The Innate Immune System: A Trigger for Many Chronic Inflammatory Intestinal Diseases

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Key Words

Chronic inflammation · Dendritic cells · Innate immunity · Macrophages · Single nucleotide polymorphisms

Abstract

Background: Mononuclear phagocytes, such as monocytes, macrophages, and dendritic cells, are important cellular components of the innate immune system that contribute to the pathogenesis of many intestinal inflammatory diseases.

Summary: While mononuclear phagocytes play a key role in the induction of inflammation in many different tissues through production of pro-inflammatory cytokines and chemokines (such as IL-1, TNF, IL-6, IL-8 and MCP-1), free oxygen radicals (also termed 'oxidative burst'), proteases (such as cathepsins) and tissue-degrading enzymes (such as metalloproteinases), resident macrophages as well as dendritic cells in the intestine display an anergic and 'tolerogenic' phenotype mediating tolerance to commensal bacteria. In recent years many single nucleotide polymorphisms (SNPs) in genes mainly expressed in the above-mentioned cell types have

been identified to convey an increased risk of autoimmune diseases. SNPs in the NOD2, ATG16L1 and TNFSF15 genes, which are involved in the function of the innate immune cells, are identified as risk factors for Crohn's disease (CD). Of note, these genes are involved in the different functions in the innate immune cells. For example, while NOD2 is required for intracellular recognition of microbial components, ATG16L1 is involved in autophagy responses against intracellular microbes. Likewise, TNFSF15 contributes to the induction of inflammatory responses by innate immune cells. Furthermore, the frequency of mutations in these genes differs by ethnicity. Genetic variations in the NOD2 and ATG16L1 genes are associated with CD in Caucasians but much less in Eastern Asian populations, whereas SNPs in TNFSF15 are dominated in Asian populations. Thus, different genetic risks may eventually lead to similar impairments in innate immune cells, thereby developing the same disease in Western and Asian patients with CD. **Key Messages:** Despite differences in risk genes, similar mechanisms associated with the innate immune system may trigger autoimmune and chronic inflammatory intestinal diseases in East and West.

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Introduction

The innate immune system plays an important role in the triggering of intestinal inflammation in diseases such as inflammatory bowel disease (IBD) [1]. Under physiological conditions it is supposed to provide the first-line response against external or internal pathogens and danger signals (e.g. ‘pathogen-associated molecular patterns’ as well as ‘danger-associated molecular patterns’). The first-line response can usually be characterized as a protective inflammatory response aimed at clearing the initial trigger. This first-line response is usually self-limiting [2].

After the initial rapid and ‘innate’ response the same cells may induce a longer-lasting adaptive and specific immune response. Following the direct destruction of pathogens bacterial components, cell detritus and damaged extracellular matrix components are phagocytosed by the cells and degraded [3]. Besides phagocytosis of extracellular material, intracellular debris is degraded by autophagy.

Cells of the innate immune system have been shown to play an important role during the initiation and chronicity of IBD [4–6]. IBD has become a prototype of an immune disease partially caused by a high number of genetic risk variation, e.g. single nucleotide polymorphisms (SNPs). Many proteins functionally important for the early immune response may be altered due to the known risk variants [7–9]. However, the risk-conveying SNPs found in large genome-wide association studies to be associated with the risk of developing either Crohn’s disease (CD) or ulcerative colitis (UC) are not disease-specific. Many of them also increase the risk of developing other autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or infectious diseases such as leprosy [10, 11].

Tissue-Specific Differentiation of Innate Immune Cells

Differentiation of macrophages from monocytes in the intestinal mucosa is accompanied by the acquisition of a typical functional phenotype [4, 5, 12, 13]. The differentiation of monocytes into macrophages normally occurs under ‘non-inflammatory’ conditions. This will lead to the differentiation of a normal intestinal macrophage after a monocyte has entered the intestinal mucosa from the blood stream [14, 15]. In IBD this differentiation is altered at sites of inflammation [16–23]. A different

phenotype with different functional properties occurs [16–24].

The mechanisms of how the various risk genes for IBD – and especially the ones that are associated with functions of the innate immune response – influence this process of tissue-specific and inflammation-modified macrophage differentiation are so far largely unclear.

A problem occurring when investigating the role of autoimmune disease risk genes in innate immune cells is that they have different distributions in mice (which are frequently used as a model) and humans. A good example are monocytes: They present 5–10% of the peripheral blood leukocytes in humans but only 2–4% of the total leukocytes in mice [25]. Therefore results obtained from mice may be not easy to transfer to the human innate immune physiology.

Circulating human peripheral blood monocytes are heterogeneous with respect to their surface protein expression [26]. Three major subsets of human monocytes have been identified especially with respect to CD14 (part of the lipopolysaccharide receptor) or CD16 (Fcγ RIII) expression [26]. Ninety percent of human monocytes display high CD14 but no CD16 surface expression (usually annotated as CD14⁺CD16[−]) representing the classical monocyte phenotype. In the remaining 10% two subtypes can be discriminated: a population with high CD14 and low (but not absent) CD16 expression (CD14⁺CD16⁺), and a population with low CD14 but high CD16 expression (CD14^{dim}CD16⁺) [26]. In mice, blood monocytes consist of two principal subsets: Ly6C^{hi}CCR2⁺CX₃CR1^{lo} inflammatory monocytes and Ly6C^{lo}CCR2[−]CX₃CR1^{hi} resident (or patrolling) monocytes [27]. The Ly6C^{hi}CCR2⁺CX₃CR1^{lo} inflammatory monocytes, which correspond to human CD14⁺CD16[−] monocytes, give rise to several tissue-resident macrophages including intestinal macrophages in both steady-state and inflammatory conditions. On the other hand, Ly6C^{lo}CCR2[−]CX₃CR1^{hi} resident monocytes, which correspond to human CD16⁺ monocytes, do not give rise to tissue macrophages but patrol blood vessels to combat against incoming pathogens as the first line of defense. Thus, the monocyte subpopulations play distinct roles in innate host defense. Although the functional differences between monocyte subsets are well documented in mice, those in human monocyte subpopulations are only partially understood [25, 26]. They may have different capacities to respond to bacterial antigens and trigger subsequent innate immune responses [25, 26]. In a recent review the different roles for the innate immune response were highlighted [28].

Fig. 1. Intestinal macrophage differentiation in healthy mucosa and CD. Peripheral blood monocytes express innate immune receptors and induce strong inflammatory responses upon stimulation with microbial antigens. Intestinal macrophages in the normal mucosa downregulate these innate receptors and upregulate certain molecules (e.g. gp96), and therefore adapt a ‘tolerogenic’

phenotype. In contrast, the differentiation of intestinal macrophages is somehow impaired in patients with IBD, particularly CD. Intestinal macrophages in CD patients respond to microbial antigens and induce inflammatory immune responses, and therefore contribute to disease pathogenesis.

Tissue macrophages usually represent up to 10–15% of the total cell number in most organs and tissues [29–31]. Intestinal macrophages and dendritic cells are mainly localized in the lamina propria [32]. Intestinal macrophages and dendritic cells represent one of the largest compartments – if not the largest – of the body’s innate immune system. They are localized preferentially at the sites of antigen entry, e.g. in the subepithelial region.

Intestinal macrophages differ in their transcriptional profile from macrophages derived from other tissues, such as Kupffer cell in the liver, alveolar macrophages or osteoclasts [33–36]. They display a specific phenotype with low expression of monocyte antigens such as CD14 or CD16 [21] and with low expression of co-stimulatory molecules such as CD80 or CD86 or pattern recognition receptors such as TLR4 or TLR2 [20, 37]. In contrast to blood monocytes or in vitro-differentiated macrophages, normal intestinal macrophages are rather irresponsive to lipopolysaccharide [37–39]. On the other hand, the

phenotype and the functional characteristics of intestinal macrophages are altered during chronic inflammation in IBD. For example, CD14-expressing macrophages, which express TLRs and are responsive to microbial components, are found to be accumulated in the intestinal mucosa of IBD patients (fig. 1) [16, 18–20, 22–24, 40, 41].

SNPs in ‘Innate Immune Genes’ in CD and UC

A concordance rate for CD of about 40% in monozygotic twins indicated a role of genetic variations for the risk to develop CD [42, 43]. The first susceptibility gene for CD to be identified was NOD2 [44–46]. Three major NOD2 genetic variants are associated with CD in Caucasians (but much less in Asian populations) [47–52]. About 20–40% of all patients – depending on the genetic background – carry variants of NOD2 in contrast to 10–15% in the healthy population. NOD2 is an intracellular

Fig. 2. Genetic variations associated with IBD that are related to intestinal macrophage/dendritic cell functions. Various ‘IBD risk genes’ have been identified. Some of them are related to the function of macrophages and dendritic cells in the intestine.

pattern recognition receptor recognizing invading bacteria that have entered the mucosal wall – a typical innate immune response protein. Muramyl dipeptide (N-acetylmuramyl-L-alanyl-D-isoglutamine, MDP), a component of the bacterial wall, is the most important ligand for NOD2 [53, 54]. NOD2 mutants associated with susceptibility to CD are impaired or deficient in their ability to bind MDP [54] and induce an innate immune response. NOD2 expression is found in intestinal macrophages and dendritic cells but not in T cells [55]. In addition NOD2 is expressed in intestinal epithelial cells [55–57], especially in mucosal Paneth cells [57–59]. MDP-NOD2 interaction is usually followed by activation of the innate immune system, reflected by an induction of α - and β -defensin secretion as a first line of defense at the mucosal barrier [60–63]. Nod2 is strongly involved in the regulation of defensin expression [62].

More than 200 other genetic polymorphisms have been reported to play a role in IBD pathogenesis [8]. An association of CD with a polymorphism in the autophagy-related 16-like 1 gene (ATG16L1) was been shown by several groups [64–66]. The ATG16L1 protein is essential in the autophagosome pathway that processes intracellular bacteria (see above). In a genome-wide association study of 3,230 CD cases and 4,829 controls the Wellcome Trust Consortium confirmed genetic risk factors including NOD2, 5q31 (IBD5)15 as well as IL23R, ATG16L1, IRGM, TNFSF15 and PTPN2 [67]. In addition 21 new

loci were found including PTPN22, ITLN1, IL12B, CDKAL1, CCR6, JAK2, C11orf30, MUC19 or STAT3 [67]. Of note, TNFSF15, which is expressed in macrophages and dendritic cells, was identified as a susceptible gene for IBD in East Asian populations [68, 69]. While NOD2 and ATG16L1 variants are less involved in Asians compared to Caucasians, genetic variants in TNFSF15 dominate in Asians. Thus, although the precise common pathways triggered by these different genetic variations remain unclear, different genetic risks may eventually lead to similar impairments in innate immune cells, thereby developing the same diseases in Western and Asian IBD patients (fig. 2).

‘IBD Risk Genes’ and Their Role in the Pathogenesis of Other Diseases

A number of excellent overview papers have been published in recent years on the impact of ‘typical IBD risk genes’ in other autoimmune or chronic inflammatory diseases [11, 70, 71].

When looking into different immune-mediated diseases, Zhernakova et al. [72] found 23 risk genes identified to play a role in two or more diseases. Many of the risk genes are involved in basic mechanisms of the innate immune system (such as NOD2). In contrast to that analysis performed in 2008, Lees et al. [11] in 2011 already

identified 51 IBD-associated genes that also showed an association with disease risks in 23 other diseases. Disease overlap was now also found for celiac disease, colorectal cancer and primary sclerosing cholangitis as well as other autoimmune diseases such as multiple sclerosis, psoriasis, atopic dermatitis, SLE or asthma [11]. The most frequently disease-associated genes found in that analysis were IL2RA and PTPN22 (5 diseases each), FCGR2A (4 different diseases) and IRF5, IL10, IL23R, IL2/IL21 and ORMDL3 (3 diseases each) [11].

Based on those data we have to assume that the underlying genetic variants cause changes in the function of proteins that may not just increase the risk of one disease but of a range of diseases. There may be some similarities between the diseases (such as celiac disease and IBD), but there also may be diseases that seem to have nothing in common at first sight (such as multiple sclerosis and IBD).

A typical example for a risk factor involved in the pathogenesis of many autoimmune diseases is the gene locus encoding protein tyrosine phosphatase non-receptor type 22 (PTPN22). Variants in PTPN22 have been associated by genome-wide association studies with several inflammatory disorders, including CD and UC [73], SLE [74], RA [75, 76] and type 1 diabetes [77]. Two major variants have been described; one SNP (C1858>T; SNP ID: rs2476601) causes the substitution of arginine 620 with a tryptophan (referred to as the 620W variant), while the other SNP (G788>C; SNP ID: rs33996649) causes a substitution of arginine 263, a glutamine residue (referred to as the 263Q variant) [78, 79].

Arginine 263 is located in the catalytic region of the PTPN22 protein. The above-mentioned substitution with glutamine results in decreased phosphatase activity [80]. The 263Q variant has been reported to reduce the risk of developing SLE and RA [81]. Similarly, it protects from the onset of UC [73]. The PTPN22 620W variant in contrast is associated with an increased risk of RA [75], SLE [74] and type 1 diabetes [77]. In contrast it surprisingly seems to reduce the risk of developing CD [73]. The PTPN22 620W variant has been reported to result in a gain-of-function variant followed by functional consequences such as an inhibition of the T cell receptor signaling cascade [82]; however, this is discussed controversially.

PTPN22 is expressed in innate and adaptive immune cells but barely present in non-hematopoietic cell types [83, 84]. Loss of PTPN22 in monocytes results in profound changes in IFN- γ -induced signaling with reduced signal transducer and activator of transcription 1

(STAT1), but strongly enhanced p38 mitogen-activated protein kinase activation, ultimately resulting in drastically enhanced IL-6 secretion together with reduced IL-12 levels [85, 86]. It causes an altered response to MDP, with increased secretion of pro-inflammatory IL-6 and IL-8 and enhanced autophagy induction [85, 86]. Dendritic cells from PTPN22-deficient mice induce higher proliferation of T cells in vitro [84].

Subsequently the PTPN22 gain-of-function variant is a typical example for a general 'autoimmune risk allele' associated with an increased risk of developing several autoimmune disorders. It plays a role in the innate as well as the adaptive immune system. Decreased protein expression of PTPN22 is mainly found in myeloid cells of the intestinal mucosa of IBD patients [86].

The Innate Immune System in Other Chronic Inflammatory Intestinal Diseases

So far we have described the role of the innate immune system mainly in IBD. Besides its involvement in IBD, the innate immune system plays a key role in the pathogenesis of other chronic inflammatory intestinal diseases. For example, CCR2⁺CD14⁺CD11c⁺ macrophages/dendritic cells accumulate in the intestinal mucosa of celiac disease patients, and these cells are believed to be involved in the immunopathology of the disease through activation of gluten-reactive T cells [87, 88]. Likewise, another report demonstrated that components of wheat, α -amylase/trypsin inhibitors, activate monocytes and macrophages through binding to TLR4 and CD14 [89]. Thus, activation of innate immune cells is linked to activation of adaptive immunity (Th1 responses) in celiac disease. The innate immune cells, such as macrophages, also contribute to irritable bowel syndrome (IBS). In this context, macrophages regulate homeostasis of the enteric nervous system (ENS) and gut motility. A recent study identified CX₃CR1⁺CD11c^{lo}MHC-II^{hi}CSF-1R⁺ macrophages residing in muscularis tissue and crosstalk with ENS neurons to maintain gut homeostasis [90]. Notably, stimulation by commensal bacteria is required to maintain this crosstalk. The importance of bacterial stimulation in gastrointestinal motility is also supported by evidence that TLR2 and TLR4 regulate gut motility [91, 92]. Although it is unclear whether the muscularis macrophages display anergic phenotypes like intestinal lamina propria macrophages, macrophages are not directly activated by microbial components in the intestinal muscular tissue. In the muscularis tissue, signals from commensal bacteria likely activate

enteric neurons, and neural mediators released from activated enteric neurons secondarily activate muscularis macrophages [90–92]. While there is evidence that activation of innate immune cells controls gut motility in rodent models, the role of innate immune cells and their activation in human IBS remain understudied. However, it has become evident that patients with IBS show dysbiosis [93]. A perturbed commensal community may compromise proper signaling toward the ENS, thereby leading to dysregulation of muscularis macrophages followed by development of IBS.

Conclusion

The innate immune system in the gut is tolerant to dietary and microbial antigens present in the intestinal lumen and in contact with the mucosa. This local tolerance is mainly controlled by intestinal macrophages and dendritic cells that adapt to the intestinal environment and

become tolerogenic phenotypes when differentiating from monocytes locally. On the other hand, this innate tolerance mechanism is somehow disturbed in certain conditions (e.g. genetic mutations), thereby leading to the development of chronic intestinal inflammation. Unraveling how malfunction of distinct genes leads to common defects in the innate immune system would advance our understanding of key pathophysiological mechanisms of intestinal inflammatory diseases common in East and West.

Disclosure Statement

G. Rogler has consulted to Abbot, Abbvie, Augurix, Boehringer, Calypso, FALK, Ferring, Fisher, Genentech, Essex/MSD, Novartis, Pfizer, Phadia, Roche, UCB, Takeda, Tillots, Vifor, Vital Solutions and Zeller, has received speaker's honoraria from Astra Zeneca, Abbott, Abbvie, FALK, MSD, Phadia, Tillots, UCB and Vifor, and has received educational and research grants from Abbot, Abbvie, Ardeypharm, Augurix, Calypso, Essex/MSD, FALK, Flamentera, Novartis, Roche, Takeda, Tillots, UCB and Zeller.

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