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Partial depletion of natural gut flora by antibiotic aggravates collagen induced arthritis (CIA) in mice

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects about 1% of the adult population and occurs twice as frequently among women than men. At present it is accepted that pathogenesis of RA is based on inflammatory response mediated by $CD4^+$ Th1 and Th17 lymphocytes. The most commonly applied model imitating RA is the collagen induced arthritis (CIA).

A growing evidence shows that there is a correlation between microbial dysbiosis and human pathology which includes autoimmunity, allergic diseases, obesity, inflammatory bowel disease (IBD), metabolic syndrome.

Methods: Collagen induced arthritis was used to study influence of natural gut flora on course of rheumatoid arthritis.

Results: Current work employing CIA model showed that partial depletion of natural gut flora with orally administered antibiotic Baytril (enrofloxacin) aggravates disease severity when compared to control mice. Observed partial depletion of both aerobic and anaerobic bacteria did not affect animal body weight. Additionally, *in vitro* study showed increased production of IFN-γ and IL-17A and decreased release of IL-4 by axillary lymph node cells (ALNC) isolated from mice treated with antibiotic and induced CIA when compared to positive control. Furthermore, treatment with antibiotic prior to CIA induction results in augmented production of IFN-γ, IL-17A and IL-6 by mesenteric lymph node cells (MLNC).

Conclusion: Presented data suggest that alteration of gut microbiota via use of enrofloxacin may play a role in modulating arthritis symptom severity in this mouse model.

Introduction

Like other mammals, a human emerges into this world from a sterile environment and in the first months their organism is gradually colonised by bacteria. Microorganisms colonise all surfaces covered by epithelia and they occur in the greatest number in the alimentary tract where it can reach 10^{14} bacterial cells [11]. The highest species diversity is observed in the large intestine and the major groups of bacteria occurring in the intestinal lumen include *Firmicutes* (e.g. Lactobacillus) and *Bacteroidetes* (e.g. Bacteroides) [20].

The microbiota which constitute the flora living on or within higher organisms are termed 'good bacteria' or 'beneficial bacteria', including commensal microorganisms as well as bacteria which benefit from living in a higher organism, but also in return offer some benefits to the latter. Studies conducted over many years have shown that the bacteria living in the alimentary tract have an essential role in the processes of food digestion, maintaining homeostasis, modulating lipid metabolism, promoting angiogenesis, supporting the immunity to infections, and maintaining the immune cell homeostasis [9,12].

The composition of natural flora is affected by a number of factors including e.g.: country of birth, prematurity, manner of delivery (natural *vs.* Caesarean section), early hospitalization, or application of antibiotics. After the natural flora is established in the early life of an individual under normal conditions, it remains unaltered throughout their life. The change of bacterial flora during

Abbreviations: ALNC, axillary lymph node cells; CFA, complete Freund's adjuvants; CIA, collagen-induced arthritis; COLL II, bovine type II collagen; IBD, inflammatory bowel disease; id, intradermal; IFA, incomplete Freund's adjuvants; IFN-γ, interferon *gamma*; IL, interleukin; MLNC, mesenteric lymph nodes cells; PP, Peyer patches; RA, rheumatoid arthritis; TGF-β, transforming growth factor *beta*; Th1, T helper 1; Th17, T helper 17; TNF-α, tumor necrosis factor-α.

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ontogeny is affected by two factors: radical change of diet and application of antibiotics [10,11].

A growing evidence shows that there is a correlation between microbial dysbiosis and human pathology which includes autoimmunity, allergic diseases, obesity, inflammatory bowel disease (IBD), metabolic syndrome and even autism [3,6,13,19,26].

The rheumatoid arthritis (RA) is one of examples of autoimmune diseases. At present it is considered that pathogenesis of RA is based on inflammatory response mediated by $CD4^+$ Th1 and Th17 lymphocytes [2]. This chronic inflammatory disease affects about 1% of the adult population and occurs twice as frequently among women than men [1]. The onset may appear at any age, but the peak of incidence comes in the 25–55 age range [5]. Because the disease affects persons in economically productive age ranges, it presents an ever-increasing economic and social problem affecting the quality of life of the patients and their ability to work.

Studies on animals are a valuable source of information on the role of the immune system in the RA pathogenesis. The most commonly used model of RA is the murine collagen-induced arthritis (CIA). This experimental procedure involves immunizing genetically susceptible mice, e.g. DBA1, with type II collagen (COLL II) in complete Freund's adjuvant (CFA) [22].

The aim of current work was to determine whether partial depletion of natural gut flora with the use of broad spectrum antibiotic Baytril (enrofloxacin) affects CIA in mice.

Materials and methods

Mice

SPF male DBA1 ($H\text{-}2^q$) mice from the breeding unit of the Department of Medical Biology, Jagiellonian University, College of Medicine were used.

Mice were maintained under specific pathogen-free conditions, and used at 10–12 weeks of age in groups of 10. All experiments were conducted according to guidelines of Jagiellonian University College of Medicine.

Reagents

Bovine type II collagen (COLL II), complete (CFA) and incomplete (IFA) Freund's adjuvants from Chondrex, Inc. Redmond, WA. RPMI 1640 and fetal calf serum (FCS) (Life Technologies, Grand Island, NY) were obtained from the manufacturer. Baytril (enrofloxacin) was from Bayer HealthCare (Shawnee Mission, KY, USA).

To measure levels of TNF-α, IL-4, IL-6, IL-10, IL-12p70, IFN-γ and TGF-β ELISA kits; BD OptEIA Set (BD Bioscience, San Diego, CA, USA) and IL-17A (eBioscience, Inc., San Diego, CA, USA) were used.

Additionally horseradish peroxidase streptavidin (Vector Laboratories, Burlingame, CA) (Sigma, St. Louis, MO) was used.

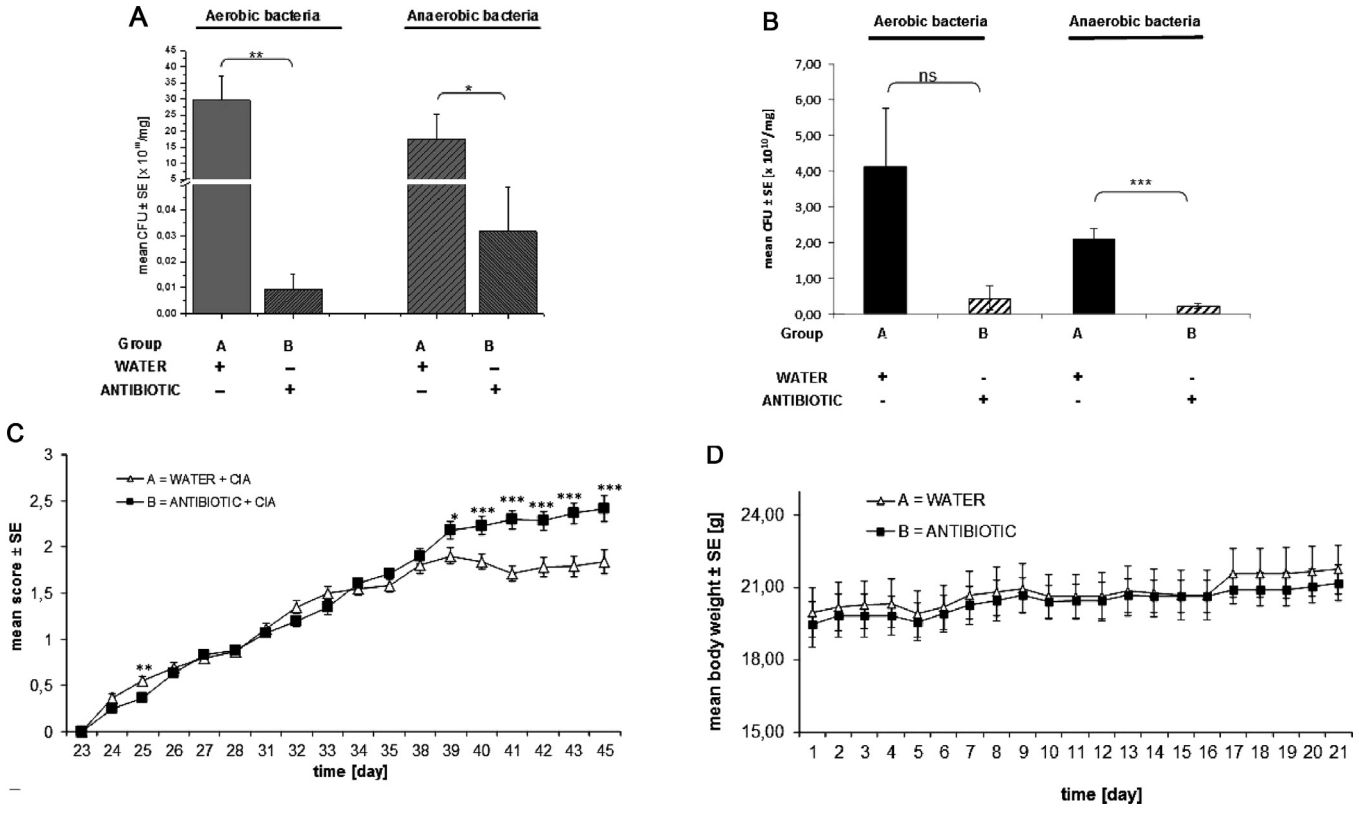

Fig. 1. Partial depletion of natural gut flora aggravates CIA. (A and B). Oral treatment with enrofloxacin partialy deplets gut flora. DBA1 mice received drinking water with enrofloxacin (0.27 mg/ml) or water alone for three weeks (Groups B and A respectively). Then, mice were sacrificed on either day "+21" or "+39" and colon content was collected and serial dilutions were cultured in general agar plates for 48 h at 37 °C in aerobic and anaerobic conditions. Total bacteria per gram of sample was calculated based on the CFU counted in each serial dilution. Data expressed as mean ± SE. $n = 6$; $^*p \leq 0.05$, $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; p = NS. (C) Depletion of gut flora aggravates CIA. Mice were orally treated with antibiotic or water alone for two weeks prior to CIA induction (Groups B and A respectively). Treatment with enrofloxacin or water was continued for another week post CIA induction. Immunization and arthritis evaluation were performed as described in Materials and methods. The clinical severity of disease was scored for each paw on a scale of 0–4, with the index being the sum of the scores for all four paws. Data expressed as mean ± SE. $n = 10$; $^*p \leq 0.05$, $^{***}p \leq 0.001$. (D) Treatment with antibiotic does not affect body weight. To determine influence of oral treatment with antibiotic, body weight of mice treated with enrofloxacin or water was monitored daily and expressed as mean ± SE (Groups B and A respectively). $n = 10$; p = NS.