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Bacillus subtilis exopolysaccharide prevents allergic eosinophilia

To the Editor,

The composition of the intestinal microbiota has been linked to the risk for developing allergic disease. Although the mechanism by which the microbiota prevents allergic sensitization is unclear, evidence suggests that it is through modulation of the immune system.¹ In this study, we investigated the ability of the spore-forming gram-positive bacterium *Bacillus subtilis* to prevent the development of allergic disease. Previous work from our laboratory established *B. subtilis* as an immune suppressive bacterium that can prevent diarrheal disease from *Citrobacter rodentium* infection.^{2,3}

In this study, we tested the ability of *B. subtilis* spores to prevent house dust mite (HDM)-induced eosinophilic inflammation using the treatment protocol shown in Figure 1A that was approved by the Midwestern University Institutional Animal Care and Use Committee. C57Bl/6 mice were orally gavaged with 10^9 WT *B. subtilis* spores or 10^9

epsH spores that are unable to produce exopolysaccharide (EPS), due to a mutation in the *eps* operon.⁴ Our previous work found that EPS was the component of *B. subtilis* required for protection from *C. rodentium*-induced inflammation.^{4,5} Following the final HDM treatment, bronchial alveolar lavage was collected from euthanized mice, by flushing the lungs with 0.8 mL PBS (containing 10% FCS, 1 mmol/L EDTA) and immune cell infiltration was assessed by DiffQuik (Dade Behring, Newark, DE, USA) staining of cytopun cells (Figure 1B-E). HDM from *Dermatophagoides pteronyssinus* (XPB82D3A2.5; Stallergenes Greer, Boston, MA, USA) alone induces a significant eosinophilia, shown as both percent and total cells per mL (Figure 1B,C). WT *B. subtilis* spore treatment significantly reduced the eosinophilia in HDM-treated mice. The *B. subtilis*-mediated protection was partially dependent on the production of EPS, evidenced by *epsH* *B. subtilis* spores being unable to significantly reduce the HDM-induced eosinophilia.

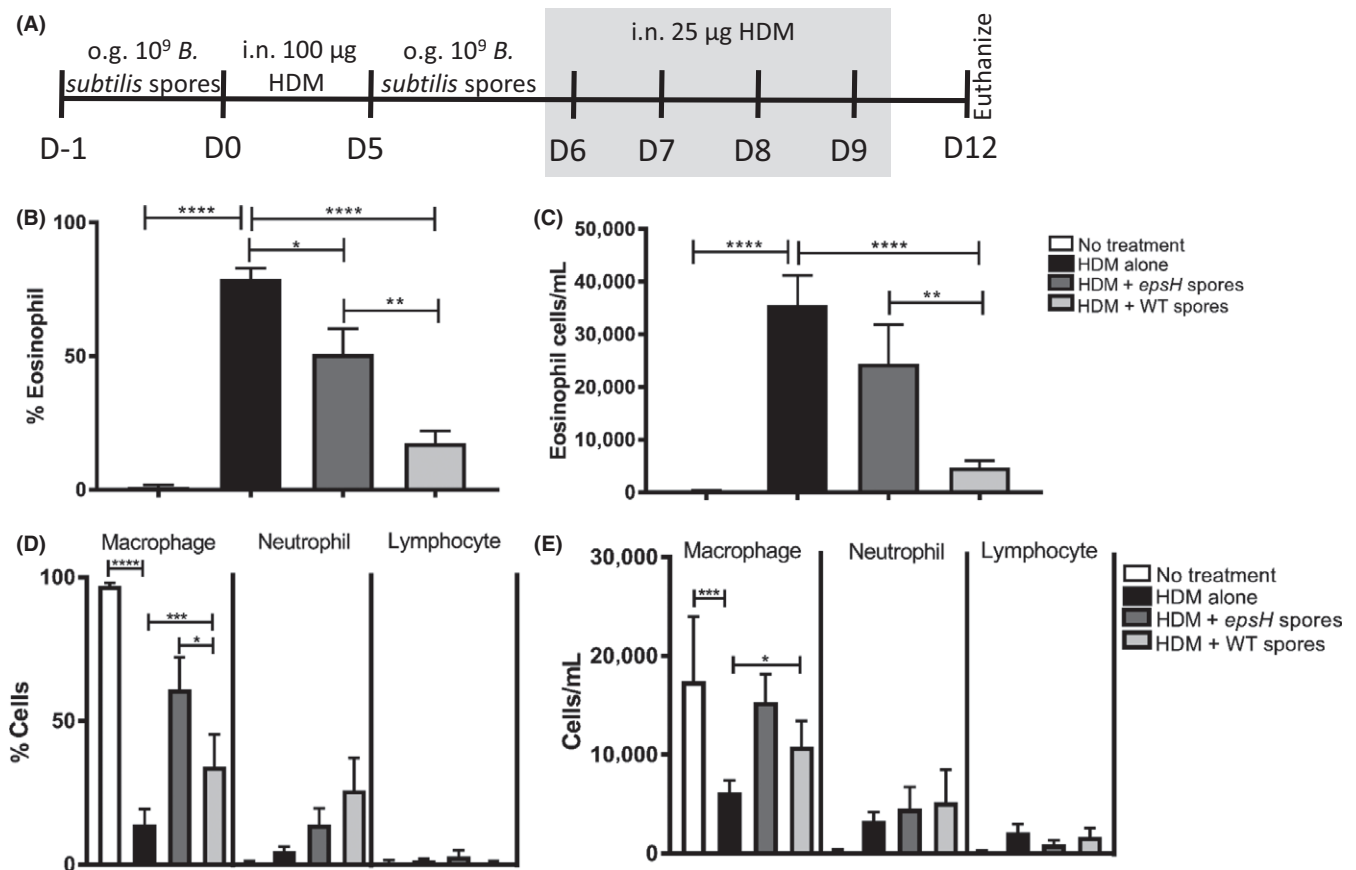


FIGURE 1 Prevention of house dust mite (HDM)-induced eosinophilia by *Bacillus subtilis*. (A) Timeline for *B. subtilis* and HDM treatment of 6-week-old C57Bl/6 mice. (B-E) Quantification of cells from bronchial alveolar lavage (BAL) cytopun and stained with DiffQuik on day 12. Data represent mean $\pm$ SEM ($n = 7-14$) from three combined experiments. **** $P < 0.0001$, *** $P < 0.0005$, ** $P < 0.005$, and * $P < 0.05$ by Student's *t* test. o.g. = oral gavage; i.n. = intranasal

To understand the mechanism of *B. subtilis* suppression of eosinophilia, we tested the effect of EPS treatment on dendritic cells. Dendritic cells are critical in the development of eosinophilia and are responsible for activating T cells. Bone marrow-derived dendritic cells (BMDC) were pretreated with or without EPS for 24 hours, followed by the presence or absence of HDM for 24 hours, and transferred intranasally into naive C57Bl/6 mice (according to protocol timeline, Figure 2A). EPS was isolated according to a previously published protocol by Jones et al. The purified EPS had undetectable nucleic acid (OD₂₆₀) and protein (OD₂₈₀). Quantification of carbohydrate content was determined by phenol-sulfuric acid method. Mice were then challenged intranasally with HDM, daily for 3 days. Dendritic cells treated with EPS in the absence of HDM failed to elicit eosinophilia (data not shown). We found that while HDM-pulsed dendritic cells elicit eosinophilia, HDM-pulsed dendritic cells treated with EPS failed to induce eosinophilia, shown as both percent and total cells per mL (Figure 2B,C). Other infiltrating immune cells including neutrophils and lymphocytes were not significantly different between the transferred dendritic cell treatment groups, while the resident macrophage population remained

significantly higher in both no treatment and EPS treatment groups (Figure 2D,E). These results suggest that *B. subtilis* EPS-treated BMDC can prevent the induction of allergic eosinophilia.

The results of these experiments support the hypothesis that bacteria can help educate the immune system to prevent allergic disease. Specifically, we show that *B. subtilis*-derived EPS can suppress dendritic cell function to elicit allergic eosinophilia. Future work is required to understand how EPS-treated dendritic cells inhibit eosinophilia. We suggest that EPS-treated dendritic cells impair Th2 cell polarization and subsequent IL-5 production, which is needed for activation of eosinophils. Probiotics have been previously identified as methods for reducing allergic eosinophilia;⁶ however, our work is unique in the fact that we required only two treatments with *B. subtilis* to prevent allergic disease and this was in the presence of a normal microbiota. Additional work will be focused on elucidating other, currently unknown, molecules or metabolites that contribute to *B. subtilis*-mediated protection that is EPS-independent. In conclusion, we have established *B. subtilis* as a novel bacterium for the treatment of allergic disease and we show *B. subtilis*-derived EPS is involved in this protection.

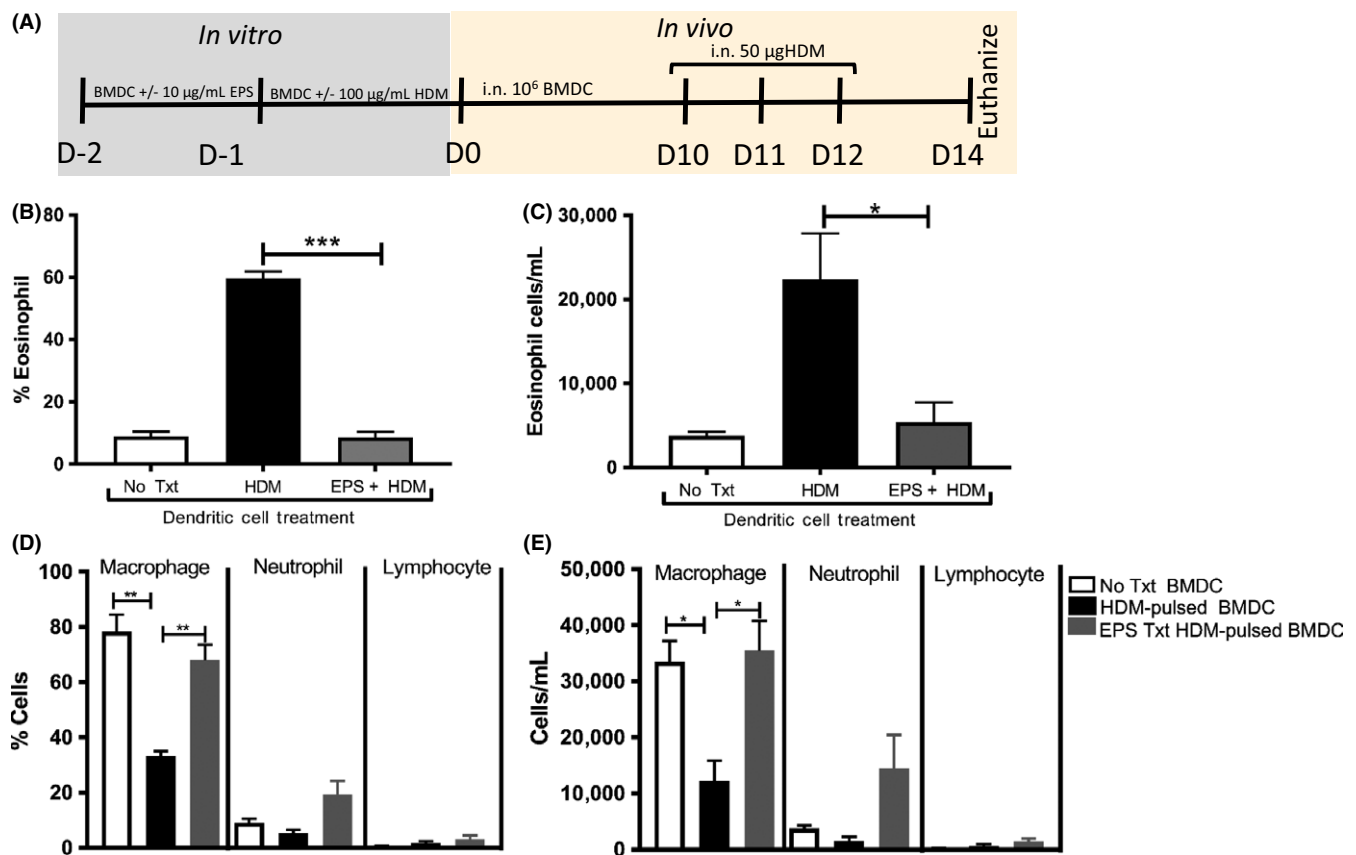


FIGURE 2 Prevention of eosinophilia by exopolysaccharide (EPS)-treated dendritic cells. (A) Timeline for bone marrow-derived dendritic cells (BMDC) incubation with exopolysaccharide and house dust mite (HDM) prior to i.n. transfer into 7–10 week-old C57Bl/6 mice. BMDC were generated from C57Bl/6 mouse bone marrow in the presence of 20 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) for 6 days to >85% CD11c⁺ purity. Dendritic cell transfer groups included (1) no treatment (No Txt) with BMDC receiving neither EPS nor HDM (2) HDM group with BMDC receiving HDM alone on D-2 and (3) EPS + HDM group of BMDC receiving EPS on D-2, followed by HDM on D-1. (B–E) Quantification of cells from BAL cytospun and stained with DiffQuik on day 14. Data represent mean ± SEM (*n* = 3). ****P* < 0.0005, ***P* < 0.005, and **P* < 0.05 by Student's *t* test. i.n. = intranasal. These data are representative of four independent BMDC transfer experiments [Colour figure can be viewed at wileyonlinelibrary.com]

AUTHOR CONTRIBUTION

JAS and KLK developed the model. JAS wrote the manuscript. JAS, RWI, SAW, and AJ performed the experiments and analyzed the data.

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CONFLICTS OF INTEREST

All authors declare that they have no relevant conflicts of interest.

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How to discontinue omalizumab in chronic spontaneous urticaria?

To the Editor,

The clinical efficacy and safety of omalizumab in chronic spontaneous urticaria (CSU) has been demonstrated in several clinical trials and real-life studies.^{1,2} The EAACI/GA²LEN/EDF/WAO urticaria guideline recommends omalizumab as an add-on third-line treatment option for patients with CSU.³ As of now, there is no consensus on when and how to discontinue omalizumab treatment in CSU patients who show complete response.

Chronic spontaneous urticaria, in most patients, is a self-limited disease. The duration of CSU is usually longer than 1 year, and

the 5-year remission rate is 34-86%.^{4,5} Because of this, omalizumab discontinuation should be tried periodically in patients with completely controlled disease in order to understand whether the disease is in remission or not.⁶ In many patients, this is done by simply stopping omalizumab treatment after 6 months or 1 year. Alternatively, discontinuation of omalizumab has been suggested to be done by prolonging treatment intervals.⁶⁻⁹ As of now, there is very little information on how well this works in real-life clinical practice.

Here, we assessed the response of CSU patients to treatment discontinuation by interval prolongation. We included 19 of 86 patients with CSU who received omalizumab treatment at the Erciyes University Allergy Clinic between 2017 and 2018. These 19 patients: (a) had been treated with omalizumab (300 mg/4 wk)

Abbreviations: AH, anti-histamines; CSU, chronic spontaneous urticaria; EAACI/GA²LEN/EDF/WAO, European Academy of Allergology and Clinical Immunology, Global Allergy and Asthma European Network, European Dermatology Forum, World Allergy Organization; IgE, immunoglobulin E; IQR, interquartile range; UCT, urticaria control test.