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Therapeutic potential of dinitrobenzene sulfonic acid (DNBS)-induced colitis in mice by targeting IL-1β and IL-18

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ABSTRACT

Interleukin (IL)-1 and IL-18 belong to the IL-1 family of ligands, and their receptors are members of the IL-1 receptor family. Both cytokines drive an extensive range of pro-inflammatory networks in many cell types using common signal transduction cascades. Anyway, differences in signaling pathways exist. With this aim in mind, we investigated by using transgenic mice the mechanisms through the simultaneous deficiency of both IL-1β and IL-18 could be more protective compared to blocking the single cytokine IL-1β or IL-18 during colitis.

Colitis was provoked in mice by instillation of dinitrobenzene sulfonic acid (DNBS) in the colon. The results indicated that single knockout (KO) mice of IL-1β or IL-18, and double KO mice of both IL-1β and IL-18 were hyporesponsive to DNBS-induced colitis compared to wild type (WT) mice, in which double KO were less sensitive than single KO mice. Moreover, treatment with Anakinra (IL-1R antagonist) also ameliorated colitis, in views of macroscopic and histological alteration, infiltration of neutrophils or Th1 cells, oxidative and nitrosative stress. Anakinra more significantly reduced cyclooxygenase (COX-2) and nuclear factor (NF-κB) levels as well as IKB-α degradation compared to blocking IL-18. On the contrary, the absence of IL-18 reduced p-ERK and p-p38 mitogen-activated protein kinase (MAPKs) in a more significant way compared to blocking IL-1β. Thus, the double KO increased the protective effects against colon inflammation maybe because different converging inflammatory pathways are being inhibited.

In conclusion, the blocking of both IL-1β and IL-18 function may be advantageous in the treatment of IBD or inflammatory diseases.

1. Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBDs) characterized by chronic inflammation of the gastrointestinal tract [1]. A disproportion of $CD4^+$ Th1 versus type 2 Th2 polarization in favor of Th1 cell subclasses appears to be a crucial pathogenic mechanism in IBD. This concept is sustained by reports of mucosal biopsies in IBD patients, proving an increased expression of proinflammatory cytokines, chemokines, and adhesion molecules [2]. The development of animal models for IBD has provided confirmation that immune dysregulation and altered cytokine secretion may have a role in the pathology of IBD [3]. Several animal models of IBDs have been advanced, between these, the model of colon inflammation induced by intrarectally injection dinitrobenzene sulfonic acid (DNBS) to normal mice, presents human Crohn's disease-like qualities, particularly prevalent nuclear factor κB (NF-κB)-dependent Th1 activation [4].

Two cytokines that are important factors in the inflammatory cascade are interleukin (IL)-1 and IL-18 [5]. Both cytokines drive an extensive range of pro-inflammatory networks in many cell types using common signal transduction cascades. In human UC enhanced production of IL-1 in the colorectal mucosa of patients has been found [6].

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis; IBDs, inflammatory bowel diseases; IL, interleukin; DNBS, dinitrobenzene sulfonic acid; NF-κB, nuclear factor κB; IFN, interferon; COX, cyclooxygenase; TNF, tumor necrosis factor; LPS, lipopolysaccharide; RA, rheumatoid arthritis; MPO, myeloperoxidase; MDA, malondialdehyde; PAR, poly-ADP-ribose; WT, wild-type; KO, knockout

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In addition, it was also reported that IL-18 is prominent at sites of inflammation in IBDs, especially in Crohn's disease, proposing a possible role for IL-18 in the persistence of IBD [7]. Thus, these cytokines are primary in acute and chronic animal models of inflammation including IBDs, and their blockade has been showed to ameliorate disease in preclinical studies. The complex relationship between different cytokines can offer another tier of immune regulation, either by providing synergy or counterregulation. For IL-18, it is well-defined that more profound Th1 responses can be caused when there is synergy with IL-12. In particular, production of interferon (IFN)-γ by T cells is intensified by stimulation with IL-12 and IL-18 together compared to cytokine alone [8]. IL-18 and IL-1 are supposed to regulate each other by both stimulating the activation of functional interleukin 1 converting enzyme (ICE or caspase 1), which in turn controls the downstream production of matured IL-1 and IL-18 [8]. Both nuclear factor (NF-κB) and mitogen-activated protein kinase (MAPKs) activation are involved in the signaling pathway of IL-1β/IL-18 [9]. Furthermore, differences in signaling pathways between IL-1 and IL-18 could exist. In particular, unlike IL-1, IL-18 itself does not induce cyclooxygenase-2 or weakly [10] so because of an evident inability of NF-κB activation [11]. Nevertheless, IL-18 remains an inflammatory cytokine that may favorably activate p38-MAPK and AP-1 pathways [11]. In addition, different studies on immune-competent cells reported that a costimulant is essential for IL-18 signal transduction and high concentrations of IL-18 are needed. Instead, IL-1 activates a variety of cells and does not require costimulants and is functional in the low picomol range [10].

Anti-inflammatory and immunosuppressive corticosteroids are used to treat acute-phase signs of IBDs, however there is a high therapeutic need for new strategies in the treatment of these autoimmune disorders. Biological agents that target IL-1 have revealed efficiency in patients with rheumatoid arthritis (RA), and extra agents targeting IL-1 or IL-18 neutralisation are in clinical development. A synthetic form of IL-1Ra, anakinra (Kineret®), is a recombinant and non-glycosylated form of human IL-1Ra that has been approved for use in arthritis by the Food and Drug Administration (FDA). Anakinra employs its physiological effects in a comparable manner to the naturally antagonist, by competitively binding to the IL-1R and neutralizing the effects of IL-1β [12]. Previous work showed that treatment with Anakinra ameliorated colitis inflammation in mice and humans [13]. Although anti- (tumor necrosis factor, TNF) treatment is now used positively in clinics, a considerable proportion of IBD patients do not react to this treatment. Thus, it will be important to also consider the efficiency of cytokines combination therapies using for example anti-IL-1 reagents together with anti-IL-18 and/or anti-IL-12 [7]. Interestingly, deficiency of both IL-1β and IL-18 (in IL-1β/IL-18 double knockout KO mice) additively prevented lipopolysaccharide (LPS)-induced mortality [14]. The harmful role of IL-1β and IL-18 was also established in mice exposed to a lethal dose of TNF, or to a lethal cecal ligation puncture (CLP) procedure [14]. In line with results from genetically deficient mice, only the combined neutralization of IL-1 and IL-18, using "anakinra" an IL-1 receptor antagonist and anti–IL-18 antibodies, conferred whole protection against endotoxin-induced lethality [14]. Furthermore, numerous studies have disclosed that by blocking the actions of IL-1 or IL-18 either by pharmacological inhibition or genetic deletion, it is possible to moderate disease parameters in hypertension [15]. Therefore, alternative treatment strategies in IBD should also be valued with regard to their influence on the innate immune system.

Based on these findings, the aim of this work was to better investigate the mechanisms through the simultaneous deficiency of IL-1β and IL-18 could be more protective and could have a synergistic effect compared to blocking the single cytokine IL-1β or IL-18 in the pathogenesis of Th1 mediated experimental colitis.

Thus, we proposed genetic studies using IL-18 KO, IL-1β/IL-18 double KO and wild-type (WT) mice, and we used an antagonist of IL-1β receptor such as anakinra (Kineret®), that is used to treat patients with RA and other autoimmune diseases [16].

2. Materials and methods

2.1. Animals

IL-1β KO and IL-1β/IL-18 double KO mice were obtained from Arturo Zychlinski (Max Planck Institute, Berlin, Germany) while IL-18 KO mice from (The Jackson Laboratory, Bar Harbor Maine USA) and control WT C57BL/6 mice were purchased from Charles River (Italy).

Animals were stored in a well-ordered environment with standard rodent chow and water. Mice were kept in stainless steel cages in an area at 22 ± 1 °C with a 12-h light–dark cycle. The mice were adapted for 1 week with libitum access to rodent standard diet and tap water. The study was permitted by the University of Messina Review Board for the care of animals. All animal experiments were conformed with regulations in Italy (D.M. 116,192 and Europe (O.J. of E.C. l 358/1 12/18/1986).

2.2. Colitis induction

Colitis was provoked in mice by an intrarectally injection of DNBS as previously indicated [17]. Briefly, isoflurane anesthetized mice. DNBS (4 mg in 100 μl of 50% ethanol per mouse) was instilled into the rectum within a catheter introduced 4.5 cm proximally to the anus. On day 4 after DNBS injection, the colon was removed and processed for all analyses.

2.3. Experimental groups

Animals were arbitrarily divided into none groups (n = 10 for each group):

- Sham WT + saline: vehicle solution (saline) was intraperitoneally (ip) administered for 4 days.
- Sham WT + Anakinra (Kineret®): Anakinra (10 mg/kg) dissolved in saline was administered ip for 4 days.
- Sham IL-18 KO + saline: saline was administered ip for 4 days.
- Sham IL-1β/IL-18 KO + saline: saline was administered ip for 4 days.
- DNBS WT + saline: mice were subjected to DNBS administration described as above, and saline was administered ip every 24 h, for 4 days, starting from 1 h after the administration of DNBS.
- DNBS WT + Anakinra: mice were subjected to DNBS administration described as above, and Anakinra (10 mg/kg) was administered ip every 24 h, for 4 days, starting from 1 h after the administration of DNBS.
- DNBS IL-18 KO + saline: mice were subjected to DNBS administration described as above, and saline was administered ip every 24 h, for 4 days, starting from 1 h after the administration of DNBS.
- DNBS IL-1β/IL-18 KO + saline: mice were subjected to DNBS administration described as above, and saline was administered ip every 24 h, for 4 days, starting from 1 h after the administration of DNBS.
- DNBS IL-1β KO + saline: mice were subjected to DNBS administration described as above, and saline was administered ip every 24 h, for 4 days, starting from 1 h after the administration of DNBS (data not shown).

The dose of Anakinra was chosen based on a previous work [13]. The data obtained from genetic deficiency of IL-1β by using IL-1β KO mice were equivalent to that obtained from pharmacological inhibition of IL-1 by using Anakinra and for this reason were not shown. In addition, because there was no significant difference between all sham groups, only data obtained from sham WT mice were showed.