Universiteit Leiden
The Netherlands

Keyhole limpet hemocyanin challenge model for studying adaptive immune system responses in early-phase clinical drug development

Saghari, M.

Citation
Saghari, M. (2024, December 17). *Keyhole limpet hemocyanin challenge model for studying adaptive immune system responses in early-phase clinical drug development*. Retrieved from https://hdl.handle.net/1887/4172487

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/4172487

Note: To cite this publication please use the final published version (if applicable).

CHAPTER VI

EVALUATION OF SINGLE-STRAIN PREVOTELLA HISTICOLA ON KLH-DRIVEN IMMUNE RESPONSES IN HEALTHY VOLUNTEERS: A RANDOMIZED CONTROLLED TRIAL WITH EDP1815

Mahdi Saghari[1,2], Pim Gal[1,2], Hendrika W. Grievink[1,3], Erica S. Klaassen[1], Ahnjili Zhuparris[1], Andrea Itano[4], Mark Bodmer[4], Duncan McHale[4], Matthijs Moerland[1,2]

1. Centre for Human Drug Research (CHDR), Leiden, the Netherlands
2. Leiden University Medical Centre (LUMC), Leiden, the Netherlands
3. Leiden Academic Centre for Drug Research (LACDR), Leiden, the Netherlands
4. Evelo Biosciences Inc., Cambridge, Massachusetts, United States

Medicine in Microecology, 2024;19. DOI: 10.1016/j.medmic.2023.100088

Read our original article here

ABSTRACT

Introduction: EDP1815 is a single-strain of *Prevotella histicola* with preclinical immunomodulatory properties. The aim of this study was to evaluate pharmacodynamic effects of EDP1815 on the immune response following immunization with keyhole limpet hemocyanin (KLH) and dermal rechallenge.

Methods: Thirty-two healthy subjects (median 30, range 18–59 years) were randomized over two cohorts to EDP1815 or placebo (12:4). Both cohorts received 8.0×10^{11} total cells daily for 28 days, reconstituted in 10 (A formulation) or 5 (B formulation) capsules. KLH-specific antibodies and circulating regulatory T cells were evaluated. Skin response after rechallenge was assessed with imaging. Immune cell subsets from blister exudates were assessed in the B cohort only. *Ex vivo* phytohemagglutinin and lipopolysaccharide whole blood challenges were performed to evaluate cytokine release. Gastrointestinal tract persistence, prevalence, and colonization of EDP1815 were assessed by fecal qPCR and microbiome assays. Data were analyzed using repeated measures analysis of covariances.

Results: There was a trend toward a treatment effect on the KLH-induced skin rechallenge response (CIELab a* estimated difference (ED) −1.50 arbitrary unit (AU), 95% CI −3.47–0.47 AU, $P = .13$ in A cohort, and average redness ED −0.14 AU, 95% CI −0.31–0.03 AU, $P = .10$ in B cohort) and, to a lesser extent, on the humoral KLH response. No notable EDP1815 effects were observed on gut persistence, microbiome, and other safety parameters.

Conclusion: Based on our findings and the clinical benefit observed in the phase 2 study in psoriasis, further investigation of the immunomodulatory effects and potential clinical benefit of EDP1815 is warranted.

INTRODUCTION

Several lines of evidence have suggested that microbes within the gastrointestinal (GI) tract interact with the systemic immune system.[1-3] Throughout the epithelium of the small intestines, immune cells can be found in both the lamina propria and associated mesenteric lymph nodes, and in aggregated lymphoid nodules also known as Peyer's patches.[1,4] The intestinal immune network in mice contains specialized regions differing in gut-associated lymphoid tissue (GALT), leukocyte populations, and antigenic composition. Similarities between murine and human intestinal immune systems have been observed.[1] Commensal microorganisms colonize the mucosal wall of the small and large intestine with variable local distribution and composition within the GI tract.[1] Conditions such as celiac disease and inflammatory bowel disease are caused by an altered intestinal immune system or gut microbiome.[1,5-7] Notably, recent advances in this field of research have suggested that intestinal disruptions to microbiota homeostasis seem not to be limited to local immunity, but can also affect more distal immune responses as observed in psoriasis,[8] rheumatoid arthritis,[9] systemic lupus erythematosus,[10] and more.[11,12] More recent trials have investigated the interaction between the intestinal microbiota of the large intestine and the systemic immune system, evaluating potentially beneficial effects of probiotics, prebiotics, and/or synbiotics.[13-17] The involved underlying mechanisms are not fully understood and are further complicated by diverse or even contrasting immunomodulatory effects when comparing administration of microbial strain mixtures to single-strain microbes.[18-21] The potential of using single strains of microbes as treatments are favored in order to advance the understanding of the interactions between the systemic immune system and the small intestine which drive tolerance of foreign food antigens.[22,23]

EDP1815 is one such single-strain microbe prepared from *Prevotella histicola*, a gram-negative, obligate, non-sporulating, commensal anaerobe isolated from a duodenal biopsy of a human donor. To date, *Prevotella* species have been identified in oral, nasopharyngeal, GI, and genitourinary mucosa in humans[24] and stool abundance ranges from <1% up to approximately 50% of total fecal microbial amount.[25] *In vitro*, EDP1815 stimulated the secretion of anti-inflammatory cytokines interleukin (IL)-10 and IL-27 from human macrophages, proinflammatory M1-biased antigen presenting cells, and dendritic cells (DCs), whilst not inducing significant levels of proinflammatory cytokines including tumor necrosis factor (TNF) and interferon gamma (IFN-γ) (unpublished data). EDP1815 reduced ear inflammation in murine keyhole limpet hemocyanin- (KLH), MC903- (vitamin D_3 analog), and imiquimod-induced skin challenge models.[26] Furthermore, *ex vivo* immunophenotyping in these mouse models showed increased regulatory T cells (Tregs).[27,28] In disease mouse models,

P. histicola decreased incidence and severity of arthritis in collagen-induced arthritis,[27] suppressed disease in experimental autoimmune encephalomyelitis,[28] delayed the onset of type 1 diabetes,[29] and suppressed T cell responses to gliadin indicating a possible future treatment modality in celiac disease.[30] Clinically, EDP1815 was considered to be safe and well tolerated in a first-in-human study and a phase 2 study in psoriasis (ClinicalTrials.gov identifier NCT04603027).[26,31]

In this clinical study the immune system of healthy volunteers was challenged with KLH immunization, a safe challenging antigen widely used for evaluation of cell-mediated responses in man.[27-38] KLH is recognized as a neoantigen that drives a cellular immune response. *In vivo,* this response can be studied by an intradermal KLH rechallenge. The resulting local inflammatory response can serve as a functional biomarker for clinical evaluation of (novel) immunomodulatory drugs in early-phase drug development.[32,34-36,43-45]

The aim of this study was to evaluate the effects of EDP1815 on the immune response following intramuscular immunization with KLH. The response to KLH immunization was evaluated by serum KLH-specific antibody concentrations and circulating Tregs, and by characterization of the skin response following intradermal KLH rechallenge, by objective imaging techniques and at the level of tissue cytokines and immune cell subsets. EDP1815 effects were also explored *ex vivo* by whole blood stimulation with Toll-like receptor 4 ligand lipopolysaccharide (LPS) and phytohemagglutinin (PHA) for lymphocyte stimulation. EDP1815 GI tract persistence, prevalence, and colonization were assessed by quantitative polymerase chain reaction assay (qPCR) using EDP1815 specific primers and 16S ribosomal RNA (rRNA) sequencing of fecal material, next to routine safety and tolerability assessments.

METHODS

This was a phase 1, randomized, placebo-controlled, double-blind, multiple dose study in thirty-two (32) healthy volunteers performed at the Centre for Human Drug Research (CHDR), Leiden, The Netherlands, based on principles of the Declaration of Helsinki. The independent Medical Ethics Committee *Medisch Ethische Toetsingscommissie van de Stichting Beoordeling Ethiek Biomedisch Onderzoek* (Assen, the Netherlands) approved the study prior to any clinical study activity. All subjects provided written informed consent before participation. The trial was registered on the Netherlands Trial Register, currently available for consultation at the International Clinical Trial Registry Platform (trial ID NL8676, https:// trialsearch.who.int). Materials and methods were executed as described previously.[46]

SUBJECTS

Healthy male and female participants were enrolled, 18 to 60 years of age with a body mass index between 18 and 35 kg/m^2, without previous exposure to KLH. Health status was verified by recording a detailed medical history, a complete physical examination, vital signs, a 12-lead electrocardiogram (ECG) and laboratory testing (including hepatic and renal panels, complete blood count, fecal calprotectin, virology and urinalysis). Subjects were excluded in case of any disease associated with immune or GI system impairment, or use of prescription medication within four weeks prior to first dose.

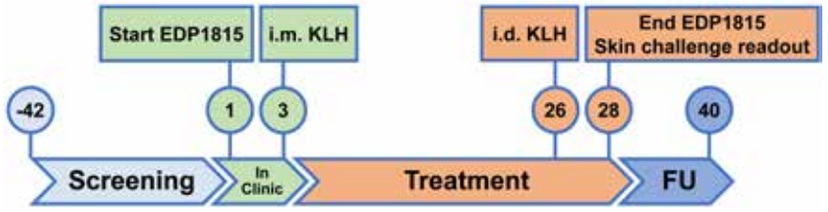
DOSE SELECTION AND REGIMEN

The doses tested were based on the results of a separate first-in-man study.[26,31] The highest dose tested contained 8.0×10^{11} total cells per dose, approximately 5 times the predicted therapeutic dose level, calculated from allometric scaling the preclinically efficacious dose level based on conversion between mouse and human gut surface area. This dose was well tolerated in humans. Participants were dosed once daily for 28 consecutive days. Participants in the second cohort received a similar dose level as the previous cohort, however, a manufacturing process change approximately doubled the EDP1815 concentration per capsule reducing the number of capsules required from 10 to 5.

STUDY DESIGN AND TREATMENTS

An overview of the study design is shown in Figure 1. Subjects were enrolled into two cohorts and randomized to either EDP1815 or placebo (12:4). The first cohort received EDP1815 freeze-dried powder in enteric-coated capsules, supplied as 8.0×10^{10} total cells per capsule, administered orally at a dose of 10 capsules daily (A formulation). The second cohort received EDP1815 freeze-dried powder in enteric-coated capsules supplied as 1.6×10^{11} total cells per capsule, administered orally at a dose of 5 capsules daily (B formulation). Compliance was confirmed by supervised administration during the in-clinic period. Administration at home was recorded by an electronic diary. Intramuscular KLH immunizations were performed in the left deltoid muscle after completion of the third EDP1815/placebo administration. KLH immunization was administered as 0.1 mg Immucothel (Biosyn Corporation, Carlsbad, CA, USA) adsorbed in 0.9 mg aluminum hydroxide (Alhydrogel, Brenntag AG, Essen, Germany) into 0.5 mL NaCl 0.9% as previously described.[42] All subjects were administered KLH (0.001 mg Immucothel) and saline in 0.1 mL NaCl 0.9% intradermally in the left and right ventral forearms, respectively, 23 days after KLH immunization. Dermal imaging was done prior to and two days after intradermal KLH administration, as in previous KLH studies.[33,34,38,41-43,47]

FIGURE 1 Study timeline. Numbers represent visit days.



i.m., intramuscular; i.d., intradermal; KLH, keyhole limpet hemocyanin; FU, follow-up.

SYSTEMIC IMMUNITY: HUMORAL RESPONSE TO KLH AND CIRCULATING REGULATORY T CELLS

The humoral response to KLH immunization was measured by anti-KLH IgM and IgG serum titers. Serum samples were analyzed by quantitative enzyme-linked immunosorbent assay (ELISA) for anti-KLH IgM and IgG as previously described.[42] Mean optical density of baseline samples was set to 1.00 and relative ratios were calculated for all subsequent samples. The percentage of circulating Tregs was evaluated by flow cytometry. Red blood cell (RBC) lysis was performed on heparinized whole blood using RBC lysis buffer (Thermo Fisher, Waltham, Massachusetts, USA). Leukocytes were stained with fluorochrome labelled antibodies (see Table S1), propidium iodide was used as viability dye (Miltenyi Biotec, Bergisch-Gladbach, Germany). Samples were measured on a MACSQuant 16 analyzer (Miltenyi Biotec), and analyzed using Flowlogic software (Inivai, Mentone, Australia). Tregs were defined as $CD4^+CD25^+CD127^-$, see Figure S1 for gating strategy.

TISSUE IMMUNITY: DERMAL RESPONSE UPON KLH INJECTION

Cutaneous blood perfusion was evaluated by laser speckle contrast imaging (LSCI; PeriCam PSI System, Perimed AB, Järfälla, Sweden) and erythema was quantified by multispectral imaging (Antera 3D, Miravex, Dublin, Ireland) as previously described.[42] Circular regions of interest at the intradermal injection sites were defined. Cutaneous blood perfusion (indicated as basal flow) was quantitatively assessed and expressed in arbitrary units (AUs). The homogeneity of cutaneous blood perfusion in the region of interest (indicated as flare), expressed as values that are +1 standard deviation (SD) from the mean basal flow within the region, was also quantitatively assessed and expressed in AUs. Erythema was quantified using the average redness and CIELab a* Antera 3D software modalities expressed as AUs. The average redness modality displays the distribution of redness using an internal software algorithm and the CIELab a* value, which is part of the CIELab color space, expresses color as a numerical value on a green–red color scale. Blister exudate was collected after blister induction and puncture in the second cohort (B formulation/placebo). Blister exudate supernatants were evaluated for immune cells ($CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, Tregs, B cells, classical monocytes, intermediate monocytes, nonclassical monocytes, DCs, natural killer (NK) cells, and neutrophils) on a MACSQuant 16 analyzer and analyzed using Flowlogic software, see Figure S2 for gating strategy. Leukocytes were stained with fluorochrome labelled antibodies (see Table S1 for a complete list), 7AAD (Miltenyi Biotec) was used as viability dye.

EX VIVO STIMULATION ASSAYS

Sodium heparinized whole blood was incubated with 10 µg/mL PHA (Sigma-Aldrich, Deisenhofen, Germany) or 2 ng/mL LPS (strain O111:B4 from *Escherichia coli*, Sigma-Aldrich) for 24 hours at 37 °C, 5% CO_2. After 24 hours, supernatant was collected and cytokines were quantified using qualified ELISA based assays (V-PLEX Proinflammatory Panel 1 Human Kit, Meso Scale Diagnostics, Rockville, Maryland, USA) by Ardena Bioanalytical Laboratory (Assen, the Netherlands). IFN-γ and IL-2 were measured in the PHA stimulated samples, TNF, IL-1β, IL-6, and IL-8 were measured in the LPS stimulated samples.

EDP1815 STOOL PERSISTENCE AND GUT MICROBIOME

Fecal concentrations of EDP1815 for stool persistence and prevalence and gut microbiome were measured by BaseClear B.V. (Leiden, the Netherlands) using validated bioanalytical methods. In short, fecal microbial DNA was extracted based on the Zymo Research (Irvine, CA, USA) fecal DNA extraction methodology. EDP1815 specific-primers and probe had been developed enabling the detection of the *P. histicola* strain. The fecal samples were analyzed using a qPCR with a lower limit of quantification of 26,400 copies/g feces. For gut microbiome analyses, extracted DNA was prepared for Illumina sequencing *via* PCR amplification of the variable region 3 and 4 of the bacterial 16S rRNA gene. After PCR purification, sample specific barcodes using Illumina Nextera XT Index kit (Illumina Inc., San Diego, CA, USA) were appended to the PCR products during a second PCR. PCR products were purified for a second time and lastly, the PCR products were equimolarly pooled and sequenced on the Illumina MiSeq platform using the MiSeq v3 sequencing kit.

SAFETY AND TOLERABILITY

Safety and tolerability were monitored by physical examination, assessment of vital signs, laboratory parameters (i.e., full blood count, biochemistry, serology, immuno-

phenotyping, circulating cytokines, fecal calprotectin, and urinalysis) and ECG data from 12-lead ECGs at regular intervals. Subjects were monitored continuously for adverse events (AEs). Participants were also asked to daily complete the Bristol Stool Scale (BSS) and questions regarding defecation pattern using an electronic diary app, to get insight in the participant's stool patterns at the time of feces sample collection.

STATISTICS

Subjects were randomized to EDP1815 or placebo in a 3:1 ratio. The placebo-treated subjects were pooled to achieve a sample size of 8 and a sample size of 12 was achieved on active treatment per group. Demographic and baseline variables were summarized by treatment. Pharmacodynamic (PD) endpoints measured at multiple time points after baseline were analyzed with a mixed effect repeated measures model with fixed factors treatment, time and treatment by time, random factor subject and the baseline value as covariate. Endpoints with one post dose measurement were analyzed with a linear model with treatment as fixed factor. Anti-KLH antibody parameters were analyzed without baseline as covariate. Skin rechallenge endpoints were analyzed with an analysis of covariance with treatment as fixed factor and the baseline and the change from baseline of the saline-injected control added as covariates. Anti-KLH IgM and IgG titers, *ex vivo* monocyte cytokine release, and immune cells in blister exudate assays required log transformation. The general treatment effect and specific contrasts were reported as estimated difference (ED) with 95% confidence interval (CI), and graphically as ED with 95% CI, as least squares mean (LSM) with 95% CI, or as mean with SD. The error terms of the mixed models are tested for normality by Shapiro-Wilk test. Fecal EDP1815 concentration was reported graphically as median with range. Fecal microbiome endpoints were analyzed using Python (Python Software Foundation, Wilmington, DE, USA). The relative abundances of all microorganisms at genus level were calculated to present the occurrence of all microorganisms relative to *Prevotella* genus in the samples. Diversity trends were analyzed using alpha diversity (Inverse Simpson's) and beta diversity (Bray-Curtis dissimilarity). The beta diversity analysis was performed solely on the ten most prevalent microorganisms based on their counts. Fecal microbiome endpoints were reported graphically as median with interquartile range for *Prevotella* relative abundance and microbiome alpha diversity. To calculate the difference between the different days (within a treatment) and between treatments for the *Prevotella* relative abundance and the alpha diversity index, independent t-tests were used. To calculate those differences for the beta diversity, a Mann-Whitney U test was used. Principal Coordinate Analysis (PCoA) was also implemented, a multidimensional scaling method, to analyze and compare the distance metrics across different days and treatments.

RESULTS

The study was conducted between December 2019 and February 2021. Thirty-two (32) subjects were enrolled and treated. All subjects completed the treatment (12 subjects EDP1815 A formulation, 12 subjects EDP1815 B formulation, and 8 subjects placebo) and the follow-up period. Baseline characteristics of all treatment groups are presented in Table 1. Treatment compliance as recorded *via* an electronic diary was 100%, no dosing days were missed.

TABLE 1 Baseline characteristics.

EDP1815 formulation	**A formulation**	**B formulation**	**Placebo**
Daily dose	**10 capsules of 8.0×10^{10} (5×) total cells**	**5 capsules of 1.6×10^{11} (5×) total cells**	
	***n* = 12**	***n* = 12**	***n* = 8**
		DEMOGRAPHICS	
Age (years)	45 (20–59)	27 (20–48)	24 (18–53)
BMI (kg/m^2)	24.5 (4.2)	23.5 (3.5)	23.1 (2.6)
Male gender (*n*)	6 (50%)	5 (41.7%)	2 (25%)
		VITAL SIGNS	
Systolic blood pressure (mmHg)	116 (11)	116 (10)	111 (9)
Diastolic blood pressure (mmHg)	70 (8)	66 (11)	65 (7)
Heart rate (BPM)	58 (9)	69 (9)	62 (13)
Temperature (°C)	36.3 (0.3)	36.9 (0.2)	36.9 (0.4)
		LABORATORY TESTS	
Leucocytes ($\times 10^9$/L)	4.85 (0.97)	5.42 (1.51)	6.07 (1.69)
Lymphocytes ($\times 10^9$/L)	1.74 (0.37)	1.75 (0.37)	1.90 (0.45)
Thrombocytes ($\times 10^9$/L)	216.1 (44.4)	243.4 (52.3)	264.0 (57.3)
ALT (IU/L)	19.2 (7.4)	15.2 (3.0)	21.4 (12.7)
AST (IU/L)	21.9 (6.1)	19.1 (5.1)	22.4 (6.0)
CRP (mg/L)	1.16 (1.21)	1.15 (1.70)	1.12 (0.96)
Fecal calprotectin (µg/g)	16.6 (9.7)	8.5 (4.8)	12.9 (13.2)

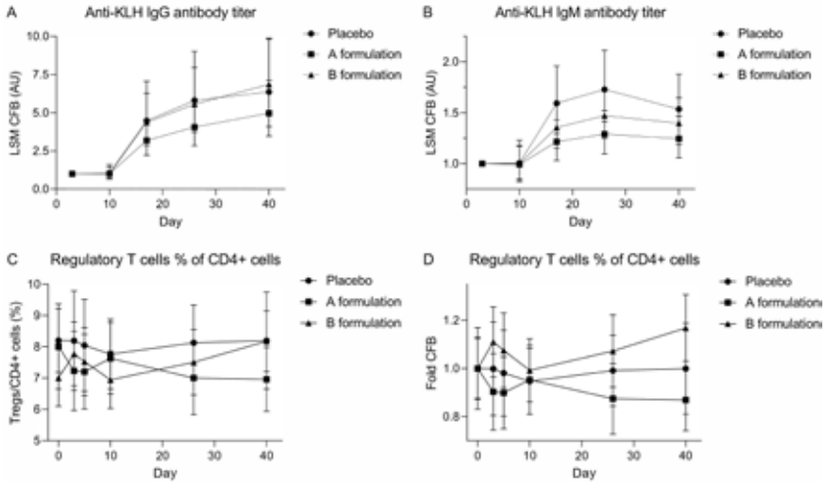
Parameters are shown as mean (standard deviation), for age as median (range), and for male gender as count (percentage). BMI, body mass index; ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein.

SYSTEMIC IMMUNITY: HUMORAL RESPONSE TO KLH AND CIRCULATING REGULATORY T CELLS

Although anti-KLH antibody levels were consistently lower in EDP1815-treated groups compared to placebo, no statistically significant EDP1815 effects were observed on

KLH-driven endpoints. Lower anti-KLH IgM (ED −17.8%, 95% CI −34.2–2.7%, $P = .08$) and IgG (ED −21.5%, 95% CI −52.1–28.7%, $P = .33$) were observed in EDP1815 A-treated participants as well as lower anti-KLH IgM (ED −10.0%, 95% CI −28.0–12.5%, $P = .34$) in EDP1815 B-treated participants compared to placebo (Table S2 and Figure 2). Although circulating Tregs as percentage of $CD4^+$ T cells was significantly decreased in the A group (ED −0.69%, 95% CI −1.23–−0.14%, $P < .05$) and significantly increased in the EDP1815 B group (ED 0.62%, 95% CI 0.05–1.19%, $P < .05$) compared to placebo (Table S2 and Figure 2), it should be noted that these percentages remained within the general range of Tregs in the CD4 population as reported in literature (5-10%).[48,49]

FIGURE 2 Anti-keyhole limpet hemocyanin (A) IgG and (B) IgM antibody titers, (C) circulating regulatory T cells as percentage of $CD4^+$ T cells, and (D) change from baseline circulating regulatory T cells as percentage of $CD4^+$ T cells over time by EDP1815 treatment group.



Data are shown as least square means change from baseline with 95% confidence interval for antibody titers and as mean with standard deviation for regulatory T cells. KLH, keyhole limpet hemocyanin; LSM, least square means; CFB, change from baseline; Tregs, regulatory T cells.

TISSUE IMMUNITY: DERMAL RESPONSE UPON KLH INJECTION

The KLH-driven skin response was overall lower in the EDP1815-treated groups compared to placebo, but none of the endpoints reached a level of significant difference (LSCI basal flow ED −8.0 AU, 95% CI −33.3–17.2 AU, $P = .52$ in A group, ED −8.4 AU, 95% CI −32.4–15.6 AU, $P = .48$ in B group and flare ED −2.4 AU, 95% CI −18.2–13.4 AU, $P = .76$ in A group, ED −4.6 AU, 95% CI −20.7–11.5 AU, $P = .56$ in B group; multispectral imaging: CIELab a* ED −0.66 AU, 95% CI −2.67–1.35 AU, $P = .50$ in A group, ED −1.50 AU, 95% CI −3.47–0.47 AU, $P = .13$ in B group and average redness ED −0.06 AU, 95% CI −0.23–0.11 AU, $P = .46$ in A group, ED −0.14 AU, 95% CI −0.31–0.03 AU, $P = .10$ in B group; Table S2 and Figure 3). The immune cells in blister exudates of placebo-treated subjects after KLH skin rechallenge consisted mainly of T cells (mean $CD3^+$ T cell count 397, SD 319, mean $CD4^+$ T cell count 288, SD 262, mean $CD8^+$ T cell count 81, SD 68, and mean Treg cell count 194, SD 189), NK cells (mean cell count 149, SD 77), and DCs (mean cell count 149, SD 77) (Table S2 and Figure 4). Scant amounts of B cells (mean cell count 12, SD 10), monocytes (mean classical monocyte count 78, SD 34, mean intermediate monocyte count 3, SD 3, and mean non-classical monocyte count 2, SD 1), and neutrophils (mean cell count 23, SD 21) were found in blister exudates of placebo-treated subjects (Table S2 and Figure 4). Although there was no clear EDP1815 treatment effect on immune cell subsets in blister exudates (Table S2 and Figure 4), definitive conclusions cannot be drawn due to very low number of cells.

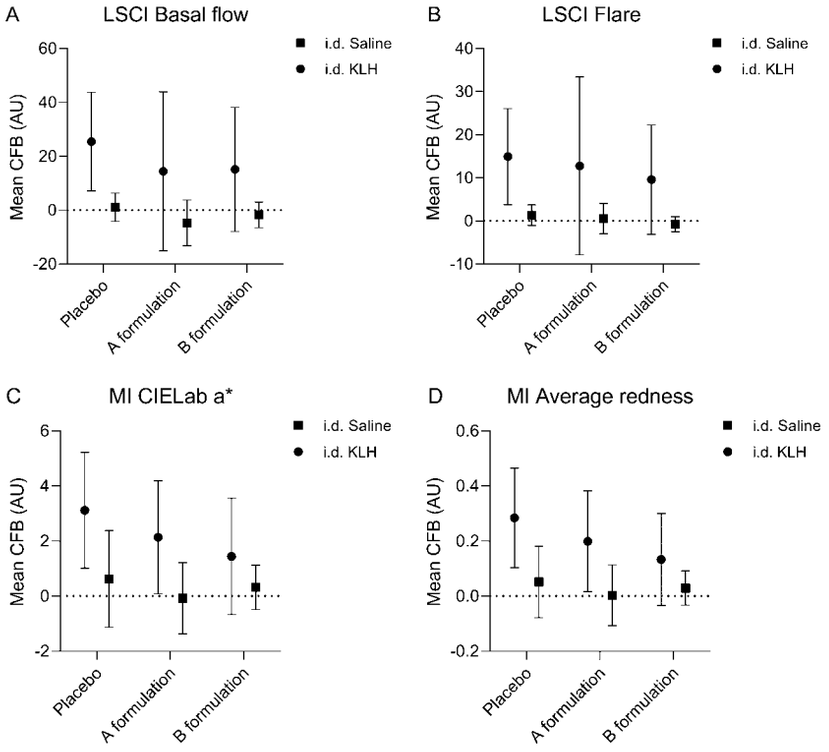
EX VIVO STIMULATION ASSAYS

EDP1815 did not significantly impact LPS- and PHA-driven cytokine responses *ex vivo*. All cytokines in supernatants from LPS-stimulated (TNF, IL-1β, IL-6, and IL-8) or PHA-stimulated (IFN-γ and IL-2) whole blood cultures were comparable across treatment groups (Table S2 and Figure 5).

EDP1815 STOOL PERSISTENCE AND GUT MICROBIOME

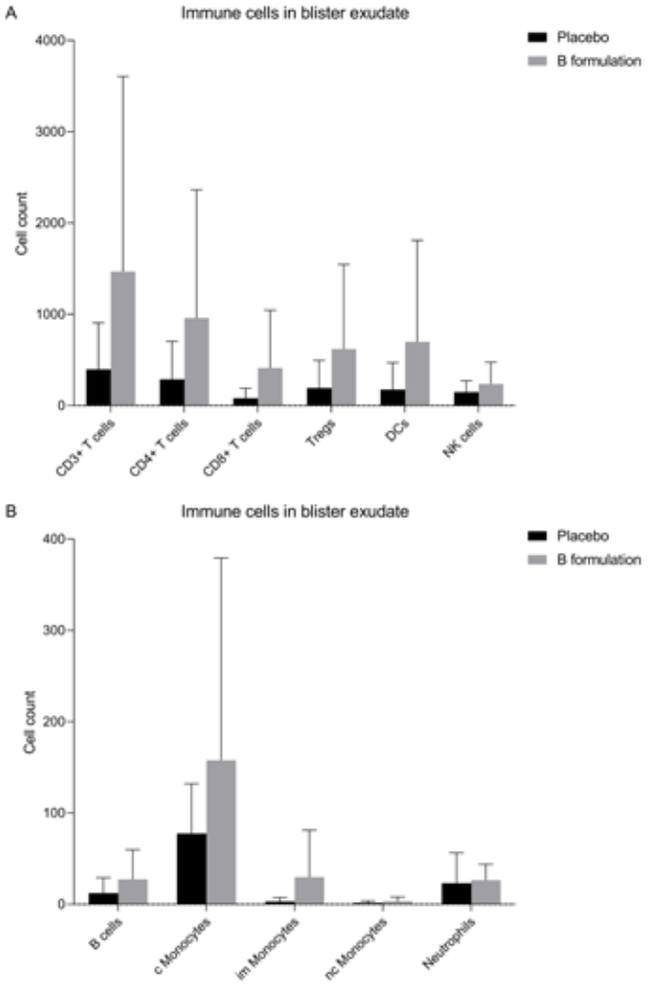
EDP1815 was detected between 18–25% on study day 26 in subjects on active treatment, returning to below the limit of quantification levels 5 days after the last EDP1815 dose (Table S3 and Figure 6). *Prevotella* was the second most abundant genus found in the top ten most abundant microbial populations over all samples combined (Table S4). *Prevotella* abundance was comparable across all treatment groups (Table S5 and Figure 6). Although a statistically significant difference was observed in microbiome alpha diversity between placebo and EDP1815 B formulation groups ($P = .02$), no differences were found within the EDP1815 B formulation group over time when compared to baseline (Table S6 and Figure 6). Furthermore, the two principal components used for the beta diversity PCoA plots combined contributed between 72% to 88% to the total score weights and no statistically significant differences were found in microbiome beta diversity (Table S7 and Figure 7).

FIGURE 3 Cutaneous blood perfusion by LSCI (A) basal flow and (B) flare, erythema by multispectral imaging (C) CIELab a* and (D) average redness after intradermal KLH and saline administration by EDP1815 treatment group.



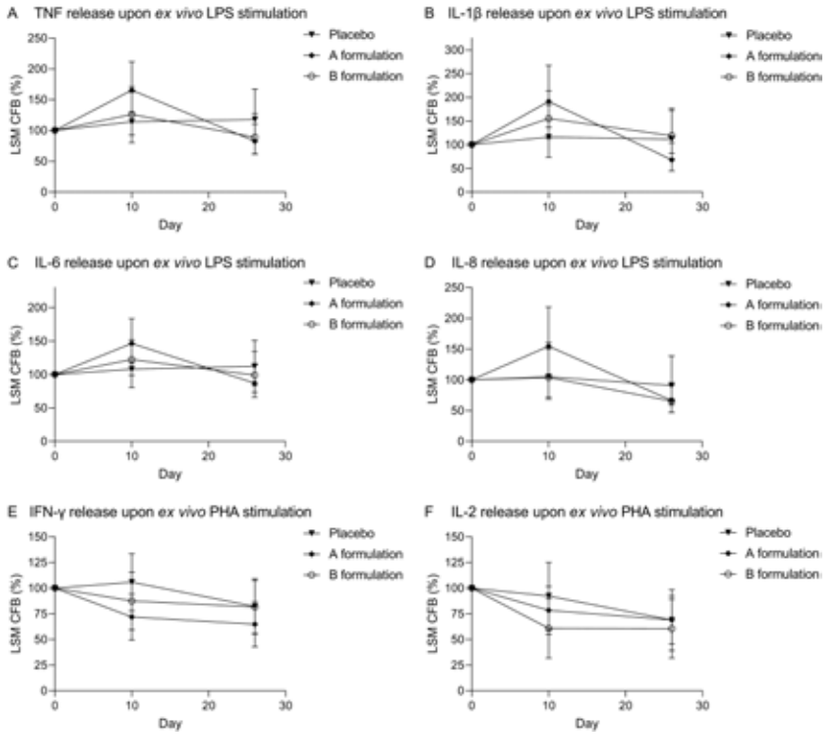
Data are shown as mean change from baseline with standard deviation. LSCI, laser speckle contrast imaging; MI, multispectral imaging; KLH, keyhole limpet hemocyanin; CFB, change from baseline; AU, arbitrary unit; i.d., intradermal.

FIGURE 4 Immune cell counts of (A) T cell subsets, dendritic cells, and natural killer cells, and (B) B cells, monocyte subsets, and neutrophils in blister exudate after intradermal KLH administration by EDP1815 treatment group.



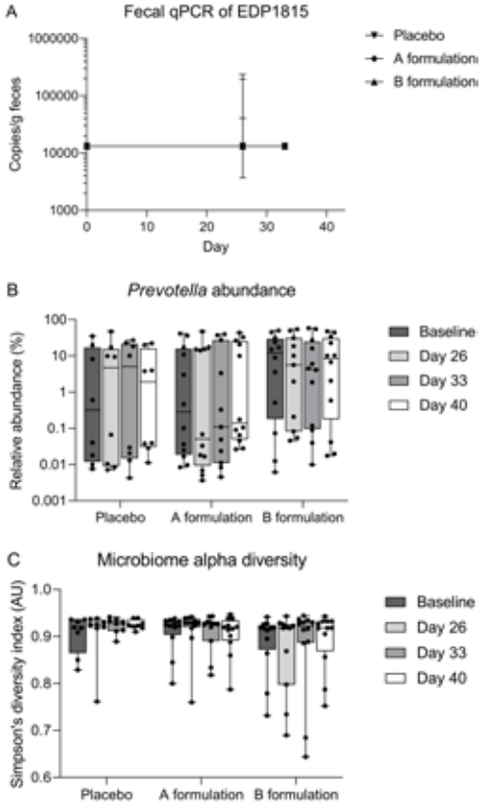
Data are shown as mean with standard deviation. c, classical; im, intermediate; nc, non-classical; DCs, dendritic cells; NK, natural killer.

FIGURE 5 Monocyte cytokine release assay of (A) tumor necrosis factor, (B) interleukin-1β, (C) interleukin-6, and (D) interleukin-8 release from whole blood cultures after *ex vivo* lipopolysaccharide stimulation and lymphocyte cytokine release assay of (E) interferon gamma and (F) interleukin-2 release from whole blood cultures after *ex vivo* phytohemagglutinin stimulation. X-axis represents number of days after initial EDP1815 dose.



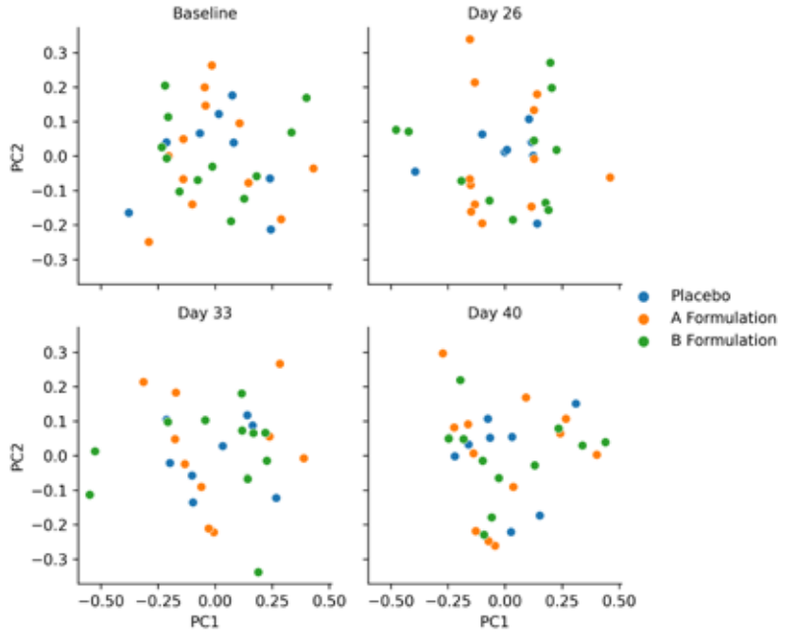
Data are shown as least squares mean change from baseline with 95% confidence interval. LSM, least squares mean; CFB, change from baseline; LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin; PHA, phytohemagglutinin; IFN-γ, interferon gamma.

FIGURE 6 (A) Fecal concentration of EDP1815 over time on log10 scale measured by quantitative polymerase chain reaction, (B) *Prevotella* relative abundance expressed on log10 scale as percentage composition of total microbiome per time point, and (C) microbiome diversity calculated using the Simpson's alpha diversity index per timepoint.



Data are shown as median with range for fecal EDP1815 concentration and as median with interquartile range for Prevotella abundance and microbiome alpha diversity. qPCR, quantitative polymerase chain reaction; AU, arbitrary unit.

FIGURE 7 Microbiome diversity calculated using a principal coordinate analysis plot of the top ten most abundant microbial populations based on the Bray-Curtis dissimilarity beta diversity index per timepoint per group.



PC, *Principal Component.*

SAFETY AND TOLERABILITY

Overall, EDP1815 treatment did not raise safety concerns. No serious AEs occurred. Most AEs were related to the GI tract (20 AEs in 11 subjects, Table S8) and all were mild and transient in nature and considered to be possibly related to treatment. The most frequently reported GI tract AEs were upper abdominal pain (5 AEs in 4 subjects) and nausea (4 AEs in 3 subjects). Subjects treated with B formulated EDP1815 also had overall slightly fewer AEs (66.7%) compared to A formulated EDP1815 and placebo-treated subjects (91.7–100%). No clinically significant changes were observed in laboratory parameters, vital signs, ECG recordings. The assessments and observations in clinical laboratory values, vital signs, and ECG recordings were similar across treatment groups. Some participants showed one or more laboratory parameters outside the normal range. These observations were incidental, not treatment-related, and judged to be of no clinical relevance. No effect of EDP1815 was observed on fecal calprotectin or the BSS and feces questionnaire.

DISCUSSION

In this study we show that various KLH-driven immune responses were overall lower in groups treated daily with EDP1815 in a dose of 8.0×10^{11} total cells, but that for none of the individual PD endpoints a level of statistical significance was reached. There was no prolonged EDP1815 persistence or colonization within the GI tract as indicated by qPCR and microbiome analyses, respectively. Treatment compliance was 100% which is in line as previously reported using a similar e-diary app.[50] Overall, EDP1815 was considered safe and well-tolerated.

In order to detect a 75% inhibition of the KLH-specific antibody response, cutaneous blood perfusion response (LSCI), and erythema response (multispectral imaging) a sample size of 12 per group was required using a parallel study design, with an α of .05 and a power of 80%.[42] Although our study was sufficiently powered for the detection of anti-KLH IgM and IgG antibody responses (minimum sample size of 4 per group required), we were underpowered to detect differences in skin rechallenge outcomes with LSCI and multispectral imaging modalities (minimum sample size of 12 per group required). We have previously shown that the T cell-dependent antibody response (TDAR) following KLH immunization is not correlated with the skin rechallenge response quantified with LSCI and multispectral imaging, suggesting that the KLH-induced humoral response likely does not drive the skin response after intradermal KLH administration.[42] In a separate study we further displayed a discrepancy between TDAR and subsequent skin rechallenge response in healthy volunteers receiving amlitelimab, an OX40L inhibitor, and subsequent KLH immunization and skin rechallenge.[43] The inhibition of the TDAR response was less pronounced compared to the inhibition of the skin rechallenge response indicating that these two responses are possibly mechanistically independent, the TDAR reflecting a B cell-induced response and the skin rechallenge reflecting a T cell-induced response.

The circulating Tregs comprised between 5-10% of detected $CD4^+$ T cells which is consistent with data described in literature.[48,49] The inconsistent but statistically significant changes in circulating Tregs observed between the treatment groups are all within the 5-10% range of total $CD4^+$ T cells. Therefore, we believe the observed variability is not clinically relevant and is most likely attributed to normal variation.

Immune cell subsets present in blister exudates after intradermal KLH rechallenge and e*x vivo* stimulation assays included substantial proportions of T cells and DCs as

anticipated. Delayed-type hypersensitivity reactions usually occur 48–72 hours after antigen rechallenge as antigen presenting cells such as DCs display antigens using major histocompatibility complex class II molecules to dermal $CD4^+$ T cells.[33,34,36,38-43,47] Proinflammatory cytokine secretion by activated $CD4^+$ T cells, such as IL-2 and IFN-γ, induces cytotoxic $CD8^+$ T cell activation and proliferation to help aid in elimination of the antigen.[51] The immune cell subsets present in blister exudates were comparable between EDP1815 and placebo-treated participants, however, the total cell count from the blister exudates was very low. For comparison, total cell counts obtained from human PBMC isolation contain roughly 1.3×10^6 cells[52] whereas we found a maximum mean total cell count of merely 2,914 cells (EDP1815 B-treated participants).

Traditional immunomodulatory therapies such as corticosteroids, non-steroidal anti-inflammatory drugs, and anti-histamines have been well-described and are successful in suppression of inflammation. However, due to lack of tissue or cell specificity they can cause a wide range of side effects, particularly after prolonged use.[53] More novel antibody-based therapies overcome this lack of specificity, however, the primary obstacle predominantly revolves around their immunogenicity and production of antidrug antibodies, leading to decreased clinical efficacy.[53] Single-strain microbes are emerging novel therapies and have been shown to be effective in preventing and ameliorating several diseases.[21] *Lactobacillus rhamnosus* GG is a single-strain microbe that has been reported to reduce the risk of antibiotic-associated diarrhea (AAD) and to prevent necrotizing colitis. *Lactobacillus helveticus* R52 has also been shown to be effective in AAD. Notably, *Bifidobacterium animalis* spp. *lactis* BB12 is a single-strain microbe that has been reported to prevent upper respiratory tract infections, indicating a systemic immune response after local GI tract exposure. Although the underlying mechanisms involved in induction of systemic immunomodulatory effects after local exposure are not fully elucidated,[22] targeting the GI tract with microbial therapies seems a plausible approach. Investigating immunomodulatory effects of compounds such as EDP1815 aids in elucidating the poorly described link between the systemic and enteric immune systems. This connection between the systems holds potential for harnessing pharmacological effects and introduces an innovative strategy for treating inflammatory diseases, possibly overcoming some of the limitations observed in traditional as well as antibody-based immunomodulatory therapies.

This clinical study did not confirm the profound and consistent immunomodulatory effects of EDP1815 on KLH-driven responses as seen in preclinical *in vitro* and *in vivo* mouse studies. There are different potential explanations for the absent translation of convincing immunomodulatory effect of EDP1815 between mice and man.

Firstly, conventional allometric scaling between mouse *vs.* human was challenging as EDP1815 exposure was believed to be restricted to the GI tract compared to systemic uptake as commonly observed in other medicinal products. The immunomodulatory action of EDP1815 was hypothesized to be solely dependent on local interplay with cells in the GI tract mucosa followed by systemic immune modulation. Stool mass and relative mucosal surface area of the GI tract are also important factors that need to be considered for allometric scaling.[54] The daily EDP1815 dose of 8.0×10^{11} total cells was possibly insufficient to generate significant immune system inhibition as the justification for dose finding was primarily hypothetical. Higher doses were not explored as daily administration of >10 capsules was impractical. Secondly, release of EDP1815 enteric-coated capsules early in the proximal small intestine is thought to be important for the mechanism of action as described before.[46] Furthermore, the EDP1815 target site, exact molecular target, as well as which EDP1815 element(s) exert immunomodulatory properties are still being elucidated. A recent publication has described an orally ingestible microdevice that entraps GI microbiota and biomarkers whilst passing through the GI tract.[55] This method of capturing GI tract biomarkers and microbiota is non-invasive and could potentially allow for site-specific sampling. Lastly, relatively unknown physiological systems and factors such as the interaction between the local and systemic immune system and the GI microbiome likely play important roles in EDP1815-induced immunomodulatory effects.

Importantly, immunosuppression with amlitelimab, an OX40L inhibitor, efficiently translates from *in vivo* animal models to a first-in-human trial including a similar KLH challenge model, and eventually to patients with atopic dermatitis.[43] Incorporating the KLH challenge model in early-phase clinical trials with novel compounds targeting the immune system is a suitable approach to evaluate desired treatment effects early on during clinical development.

Microbial interventions are used for prevention and treatment of several immune related diseases. The interaction between the systemic and GI immune system is currently not fully understood. Although oral EDP1815 did not evoke consistent significant systemic immune modulation, there was a trend toward a treatment effect on the KLH-induced skin rechallenge response and, to a lesser extent, on the humoral KLH response. Based on our clinical data and data from other EDP1815 trials,[26] it is premature to conclude that EDP1815 is not effective in inhibiting (KLH-driven) immune responses in man. We advocate more extensive and mechanistic EDP1815 research, including facilitation of higher doses of EDP1815. Currently, both EDP1815 in early-release enteric-coated capsule formulation as well as a microbial extracellular vesicle are being investigated in a similar setup as in our study.

REFERENCES

1 Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol. 2014;14(10):667-685. doi:10.1038/nri3738

2 Cresci GA, Bawden E. Gut Microbiome. Nutr Clin Pract. 2015;30(6):734-746. doi:10.1177/0884533615609899

3 Heintz-Buschart A, Wilmes P. Human Gut Microbiome: Function Matters. Trends Microbiol. 2018;26(7):563-574. doi:10.1016/j.tim.2017.11.002

4 Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol. 2003;3(4):331-341. doi:10.1038/nri1057

5 Meresse B, Malamut G, Cerf-Bensussan N. Celiac Disease: An Immunological Jigsaw. Immunity. 2012;36(6):907-919. doi:10.1016/j.immuni.2012.06.006

6 Chassaing B, Darfeuille–Michaud A. The Commensal Microbiota and Enteropathogens in the Pathogenesis of Inflammatory Bowel Diseases. Gastroenterology. 2011;140(6):1720-1728.e3. doi:10.1053/j.gastro.2011.01.054

7 Nguyen HTT, Dalmasso G, Müller S, Carrière J, Seibold F, Darfeuille–Michaud A. Crohn's Disease–Associated Adherent Invasive Escherichia coli Modulate Levels of microRNAs in Intestinal Epithelial Cells to Reduce Autophagy. Gastroenterology. 2014;146(2):508-519. doi:10.1053/j.gastro.2013.10.021

8 Sikora M, Stec A, Chrabaszcz M, et al. Gut Microbiome in Psoriasis: An Updated Review. Pathogens. 2020;9(6):463. doi:10.3390/pathogens9060463

9 Gupta VK, Cunningham KY, Hur B, et al. Gut microbial determinants of clinically important improvement in patients with rheumatoid arthritis. Genome Med. 2021;13(1):149. doi:10.1186/s13073-021-00957-0

10 Zhang L, Qing P, Yang H, Wu Y, Liu Y, Luo Y. Gut Microbiome and Metabolites in Systemic Lupus Erythematosus: Link, Mechanisms and Intervention. Front Immunol. 2021;12:686501. doi:10.3389/fimmu.2021.686501

11 Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. Nat Immunol. 2013;14(7):646-653. doi:10.1038/ni.2604

12 Belkaid Y, Hand TW. Role of the Microbiota in Immunity and Inflammation. Cell. 2014;157(1):121-141. doi:10.1016/j.cell.2014.03.011

13 Liu Y, Alookaran J, Rhoads J. Probiotics in Autoimmune and Inflammatory Disorders. Nutrients. 2018;10(10):1537. doi:10.3390/nu10101537

14 Askari G, Ghavami A, Shahdadian F, Moravejolahkami AR. Effect of synbiotics and probiotics supplementation on autoimmune diseases: A systematic review and meta-analysis of clinical trials. Clin Nutr. 2021;40(5):3221-3234. doi:10.1016/j.clnu.2021.02.015

15 Bock PM, Telo GH, Ramalho R, et al. The effect of probiotics, prebiotics or synbiotics on metabolic outcomes in individuals with diabetes: a systematic review and meta-analysis. Diabetologia. 2021;64(1):26-41. doi:10.1007/s00125-020-05295-1

16 Chang YS, Trivedi MK, Jha A, Lin YF, Dimaano L, García-Romero MT. Synbiotics for Prevention and Treatment of Atopic Dermatitis. JAMA Pediatr. 2016;170(3):236. doi:10.1001/jamapediatrics.2015.3943

17 Morshedi M, Hashemi R, Moazzen S, Sahebkar A, Hosseinifard ES. Immunomodulatory and anti-inflammatory effects of probiotics in multiple sclerosis: a systematic review. J Neuroinflammation. 2019;16(1):231. doi:10.1186/s12974-019-1611-4

18 Fong FLY, El-Nezami H, Mykkänen O, Kirjavainen P V. The Effects of Single Strains and Mixtures of Probiotic Bacteria on Immune Profile in Liver, Spleen, and Peripheral Blood. Front Nutr. 2022;9:773298. doi:10.3389/fnut.2022.773298

19 Chapman CMC, Gibson GR, Rowland I. In vitro evaluation of single- and multi-strain probiotics: Inter-species inhibition between probiotic strains, and inhibition of pathogens. Anaerobe. 2012;18(4):405-413. doi:10.1016/j.anaerobe.2012.05.004

20 Fredua-Agyeman M, Stapleton P, Basit AW, Gaisford S. Microcalorimetric evaluation of a multi-strain probiotic: Interspecies inhibition between probiotic strains. J Funct Foods. 2017;36:357-361. doi:10.1016/j.jff.2017.07.018

21 McFarland L V. Efficacy of Single-Strain Probiotics Versus Multi-Strain Mixtures: Systematic Review of Strain and Disease Specificity. Dig Dis Sci. 2021;66(3):694-704. doi:10.1007/s10620-020-06244-z

22 Ramani K, Cormack T, Cartwright ANR, et al. Regulation of Peripheral Inflammation by a Non-Viable, Non-Colonizing Strain of Commensal Bacteria. Front Immunol. 2022;13:768076. doi:10.3389/fimmu.2022.768076

23 Bodmer M, Itano A, McInnes I. Harnessing the small intestinal axis to resolve systemic inflammation. Front Immunol. 2022;13. doi:10.3389/fimmu.2022.1060607

24 Tett A, Pasolli E, Masetti G, Ercolini D, Segata N. Prevotella diversity, niches and interactions with the human host. Nat Rev Microbiol. 2021;19(9):585-599. doi:10.1038/s41579-021-00559-y

25 Vandeputte D, Kathagen G, D'hoe K, et al. Quantitative microbiome profiling links gut community variation to microbial load. Nature. 2017;551(7681):507-511. doi:10.1038/nature24460

26 Itano A, Maslin D, Ramani K, et al. Clinical translation of anti-inflammatory effects of Prevotella histicola in Th1, Th2, and Th17 inflammation. Front Med. 2023;10. doi:10.3389/fmed.2023.1070433

27 Marietta E V., Murray JA, Luckey DH, et al. Suppression of Inflammatory Arthritis by Human Gut-Derived Prevotella histicola in Humanized Mice. Arthritis Rheumatol. 2016;68(12):2878-2888. doi:10.1002/art.39785

28 Mangalam A, Shahi SK, Luckey D, et al. Human Gut-Derived Commensal Bacteria Suppress CNS Inflammatory and Demyelinating Disease. Cell Rep. 2017;20(6):1269-1277. doi:10.1016/j.celrep.2017.07.031

29 Marietta E, Horwath I, Meyer S, et al. Administration of Human Derived Upper gut Commensal Prevotella histicola delays the onset of type 1 diabetes in NOD mice. BMC Microbiol. 2022;22(1):8. doi:10.1186/s12866-021-02406-9

30 Bodkhe R, Marietta E V., Balakrishnan B, et al. Human gut-derived commensal suppresses generation of T-cell response to gliadin in humanized mice by modulating gut microbiota. Anaerobe. 2021;68:102237. doi:10.1016/j.anaerobe.2020.102237

31 ClinicalTrials.gov [Internet]. Identifier NCT04603027, A Phase 2 Study Investigating the Effect of EDP1815 in the Treatment of Mild to Moderate Plaque Psoriasis; 2022 Dec 19 [cited 2022 Dec 19]. https://clinicaltrials.gov/ct2/show/NCT04603027

32 Palestine AG, Roberge F, Charous BL, Lane HC, Fauci AS, Nussenblatt RB. The effect of cyclosporine on immunization with tetanus and keyhole limpet hemocyanin (KLH) in humans. J Clin Immunol. 1985;5(2):115-121. doi:10.1007/BF00915009

33 Smith A, Vollmer-Conna U, Bennett B, Wakefield D, Hickie I, Lloyd A. The relationship between distress and the development of a primary immune response to a novel antigen. Brain Behav Immun. 2004;18(1):65-75. doi:10.1016/S0889-1591(03)00107-7

34 Boulton C, Meiser K, David OJ, Schmouder R. Pharmacodynamic Effects of Steady-State Fingolimod on Antibody Response in Healthy Volunteers: A 4-Week, Randomized, Placebo-Controlled, Parallel-Group, Multiple-Dose Study. J Clin Pharmacol. 2012;52(12):1879-1890. doi:10.1177/0091270011427908

35 Bingham CO, Looney RJ, Deodhar A, et al. Immunization responses in rheumatoid arthritis patients treated with rituximab: Results from a controlled clinical trial. Arthritis Rheum. 2010;62(1):64-74. doi:10.1002/art.25034

36 Valdez H, Smith KY, Landay A, et al. Response to immunization with recall and neoantigens after prolonged administration of an HIV-1 protease inhibitor-containing regimen. AIDS. 2000;14(1):11-21. doi:10.1097/00002030-200001070-00002

37 Ferbas J, Belouski SS, Horner M, et al. A novel assay to measure B cell responses to keyhole limpet haemocyanin vaccination in healthy volunteers and subjects with systemic lupus erythematosus. Br J Clin Pharmacol. 2013;76(2):188-202. doi:10.1111/bcp.12172

38 Boelens PG, Fonk JCM, Houdijk APJ, et al. Primary immune response to keyhole limpet haemocyanin following trauma in relation to low plasma glutamine. Clin Exp Immunol. 2004;136(2):356-364. doi:10.1111/j.1365-2249.2004.02447.x

39 Rentenaar RJ, Van Diepen FNJ, Meijer RT, et al. Immune responsiveness in renal transplant recipients: Mycophenolic acid severely depresses humoral immunity in vivo. Kidney Int. 2002;62(1):319-328. doi:10.1046/j.1523-1755.2002.00425.x

40 Grant RW, Mariani RA, Vieira VJ, et al. Cardiovascular exercise intervention improves the primary antibody response to keyhole limpet hemocyanin (KLH) in previously sedentary older adults. Brain Behav Immun. 2008;22(6):923-932. doi:10.1016/j.bbi.2008.01.006

41 Smith TP, Kennedy SL, Fleshner M. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. J Appl Physiol. 2004;97(2):491-498. doi:10.1152/japplphysiol.01404.2003

42 Saghari M, Gal P, Ziagkos D, et al. A randomized controlled trial with a delayed-type hypersensitivity model using keyhole limpet haemocyanin to evaluate adaptive immune responses in man. Br J Clin Pharmacol. 2021;87(4):1953-1962. doi:10.1111/bcp.14588

43 Saghari M, Gal P, Gilbert S, et al. OX40L Inhibition Suppresses KLH-driven Immune Responses in Healthy Volunteers: A Randomized Controlled Trial Demonstrating Proof-of-Pharmacology for KY1005. Clin Pharmacol Ther. 2022;111(5):1121-1132. doi:10.1002/cpt.2539

44 Yang J, Lickliter JD, Hillson JL, et al. First-in-human study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of ALPN-101, a dual CD28/ICOS antagonist, in healthy adult subjects. Clin Transl Sci. 2021;14(4):1314-1326. doi:10.1111/cts.12983

45 Poirier N, Blancho G, Hiance M, et al. First-in-Human Study in Healthy Subjects with FR104, a Pegylated Monoclonal Antibody Fragment Antagonist of CD28. J Immunol. 2016;197(12):4593-4602. doi:10.4049/jimmunol.1601538

46 Saghari M, Gal P, Grievink HW, et al. Impact of oral administration of single strain Lactococcus lactis spp. cremoris on immune responses to keyhole limpet hemocyanin immunization and gut microbiota: A randomized placebo-controlled trial in healthy volunteers. Front Immunol. 2022;13. doi:10.3389/fimmu.2022.1009304

47 Smith AJ, Vollmer-Conna U, Bennett B, Hickie IB, Lloyd AR. Influences of Distress and Alcohol Consumption on the Development of a Delayed-Type Hypersensitivity Skin Test Response. Psychosom Med. 2004;66(4):614-619. doi:10.1097/01.psy.0000130962.28801.af

48 Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol. 2006;6(4):295-307. doi:10.1038/nri1806

49 Oleinika K, Nibbs RJ, Graham GJ, Fraser AR. Suppression, subversion and escape: the role of regulatory T cells in cancer progression. Clin Exp Immunol. 2012;171(1):36-45. doi:10.1111/j.1365-2249.2012.04657.x

50 Rijsbergen M, Niemeyer-van der Kolk T, Rijneveld R, et al. Mobile e-diary application facilitates the monitoring of patient-reported outcomes and a high treatment adherence for clinical trials in dermatology. J Eur Acad Dermatology Venereol. 2020;34(3):633-639. doi:10.1111/jdv.15872

51 King T. 2 - Inflammation, Inflammatory Mediators, and Immune-Mediated Disease. In: King TC, Ed. Elsevier's Integrated Pathology. Philadelphia-Mosby; 2007:21-57.

52 Corkum CP, Ings DP, Burgess C, Karwowska S, Kroll W, Michalak TI. Immune cell subsets and their gene expression profiles from human PBMC isolated by Vacutainer Cell Preparation Tube (CPTTM) and standard density gradient. BMC Immunol. 2015;16(1):48. doi:10.1186/s12865-015-0113-0

53 Strzelec M, Detka J, Mieszczak P, Sobocińska MK, Majka M. Immunomodulation—a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. Front Immunol. 2023;14. doi:10.3389/fimmu.2023.1127704

54 H. Karasov W, Douglas AE. Comparative Digestive Physiology. In: Comprehensive Physiology. Vol 3. Wiley; 2013:741-783. doi:10.1002/cphy.c110054

55 Chen L, Gruzinskyte L, Jørgensen SL, Boisen A, Srivastava SK. An Ingestible Self-Polymerizing System for Targeted Sampling of Gut Microbiota and Biomarkers. ACS Nano. 2020;14(9):12072-12081. doi:10.1021/acsnano.0c05426