



**HAL**  
open science

## Protective effect of intraluminal nitric oxide in experimental colitis in rats

Florence Lamine, Vassilia Theodorou, Pauline Anton, Sabine Roy, Rafael Garcia Villar, Jean Fioramonti, Lionel Bueno

► **To cite this version:**

Florence Lamine, Vassilia Theodorou, Pauline Anton, Sabine Roy, Rafael Garcia Villar, et al.. Protective effect of intraluminal nitric oxide in experimental colitis in rats. Digestive Disease Week / 101th Annual meeting of the AGA, May 2000, San Diego, California, United States. 2000, Gastroenterology 118 (4 part 2). hal-01600592

**HAL Id: hal-01600592**

**<https://hal.science/hal-01600592v1>**

Submitted on 4 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

4229

**ON THE MECHANISMS UNDERLYING THE PROTECTIVE ROLE OF M-CSF DEPENDENT MACROPHAGES IN EXPERIMENTAL COLITIS IN MICE.**

Francesca Galeazzi, Cory M. Hogaboam, Bruce A. Vallance, Stephen M. Collins, Univ of Padova, Padova, Italy; Univ of Michigan, Ann Arbor, MI; McMaster Univ, Hamilton, ON, Canada.

Macrophages play a critical role in host defence and cell-mediated immunity. Previously, we have shown that the absence of infiltrating macrophages resulted in a more severe colitis induced by hapten in mice. However, the mechanisms underlying this unexpected finding are unknown. In the present study, we explored this observation by studying colitis in mice lacking mature macrophages as a result of a spontaneous mutation in the macrophage-colony stimulating factor (M-CSF) encoding gene. Macrophage-deficient mice (*op/op*), as well as their M-CSF expressing littermates (*op/+*), received 3 mg of the hapten dinitrobenzenesulfonic acid (DNBS) in 50% ethanol intrarectally. Mice were euthanized at days 1 and 3 post colitis and were evaluated for: macroscopic damage of the colon, for myeloperoxidase activity (MPO), for the presence of F4/80+ve macrophages by immunohistochemistry, and for levels of the chemokine MIP-1 $\alpha$ , which is involved in the recruitment of inflammatory. In parallel experiments, mice received daily intraperitoneal injections of human recombinant M-CSF (hr M-CSF) prior to and during the colitis. In *op/+* mice, DNBS-induced colitis was accompanied by an abundant transmural macrophagic infiltrate, while in M-CSF deficient mice only a few F4/80+ cells were evident. The severity of colitis was substantially greater in M-CSF deficient mice compared to *op/+* mice at both days 1 and 3 post colitis (macroscopic score day 1 in *op/+* mice = 4.4+0.9, in *op/op* mice = 7.2+0.8; at day 3 in *op/+* mice = 3.3+0.5, in *op/op* mice = 7.3+0.5). Also, MPO activity was significantly higher in *op/op* mice at day 1 post colitis (*op/+* mice = 1.9+0.3, *op/op* mice = 3.7+1.0). Colitis was accompanied by an increase in MIP-1 $\alpha$  levels, which were 10 times higher in *op/op* mice compared to *op/+* mice at both days 1 and 3 post colitis. Treatment with hrM-CSF restored the macrophage infiltrate, reversed the increase in MPO activity observed in *op/op* mice, and ameliorated the macroscopic damage at day 3 but not at day 1 post colitis. Thus, in the absence of macrophages, there is a compensatory local overproduction of MIP-1 $\alpha$ , leading to an exaggerated neutrophilic infiltration and a greater degree of colonic damage. The ability of hrM-CSF to improve damage in *op/op* mice on day 3, but not on day 1 post colitis, indicates that additional factors are required for the early influx of macrophages to the inflamed bowel. Supported by MRC Canada.

4230

**DIFFERENCES IN MRNA STABILITY DETERMINE THE KINETICS OF ENA-78 AND IL-8 PROTEIN PRODUCTION BY CACO-2 CELLS.**

Andrew C. Keates, Sarah Keates, Michel Warny, Ciaran P. Kelly, Beth Israel Deaconess Med Ctr & Harvard Med Sch, Boston, MA.

Background and Aim: Cytokine-stimulated Caco-2 intestinal epithelial cells produce the neutrophil-activating C-X-C chemokines ENA-78 and IL-8. We have reported previously that ENA-78 production by Caco-2 cells is delayed and markedly prolonged compared to IL-8. This is associated with a delayed and prolonged increase in steady state ENA-78 mRNA levels. The aim of this study was to determine whether the different kinetics of expression of these chemokines by intestinal epithelial cells is transcriptionally regulated. Method: ENA-78 and IL-8 luciferase reporter constructs were prepared, and transfected into Caco-2 cells stimulated with IL-1b (25ng/ml, for 0-6 hr) to examine the kinetics of ENA-78 and IL-8 gene transcription. NF- $\kappa$ B is the main transcription factor regulating both ENA-78 and IL-8 gene expression. Hence, electrophoretic mobility shift assays (EMSA) were performed to examine the time course of NF- $\kappa$ B activation and binding to ENA-78- and IL-8-specific kB probes. To determine the half-life of ENA-78 and IL-8 mRNA, IL-1 $\beta$ -treated Caco-2 cells (25ng/ml; 3h) were incubated with actinomycin D (10 $\mu$ g) for 0-8h. The fraction of mRNA remaining at each time point was then assessed by RT-PCR. Results: In IL-1b-treated Caco-2 cells, ENA-78 reporter gene activity was elevated after 3 h and maximal at 6 h (11-fold induction). An identical time course was seen using the IL-8 reporter but with greater maximal reporter gene activation (36-fold). Similarly, EMSA showed binding of activated NF- $\kappa$ B to the ENA-78- and IL-8-specific probes from 30 min to 6 h after IL-1b stimulation. Analysis of mRNA stability indicated that the  $t_{1/2}$  for IL-8 mRNA decay was 45 min. In contrast, under the same experimental conditions, ENA-78 mRNA was substantially more stable with a  $t_{1/2}$  of > 8 h. Summary and Conclusion: Our findings indicate that the differential regulation of ENA-78 and IL-8 protein production in Caco-2 cells is not due to differences in NF- $\kappa$ B activation and nuclear binding or in NF- $\kappa$ B-regulated gene transcription. Instead, we find that ENA-78 mRNA is remarkably stable, whereas, IL-8 mRNA is unstable. This novel finding indicates a post-transcriptional mechanism (mRNA stability) for the prolonged production of ENA-78 by IL-1 $\beta$ -stimulated Caco-2 cells. This mechanism may also account for the relative abundance of ENA-78 mRNA and protein observed in normal human colonic epithelial cells and their up-regulation in chronic ulcerative colitis.

4231

**INTESTINAL TREFOIL FACTOR INDUCES IMMUNE AND INFLAMMATORY RESPONSE: UPREGULATION OF IL-8 AND IL-1B IN EPITHELIAL AND MONOCYTTIC CELLS VIA NF $\kappa$ B.**

Koichi K. Kinoshita, Daniel K. Podolsky, MA Gen Hosp, Harvard Med Sch, Boston, MA.

Background : Intestinal trefoil factor (ITF) is the most widely expressed peptide of the trefoil factor family. It is especially abundant at sites of ulceration including those associated with peptic ulcer and inflammatory bowel disease throughout the small and large intestinal mucosa. Trefoil peptides play a critical role in mucosal protection against injurious chemical and biological agents and enhance epithelial restitution after injury occurs. Recently, inflammatory cytokine and chemokine gene upregulation via NF $\kappa$ B transcription factor following mucosal injury has been reported. This study was undertaken to examine effects of exogenous ITF on NF $\kappa$ B signal transduction pathways in epithelial cells and monocytes. Methods: Human ITF was expressed from *Pichia pastoris* using derivatives of the pPIC9 expression vector and purified by sequential chromatography. ITF was added to the rat nontransformed intestinal epithelial cells IEC-6 and human monocytes U937. NF $\kappa$ B binding of p65 and p50 protein was determined by electrophoretic mobility shift assay (EMSA) and Western blot analysis of nuclear proteins. IL-8 (CINC) promoter activity was assessed following transient transfection of a CINC promoter-luciferase reporter construct. mRNA expression was measured by Northern blot and RNase protection assay (RPA). ELISA was performed to detect secreted IL-1 $\beta$  in culture media. Results: ITF induced NF $\kappa$ B protein-DNA complexes consisting of p65/p50 heterodimers in nuclear extracts of both IEC-6 and U937 cells as assessed by EMSA. This induction was accompanied by phosphorylation and degradation of cytoplasmic I $\kappa$ B- $\alpha$ . ITF enhanced NF $\kappa$ B site-dependent CINC promoter activity up to 2-folds in IEC-6 cells. Consistent with the observed activation of NF $\kappa$ B, ITF also strongly upregulated CINC mRNA content as assessed by Northern blot analysis. RPA conferred upregulation of IL-1 $\beta$  message and a concomitant production of IL-1 $\beta$  protein was found by ELISA in U937 cells. Conclusion: ITF induced both epithelial IL-8 and monocytic IL-1 $\beta$  upregulation through activation of NF $\kappa$ B signal pathway. Given that both IL-1 $\beta$  and IL-8 are major early responsive cytokines involved in inflammation in the gastrointestinal tract, these results suggested that ITF may play an important mucosa-host defending role coordinating with an immune and inflammatory response to mucosal injury.

4232

**PROTECTIVE EFFECT OF INTRALUMINAL NITRIC OXIDE IN EXPERIMENTAL COLITIS IN RATS.**

Florence Lamine, Vassilia Theodorou, Pauline Anton, Sabine Roy, Rafael Garcia-Villar, Jean Fioramonti, Lionel Bueno, INRA, Toulouse, France; Esap, Toulouse, France.

The role of nitric oxide (NO) in inflammatory gut processes is still discussed. A decrease of endogenous NO production by NO synthases inhibition improves experimental colitis. However, according to its ubiquitous role, a protective action of exogenous NO in inflammation can be suggested. Aims: This study was aimed at evaluating the effect of exogenous NO in trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. Methods: Eight groups of 8 male Wistar rats (200-250 g) were used. Under anesthesia (acepromazine 0.2 mg/kg; ketamine 6mg/kg), animals were equipped with an intracolonic catheter (+2 cm from the caeco-colonic junction) exteriorized in the dorso-scapular region. Six days after surgery, rats received intracolonic instillation of TNBS (80mg/kg in 200 $\mu$ L ethanol 50% V/V) or saline (NaCl 0.9%). Four hours after TNBS or saline instillation, animals were infused continuously (0.25 ml/h; 24h/d) during 4 days with a NO donor, sodium nitroprusside (SNP; 2 mg/kg/d), or a NO scavenger, hemoglobin (Hb; 200 mg/kg/d), or SNP (2 mg/kg/d) plus Hb (200 mg/kg/d) or their vehicle (saline). At the end of the different treatments, animals were sacrificed and both myeloperoxidase (MPO) activity and macroscopic damage scores (MDS; according to Wallace et al. 1992) were determined on colonic samples. Results: In TNBS instilled rats, MPO activity and MDS were significantly increased ( $p < 0.05$ ) compared to control animals: 5467 $\pm$ 1760 vs 102 $\pm$ 86 U/g protein and 6.6 $\pm$ 0.8 vs 0, respectively. In colitic rats, SNP significantly reduced ( $p < 0.05$ ) MPO activity and MDS to 2716 $\pm$ 768 U/g protein and 2.1 $\pm$ 1.1, respectively. Infusion of Hb simultaneously with SNP abolished this reduction of inflammation (MPO: 4980 $\pm$ 1591 U/g protein; MDS: 6.8 $\pm$ 1.4). SNP had no effect in non-colitic rats and Hb alone had no effect in both non-colitic and colitic rats. Conclusion: NO released intraluminally by a NO donor reduces TNBS-induced colitis in rats, suggesting a protective role of exogenous NO in colonic inflammation.