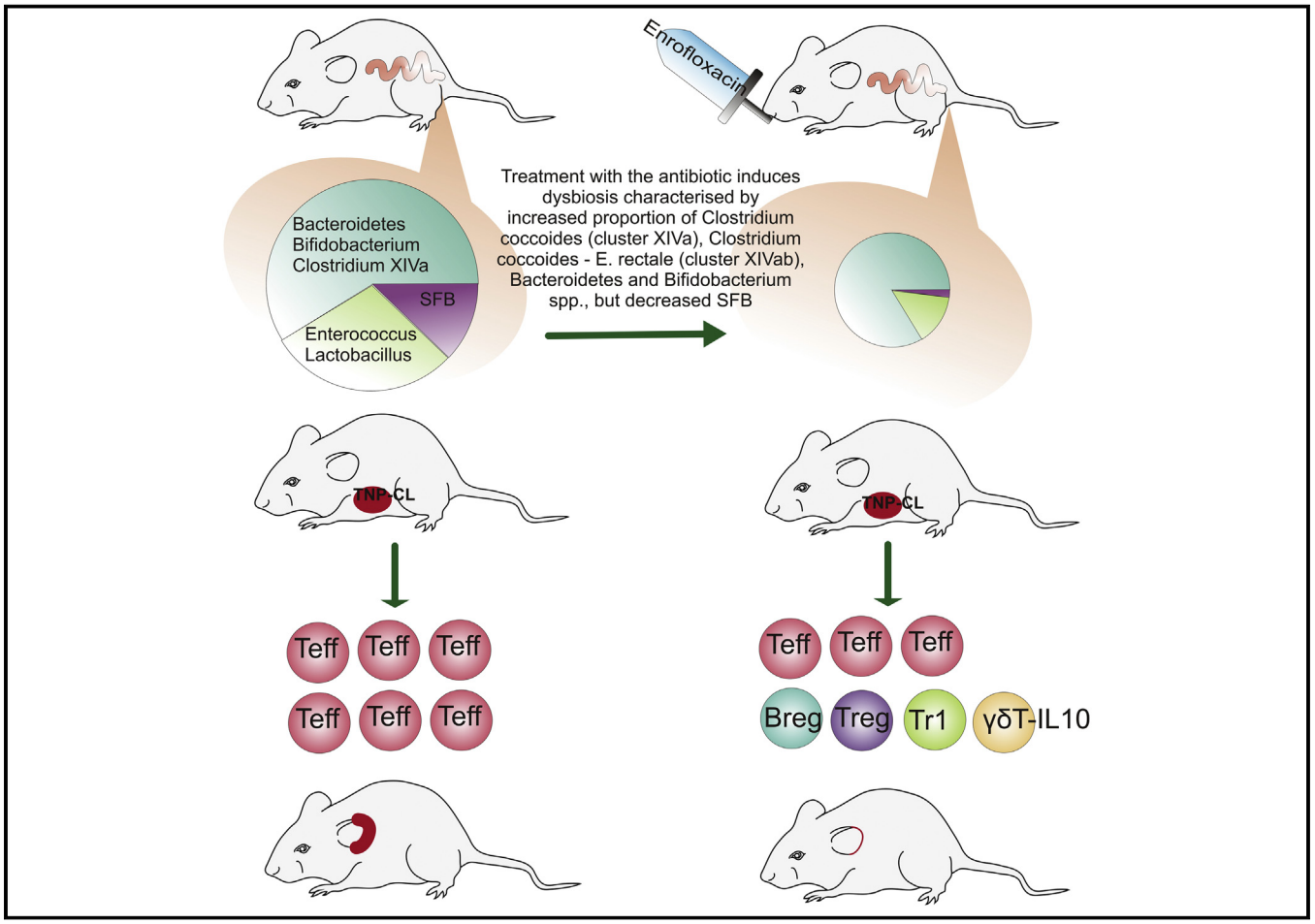
Broad spectrum antibiotic enrofloxacin modulates contact sensitivity through gut microbiota in a murine model

Anna Strzępa, PhD,[a] Monika Majewska-Szczepanik, PhD,[a] Francis M. Lobo, MD,[b] Li Wen, MD, PhD,[c] and Marian Szczepanik, PhD[a] *Krakow, Poland, and New Haven, Conn*

GRAPHICAL ABSTRACT



From [a]the Department of Medical Biology, Faculty of Health Sciences, Jagiellonian University Medical College, Krakow; [b]the Section of Allergy and Clinical Immunology, and [c]the Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven.

Supported by grants from National Science Center in Poland (2011/01/B/NZ6/00300 and K/ZDS/004650 to M.S.) and National Institutes of Health (DK088181 and DK092882 to L.W., and K/DSC/002879 to A.S.).

Disclosure of potential conflict of interest: A. Strzępa has received a grant from Ministerstwo Nauki i Szkolnictwa Wyższego. L. Wen has received a grant from the National Institute of Health. M. Szczepanik has received grants from Ministerstwo Nauki i Szkolnictwa Wyższego and the National Science Centre. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 10, 2016; revised November 10, 2016; accepted for publication November 23, 2016.

Available online January 24, 2017.

Corresponding author: Marian Szczepanik, PhD, Department of Medical Biology, Jagiellonian University Medical College, ul. Kopernika 7a, 31-034 Krakow, Poland. E-mail: mmszczep@cyf-kr.edu.pl. Or: Li Wen, MD, PhD, Department of Internal Medicine, Section of Endocrinology, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06520-8013. E-mail: li.wen@yale.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.11.052

Background: Medical advances in the field of infection therapy have led to an increasing use of antibiotics, which, apart from eliminating pathogens, also partially eliminate naturally existing commensal bacteria. It has become increasingly clear that less exposure to microbiota early in life may contribute to the observed rise in "immune-mediated" diseases, including autoimmunity and allergy.
Objective: We sought to test whether the change of gut microbiota with the broad spectrum antibiotic enrofloxacin will modulate contact sensitivity (CS) in mice.
Methods: Natural gut microbiota were modified by oral treatment with enrofloxacin prior to sensitization with trinitrophenyl chloride followed by CS testing. Finally, adoptive cell transfers were performed to characterize the regulatory cells that are induced by microbiota modification.
Results: Oral treatment with enrofloxacin suppresses CS and production of anti–trinitrophenyl chloride IgG1 antibodies. Adoptive transfer experiments show that antibiotic administration favors induction of regulatory cells that suppress CS. Flow cytometry and adoptive transfer of purified cells show that antibiotic-induced suppression of CS is mediated by TCR $\alpha\beta^+CD4^+CD25^+FoxP3^+$ Treg, $CD19^+B220^+CD5^+$ IL-10^+, IL-10^+ Tr1, and IL-10^+ TCR $\gamma\delta^+$ cells. Treatment with the antibiotic induces dysbiosis characterized by increased proportion of *Clostridium coccoides* (cluster XIVa), *C coccoides–Eubacterium rectale* (cluster XIVab), *Bacteroidetes,* and *Bifidobacterium* spp, but decreased segmented filamentous bacteria. Transfer of antibiotic-modified gut microbiota inhibits CS, but this response can be restored through oral transfer of control gut bacteria to antibiotic-treated animals.
Conclusions: Oral treatment with a broad spectrum antibiotic modifies gut microbiota composition and promotes anti-inflammatory response, suggesting that manipulation of gut microbiota can be a powerful tool to modulate the course of CS. (J Allergy Clin Immunol 2017;140:121-33.)

Key words: *Contact sensitivity, gut microbiota, immunoregulation, dysbiosis, regulatory cells*

Abbreviations used
AD: Atopic dermatitis
ALNC: Axillary and inguinal lymph node cell
Breg: Regulatory B
CS: Contact sensitivity
EAE: Experimental autoimmune encephalomyelitis
FACS: Fluorescence-activated cell sorting
i.p.: Intraperitoneal
i.v.: Intravenous
MLNC: Mesenteric lymph node cell
MPO: Myeloperoxidase
PP: Peyer patch
SFB: Segmented filamentous bacteria
SPLC: Splenocyte
T_H: T helper lymphocyte
Treg: Regulatory T
TNP-Cl: Trinitrophenyl chloride

Advances in medicine in the field of infection therapy over the last several decades have led to a drastically increasing application of antibiotics, which, apart from eliminating pathogens, also partially eliminate naturally existing commensal bacteria. In addition, unlabeled broad-spectrum antibiotics are often present in the food supply, which also can change the composition of gut microbiota. A study in a group of healthy human volunteers showed that treatment with ciprofloxacin for 5 days decreases diversity, richness, and evenness of fecal microbiota during antibiotic treatment.[1] Although the microbiota mostly returned to pretreatment composition following ciprofloxacin treatment, several bacterial taxa failed to recover, indicating that changes to the microbiota can persist following a short course of oral antibiotics.[2] Similar to human studies, mouse models have revealed that treatment with ampicillin, cefoperazone, vancomycin, or clindamycin causes long-lasting changes in the gut microbiota that persist after cessation of antibiotic treatment.[3-5]

It has become increasingly clear that less exposure to microbiota early in life may contribute to the observed rise in the diseases characterized by immune dysregulation, including allergy, autoimmunity, metabolic disorders, and even neoplastic diseases.[6] A number of studies have demonstrated the increased risk of developing asthma and food allergy in children who have been exposed to antibiotics in the first year of life.[7-9] Similar findings are also observed in animal studies, such as in the non-obese-diabetic mouse, which is known to be very sensitive to the change of the environment, such that a cleaner environment enhances the rate of onset and incidence of type 1 diabetes.[10] This is also the case in animal models of allergic asthma.[11,12] Studies carried out in recent years have confirmed a strong connection between the natural gut microbiota and the immune response. However, this interaction may differ in various experimental models. In an animal model of multiple sclerosis, for instance, a strong inhibition of inflammation has been observed in mice treated orally with an antibiotic.[13] Similar observations have been reported in an animal model of human rheumatoid arthritis.[14]

Atopic dermatitis (AD) is a prominent clinical example of an immune-mediated disease that is affected by environmental factors. AD has become a considerable clinical problem, having nearly tripled in prevalence over the last 30 years in developed countries. The disease afflicts 15% to 30% of children and about 2% to 10% of adults.[15] Yet another form of skin disease with an underlying immune-mediated hypersensitivity reaction is contact sensitivity (CS) to haptens. Allergic contact dermatitis, as opposed to AD, is a typical CS response in humans and has become a significant cause of morbidity. Indeed, contact dermatitis linked to exposure to chemical substances in the workplace constitutes about 30% of all occupational diseases and represents a severe social and economic problem.[16]

The hygiene hypothesis has been advanced as an explanation for the dramatic rise in the prevalence of allergic diseases in industrialized societies. Supporters of this hypothesis note that decreasing microbial exposure of many types, including bacteria, parasites, and viruses, has been associated with the rise in incidence of allergic diseases and autoimmune disorders.[17] Increasing evidence suggests that gut microbes, which represent an enormous source of interaction between microbes and immunity, play a very important role in various health problems.[6] However, it is not clear what effects are exerted by natural gut microbiota on the course of CS responses. Given the common use of antibiotics in industrialized societies, in the setting of ever-increasing exposure to potential CS-generating haptens in