate that in the presence of TGF-β, IL-6 may redirect their differentiation to pro-inflammatory TH17 cells (104, 105). This finding raises a link with mucosal microbial interaction: TLR signaling is an effective mode of IL-6 induction and is known to suppress regulatory CD4$^+$ T cell activation (106). Moreover, TLR-induced IL-6 renders DCs resistant to the action of regulatory CD4$^+$ T cell (107).

Mucosal B cells contribute in two contexts to mucosal homeostasis. As previously summarized, secretory immunoglobulin is an important component of the mucosal barrier, providing a relatively noninflammatory mechanism to impede translocation of luminal microbiota and bioactive antigens. Second, B cells contribute to immunoregulation in the mucosa and other sites, most commonly through a process requiring B cell sufficiency for IL-10 and CD1d (51, 108). Although the direct cellular target of this regulation is not well known, recent work has implicated CD8 and NK T cells as candidates. Note that this regulation involves attenuation and augmentation of TH1- and TH2-induced inflammation, respectively. This is reminiscent of the action of invariant NK T cells and suggests that these cell types may be partners in this mode of immunoregulation.

It is not surprising that, with these distinctive antigenic selection and regulatory properties, mucosal lymphocytes are programmed to home and cycle between mucosal sites. Homing of all lymphocytes to the intestine and mucosa-associated lymphoid compartment is facilitated by α4β7 and expression of mucosal addressin cell adhesion molecule-1 and other vascular counterligands (109). For T lymphocytes, mucosal localization in the human correlates with expression of CXC-type chemokine receptor 3 (CXCR3), CCR5, and CCR2 (110), and this is supported by impaired intestinal CD4$^+$ T cell recruitment in mice bearing CCR2 or CCR5 null mutations (111). Regional selectivity for the small intestine is conferred by CCR9 (112, 113). Mucosal IgA-committed B cells are additionally distinguished by expression of CCR10 and CXCR4 (114, 115), whether destined for the small or large intestine. Notably, expression of these regional markers is observed in blood lymphocytes with prior mucosal experience and possibly in between periods of mucosal residence. Thus, phenotype and functional assessment of these peripheral cells may be useful for determining the biologic state of these mucosal microenvironments.

INFLAMMATORY BOWEL DISEASE HOST EFFECTOR MECHANISMS

A cardinal feature of active human IBD is the presence of chronic mucosal inflammation. What cell types drive this inflammatory response, and by what underlying stimuli? In CD, CD4$^+$ T cells from involved lamina propria and mesenteric nodes are notable for T-bet (T-box expressed in T cells) expression and strongly polarized production of IFN-γ. In these tissues, pathways promoting TH1 differentiation and activation are increased, including macrophage and DC production of IL-12, IL-18, IL-21, and TL1A (116–120). Such findings are observed in mouse models with features of CD, and a therapeutic response could be achieved with several agents targeting CD4$^+$ T cells or the IFN-γ axis (5). On the basis of such observations, CD has been considered a TH1 disease. On a patient population basis, no recurrent antigen (microbial or other) has been associated with these TH1 cells, although a novel flagellin protein has emerged as a recent candidate (121).

In 2004, a phase I/IIa clinical trial of anti-IFN-γ in CD patients reported a significantly higher response rate (disease activity) for antibody versus placebo at seven weeks, although this response was not durable relative to placebo at later times (122). A

new trial using a fully humanized anti-IFN-γ (fontolizumab) and a fuller dosing design is now underway (123). Taken together, these in vitro and in vivo findings support the idea that an IFN-γ response, presumably derived from $CD4^+$ T cells, promotes CD inflammation. However, both the observational and therapeutic studies reveal patient heterogeneity with respect to cellular activity and response to treatment. This may reflect either heterogeneity in the underlying disease biology among patients or heterogeneity with regard to IFN-γ at different points in a patient's disease course. IL-23 (a powerful inducer of IL-17 expression) may be selectively involved in colitis induced even in the absence of adaptive immunity, a process augmented by IFN-γ (presumably of NK cell origin) (124). Indeed, much interest has emerged on the inflammatory action of IL-17, derived from the TH17 subset of $CD4^+$ T cells, as an alternate effector mechanism in immune colitis (78, 125).

In UC, T cells from involved lamina propria produced significantly greater amounts of IL-13 (and IL-5) and little IFN-γ, compared with control cells and with the reciprocal cytokine production by equivalent specimens from CD (126). Using an in vitro stimulation assay, IL-13-producing cells were positive for the NK marker (CD161) and dependent on target cells bearing CD1d (but not restricted to invariant NK T cell antigens). Epithelial damage by these cells was associated with their IL-13-induced cytotoxicity to human tumor-29 epithelial cells and with direct receptor-mediated IL-13 signaling that impairs epithelial cell barrier integrity (127). In the murine oxazolone colitis model, IL-13 was elevated, and this was due to production of NK T cells by direct cellular measurement, and by the reversal of both IL-13 and disease activity through NK T cell depletion. Administration of neutralizing IL-13Rα2-Fc prevented colitis, demonstrating that IL-13 was the proximate mediator of inflammation. Taken together, these findings suggest that NK T cells and IL-13 are effector candidates for some patients with UC.

INFLAMMATORY BOWEL DISEASE GENOMICS

A powerful window on such underlying host traits for IBD has emerged with the genome-wide search for IBD susceptibility loci (8). This is a burgeoning area of research, and this review focuses on selected loci either well defined molecularly or associated with biologic heterogeneity of IBD. Most extensively studied is the *IBD1* locus on chromosome 16, which reflects coding polymorphisms or truncations of the *CARD15/NOD2* gene (128, 129). This protein encodes an intracellular peptidoglycan receptor that activates CARD and NF-κB-dependent pathways of innate immune cellular activation. The preponderance of evidence relates *CARD15* alleles to reduced microbial sensing and response by macrophages and epithelial (Paneth) cells (45). However, evidence for augmented microbial responsiveness has also been reported, either through biochemical or cross-talk mechanisms, and the molecular pathogenesis has been remarkably challenging to pin down in animal models (130).

There are three predominant disease-related *CARD15* allelisms, and these exclusively affect CD susceptibility, typically in the heterozygote context. Thus, *CARD15* pertains to the distinctive disease biology of CD. However, CARD15 is estimated to contribute to 10–30% of disease risk in European but not Japanese heritage populations, so it represents only one genetic path to CD susceptibility (8). Nonetheless, these *CARD15* alleles have proven informative in dissecting alternate CD phenotypes and in prognosis, notably prediction of fibrostenosing ileal disease (131–133).

IBD5 (a 250-kb haplotype in the 5q31 cytokine gene cluster) confers CD disease susceptibility independent of *CARD15* (134). Alleles of many candidate loci exist in tight linkage in this region, so that a definition of the definitive genes is challenging. Notable prospects are *OCTN1/SLC22A4* and *OCTN2/SLC22A5*, representing organic cation transporters for epithelium,

macrophages, and other cell types. These tightly linked genes have been associated with CD susceptibility and perhaps perianal penetrating disease, particularly in the context of *CARD15* (135, 136). Coding or promoter alleles of these two regions affect their expression and function, respectively, as measured by reduced carnitine transport.

As noted above, epithelial integrity forms an innate barrier to bacteria and their products. P-glycoprotein (encoded by the multidrug resistance-1 gene, *MDR1*) is widely expressed in intestinal epithelial cells and forms a barrier to bacteria-dependent intestinal inflammation, bacterial invasion, and incursion of microbial products (137). The human *MDR1* single nucleotide polymorphism C3435T is associated with lower intestinal P-glycoprotein expression, and this and perhaps other *MDR1* polymorphisms promote UC susceptibility and disease course (138–140).

Haplotypes bearing coding allelisms of DLG5a (discs large *Drosophila* homologue 5) scaffolding protein, associated with epithelial junction formation, may be associated with both UC and CD susceptibility and disease course (135, 141). However, this region of chromosome 10q23 is genetically complex, and presently there is divergence among studies and populations with respect to disease association and functional allelisms of DLG5. The major histocompatibility locus has long been a candidate for IBD susceptibility. Although attention had focused previously on the HLA class 1 and 2 genes, the locus also includes TNF-α and lymphotoxin-α genes. Certain haplotypes in this region may predict responsiveness to anti-TNF (infliximab) therapy in CD (142).

CANDIDATE MICROBIAL PATHOGENS AND MUCOSAL DYSBIOSIS

The emergence of *Helicobacter pylori* in chronic inflammatory disease of the upper gastrointestinal tract is an important precedent in the search for microbial pathogens for diseases such as IBD. In CD, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is a recurrent candidate for several reasons: its association with epidemic bovine colitis, the presence of anti-MAP antibodies and MAP sequence by polymerase chain reaction (PCR) CD patients, and some reports that antimycobacterial drugs ameliorate disease (143–146). However, other groups were unable to detect these microorganisms, and the prevalence of disease-associated anti-MAP antibodies is variable. Only limited evidence has been presented for anti-MAP T cell responses in CD patients, and the effectiveness of anti-TNF therapy weighs against a mycobacterial etiology because such treatment is expected to reduce host resistance. Antimycobacterial chemotherapy has not reached clinical endpoints in some studies and has not been related to reduction in the load of MAP organisms (147–152).

Other microorganisms have also been associated with CD by increased tissue levels of organism, host antibody levels, and partial effectiveness of pertinent antibiotics (153, 154). Of particular interest is a set of novel bacteria inferred from the isolation of distinctive flagellin molecular phylotypes associated with CD (121, 155, 156). Antibodies to the CBir1 flagellin (a flagellin molecular clone isolated from enteric bacteria of the C3H/HeJBir mouse strain) are elevated in many IBD mouse models, as well as in the majority of CD patients. Moreover, anti-CBir-1 T cells were colitigenic in mouse transfer models. This candidate is particularly appealing in view of the role of TLR5 in flagellin recognition. TLR5 can augment the adaptive immune response to flagellin as an adaptive immune antigen (157, 158). Analogous to TLR9 and systemic lupus erythematosis, the combination of antigen and TLR ligand creates a challenge for immunoregulation that may enable immunopathology in the presence of host susceptibility traits (159, 160). Indeed, polymorphisms of TLR5 have been associated with CD risk (161).

A second mode of candidate pathogenicity is exemplified by adherent-invasive *Escherichia coli* (AIEC) in CD (162). Among mucosa-isolated organisms, *E. coli* is distinguished by an increased abundance of isolates from CD showing adherence to human intestinal cell lines (85% from CD tissue versus 40% from normal tissue). These isolates are prevalent in ileal but not colonic tissues. Conversely, AIEC strains adhere more efficiently to native enterocytes from ileum versus colon and from CD versus normal ileum. Like various enteropathogenic *E. coli*, AEIC are invasive in intestinal and macrophage cell lines, but are distinguished by their prolonged intracellular survival and growth owing to their capacity to escape endocytic residence and enter a cytoplasmic compartment (163). Genetic analysis of a model strain, LF82, has uncovered a series of virulence traits required for the adherent-invasive phenotype (**Figure 5**). Enterocyte or macrophage interaction with AIEC triggers a molecular inflammatory response, selectively involving TNF-α activation (164).

A third mode of commensal pathogenicity was reported by Swidsinski and colleagues, who observed increased abundance of bacteria penetrating the colonic mucous layer in IBD patients (21, 22). Using 16S rDNA fluorescence in situ hybridization, these penetrating bacteria were highly divergent phylogenetically. This finding may reflect shared virulence traits conferred by horizontal gene transmission (165). However, it is more likely that this observation could represent a defective mucosal barrier function(s) that permits such penetration and provides a common underlying disease susceptibility trait in this subset of IBD patients. Thus, it may be pertinent that isolated knockout of MUC2 alone is sufficient to cause chronic colitis (27). Similarly, the observations in CD patients of MAP bacteremia (166) or the presence of elevated levels of *E. coli* 16S in lamina propria (149) may be examples of impaired mucosal barrier or microbial clearance in these patients.

Note that CD patients are distinguished from UC and non-IBD patients by the presence of serum IgG or IgA antibodies to a variety of microbial antigens (154, 156, 167, 168). Some of the more widely used antigens include ASCA (antibodies to the cell wall polysaccharide of *Saccharomyces cerevisiae*), OmpW and OmpC (TonB-linked outer membrane proteins of *Bacteroides caccae* and *E. coli*), and PfiT (I2) (product of *Pseudomonas fluorescens*, a common dietary contaminant). Antibodies to one or more of these products

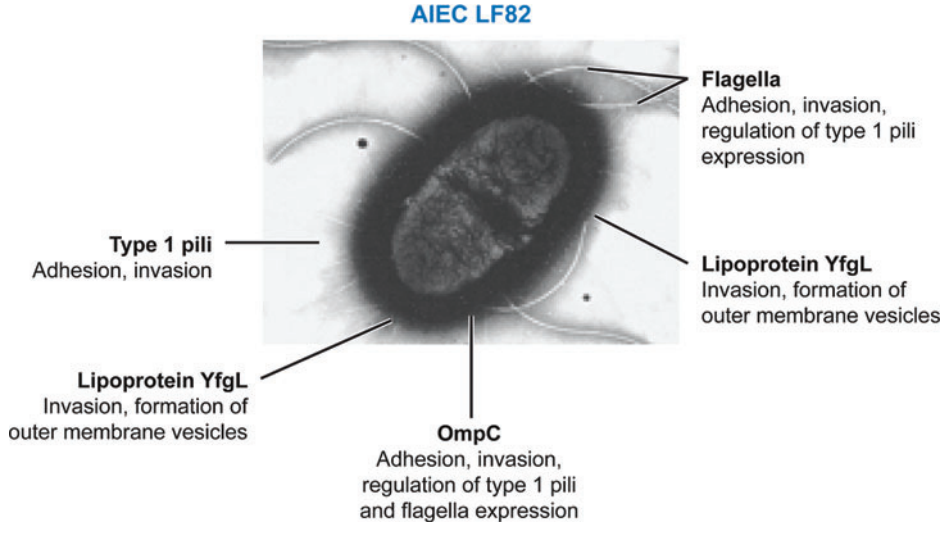

Figure 5

Virulence traits of adherent-invasive *Escherichia coli* (AIEC). Adapted from Reference 164.

are a feature of the majority of CD patients, compared to low levels of these antibodies in UC or non-IBD patients. In multiplex families, a significant familial concordance is observed for seropositivity to individual molecules, including ASCA or OmpC, and such concordance is unaffected by first-order relatives (153, 169). It is unclear whether any of these organisms plays a causal role in CD biology. However, these findings suggest that formation of these antibodies may reflect a shared disease biology. For example, discrete impairments of host barrier or microbial clearance may permit elevated antigenic encounter to selected organisms with traits that advantage these impairments. This may account for the utility of antibody algorithms to predict disease severity, progression, and response to antibiotic treatment (156, 170, 171). The possibility that seropositivity in unaffected first-order relatives represents a preclinical state is supported by longitudinal studies of 18-year-old military transcripts (172). If validated further, these may represent predisease biomarkers for therapies to avert progression to a clinical state, such as the strategies now employed for intervention in families with type 1 diabetes risk.

Therapeutically, there is much interest in antibiotics, or pre- and probiotics, that may beneficially modulate the enteric microbial community (4, 80, 173). Thus far, antibiotics (e.g., metronidazole or ciprofloxin) have proven useful for the treatment of relapse and pouchitis, but not for the maintenance of remission. The probiotic *Lactobacillus* GG (the GG strain of *Lactobacillus rhamnosus*) has efficacy for maintenance in UC, but curiously lacks clinical utility in CD. These limitations suggest heterogeneity among patients and disease subsets for microorganisms targeted by these agents. Accordingly, advances in such treatments will require the identification of meaningful organisms and their products and of innovative microbial or inflammatory biomarkers for treatment selection and endpoint of therapy.

ETIOLOGIES OF INFLAMMATORY BOWEL DISEASE AND THERAPEUTIC OUTLOOK

We have summarized the elegant interplay of intestinal microbiota, mucosal barrier function, innate mechanisms of microbial clearance, and immunoregulation, which averts destructive inflammation despite the constant presence of resident microbiota and bioactive dietary intake (**Figure 6**). The pathogenesis of IBD, a chronic and relapsing state of destructive inflammation, is the plausible consequence when one or more of these collaborating elements bear functional abnormalities (**Table 3**). In this review, we hope to have conveyed the principle that IBD is a scope of diseases—distinguished by environmental and genetic factors—which share a common endpoint of chronic intestinal inflammation. Accordingly, it is likely that the predominant etiologic factors, and hence the most effective therapeutic targets, will differ not only between UC and CD patients, but also between subsets of UC or CD patients. How do we identify which of these potential etiologic factors indeed cause IBD in a particular patient? In cancer patients with no underlying IBD susceptibility, treatment with anti-CTLA4 (which globally abrogates regulatory $CD4^+$ T cells) causes rapid and penetrant (>30%) induction of an IBD-like syndrome (174). This suggests that immunoregulation of $CD4^+$ T cells and their inflammatory effector pathways may alone be a critical factor in IBD disease susceptibility in humans. Moreover, most (but not all) of the divergent genetic and microbial murine models of intestinal chronic inflammation clearly reflect

Table 3 Candidate etiologies for inflammatory bowel disease

Impairment of mucosal barrier function
Defective innate immune control mucosal microorganisms
Mucosal dysbiosis or specific microbial pathogens
Defective mucosal immunoregulation

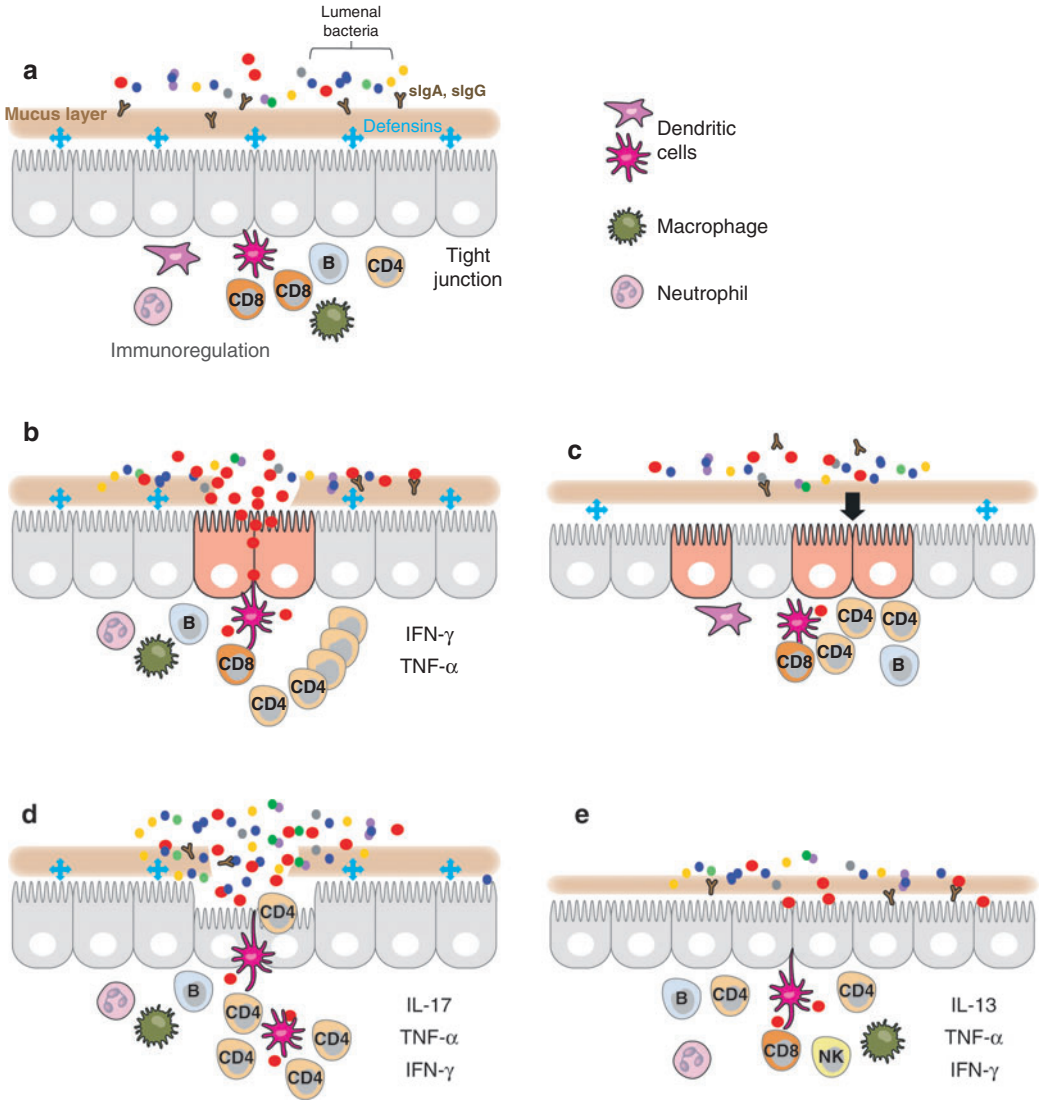

Figure 6

Microbial-host interactions at the mucosal interface and inflammatory bowel disease susceptibility. (*a*) Mucosal barrier integrity, efficient microbial clearance, and immune regulation maintain mucosal homeostasis. (*b*) Pathogenic microorganisms break the mucosal barrier and stimulate the colitigenic $CD4^+$ T cell population, which leads to a destructive immune response and tissue damage. (*c*) Impaired microbial clearance permits microbial accumulation and host response, by traits that reduce Paneth cell production of antimicrobial products, levels or activity of innate hemopoeitic cell types, or impaired microbial-sensing mechanisms by these cell types. (*d*) Owing to host genetic traits impairing mucosal immunoregulation, there is a vicious circle of intensified antimicrobial immune-mediated inflammation, tissue damage, and augmented microbial penetration (typical Crohn's disease–like inflammation). (*e*) Genetic and environmental factors impairing mucosal barrier function (mucus abundance and composition, tight junction integrity, secretory immunoglobulin levels) permit adherence and penetration of resident microbiota and superficial mucosal inflammation (ulcerative colitis–like inflammation). sIgA, lumen-secreted IgA; sIgG, lumen-secreted IgG; B, B cell; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin; NK, natural killer cell.

a critical role for $CD4^+$ T cells. However, current immunosuppressive human IBD therapies (azathioprine, methotrexate, glucocorticoids, and TNF-α or α4 integrin antibodies) are variably effective and certainly do not specifically target the $CD4^+$ T cell effector pathway (175).

A concept to bridge these gaps is the "national guard" scenario. Under homeostatic conditions, barrier and innate immune activity reduce antimicrobial sensing and effector responses akin to the effective but measured levels of neighborhood policing. However, animal models teach us that IBD-like disease can result from many combinations of moderate impairments in host policing (barrier function, innate immunity, immunoregulation) and microbial dysbiosis (mucosal colonization with organisms expressing virulence and pro-inflammatory traits that probe host policing deficits) (4). In this context, the $CD4^+$ T cell response may be akin to the national guard response: potent, destructive, and deployed when microbial bioactivity is sufficient for adaptive immune effector recruitment. Accordingly, although $CD4^+$ T cell responses may indeed be the center of IBD-associated inflammation, their recruitment may be secondary to those underlying host and/or microbial community disorders. If true, then definitive therapy for IBD will require strategies to determine which defects exist in the underlying etiologic factors: dysbiosis, mucosal barrier integrity, innate immune microbial clearance, and/or immunoregulation. Biomarkers to diagnosis these defects, and therapies to correct them, comprise the opportunity to definitively characterize and cure this category of intestinal diseases.

DISCLOSURE STATEMENT

JB reports equity in Santarus Corporation and Prometheus Corporation and has served as a consultant to Genentech, Inc.

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