Host genotype, intestinal microbiota and inflammatory disorders

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Abstract

Intestinal microbiota may influence human physiology and disease risk due to the role it plays in mediating appropriate immune responses to harmful and innocuous antigens. Colonisation of the intestine in early life seems particularly important as it is the main environmental stimulus for immune system maturation. This is a dynamic process, which depends on both environmental and genetic factors. The pathogenesis of inflammatory bowel disease, such as Crohn's disease, involves genetic polymorphisms (e.g. *CARD15*/nucleotide-binding oligomerisation domain 2) related to an excessive inflammatory response to commensal microbiota and to its unbalanced composition. Atopic diseases have also been linked to imbalances in microbiota and to related genetic factors (e.g. *TLR4* and *CD14* genes), although these associations are still controversial. Research into the relationship between the genetic risk of developing celiac disease (human leukocyte antigen (HLA)-DQ2/DQ8) and the early colonisation process in infants at family risk of the disease has also reported that the HLA-DQ genotype could influence staphylococcal colonisation. Future observational studies considering both host genetics and microbiota could be critical in defining the complex host–microbe interactions and the respective role each plays in inflammatory disorders.

Key words: Intestinal microbiota: Genotype: Host–microbe interactions: Celiac disease: Atopic disease: Inflammatory bowel disease

The human intestinal tract is inhabited by a vast number of micro-organisms that may influence host physiology and disease risk. Intestinal colonisation constitutes the main exposure to environmental antigens, thus providing experience and maturity to the immune system, which in turn could determine the risk of immune-mediated disorders[1]. Upon birth, the infant gut is rapidly colonised by a consortium of micro-organisms that co-evolve with the host and its environment. This is a dynamic process, influenced by environmental factors (e.g. the mode of delivery, microbial load from the environment, antibiotic intake, feeding practices, etc.) and the host genotype[2,3]. One of the main environmental factors influencing early intestinal colonisation is the type of milk feeding. In general, *Bifidobacterium* spp. are dominant in breast-fed infants (up to 90% of the total faecal bacteria), while a more diverse adult-like microbiota is found in formula-fed infants[4,5]. A large-scale population study has also reported that exclusively formula-fed infants are more often colonised with *Escherichia coli, Clostridium difficile, Bacteroides* spp. and *Lactobacillus* spp., compared with breast-fed infants[2]. Moreover, *Bifidobacterium* species colonisation is delayed in formula-fed compared with breast-fed babies[6]. In addition, Van de Merwe *et al.*[7] have demonstrated that the faecal microbiota of monozygotic twins was much more similar than that of dizygotic twins, suggesting the influence of the genotype on its composition. The same observation has been reported in adults with varying degrees of relatedness[8] and in identical twins, fraternal twins and unrelated controls[9]. Palmer *et al.*[10] also studied compositional variation in the intestinal microbiota during the first month of life, evaluating its similitude with the vaginal, breast milk and stool samples from mothers and fathers. Correlations between the faecal bacterial composition, breast milk and vaginal samples have been observed, suggesting that the initial bacterial colonisation stages are determined by the specific bacteria to which the infant is exposed. However, these initial colonisers do not persist indefinitely, as evidenced by the significantly higher correlation between the taxonomic profiles of infants and parents, which may partially relate to dominance of the genetic background. Therefore, although most studies have only investigated the role that environmental factors (e.g. diet, antibiotics, etc.) play in shaping the gut microbiota, there is emerging evidence that studies on interactions between environmental factors and predisposing genes could be more relevant in discerning their respective influence on disease risk (Fig. 1)[3].
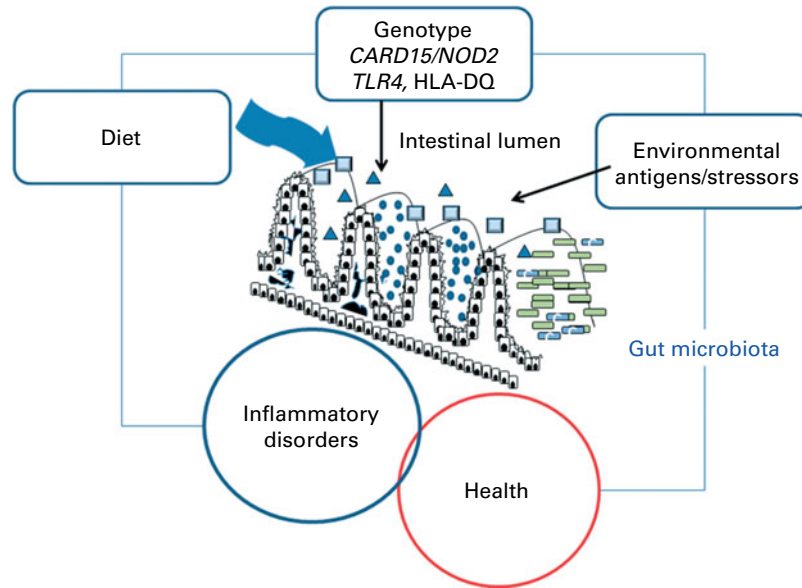
Fig. 1. Schematic representation of environmental, genetic and gut microbiome interactions that may be involved in the risk of developing inflammatory disorders.

Relationship between genotype and gut microbiota in inflammatory diseases

It has been established that the pathogenesis of inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, involves genetic risk factors[11,12] and imbalances in the intestinal microbiota[13,14], which seem to result from alterations in host−microbe interactions, leading to an excessive inflammatory response to commensal microbiota[15]. In this context, unlike conventional mice, germ-free mice have been shown not to develop experimental colitis[16], and deletions in immunoregulatory genes (e.g. IL-10- or transforming growth factor-β-deficient mice) trigger colitis via a deregulated immune response to normal gut microbiota[17]. More recent animal studies also indicated that genes coding for inflammasome-related proteins (e.g. nucleotide-binding and oligomerization domain-like receptor (NLR) P6 (NLRP6)), which are involved in recognition of microbial or other damage signals, influence intestinal microbiota composition and development of colitis[18]. In particular, NLRP6 inflammasome-deficient mice showed reduced IL-18 production by epithelial cells, exacerbation of colitis induced by exposure to dextran sodium sulphate and altered faecal microbiota characterised by increased representation of the phyla Bacteroidetes (Prevotellaceae) and TM7. The transplantation of the altered microbiota into neonatal or adult wild-type mice also exacerbated colitis via induction of cytokine production[18]. In animal studies, genes related to IgA production and secretion have been demonstrated to be critical for the regulation of intestinal microbiota composition[19], which could contribute to intestinal inflammatory conditions. Activation-induced cytidine deaminase (AID) plays an essential role in class-switch recombination and somatic hypermutation of Ig genes. AID deficiency, which results in absence of hypermutated IgA, accumulation of IgM and hyperplasia of isolated lymphoid follicles, has also been associated with permanent expansion of anaerobes, including segmented filamentous bacteria in the small intestine; however, reconstitution of lamina propria IgA production in AID$^{-/-}$ mice led to restoration of the normal composition of intestinal microbiota and abolished the local and systemic activation of the immune system. Segmented filamentous bacteria have been shown to contribute to triggering intestinal inflammation in animal models of IBD[20].

Studies with monozygotic twins have also helped to unravel the respective contribution of host genetics and commensal bacteria to Crohn's disease in human subjects[15]. While healthy twins and twins concordant with the disease presented similar microbial community profiles, these similarities were not observed in discordant twins, suggesting that the disease implies a particular microbiota structure[15]. In addition, polymorphisms in the caspase activation recruitment domain 15 (CARD15) gene encoding nucleotide-binding oligomerisation domain 2 (NOD2) and functional variants of the Toll-like receptor (TLR)-5 and CD14 antigen (CD14) genes have been associated with the development of Crohn's disease in human subjects[21]. A recent study has also reported that the NOD2 genotype was associated with shifts in human ileal microbiota[22]. IBD phenotype, C. difficile and NOD2 genotype were associated with shifts in overall microbial composition. Moreover, IBD phenotype and NOD2 genotype were associated with shifts in the relative proportion of the Clostridium coccoides–Eubacterium rectale group, and IBD phenotype, smoking and IBD medications were associated with shifts in the relative proportion of Faecalibacterium prausnitzii[22].

These genetic polymorphisms are involved in host innate immunity, establishing a dialogue with the host and transient microbiota. NOD2 is an intracellular sensor for bacterial cell wall components (muramyl dipeptide of the peptidoglycan) that also interacts with TLR, playing an indispensable role both in triggering the host's inflammatory response to infection and in developing autoimmunity. CD14 and TLR-4 are

involved in the recognition of the lipopolysaccharide of Gram-negative bacteria, which upon binding can activate the NF-κB, producing inflammatory cytokines. Animal studies have been performed on the molecular mechanism by which polymorphisms in the NOD2-encoding gene could increase susceptibility to Crohn's disease in human subjects. These studies indicate that mice bearing NOD2 transgene, and thus having cells with increased NOD2 function, display diminished responses to TLR stimulation and are resistant to experimental colitis induction. In contrast NOD2-deficient mice displayed increased responses to TLR stimulation, as may be the case in genetically susceptible subjects[23]. Furthermore, cells could become unresponsive to TLR stimulation due to pre-stimulation with the NOD2 ligand, because such pre-stimulation leads to the production of inhibitory factor (interferon regulatory factor (IRF)4) of the TLR-induced inflammatory pathways. Moreover, when muramyl dipeptide was administered to normal mice, IRF4 was induced, preventing experimental colitis. Therefore, NOD2 polymorphisms could increase susceptibility to Crohn's disease due to the suppression of TLR homeostasis, which would trigger a pathogenic response to the commensal microbiota signatures[23].

Clinical studies have also linked the defective expression of α- and β-defensin, which are antimicrobial peptides essential in host defence and maintenance of immune homeostasis, to impaired regulation of micro-organisms by the intestinal mucosa of Crohn's disease patients[24].

In recent years, genome-wide association studies have revealed relationships between a large number of additional genomic loci (e.g. IL 23 receptor (*IL23R*), fucosyltransferase 2 (*FUT2*), IRF5 (*7q32*), DNA methyltransferase 3A (*DNMT3A*), etc.) and IBD in human subjects[25,26]. Some of these loci could also be involved in bacterial sensing and immune reactions and could contribute to explaining the relationship between these disorders and intestinal dysbiosis in the future.

Atopic diseases have also been related to imbalances in the human intestinal microbiota and to genetic factors, although their association with genes related to host–microbe interactions (e.g. *TLR4* and *CD14*) remains controversial. In this context, a prospective study of 957, 1 month-old infants, genotyped for *TLR4* and *CD14* gene polymorphisms, investigated the relationships between faecal *E. coli*, genotype and the onset of atopic sensitisation, determined by assessing IgE-specific antibodies against different food and environmental antigens[27]. Results associated *E. coli* colonisation with reduced risk of sensitisation in children with a particular genotype (*TLR4 SNP rs10759932*), while no associations were detected when the host and microbial factors were analysed separately, without taking into account their interactions[27].

Relationship between genotype and gut microbiota in infants at risk of celiac disease

Unlike IBD and atopic disorders, the role of microbiota in celiac disease (CD) has only been investigated relatively recently[28]. CD is an autoimmune disorder characterised by a permanent intolerance to gluten proteins. The disorder has been associated with the expression of DQ2 and DQ8 molecules, encoded by the human leukocyte antigen (HLA)-DQ genes, mainly expressed in antigen-presenting cells. Deamidated gluten peptides bind to DQ2/DQ8 molecules to be presented to $CD4^+$ T cells, which then trigger a T-helper 1 and T-helper 17 responses, leading to the destruction of the architecture of the epithelial mucosa (revised by Di Sabatino & Corazza[29]). This inflammatory disorder has also been associated with imbalances in the intestinal microbiota of CD patients, characterised by decreased numbers of *Bifidobacterium* spp. and increased numbers in *Bacteroides* spp.[30,31]. However, intestinal dysbiosis is not completely restored in patients treated with a gluten-free diet, suggesting that some of the alterations are not only secondary to the inflammatory milieu, but can also be linked to the genotype, predisposing to the disease. In murine models using mice with different MHC, but otherwise identical backgrounds, the MHC genes are revealed to represent one of the genetic factors exerting a pronounced influence on faecal microbiota composition[32]. Regarding CD, there is only one human study in which the HLA-DQ genotype and intestinal microbiota composition have been related. The present study included 164 healthy newborns, with at least one first-degree relative with CD, which showed that the intestinal microbiota composition is influenced by both the milk-feeding type and HLA-DQ genotype. Infants at high genetic risk of developing CD had reduced numbers of *Bifidobacterim* spp. and increased numbers of *Bacteroides* spp., in comparison with those at low genetic risk, although these differences were attenuated by breast-feeding. In addition, increased numbers of *Staphylococcus* spp. were found in infants at high genetic risk of developing CD, regardless of the milk-feeding type, suggesting that the HLA-DQ genotype plays a more prominent role in its colonisation.

Previous studies into the mitogenic activity of staphylococcal superantigens show that they are able to bind to MHC class II molecules. This binding activates T cells, triggering a massive release of pro-inflammatory cytokines (revised by Proft & Fraser[33]). Non-specific T-cell activation without prior processing of antigens is a unique property of superantigens[34]. In this regard, Schiffenbauer *et al.*[35] showed that mice recovering from encephalomyelitis, an autoimmune syndrome, relapsed when autoreactive T cells were stimulated by staphylococcal superantigens administration. Staphylococcal enterotoxin B also increased systemic inflammation in HLA class II transgenic mice[36]. However, the possible role played by staphylococci or their superantigens in the risk of developing CD has yet to be established.

In addition, genome-wide association studies in CD are leading to the identification of non-HLA genes related to immune dysfunction (e.g. *4q27* region containing *IL-2* and *IL-21* genes), which are partly shared with other autoimmune diseases[37]. This information could be also of significance in defining host–microbe interactions that could influence the risk of developing CD in the future.

Perspectives

In the light of existing evidence, future observational studies considering both host genetics and microbiota could be

critical in defining the complex host−microbe interactions and the respective role each plays in chronic inflammatory and autoimmune disorders in human subjects. In this context, genome-wide association studies could help select the potential candidate genetic loci related to microbe colonisation and signalling via interactions with the host intestinal epithelium and gut-associated lymphoid tissue. Evidence from human observational studies could also serve as the basis for designing mechanistic studies in genetically modified animal models that explain associations established in human subjects. Altogether, this could contribute to uncovering adverse and mutually beneficial host−microbe interactions and identifying modifiable disease risk factors.

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