Impaired innate immunity in Crohn’s disease

Mònica Comalada¹ and Maikel P. Peppelenbosch²

¹ Department of Pharmacology, School of Pharmacy, University of Granada, Campus Universitario La Cartuja s/n, Granada 18071, Spain
² Department of Cell Biology, University Medical Center Groningen. University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

The aetiology of Crohn’s disease – a chronic intestinal disorder that involves an immune response against the commensal bacterial flora – remains fiercely debated. Two hypotheses exist: (i) those who think that the disease is caused by genetic defects that produce exaggerated innate responses to the flora, leading to excessive inflammation; and (ii) those who think that the genetic defects cause diminished inflammatory responses, in turn leading to uncontrolled accumulation of the inducer stimuli and, thus, activation of the adaptive immune system. Importantly, Marks and colleagues have recently investigated the immune response of Crohn’s disease patients directly, convincingly showing impaired innate immunity.

Impaired innate immunity lets the mucosal immune system go berserk

Approximately $2 \times 10^{15}$ bacteria reside in the gastrointestinal tract. This astonishing microbiological pressure represents an extraordinary challenge to the mucosal immune system, which has to perform a balancing act between appropriate responsiveness to pathogenic organisms and tolerance for harmless organisms. When this balance in mucosal immune-system responsiveness is tilted towards an exaggerated or uncontrolled reaction against the commensal flora, Crohn’s disease results. Despite many important findings in recent years, it is not fully understood why the mucosal immune response is over-reactive in patients with this inflammatory bowel disease, or how current medical treatment affects the mucosal immune system.

What has become evident is that alterations in innate immunity are crucially important for the pathogenesis of Crohn’s disease. Thus, one school of thought for the pathogenesis of Crohn’s disease postulates that predisposing mutations increase the activity of the innate-immune system, with intestinal inflammation as a consequence (Figure 1a). A second school of thought, however, sees an inadequate innate response to bacterial invasion as the main cause of Crohn’s disease. Such inadequate innate immunity would lead to the accumulation of potential immune inducers (commensal bacteria), leaving to the secondary lines of defence of the body (i.e. adaptive immunity) the task of bacterial resolution. Adaptive immunity, however, is by nature much less precisely controlled compared with innate responses. In turn, this less-regulated response produces the intestinal inflammation typical of Crohn’s disease (Figure 1b). Thus, these two mutually exclusive views coexist explaining how genetic predisposition might develop into intolerance of the intestinal commensal flora.

Importantly, in a recent study, Marks et al. [1] has compared the inflammatory responses in healthy controls with those in Crohn’s disease patients by subjecting monocyte-derived macrophages to pro-inflammatory signals and by quantifying neutrophil recruitment and cytokine production. These authors employed a highly innovative model of acute intestinal trauma (two sequential endoscopic intestinal biopsies at the same place taken six hours apart). Importantly, in the second biopsies, a lower cytokine production and an abnormally low neutrophil accumulation was observed in patients with Crohn’s disease compared with healthy controls. It is difficult to interpret these data in any other way than with the hypothesis that patients who suffer from Crohn’s disease have an impaired acute immunity. These results were further confirmed by a lower immune response induced by a local skin inflammatory challenge in the same Crohn’s disease patients when compared with healthy controls [1]. Thus, these data would strongly support the notion that a weak innate response would lead to an inadequate reaction to bacterial challenge of the mucosal barrier, provoking the accumulation of potential bacterial antigens in tissues, a more-uncontrolled secondary reaction and a chronic immune response in the gut. Therefore, it seems that the above-mentioned second school of thought prevails in the light of these data (Figure 1b).

Genetic basis of insufficient mucosal defence against intestinal bacteria

Genetics largely supports the hypothesis of insufficient bacterial immunity (Table 1). Inactivating mutations in pattern-recognition receptors of the innate-immune system that recognize evolutionary conserved pathogenic motifs are associated with Crohn’s disease [2]. Important examples are the mutations in NOD2 [3,4], which is an intracellular receptor for gram-positive and gram-negative bacteria muramyl dipeptide (MDP), and the inactivating mutations in the lipopolysaccharide receptor toll-like receptor 4 (TLR4) [5], which is an essential mediator of the innate response. Especially the role of NOD2 in Crohn’s disease has aroused wide-spread attention from both sides in the interesting debate on the underlying cause of Crohn’s disease. NOD2 mutations are mainly associated with ileal disease [6,7]. Upon binding to its cognate ligand,
NOD2 activates the nuclear factor-κB (NF-κB) pathway of pro-inflammatory transcription. In vitro studies using cell lines that were transfected with the Crohn’s disease-associated NOD2 mutants result in a loss of NF-κB activity [4]. However, Crohn’s disease is characterized by an increased NF-κB activity, prompting further studies to understand the role of NOD2 mutations in disease. Watanabe et al. [8] observed reduced responses of splenic macrophages to MDP in NOD2−/− mice but found that peptidoglycan (another conserved structural motif that is present in gram-positive bacteria) stimulation led to elevated levels of inflammatory cytokines. Importantly, peptidoglycan is the ligand of TLR2 and, thus, this finding would suggest a NOD2-mediated negative regulation of TLR signalling. Therefore, loss of NOD2 function (as a consequence of the Crohn’s disease-associated mutation) would result in increased innate immunity to bacterial stimulation, predisposing to intolerance for the commensal intestinal microbiota, with intestinal inflammation as a consequence.

Kobayashi et al. [5] suggested that a loss-of-function NOD2 allele might affect epithelial cells rather than macrophages: NOD2−/− mice developed more-severe

**Figure 1.** (a) In this model, NOD2 should downregulate TLR signalling by an unknown mechanism. The mutated NOD2 protein loses this negative regulation of TLR signalling (as a consequence of the Crohn’s disease-associated mutation), hence resulting in increased innate immunity to bacterial stimulation and predisposing to intolerance for the commensal intestinal microbiota, with intestinal inflammation as a consequence. (b) In this other model, both signalling pathways (NOD2 and TLR) are required for optimal bacterial defence. The mutated NOD2 receptor does not contribute to the pro-inflammatory gene transcription in response to bacteria, resulting in an inadequate innate response to bacterial invasion and enabling the accumulation of commensal bacteria. The poor innate response leads to the formation of the granulome and, thus, the activation and perpetuation of a deregulated secondary adaptive response. The work by Marks et al. [1] provides strong support for this hypothesis.

**Table 1. Genetic predisposition implicated in Crohn’s disease**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Gene</th>
<th>Function</th>
<th>Allelic variants</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD2</td>
<td>CARD15</td>
<td>Intracellular receptor for bacterial MDP</td>
<td>R702W G908R 1007fs</td>
<td>[3,4]</td>
</tr>
<tr>
<td>TLR4</td>
<td>TLR4</td>
<td>Extracellular receptors for bacterial PAMPs</td>
<td>A299G</td>
<td>[5]</td>
</tr>
<tr>
<td>TLR9</td>
<td>TLR9</td>
<td></td>
<td>−1237 T → C 2848 G → A</td>
<td>[14]</td>
</tr>
<tr>
<td>PPARγ</td>
<td>PPARγ</td>
<td>Nuclear receptor for lipidic compounds. Immune modulator</td>
<td>AKR</td>
<td>[15]</td>
</tr>
<tr>
<td>OCTN</td>
<td>OCTN1</td>
<td>Organic cation transporters</td>
<td>1672T −207C</td>
<td>[16]</td>
</tr>
<tr>
<td>OCTN2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CARD15, caspase recruitment domain family, member 15; OCTN, organic cation transporter; PPARγ, peroxisome proliferative activated receptor, γ; TLR, Toll-like receptor.*
infection to *Listeria monocytogenes* when the pathogen was given orally compared with systemic administration. This suggests that *NOD2* mutations are correlated with a defective bacterial sensor mechanism (diminished cryptdin expression), enabling the bacteria (commensals) to enter and accumulate the lamina propria more easily. This, together with the finding of specific NOD2 expression in intestinal crypt epithelial cells in wild-type but not in *NOD2*−/− mice, supported the hypothesis depicted in Figure 1b. Such epithelium-mediated impaired local control of pathogenic bacteria would result in an inflammatory condition, and this hypothesis is now bolstered by the data of Marks *et al.* [1]. Interestingly, Maeda *et al.* [9] observed increased interleukin (IL)-1β and other cytokine production as a consequence of increased NF-κB activity in *NOD2*−/− mice, supporting an enhanced immune reaction as a consequence of such mutations. However, Maeda *et al.* [9] also showed increased macrophage apoptosis, which is clearly an immunosuppressive effect. Irmler *et al.* [10] and others have shown that IL-1β production and macrophage apoptosis converge because both are stimulated by IL-1β-converting enzyme (ICE), which might explain the observed phenomenon on a molecular level. Thus, the work by Maeda *et al.* [9] might provide the link between defective innate immunity and a stimulation of the acquired immune system.

**Importance for clinical findings**

Such a scheme fits well with the observation that infliximab, the most-effective drug that is available for the clinical management of Crohn’s disease, exerts its action in this disease by inducing apoptosis in the T-cell compartment [i.e. by inhibiting secondary immunity rather than innate responses through direct inhibition of soluble tumor necrosis factor-α (TNF-α) bioactivity] [11]. Notably, however, an inadequate constitutive weak innate response is not inconsistent per se with a negative role for the NOD2 receptor in impairing TLR signalling, but it raises questions regarding the importance of this phenomenon for the pathogenesis of Crohn’s disease. In the light of this discussion, it is important to note that no functional differences were detected between patients with either compound heterozygous or homozygous *NOD2* mutations and patients with Crohn’s disease who did not have these mutations [1]. This shows that the diminished weak innate immunity within the set of Crohn’s disease patients is not specific to patients with *NOD2* mutations but is generally observed within this patient group. Hence, the impaired immune responses in these patients are not an epiphenomenon but probably reflect a fundamental property of the disease, and is probably causative in predisposing to Crohn’s disease. Nevertheless, in genetically identical twins, disease might be limited to one sibling and, thus, the weak immune responses observed in patients are by themselves not yet sufficient for acquiring Crohn’s disease. Other environmental factors remain essential to the pathogenesis as well.

**Concluding remarks and further questions**

An important question is whether weak innate immunity (in contrast to acquired immunity) is in general associated with the development of autoimmunity. Although this possibility has not been exhaustively tested, the available evidence is mixed. However, if a causal relationship between weak immunity and autoimmunity is relevant for other diseases in addition to Crohn’s disease, it would be limited to T helper (Th1)-type autoimmunity. For instance, in asthmatic disease – an archetypical Th2 disease – airway hyper-responsiveness shows a strict correlation with non-invasive markers of airway inflammation, which is apparently inconsistent with a role for weak innate immunity in this disease [12]. Thus, the Th1–Th2 dichotomy in responses might also be reflected in autoimmune-disease pathogenesis, with Th1-mediated autoimmunity being aggravated by a constitutively weak immune response, but Th2-mediated autoimmunity being ameliorated by such weak innate responses. Further work, however, will be essential to prove this notion.

**References**


1471-4914/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.smmed.2006.07.005