of Dermatology and Allergology, Philipps-Universität Marburg, Baldingerstraße, 35043 Marburg, Germany.
Email: wpfuetzn@med.uni-marburg.de

ORCID

Michèle Myriam Rauber https://orcid.org/0000-0001-8022-7421
Christian Möbs https://orcid.org/0000-0002-5197-7669
Rudolf Valenta https://orcid.org/0000-0001-5944-3365
Wolfgang Pfützner https://orcid.org/0000-0002-8721-724X

REFERENCES

1. Curin M, Khaitov M, Karaulov A, et al. Next-generation of allergen-specific immunotherapies: molecular approaches. *Curr Allergy Asthma Rep*. 2018;18(7):39.
2. Niederberger V, Neubauer A, Gevaert P, et al. Safety and efficacy of immunotherapy with the recombinant B-cell epitope-based grass pollen vaccine BM32. *J Allergy Clin Immunol*. 2018;142(2):497-509.
3. Focke-Tejkl M, Weber M, Niespodziana K, et al. Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy. *J Allergy Clin Immunol*. 2015;135(5):1207-1217.
4. Frew AJ, Powell RJ, Corrigan CJ, Durham SR, Group UKIS. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol*. 2006;117(2):319-325.
5. Marth K, Breyer I, Focke-Tejkl M, et al. A nonallergenic birch pollen allergy vaccine consisting of hepatitis PreS-fused Bet v 1 peptides focuses blocking IgG toward IgE epitopes and shifts immune responses to a tolerogenic and Th1 phenotype. *J Immunol*. 2013;190(7):3068-3078.
6. Möbs C, Ipsen H, Mayer L, et al. Birch pollen immunotherapy results in long-term loss of Bet v 1-specific TH2 responses, transient TR1 activation, and synthesis of IgE-blocking antibodies. *J Allergy Clin Immunol*. 2012;130(5):1108-1116.
7. Renand A, Shamji MH, Harris KM, et al. Synchronous immune alterations mirror clinical response during allergen immunotherapy. *J Allergy Clin Immunol*. 2018;141(5):1750-1760.
8. Zieglmayer P, Focke-Tejkl M, Schmutz R, et al. Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy. *EBioMedicine*. 2016;11:43-57.
9. Prickett SR, Rolland JM, O'Hehir RE. Immunoregulatory T cell epitope peptides: the new frontier in allergy therapy. *Clin Exp Allergy*. 2015;45(6):1015-1026.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.14003

Effect of *Lactobacillus plantarum* YIT 0132 on Japanese cedar pollinosis and regulatory T cells in adults

To the editor,

Japanese cedar pollinosis (JCPsis) is a major health problem that has increased over recent decades and impairs daily activities.[1] Besides the standard treatments specified in the guidelines,[2] some strains of lactic acid bacteria (LAB), such as *Bifidobacterium* and *Lactobacillus*, have been shown to improve pollinosis symptoms.[3,4] Although LAB are believed to induce regulatory T (Treg) cells, thereby alleviating allergic symptoms,[4] whether LAB augment Treg cell abundance, together with clinical symptoms, remains unclear.

Lactobacillus plantarum YIT 0132 (LP0132), derived from fermented food, induces a high level of IL-10 secretion from murine macrophages.[5] Previously, LP0132-fermented citrus juice, which contained approximately 8.0×10^{10} heat-killed LP0132 cells/100 mL, showed therapeutic potential to improve JCPsis-associated symptoms.[5] In this trial, we further evaluated the efficacy of this beverage for the treatment of JCPsis, in association with immunological parameters including peripheral Treg cells in the pollen season.

We randomly assigned 100 adults with JCPsis to consume 125 mL of fermented citrus juice containing heat-killed LP0132 (LP0132, n = 50) or unfermented citrus juice (placebo, n = 50) for 8 weeks from mid-February to mid-April 2017, from the beginning to the end of the pollen season (Figure S1). Throughout the trial period, the subjects were asked to avoid taking LAB-containing food and keep daily records of their consumption of beverages, medications, and medical visits, and to perform a weekly self-assessment of JCPsis symptoms based on the Japanese Rhino-conjunctivitis Quality of Life Questionnaire.[2] The primary outcome was total symptom score (TSS) in March (predicted peak season). Peripheral blood samples were obtained at enrollment, in late March, and during follow-up to evaluate the percentage of CD4 + CD25 + Foxp3 + Treg cells. Detailed methods and statistical analyses are described in supporting information. *P*-values <.05 were considered statistically significant. The trial protocol was approved by the Ethics Committee of Shimoshizu National Hospital, in accordance with the Declaration of Helsinki, and was registered at the University Hospital Medical Information Network (UMIN000025924). Written-informed consent was obtained from all subjects.

Correction added on 04 October 2019, after first online publication on 27 August 2019 : Title and supporting information have been updated in this version

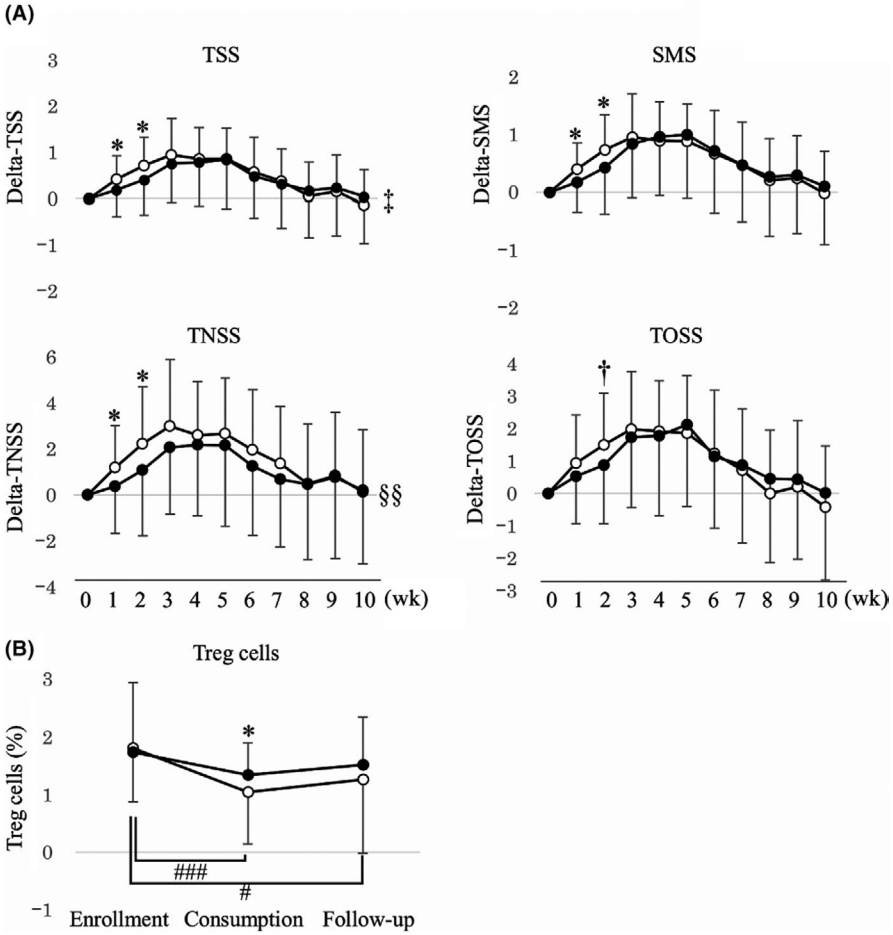

FIGURE 1 Changes in (A) total symptom score (TSS), symptom medication score (SMS), total nasal symptom score (TNSS), and total ocular symptom score (TOSS), and (B) Treg cells in the trial period. Data are expressed as the mean ± SD. –●–: LP0132, –○–: Placebo. **P* < .05, †*P* < .1 compared with the placebo group (Student's *t* test). ‡*P* < .1, §§*P* < .01 compared with the placebo group during the consumption period (weeks 0-8) (two-way ANOVA). #*P* < .025, ###*P* < .0005 compared with enrollment (paired *t* test with Bonferroni's correction)

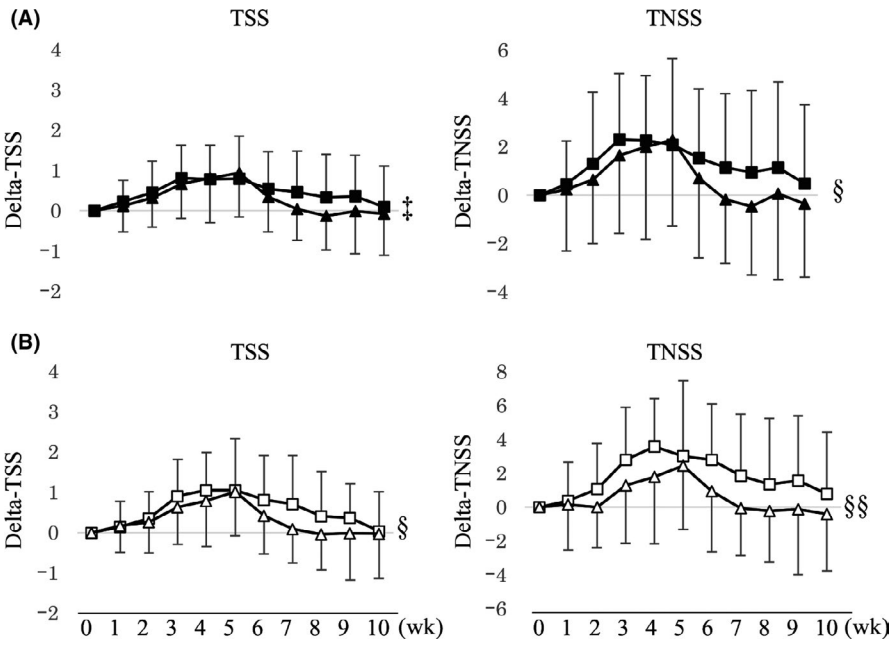

FIGURE 2 Changes in total symptom score (TSS) and total nasal symptom score (TNSS) based on increased and decreased Treg abundance between enrollment and the consumption period in the LP0132 group (A) –▲–: increased Treg subgroup (n = 17), –△–: decreased Treg subgroup (n = 33), and after propensity score matching (B) –■–: increased Treg-matched subgroup (n = 14), –□–: decreased Treg-matched subgroup (n = 14). Data are expressed as the mean ± SD. ‡*P* < .1, §*P* < .05, §§*P* < .01, compared between subgroups during the consumption period (weeks 0-8) (two-way ANOVA)

All subjects completed the trial (Figure S2), and no significant adverse events were reported in either group. The baseline characteristics were comparable, except for the higher symptom medication score (SMS) and medication score (MS) in the LP0132 group (Table S1). Average TSS in March (weeks 3-6) did not differ significantly between both groups; however, the LP0132 group had a significantly

lower total nasal symptom score (TNSS) than the placebo group during the entire consumption period (weeks 0-8), and they had a significantly lower TSS, SMS and TNSS at weeks 1 and 2 compared to the placebo group (Figure 1A). The percentage of Treg cells decreased significantly from enrollment to late March only in the placebo group, and the percentage of Treg cells in late March was significantly higher in the LP0132 group than in the placebo group (Figure 1B). Other immunological parameters did not differ significantly between both groups at each timepoint (Table S2). Although MS in the LP0132 group was significantly higher throughout the consumption period, propensity score matching analysis adjusting for MS from weeks 0 to 10 (36 subjects from each group) was consistent with these results (Figure S3).

As a predetermined exploratory analysis, based on the changes in the percentage of Treg cells from enrollment to the consumption period, the subjects in the LP0132 group were divided into increased Treg and decreased Treg subgroups to compare the symptom scores. The increased Treg subgroup (n = 17) had a significantly lower TNSS than the decreased Treg subgroup (n = 33) during the consumption period (Figure 2A). Propensity score matching analysis adjusting for MS from weeks 0 to 10 (14 subjects from each subgroup) was consistent with these results (Figure 2B). In contrast, the same analysis in the placebo group did not show a significant difference between the increased Treg (n = 9) and decreased Treg (n = 41) subgroups.

Despite the negative result for the primary outcome, this is the first clinical trial to demonstrate the ability of LAB to suppress the reduction in Treg cells in the peak pollen season. In a recent trial, LAB increased the percentage of Treg cells from the baseline, but the changes were not significant compared with placebo.[6] Treg cells are reported to be reduced in patients with allergic rhinitis[7] and increased by immunotherapy with a clinical response.[8] The results of the present study suggest that Treg cells may be reduced with the severity of pollinosis due to exposure to pollen. However, few studies have observed this fluctuation. Thus, further studies are needed to examine whether circulating Treg cells decrease in the pollen season and LAB are capable of inhibiting this seasonal change in Treg cells.

Notably, the increase in Treg cells in the pollen season was associated with milder symptoms in the LP0132 group. LP0132, like other LAB administered for JCPsis,[3] alleviated JCPsis symptoms only in the early phase, whereas symptom scores in this phase did not differ between the increased Treg and decreased Treg subgroups in the LP0132 group. From these findings, we speculate that LP0132 may have an immunological effect, other than inducing the expansion of Treg cells, which may contribute to the alleviation of JCPsis symptoms in the early phase.

This study has several limitations. In this trial, the specific subtypes of Treg cells induced by LP0132 to alleviate JCPsis symptoms were not examined. For instance, experiments suggest that CD4 + CD25 + Foxp3 + RORγt + Treg cells induced by intestinal microbiota may suppress Th2 immune responses.[9] IL-10 levels in the blood samples were not measured to validate the LP0132 signature, which we will perform in a future study to further elucidate the mechanism underlying the action of LP0132. Propensity score matching to adjust for MS may have missed unrecognized backgrounds, which could lead to the misinterpretation of our results. However, we did not find any factors that were different between the matched groups.

In summary, citrus juice fermented with LP0132 alleviated JCPsis symptoms in the early season and suppressed reduction in circulating Treg cells in the peak season. The increase in Treg abundance from enrollment was associated with milder JCPsis symptoms.

ACKNOWLEDGMENTS

We thank all participants in this trial. We also thank all staff of Shimoshizu National Hospital for clinical support, N. Sahashi of Toho University for providing data on pollen dispersion, T. Uetake and K. Fujiura of CX Wellness Co., Ltd. for support with subject management and data collection, and M. Miyagawa of Takumi Information Technology Corporation, J. Nakayama of LSI Medience Corporation, S. Nakamura of Riken, C. Nonaka, K. Nakamori, and T. Igarashi of Yakult Honsha Co., Ltd., and E. Kotaki of Yakult Central Institute for helpful advice. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

This trial was conducted based on a collaboration between Yakult Central Institute at Yakult Honsha Co., Ltd. and Shimoshizu National Hospital. NK, SK, KM, and NHM are employees of Yakult Honsha Co., Ltd. The other authors declare no conflicts of interest.

FUNDING INFORMATION

This trial was funded by Yakult Honsha Co., Ltd.

Shuichi Suzuki[1]
Norihiro Kubota[2]
Sayaka Kakiyama[2]
Kouji Miyazaki[2]
Kazuki Sato[1]
Naomi Harima-Mizusawa[2]

[1]*Department of Clinical Research, Shimoshizu National Hospital, Chiba, Japan*
[2]*Yakult Central Institute, Tokyo, Japan*

Correspondence
Shuichi Suzuki, Department of Clinical Research, Shimoshizu National Hospital, Yotsukaido, Chiba 284-0003, Japan.
Email: seeyou@msj.biglobe.ne.jp

ORCID

Shuichi Suzuki https://orcid.org/0000-0003-4295-4630

Norihiro Kubota https://orcid.org/0000-0002-4682-2750

REFERENCES

1. Yamada T, Saito H, Fujieda S. Present state of Japanese cedar pollinosis: the national affliction. *J Allergy Clin Immunol*. 2014;133(3):632–639.
2. Okubo K, Kurono Y, Ichimura K, et al. Japanese guidelines for allergic rhinitis 2017. *Allergol Int*. 2017;66(2):205-219.
3. Güvenç IA, Muluk NB, Mutlu FŞ, et al. Do probiotics have a role in the treatment of allergic rhinitis? A comprehensive systematic review and meta-analysis. *Am J Rhinol Allergy*. 2016;30(5):157-175.
4. Martens K, Pugin B, De Boeck I, et al. Probiotics for the airways: Potential to improve epithelial and immune homeostasis. *Allergy*. 2018;73(10):1954-1963.
5. Harima-Mizusawa N, Kano M, Nozaki D, Nonaka C, Miyazaki K, Enomoto T. Citrus juice fermented with *Lactobacillus plantarum* YIT 0132 alleviates symptoms of perennial allergic rhinitis in a double-blind, placebo-controlled trial. *Beneficial Microbes*. 2016;7(5):649-658.
6. Dennis-Wall JC, Culpepper T, Nieves C Jr, et al. Probiotics (*Lactobacillus gasseri* KS-13, *Bifidobacterium bifidum* G9–1, and *Bifidobacterium longum* MM-2) improve rhinoconjunctivitis-specific quality of life in individuals with seasonal allergies: a double-blind, placebo-controlled, randomized trial. *Am J Clin Nutr*. 2017;105(3):758-767.
7. Palmer C, Mulligan JK, Smith SE, Atkinson C. The role of regulatory T cells in the regulation of upper airway inflammation. *Am J Rhinol Allergy*. 2017;31(6):345-351.
8. Varona R, Ramos T, Escribese MM, et al. Persistent regulatory T-cell response 2 years after 3 years of grass tablet SLIT: Links to reduced eosinophil counts, sIgE levels, and clinical benefit. *Allergy*. 2019;74(2):349-360.
9. Sefik E, Geva-Zatorsky N, Oh S, et al. Individual intestinal symbionts induce a distinct population of RORgamma(+) regulatory T cells. *Science*. 2015;349:993-997.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

DOI: 10.1111/all.14004

Demonstration of human mast cell progenitors in the bone marrow

To the editor

Mast cells are tissue-resident cells widely recognized for their role in asthma and allergy. In inflammatory diseases, mast cells accumulate and become activated in particular sites of affected tissues such as, for example in the bronchial smooth muscles of allergic asthmatics.[1] Using in vivo models, we and others have shown that these inflammation-induced mast cells originate from mast cell progenitors (MCp).[2] For decades, human mast cells have been differentiated in vitro from uncommitted progenitor cells isolated from, for example peripheral blood and bone marrow (BM). In the current study, a population of MCp in the BM of healthy adults was identified, which was more frequent, and had lower surface expression of integrin β7 than the recently described MCp population in the blood.[3] Integrin β7 is required for transmigration of mouse MCp into the lung.[4] Thus, we speculate that integrin β7 is upregulated on the human MCp upon release from the BM in preparation for their transmigration from the blood to the peripheral tissues.

Using flow cytometry, BM samples from healthy donors were investigated for the possible presence of a MCp population. In comparison with peripheral blood samples from the same individuals (n = 11), the BM was enriched in lineage (Lin)$^-$ (CD4$^-$ CD8$^-$ CD19$^-$ CD14$^-$) cells, Lin$^-$ CD34^{hi} cells and Lin$^-$ CD34^{hi} CD117$^+$ cells (Figure 1A). A population of Lin$^-$ CD34^{hi} CD117$^+$ FcεRI$^+$ cells was found in healthy BM (Figure 1D), which was similar but 14-fold more frequent than the known MCp population in peripheral blood (Figure 1B and I). In one set of experiments, Lin$^-$ CD34^{hi} CD117$^+$ FcεRI$^+$ cells from the BM and blood from seven donors were isolated by fluorescence-activated cell sorting (FACS) and either cultured in a myeloid-erythroid cytokine cocktail for 7 days or analysed directly. The primary BM Lin$^-$ CD34^{hi} CD117$^+$ FcεRI$^+$ cells demonstrated immunofluorescence for mast cell tryptase (Figure 1E). Re-analysis of the respective progeny cells by flow cytometry revealed that all cells had an intermediate CD117 expression after culture and that a proportion of them had lost FcεRI surface expression (Figure 1F and J). The loss of FcεRI expression upon in vitro culture of the human peripheral blood MCp during the same culture conditions was reported previously,[3] and FcεRI$^-$ mast cells have been demonstrated in vitro[5] and shown residing in the alveolar parenchyma in vivo.[6] Regardless, there was no difference in the expression of FcεRI between the BM and blood progenies (39.8% ± 10.2% and 52.7% ± 12.0%, respectively). The BM progeny cells had a similar mast cell-like morphology, determined by May-Grünwald-Giemsa staining (Figure 1G), as the blood progeny cells (Figure 1K). Further, the progeny cells from both sources expressed various levels of tryptase (Figure 1H and L). Altogether,