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SUPPORTING INFORMATION

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Prevention of allergic airway and gut inflammation in humanized mice by lactobacilli, bifidobacteria, and butyrate

To the Editor,

Probiotic bacteria and their metabolites, particularly the short chain fatty acid butyrate, possess beneficial immunomodulatory and anti-inflammatory properties and have been shown to protect against gastrointestinal and respiratory diseases including IgE-mediated allergic inflammation.[1–3] However, underlying mechanisms such as modulation of dendritic cell maturation and induction of regulatory T (Treg) cells are complex and not fully understood.[2,4] Moreover, to achieve a strong immune-modulatory response, it is crucial to provide multiple bacteria in optimal concentration and simultaneously. Here, we investigated for the first time a well-defined commercially available probiotic formulation (BactoFlor® 10/20, BF), consisting of 10 large and small intestine-specific strains of Lacto- and Bifidobacteria and the prebiotic carbohydrate inulin, in comparison to butyrate in a well-established and valid humanized mouse model of allergen-induced gut and lung inflammation.[5]

Nonobese diabetic severe-combined-immunodeficient $\gamma c^{-/-}$ (NSG) mice were intraperitoneally injected with PBMC from highly-sensitized birch or grass pollen allergic human donors together with the respective allergen or PBS as control. During the whole experiment, mice were orally treated with BF or butyrate as described in the Appendix S1. In this model, sufficient numbers of human cells are detectable 3 weeks after PBMC transfer, and inflammation of the gut and lungs can be measured by high-resolution video mini-endoscopy, evaluating the parameters translucency, granularity, fibrin production, vascularity, and stool, or by invasive body plethysmography after rectal or intranasal allergen challenge, respectively.[5] Figure 1A,B shows that the strong inflammation of the gut in PBMC plus allergen-treated mice was completely prevented by oral administration of BF or butyrate. In addition, disturbed intestinal barrier integrity, as measured by FITC-dextran assay, was significantly restored (Figure 1C). Analysis of lung allergic inflammation further revealed reduced lung airway resistance, reduced mucosal hypertrophy, and reduced mucus-producing goblet cells in BF- or butyrate-treated mice (Figure 1D,E). Interestingly, despite equal numbers of human $CD45^+$ cells in all organs, an increased migration of $FoxP3^+$ Treg cells into the lung and gut tissue was found in BF- as well as butyrate-treated mice after respective allergen challenge (Figure 1F). In the spleen, a slight enhancement of $CD4^+FoxP3$ expression was also detected without reaching significance (Figure 1G). Engraftment of BF- or butyrate-pretreated PBMC also significantly reduced gut and lung inflammation, but less pronounced as compared to oral treatment (Figure S1A–D). This superior protective effect of orally applied probiotics/butyrate suggests that besides their immunomodulatory effect on PBMC, they also directly affect the mucosal epithelium and barrier. Moreover, a slight but not significant increase in butyrate concentration was observed in stool of BF-treated mice (Figure S2). This implicated that other microbiota-derived metabolites than butyrate, possibly tryptophan metabolites or retinoid acid, might also be involved in the induction of the regulatory immune response.[2,6]

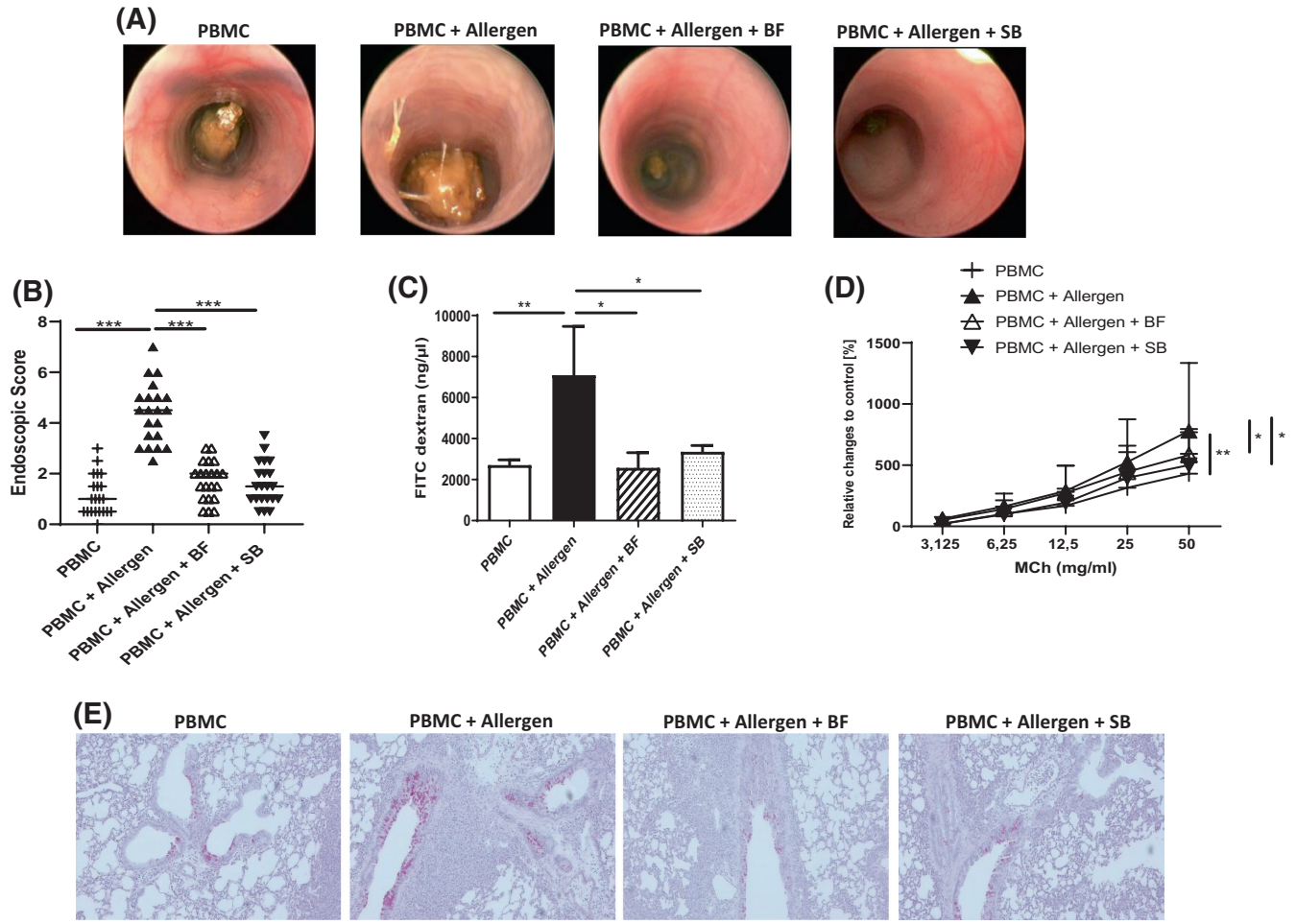

FIGURE 1 Prevention of allergen-induced gut and lung inflammation in BactoFlor® 10/20 (BF)- or sodium butyrate (SB)-treated humanized NSG mice. NSG mice were transplanted with PBMC from birch or grass pollen allergic donors together with the respective allergen, and orally treated with BF or butyrate or left untreated throughout the whole experiment. On day 21, mini-endoscopy was performed 4 h after rectal allergen challenge. On day 23, mice were intranasally challenged with the allergen and AHR was measured by invasive body plethysmography 24 h later. Shown are representative endoscopic pictures (A), the single values + mean of the endoscopic score based on the parameters granularity, vascularity, translucent structure, fibrin production, and stool consistency (B), the means ± SEM of FITC-Dextran in mouse sera (C), the means ± SEM of the relative changes of AHR to baseline value (D), representative pictures of PAS staining of the lungs (magnification ×200) (E), micrographs of antihuman CD45 (magnification ×100) and FoxP3 (magnification ×200) in lung (upper panels) and colon (lower panels) (F), and flow cytometric staining of human $CD45^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $FoxP3^+$ cells in spleen (G) of 10 independent experiments/allergic donors PBMC (2 for C) with 2–3 mice per group. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$.

To analyze whether Treg cells are responsible for the protective effect of BF and butyrate, $CD25^{high}$-expressing cells were depleted from the PBMC prior to injection (Figure S3). In these $CD25^{neg}$PBMC engrafted NSG mice, gut and lung inflammation was only marginally reduced by BF or butyrate (Figure 2A–D). This is in accordance with former studies demonstrating the strong Treg cell dependency in prevention of allergic diseases.[5] Notably, neutralization of allergy promoting group 2 innate lymphoid cells (ILC2), which are known to be controlled by Treg,[1] by anti-ST2 mAbs restored the protective effect of BF and butyrate in Treg-depleted mice (Figure S4). Other cell types might also be involved which is currently under investigation.

Taken together, our study support the relevance of Treg cells regarding the combined effect of defined beneficial intestinal microbial species and their products on allergic intestinal and airway inflammation. Hence, it advances our current understanding of the

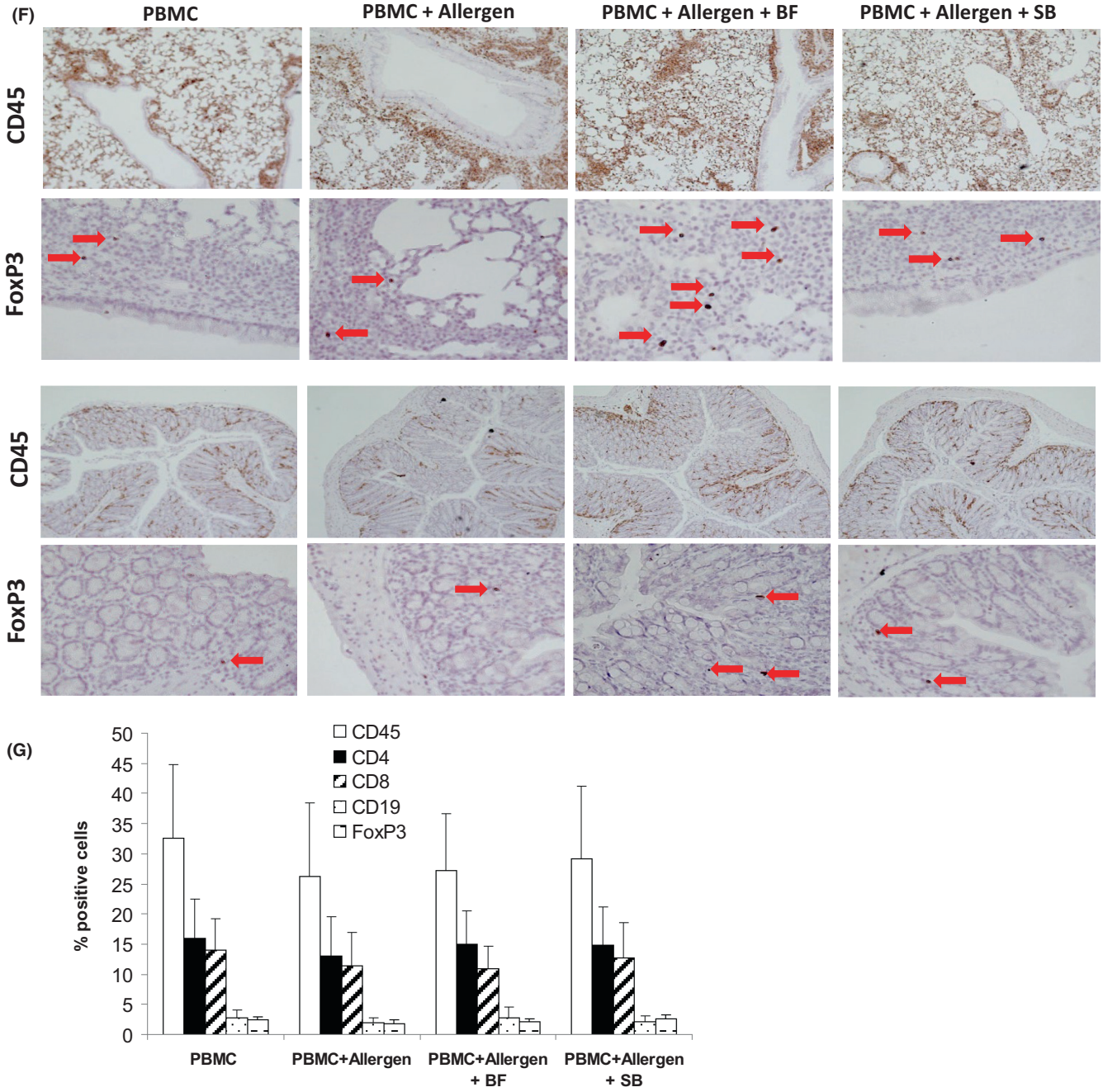

FIGURE 1 (Continued)

previously suspected adjuvant effect of certain probiotics in allergic desensitization. Our results may also be applicable to other inflammatory diseases of the gastrointestinal and respiratory tract.

AUTHOR CONTRIBUTIONS

DS, JS, and IB generated the idea and supervised the work; RK, BW, FS, SS, and IB performed laboratory assays and contributed to data analysis; RK, DS, and IB wrote the manuscript; all authors have read and approved the manuscript.

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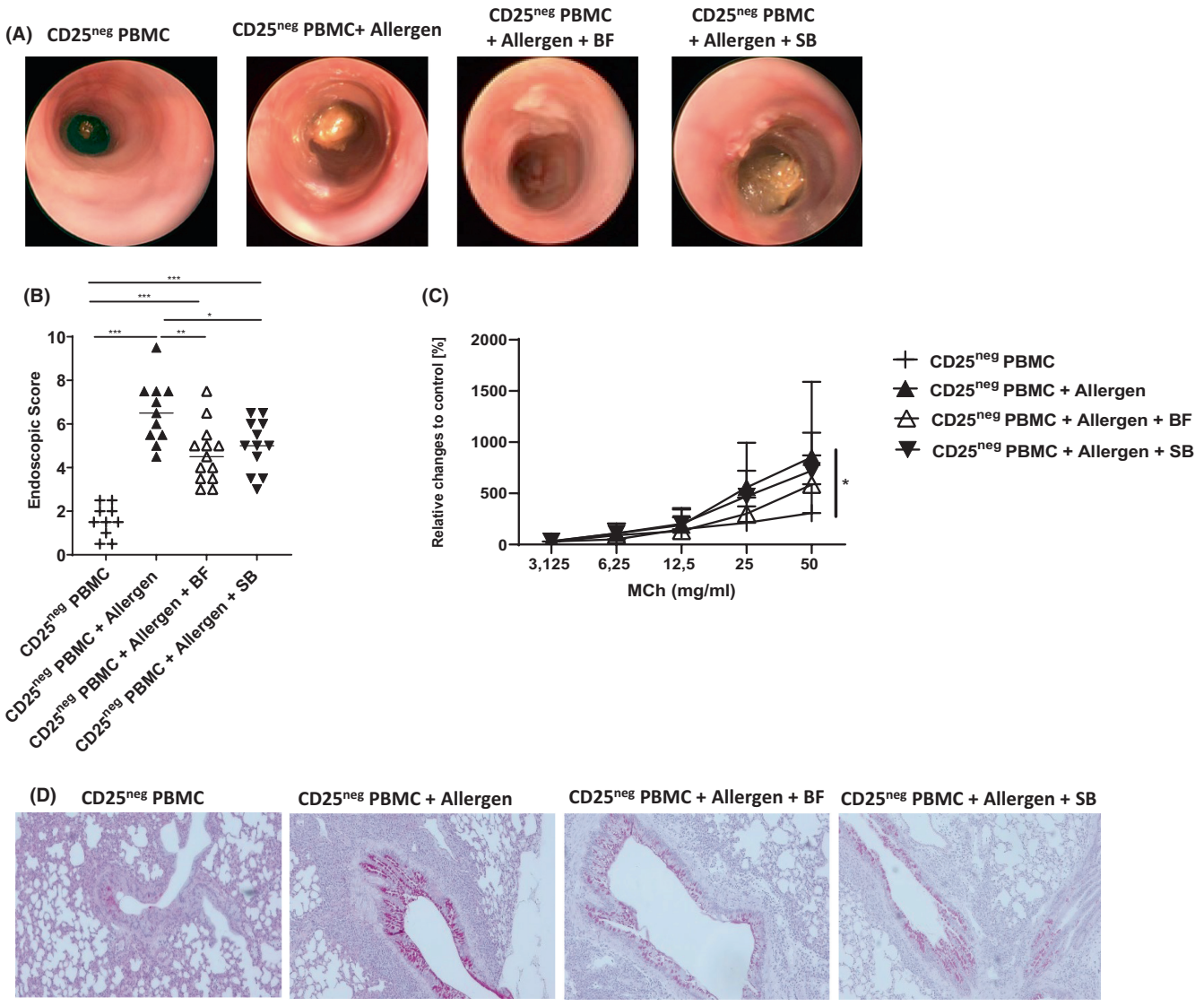

FIGURE 2 Loss of protective effect of orally administered BactoFlor® 10/20 (BF) or sodium butyrate (SB) after injection of Treg cell-depleted CD25^{neg}PBMC. NSG mice were treated as described in Figure 1 using Treg cell-depleted CD25^{neg}PBMC. Shown are representative endoscopic pictures (A), the single values + mean of the endoscopic score (B), the means ± SEM of the relative changes of AHR to baseline value (C), and representative pictures of PAS staining of the lungs (magnification ×200) (D) of six independent experiments/allergic donors PBMC with 2–3 mice per group. *$p < .05$, **$p < .01$, ***$p < .001$.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicting financial interests to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Anti-rubella IgG serum levels predict risk for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) breakthrough infections

To the Editor,

SARS-CoV-2 breakthrough infections among vaccinated individuals are common.[1] The identification of people at risk for such breakthrough infections remains challenging.[2] In this regard, age and pre-existing comorbidities have been shown to significantly influence the risk for SARS-CoV-2 breakthrough infections.[3] Immunological factors have long been recognized to play a central role in the prevention of viral infections; however, we were unable to predict SARS-CoV-2 breakthrough infections in the extensively studied CoV-ADAPT cohort using a machine learning approach based on SARS-CoV-2-specific, longitudinal, immunological data from different time points prior to infection.[4] Recently, low anti-rubella IgG titers were shown to be associated with low SARS-CoV-2 anti-RBD IgG responses following vaccination with BNT162b2.[5] Hence, the aim of this study was to investigate whether lower anti-rubella IgG serum levels predispose for SARS-CoV-2 breakthrough infections and add predictive value.

This study was carried out using serum material from the well-defined CoV-ADAPT cohort.[4,6,7] The inclusion criteria were described previously.[6] Anti-rubella IgG levels were measured in a total of 234 samples—collected from 134 participants prior to a potential SARS-CoV-2 breakthrough infection (Table S1)—from T1 (<2 weeks before the second SARS-CoV-2 vaccination) and T3 (3–7 months after the second vaccination) using the Rubella IgG assay (Abbott Laboratories, Abbott Park, USA) on the Architect i2000SR (Abbott Laboratories). Measurements were then linked to the respective breakthrough status at later stages between T3 and T4 (3–7 months after the third vaccination). Details regarding the

Moritz M. Hollstein and Sascha Dierks share first authorship.

Luise Erpenbeck and Moritz Schnelle are joint senior authors.