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Inflammatory signal transduction in Crohn's disease and novel therapeutic approaches

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Abstract

Although signal transduction phenomena are well understood *in vitro*, their relevance for physiology and pathophysiology remains obscure. Recent years, however, have seen a plethora of attempts to link cell biochemistry and disease. At the Luxembourg conference it was attempted to link basal science to disease. In my presentation I focused on our research on stress activated kinases and other signalling elements in this respect in a variety of pro-inflammatory conditions. Knowledge of these pathways has now led to new therapeutic approaches, especially in Crohn's disease, which will be the subject of this commentary.
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1. Crohn's disease

Crohn's disease is an inflammatory chronic disorder of the digestive tract that is usually localised in the terminal ileum and the colon [1]. Clinical features consist of abdominal pain, diarrhoea and weight loss, and the disease typically starts in early adult life [2]. The incidence of Crohn's disease has increased in the past decades, and the current prevalence in the northern part of the world is 5–8‰ [2]. Crohn's disease and ulcerative colitis are considered as the inflammatory bowel diseases. Ulcerative colitis differs from Crohn's disease with regard to genetic background, immunological and clinical features, and this condition is not further addressed in this commentary. We will here discuss briefly the present views on etiology and treatment of Crohn's disease. The current notions on the most important cellular and molecular interactions that are involved in physiological immune responses will be considered in more detail (Fig. 1).

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Abbreviations: IBD, inflammatory bowel disease; LPS, lipopolysaccharides; Th, T helper; IL, interleukin; TGF-β, transforming growth factor β; IFN-γ, interferon-γ; TNF-α, tumour necrosis factor.

2. Etiology of Crohn's disease

Accumulating evidence suggests that Crohn's disease represents the outcome of three interactive factors: host susceptibility, enteric microflora and the mucosal immune system [3]. Host susceptibility is determined by environmental and genetic factors. Potentially relevant environmental factors include smoking and diet [4]. Genetic factors associated with IBD have been extensively studied and the first susceptibility gene for Crohn's disease has recently been identified. Mutations in the NOD2 gene, which encodes a protein with homology to plant disease resistance gene products, were found in 40–50% of Crohn's disease patients [5–7]. Homozygosity for a functionally relevant mutation within this NOD2 gene confers an extremely high risk for the development of Crohn's disease, and is considered to be the causative genetic defect in about 15% of the patients. It is tempting to speculate that NOD2 is involved in the defense against certain luminal bacteria: it is expressed by antigen presenting cells, such as monocytes and dendritic cells[1] and, following stimulation by bacterial LPS, activates nuclear factor-κB, a family of signal transduction proteins that regulates expression of

[1] Braat H, unpublished results.

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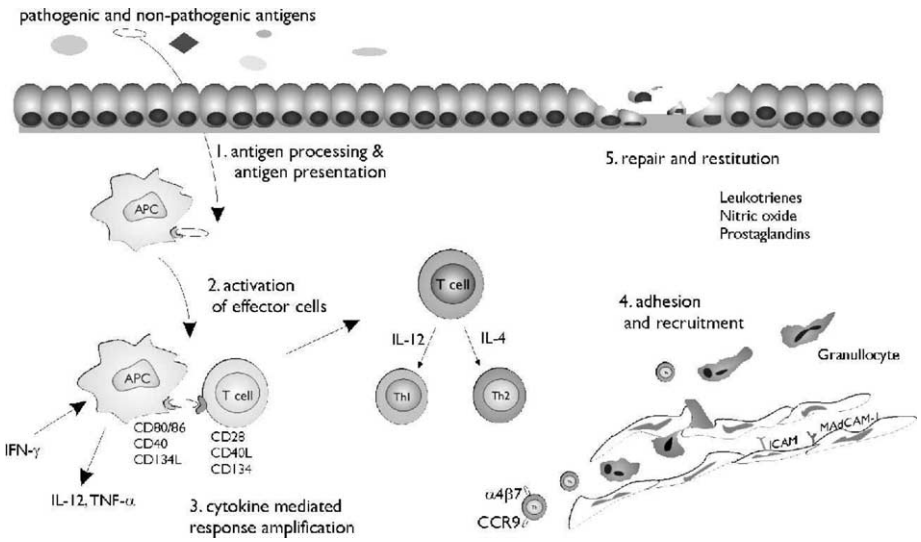

Fig. 1. Immune response in Crohn's disease and therapeutic targets for biological agents. Under inflammatory conditions, migrating dendritic cells transport antigens to regional lymph nodes. Presentation of antigens to naïve T cells in the presence of IL-12 results in the generation of Th1 effector cells that migrate into the intestine to mediate an inflammatory response. Targets for biological agents may be divided to according to the different phases of an immune response: (1) antigen and antigen presentation, (2) activation of effector T cells, (3) cytokine-mediated response amplification, (4) adhesion and recruitment, (5) repair and restitution.

many genes (i.e. TNF-α and IL-1β) involved in immune and inflammatory responses [5,8]. It is possible that early recognition of (pathogenic) bacteria is defective in Crohn's disease patients with mutant NOD2, leading to an abnormal T cell-mediated response, tissue inflammation and aberrant cytokine production. However, the precise functional consequences of the NOD2 mutations that are involved in the pathogenesis of Crohn's disease are not clear and need to be further characterised.

Many experimental and clinical studies have demonstrated that the enteric microflora is an essential cofactor in driving mucosal inflammation: germ-free mice do not develop experimental colitis [9,10], and diversion of the fecal stream following formation of a loop ileostomy has a beneficial effect in patients with Crohn's disease [11]. More direct evidence comes from studies by Duchmann *et al.*, who showed that mucosal, but not peripheral blood, $CD4^+$ T cells from IBD patients proliferate when exposed to autologous intestinal bacteria [12,13].

The mucosal immune system is activated upon exposure to a pathogenic microorganism. Initially, innate immune cells, such as macrophages, dendritic cells and natural killer cells are recruited to eliminate pathogens by uptake and destruction or degradation [14]. Macrophages and dendritic cells are activated and can present the products of pathogen degradation to antigen reactive T cells resulting in an adaptive immune response. T cells, in particular $CD4^+$ T cells, play multiple roles as effector and regulatory cells in intestinal inflammation: a subset of naïve $CD45RB^{high}$ $CD4^+$ cells [15] has been shown to induce experimental colitis after transfer to severe combined immunodeficient (SCID) mice [16]. In addition, the number of activated mucosal T cells secreting pro-inflammatory cytokines that mediate interactions between cells, such as IFN-γ, is increased in active Crohn's disease [17,18]. Finally, $CD4^+$ cell depletion ameliorates inflammation in experimental colitis [19] and in Crohn's disease [20]. By comparison, whereas $CD8^+$ cells are important in first line defence in the epithelium [21], they do not appear to play a decisive pathologic role in intestinal inflammation [22,23].

Based on these observations, the current working model of the pathophysiology of Crohn's disease is that of an uncontrolled abnormal immune response of $CD4^+$ cells to enteric bacteria in genetically susceptible individuals.

3. Therapy of Crohn's disease

Currently there is no curative treatment for patients with Crohn's disease. For several decades corticosteroids were the only potent anti-inflammatory agents available for treatment of active Crohn's disease [24]. Corticosteroids act rapidly and have a wide range of anti-inflammatory activities, but do not alter the natural course of the disease, do not consistently heal intestinal ulcers, and are associated with many side effects [25]. The use of immunomodulatory therapy, as azathioprine and methotrexate has provided alternative therapeutic options [26–28]. However, the administration of these agents is limited by inconstant efficacy, a relatively slow onset of action, inadequate selectivity and substantial short- and long-term toxicity.

New biological therapeutic strategies are based on the increased knowledge of the pathophysiology of Crohn's disease. Biological agents include recombinant cytokines, cytokine receptor antagonists, chimeric and humanised antibodies, antisense oligonucleotides and viral as well

as non-viral vectors for gene transfer. Presently, of this growing range of biologic therapies antagonism of TNF-α with the monoclonal TNF-α antibody infliximab is the only approach that has been approved for clinical use. Infliximab is effective and well tolerated in patients with moderate to severe Crohn's disease that is resistant to conventional treatment [29] and can maintain remission up to 44 weeks if administered by repeated intravenous infusions [30]. The rapid healing of enterocutaneous fistulas is a good example of the anti-inflammatory activity of infliximab in Crohn's disease [31]. Traditional therapeutic agents have limited mucosal healing effects, and in the older studies endoscopic severity of ileocolonic Crohn's disease did not correlate well with therapeutic effects as measured by clinical parameters. However, the clinical improvement following infliximab therapy has been consistently associated with mucosal healing [32,33]. The long-term efficacy and safety as well as the precise mode of action of infliximab are currently under investigation. However, because of the immunogenicity of monoclonal antibodies and risk of side effects such as opportunistic infections and malignancy, infliximab may be best suited as a bridge to more long-term maintenance strategies. Furthermore, gene therapy has emerged as a new and promising method for maintenance of remissions in chronic inflammatory diseases, but its clinical application is still far away.

4. Intestinal immune response in physiological conditions: peripheral tolerance

Although the intestinal epithelium forms a physical barrier for pathogens, bacterial antigens are continuously sampled by gut mucosal dendritic cells, the major intestinal antigen presenting cells [34], and presented to T cells [35]. The mucosal immune system needs to discriminate pathogens from dietary antigens and commensal bacteria present in the gut lumen. Indeed, a large pool of immune cells is present within the mucosal epithelium and lamina propria. The continuous presence of potential pathogens in close proximity to immune cells must be tightly controlled to ensure that pathologic inflammation does not develop. Control is provided by mechanisms of tolerance. The concept of tolerance includes, by definition, any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a non-injurious class of immune response [36]. Central tolerance refers to thymic deletion of self-reactive cells, which is not complete and self-reactive cells escape to the periphery. Several mechanisms are involved in maintaining peripheral tolerance, i.e. *anergy* (functional inactivity), *apoptosis* (programmed cell death) and the involvement of *tolerogenic dendritic cells* or *regulatory T cells* [37]. These mechanisms can interfere during all phases of the immune response and are probably all necessary to control (1) self-reactive cells, (2) cells reactive to non-pathogenic microorganisms and (3) cells that become activated following a normal immune response to a foreign antigen.

5. Physiological immune response to pathogens

The intestinal mucosa contains a large number of dendritic cells for uptake, processing and transport of antigens to the regional lymph nodes. Dendritic cells critically steer immune responses through interactions with T cells, requiring close proximity and crosstalk of these two cell types. The decision between immunity and tolerance is taken by dendritic cells: they induce the development of naïve T cells into effector or regulatory T cells, or can induce anergy/apoptosis of T cells [38–40].

Dendritic cells express pattern recognition receptors (e.g. Toll-like receptors) [41] that recognise pathogen-associated molecules (e.g. LPS) from invading microbes. Such activation of pattern recognition receptors generally increases the immune-stimulatory capacities of dendritic cells. After antigen uptake, activated and maturing dendritic cells start to express the chemokine receptor CCR7. Dendritic cells migrate to the lymph nodes, where specialised high endothelial venules express the CCR7 ligand CCL2 [42]. CCR7 is also present on naïve T cells [43,44] and directs, together with the lymph node homing receptor L-selectin [45] the migration of naïve T cells to the regional lymph nodes. The passage across the high endothelial venules is a multistep process that involves selectin-supported rolling, followed by a triggering event, and firm integrin-mediated adhesion [46,47]. Chemokines are a family of leukocyte chemoattractants that regulate both inflammatory cell recruitment and homeostatic trafficking of leukocytes.

When dendritic cells present antigens to naïve T cells in a lymph node, the T cells differentiate into effector/memory $CD4^+$ or cytotoxic $CD8^+$ cells. Based on their cytokine secretion profile, $CD4^+$ T cells differentiate into at least two subsets of helper cells, Th1 and Th2 cells [48]. Th1 cells produce the pro-inflammatory cytokines IL-2 and IFN-γ and protect against intracellular pathogens (cellular response), whereas Th2 cells selectively secrete IL-4, IL-5 and IL-13, and counter extracellular pathogens through production of antibody by B cells (humoral response). Although both subsets of T helper cells can also produce the two pivotal immunoregulatory cytokines, IL-10 and TGF-β, regulatory T cells, which are discussed more fully below, are the main producers of these cytokines. The cytokine microenvironment to which naïve T cells are exposed is the primary determining factor for the differentiation towards a Th1 or Th2 cell type [49]: IL-12 with support of IL-18, both produced by antigen presenting cells, induce Th1 differentiation [50,51] and IL-4, produced by NK cells, mast cells, basophils and mature $CD4^+$ cells [52], drives Th2 differentiation [53]. Others factors

such as antigen density [54], major histocombatibility complex haplotypes [55], type of antigen presenting cell [56], and costimulatory factors [57] also influence the selective development of Th1/Th2 cells and regulatory T cells.

After local priming of T cells in the presence of—high levels of—IL-12 and IL-18, the differentiated effector Th1 cells and memory T cells recirculate preferentially to the intestinal mucosa. The integrin α4β7, expressed on these T cells, is the principal gut homing receptor and functions at several steps in the adhesion cascade by interacting with the mucosal addressin MAdCAM-1 present on endothelial cells [58,59]. In addition, the chemokine TECK and its receptor CCR9 are implicated in the migration of $\alpha4\beta7^{+}$ memory T cells to the small intestine [60]. Once arrived in the intestine the Th1 effector cells mount an inflammatory response on encounter of the antigen, and this response can be amplified by freshly recruited immature dendritic cells. After elimination of the pathogen, the bulk of the effector T cells become redundant and most of them disappear, in large part through apoptosis. However, a small proportion survives to become long-lived memory cells [61]. Thus, whereas induction of anergy and apoptosis serve to reduce the number of T cells that can respond to mucosal antigens, this mechanism is not sufficient for elimination of all potentially reactive T cells. Cells that escape these mechanisms of peripheral tolerance need to be controlled by tolerogenic dendritic cells and/or regulatory T cells.

6. What is wrong with the mucosal immune system in Crohn's disease?

Clinical and experimental studies have provided evidence for several defects of the mucosal immune response in Crohn's disease. Based on the predominance of IL-2 and IFN-γ secreting mucosal T cells and the specific expression of Th1 driving cytokines IL-12 and IL-18 in the intestinal mucosa, Crohn's disease is considered to be a prototype Th1 disease [18,62–64]. Apparently, mechanisms of peripheral tolerance are not effective in down-regulating this Th1 cell-mediated inflammation, and two studies have reported that mucosal T cells in patients with Crohn's disease are resistant to apoptosis [65,66]. Our laboratory has recently demonstrated that the number of dendritic cells producing IL-12 and IL-18 in the intestinal mucosa is increased in patients with Crohn's disease, indicating the enhanced activation of the immune system in the intestine.[2]

In the intestinal mucosa of healthy individuals, $CD4^{+}$ cells have been shown to contribute to down-regulation of effector T cells, via production of IL-10 and TGF-β [67]. Conflicting reports exists concerning suppressor/regulatory cell activity by lamina propria cells [68,69] and peripheral blood mononuclear cells [70,71] in Crohn's disease patients. Crohn's disease is characterised by a relatively reduced production of bioactive IL-10 in the lamina propria, and the resulting IL-10 concentrations seem insufficient to down-regulate pro-inflammatory cytokines [72,73]. A low ileal IL-10 concentration is associated with early endoscopic recurrence of disease after surgery in patients with Crohn's disease [74]. Our laboratory has recently found that mucosal memory ($CD45RB^{low}$ $CD45RO^{+}$) $CD4^{+}$ cells from patients with Crohn's disease are less abundant than in controls and produce less IL-10 [75]. Conversely, increased TGF-β1 expression was found in affected mucosa of patients with active Crohn's disease [76,77], and it was shown that mucosal T cells from IBD patients were insensitive to the regulatory effects of TGF-β because they overexpress SMAD7, an inhibitor of TGF-β signalling [78].

These data suggest that in Crohn's disease several defects in the mechanisms of peripheral tolerance, including resistance to T cell apoptosis and disrupted regulatory T cell function can exist leading to a final common pathway of Th1-mediated intestinal inflammation. It is logical to assume that interference with underlying signalling pathways will have clinical benefit in Crohn's disease.

7. MAP kinases

MAP kinases are a family of evolutionary well-conserved proteins that are expressed in all cells, and regulate survival, growth and inflammation. MAP kinases are activated by phosphorylation of both a threonine and serine residue and subsequently phosphorylate downstream kinases and transcription factors. This lead to altered transcription of genes that regulate growth, apoptosis or inflammation, or altered post-transcriptional events like translation or mRNA degradation. In mammalian cells, three main MAP kinase pathways are of importance, i.e. p38 MAP kinase, p42/p44 MAP kinase and the c-Jun-N-terminal kinase. Of these, the p38 MAP kinase pathway has attracted most attention as a therapeutic target, because it was shown to mediate cellular responses to stress, induced by signals such as cytokines and bacterial components. Moreover, the p38 MAP kinase pathway is activated in LPS-infused human volunteers [79] and blocking p38 MAP inhibitors in such volunteers strongly reduced clinical symptoms [80].

More recently, the other MAP kinases have been reported to mediate inflammation and we investigated whether inhibition of MAP kinases was beneficial in Crohn's disease. Inhibition of JNK and p38 MAPK activation with CNI-1493, a guanylhydrazone, was tested in 12 patients with severe Crohn's disease. Colonic biopsies displayed enhanced JNK and p38 MAPK activation. CNI-1493 inhibition of both JNK and p38 phosphorylation was observed *in vitro*. Treatment resulted in diminished JNK phosphorylation and TNF production as well as

[2] te Velde A, unpublished results.

significant clinical benefit and rapid endoscopic ulcer healing. No serious adverse events were noted. A CDAI decrease of 120 at week 4 ($P = 0.005$) and 146.5 at week 8 ($P = 0.005$) was observed. A clinical response was seen in 67% of patients at 4 weeks and 58% at 8 weeks. Clinical remission was observed in 25% of patients at week 4 and 42% at week 8. Endoscopic improvement occurred in all but one patient. Response was seen in three of six infliximab failures, two of which showed remission. Fistulae healing occurred in four of five patients, and steroids were tapered in 89% of patients [81]. In conclusion, knowledge of signal transduction pathways has now led to the realisation that inflammatory MAPKs are critically involved in the pathogenesis of Crohn's disease and their inhibition provides a novel and powerful therapeutic option.

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