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Short communication

Retinoic acid decreases the severity of *Salmonella enterica* serovar Typhimurium mediated gastroenteritis in a mouse model

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ABSTRACT

Gastroenteritis is a global burden; it's the major cause of morbidity and mortality both in adults and children of developing countries. *Salmonella* is one of the leading causes of bacteria-mediated gastroenteritis and due to its increasing multidrug antibiotic resistance; *Salmonella*-mediated gastroenteritis is difficult to control. Retinoic acid, the biologically active agent of vitamin A has an anti-inflammatory effect on experimental colitis. In this study we have shown All trans retinoic acid (ATRA) treatment down regulates *Salmonella*-mediated colitis in a murine model. Macroscopic signs of inflammation such as decrease in body weight and cecum weight, shorter length of proximal colon and pathological score of colitis were observed less in ATRA treated mice than in a vehicle control group. ATRA treatment not only reduced pro-inflammatory cytokine responses, such as TNF-α, IL-6, IL-1β, IFN-γ and IL-17 production but also increased IL-10 response in the supernatant of intestinal tissue. Results also suggested that ATRA treatment enhances the number of FoxP3-expressing T regulatory cells in MLN and also decreases bacterial load in systemic organs. We concluded that ATRA treatment indeed reduces *Salmonella* Typhimurium-mediated gastroenteritis in mice, suggesting it could be an important part of an alternative therapeutic approach to combat the disease.

1. Introduction

Non-typhoidal *Salmonella,* such as *Salmonella* enteric serovar Typhimurium and *Salmonella* Enteriditis are important food borne pathogens that causes gastroenteritis, bacteraemia and shows symptoms of diarrhea and abdominal pain (Majowicz et al., 2010). Gastroenteritis is a major cause of morbidity and mortality in adults and children in both developed and developing countries (Ifeanyi et al., 2014). *S. enterica* serovar Typhimurium (*S.* Typhimurium) triggers acute intestinal inflammation in the terminal ileum and colon in humans (Ifeanyi et al., 2014; Jones and Falkow, 1996). Following the pretreatment with streptomycin, *S.* Typhimurium causes acute intestinal inflammation in mice by using its type III secretion system (T3SS-1) to enter the intestinal mucosa (Barthel et al., 2003). A rapid and robust defense is activated in the host intestine with the invasion of *S.* Typhimurium. Activation of host defense, especially innate immunity by pathogen associated molecular patterns of *Salmonella*, results in rapid pro-inflammatory cytokine production by the activation of TLRs, NOD-like receptors and NLRP. Activation of host defense, especially innate immunity by pathogen associated molecular patterns of *Salmonella*, results in rapid pro-inflammatory cytokine production through the activation of TLRs, NOD-like receptors and NLRP (Jones and Falkow, 1996; Sivick et al., 2014; Nishimori et al., 2012). Activation of a pro-inflammatory CD4+ T cell response, especially Th1 and Th17 immune responses, is also an important factor in intestinal inflammation due to *Salmonella* infection (Keestra et al., 2011; Godinez et al., 2008; Noto Llana et al., 2012). Increase in the number of multidrug resistant bacteria has limited the choice for drugs in treatment (Elhadi et al., 2013). Therefore an alternative therapeutic approach is needed to manage the present situation.

Vitamin A and its metabolites such as all trans retinoic acid (ATRA) are biologically active agents that have a broad range of functions involving immune cell differentiation and maintaining immune homeostasis (Hall et al., 2011a,b). Vitamin A supplementation is known to reduce infant mortality and severity of pathogen induced diarrhea consistently (Sommer et al., 1986). Vitamin A supplements reduced fecal pro-inflammatory cytokine response due to enteropathogenic *Escherichia coli* infection in Mexican children (Long et al., 2006, 2011). Despite such observations, the role

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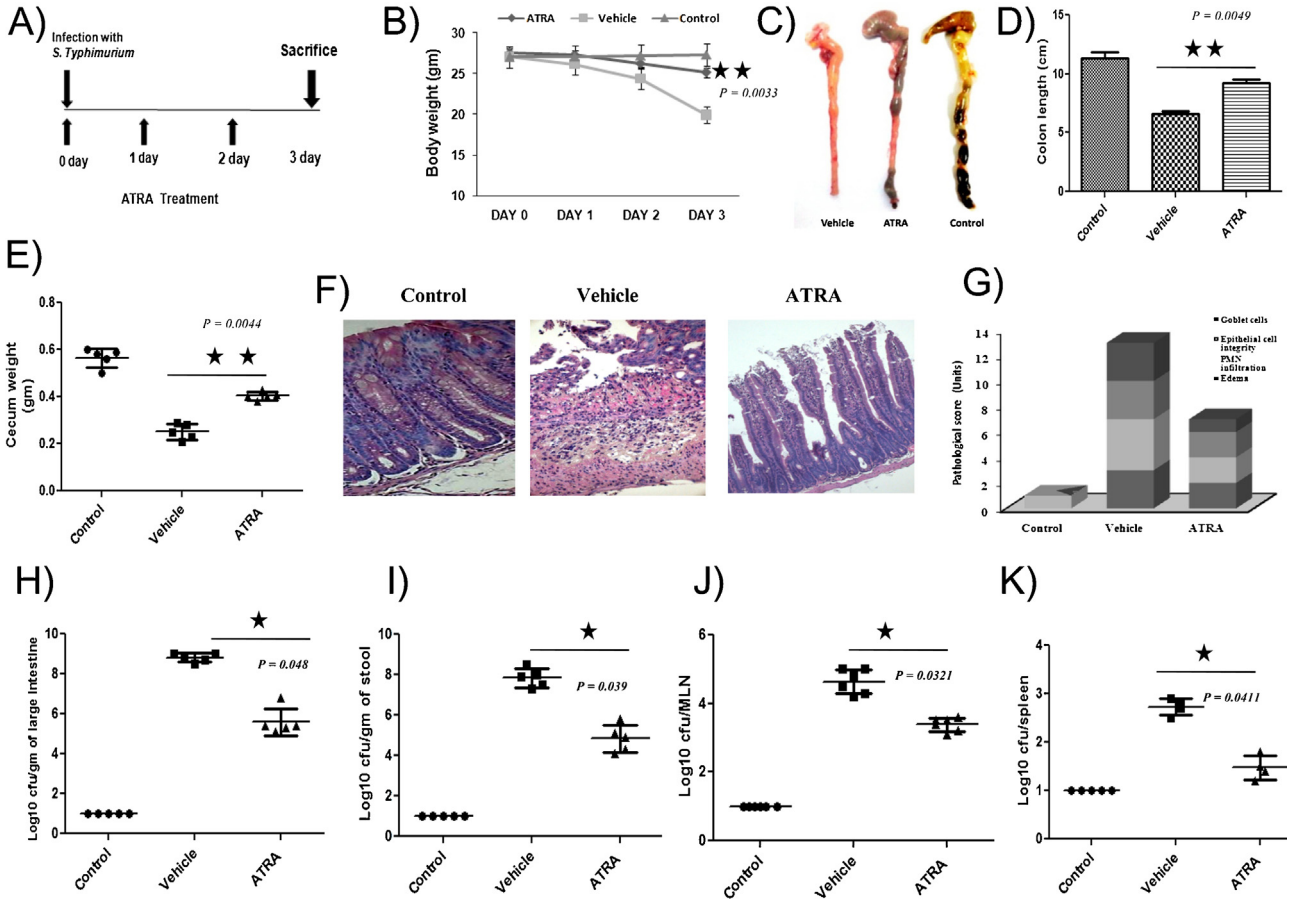

Fig. 1. All trans retinoic acid down regulates *Salmonella* Typhimurium mediated intestinal inflammation: As per as schematic diagram of animal experiment (A), streptomycin pre-treated two groups of mice were orally infected with *Salmonella* Typhimurium SL 1344,one group of mice were treated with vehicles (200 μl DMSO) as a positive control, other group were treated with ATRA (200 μg/200 μl DMSO) intraperitoneally. Acute colitis symptoms, such as body weight (B), Colon length (C, D) Cecum weight (E) of vehicle treated group were significantly decreased as compared to ATRA treated group. Histopathological analyses (F,G). HE-stained sections of cecal tissue were scored with respect to edema in the submucosa (black), PMN infiltration (medium gray), reduction in the number of goblet cells (dark gray), and desquamation/erosion/ulceration of the epithelial layer (light gray) was acute in vehicle control group than ATRA treated group. After three days of infection, bacterial load in different organs, such as large intestine (H), stool (I) and mysentric lymph node/MLN (J) and spleen (K) were significantly lower in ATRA treated than in vehicle control group. Data are presented as the mean ± SEM ($n = 5$ in each group). ($^{*}p < 0.05$, $^{**}p < 0.005$).

of Vitamin A is unclear in perspective of intestinal inflammation. However, several studies revealed that more than 15% of children with inflammatory bowel disease (IBD) have low serum levels of Vitamin A at the time of diagnosis (Bousvaros et al., 1998).

All trans retinoic acid (ATRA) is an active metabolite of vitamin-A having an anti-inflammatory role and down regulates LPS mediated endotoxicity as well as protein mediated pro-inflammatory cytokines level (Na et al., 1999; Liu et al., 2005). On the other hand ATRA also has some role in the T cell immune response (Menning et al., 2010). The role of ATRA on oral tolerance via generation of innate regulatory T cells is well established and treatment with ATRA reduces pro-inflammatory Th1 and Th17 immune responses by generating a regulatory T immune response in Disodium sulphonate (DSS) and TNBS mediated colitis in a murine model (Bai et al., 2009, 2010). For the above reasons, recent studies showed that ATRA is a potent therapeutic agent for inflammatory diseases such as arthritis, acne, and airway infections (Kwok et al., 2012; Jalian et al., 2008; Wu et al., 2013).

In our present study: in order to observe the effect of ATRA on *Salmonella* Typhimurium colitis, we first developed streptomycin treated *Salmonella* Typhimurium mediated colitis in mice model as previously described (Barthel et al., 2003). Infected mice were intraperitoneally treated with ATRA and observed its effectiveness on *Salmonella* mediated gastroenteritis by measuring intestinal histo-pathological changes, pro-inflammatory cytokine levels and T-regulatory cells generation. This study may help in building a supportive therapy for human medical care in near future.

2. Methods and materials

2.1. Bacterial strains

The naturally streptomycin-resistant wild-type strain *S. enterica* serovar Typhimurium SL1344 (Barthel et al., 2003) were grown for 12 h at 37 °C in Luria–Bertani broth supplemented with 0.3 M NaCl, diluted 1:20 in fresh medium, and sub cultured for 4 h under mild aeration. Bacteria were washed twice in ice-cold phosphate-buffered saline (PBS) and then suspended in cold PBS (2×10^8 CFU/50 μl).

2.2. Mice

Pathogen free 5–6 weeks adult male Balb/c mice (18–20 gm) were taken and maintained under specific pathogen free condition in ventilated cages equipped with steel grid floors and autoclaved straw at Animal house (NICED) where they received sterilized food