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Review

The TNBS-induced colitis animal model: An overview

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HIGHLIGHTS

- We sought to present the animal model of TNBS induced colitis and outline the pathogenesis, pathophysiology, clinical course and pathological characteristics of the model.
- Furthermore, we describe the differences among those protocols regarding types of animals and colitis induction.
- The MEDLINE database was thoroughly searched using the keywords: TNBS, colitis, Crohn's disease, animal model.
- Two investigators independently reviewed the abstracts and appropriate articles were included in this review.
- The aim of this study was to thoroughly present an updated review of the TNBS-induced colitis protocols that are implemented by researchers.

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ABSTRACT

Background: Despite recent advances the pathogenesis of Crohn's disease remains incompletely understood. A variety of animal models have been utilized in an effort to provide further insights and develop more therapeutic options. In order to simulate, to an extent, the pathogenesis and the clinical course of the disease, TNBS induced colitis is often used. Various approaches for inducing TNBS -colitis have been described in the literature.

Methods/results: In this review, we sought to present the animal model of TNBS induced colitis and outline the pathogenesis, pathophysiology, clinical course and pathological characteristics of the model. Furthermore, we describe the differences among those protocols regarding types of animals and colitis induction.

Data sources: The MEDLINE database was thoroughly searched using the keywords: TNBS, colitis, Crohn's disease, animal model. Two investigators independently reviewed the abstracts and appropriate articles were included in this review. Additional articles were gathered and evaluated.

Conclusion: The aim of this study was to thoroughly present an updated review of the TNBS-induced colitis protocols that are implemented by researchers.

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1. Introduction

Crohn's disease is a chronic, relapsing immunologic disorder that primarily affects the gastrointestinal tract [1] The estimated incidence of the disease approximates 20.2 cases per 100.000 persons in Northern Europe and USA and it should be emphasized that incidence depends on the exact geographic region. In the modern era, intense research has provided insight on the pathogenesis of the disease and the current understanding relates Crohn's to a dysregulated immune response towards gut microbiota in genetically predisposed persons. Despite recent advances, the exact pathogenesis remains not well defined. Thus, Crohn's disease is still an incurable, life-long disease that warrants better understanding and more efficient treatment.

While clinical research findings are more easily extrapolated and integrated to clinical practice, basic research and especially suitable animal models have provided valuable insights to the molecular level and have allowed researchers to manipulate genetic factors in order to study their role. However, conventional animal models that are not so sophisticated, like the modern genetically engineered rodents that develop spontaneous disease, seem to still carry a significant role in studying the pathways that lead to overt Crohn's disease. Among them, hapten reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis introduced in 1989 by Morris et al. bears a pivotal role especially in the preclinical testing of various chemical or natural compounds in terms of their anti-inflammatory and/or anti-oxidative effects. Briefly, TNBS colitis belongs to the group of chemically induced colitis animal models that includes among others DSS colitis, and oxazolone colitis [2–5]. Those three are the most commonly utilized animal models of that category and have shown a significant consistency that reflects to their extensive use during the last decades.

Since the first report of the model in 1989, a plethora of variations and modifications in technical aspects of the main protocol have been described. Our aim was to search meticulously, identify and present all variations of TNBS-induced colitis and further discuss their impact. This review could be of significant benefit for the researcher who plans to perform an experiment involving TNBS–induced colitis as well as for the scientist who needs a critical appraisal of the different protocols utilized in literature.

1.1. Pathogenesis

The exact mechanisms that mediate pathogenesis in that particular model remain elusive. As it has already been emphasized TNBS colitis does not recapitulate the aetiopathogenesis of Crohn's disease. However, the relevance of the model to Crohn's disease in terms of pathogenesis is evident by the involvement of NOD2 (a key CD susceptibility gene) in the pathogenesis of TNBS colitis [6]. Specifically, the administration of plasmid carrying intact NOD2, but not plasmid carrying associated frame-shift-mutated NOD2, makes mice more resistant to this colitis [7].

1.2. Pathophysiology of TNBS-induced colitis

Based on the original report by Morris et al. [8] ethanol and TNBS (Trinitrobenzenesulfonic acid) at a dose of 100 mg/kg are co-administered intra-rectally to rats. Ethanol is used as a means to effectively disrupt intestinal barrier and enable the interaction of TNBS with colon tissue proteins [9,10]. Trinitrobenzenesulfonic acid, a classical skin contactant serves as a hapten and as Little et al. suggested as early as 1966 [11], when coupled with proteins with high molecular weight can elicit significant immunologic responses by rendering those proteins immunogenic to the host immune system. A single administration of the combined substances leads to the development of an excessive cell mediated immune response reflected by acute Th1 inflammation [12].

The phenotype of Th1 inflammation includes a dense colonic tissue infiltration by CD4 T cells and the secretion of various potent pro-inflammatory cytokines [13]. The most characteristic cytokines in that network include TNF-a and IL-12 [14]. The clinical importance of anti-IL-12p40 mAbs in the treatment of colitis was suggested for the first time in 1995 after implementing a TNBS colitis model [9]. That particular study triggered physicians almost 10 years later to conduct a human trial of anti-IL-12p40 mAb therapy in patients with active disease [15]. Interestingly, while colitis developed in the TNBS model has been shown to be mediated and exacerbated by IL-12p70, this is not the case in other IBD models [16]. Likewise, while IL-23 receptor (IL-23R) is known as a major IBD susceptibility gene [17,18] in the TNBS model the same receptor seems to exert a protective role [19].

Alternatively, IL-23 serves as the key factor of maintaining Th17 cells and inducing IL-17-producing innate lymphoid cells [20]. IL-17 receptor KO mice are shown to resist against TNBS colitis. Recent findings point towards a prominent role of Th17 cells in the pathogenesis of TNBS-induced colitis. Specifically, absence of the IL-17 receptor renders mice resistant to trinitrobenzene sulfonic acid (TNBS)-induced acute colitis. In an acute trinitrobenzenesulfonic acid model of colitis, Zhang et al. [4] showed that IL-17 protein levels are increased in wild-type mice treated with TNBS. Il-17R$^{(-/-)}$ mice that were treated with TNBS developed only mild inflammation with less infiltration from neutrophils, and a reduction in the amount of weight loss compared to wildtype mice. Measurement of IL-6 protein levels revealed a reduction in the amount of IL-6 produced in Il-17R$^{(-/-)}$ mice substantiating the lack of IL-17 signaling. These data reflect the importance of IL-17 signaling in the development of acute colitis in mice treated with TNBS. In the same molecular context, IL-23 a cytokine that is secreted by Th17 as well as mucosal T follicular helper cells (Tfh) seems to exert a significant protective role. In the histopathological level, the prominent feature of TNBS-colitis includes the