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Effect of probiotic on innate inflammatory response and viral shedding in experimental rhinovirus infection – a randomised controlled trial

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Supplementary materials and methods

S1. Complete inclusion/exclusion criteria

Inclusion criteria

Inclusion criteria at enrolment:

- subject must be 18-60 years of age;
- subject must read and sign a copy of the approved consent form.

Inclusion criteria at day -28:

- female subjects must be using an effective birth control method;
- subject must have a serum neutralizing antibody titre of less than or equal to 1:4 to rhinovirus type 39.

Inclusion criteria at challenge (day 0):

- female subjects must be using an effective birth control method.

Exclusion criteria

Exclusion criteria at day -28:

- antibiotic use within 3 months prior to day -28;
- female subjects with a positive urine pregnancy screen;
- history of use of probiotics in the preceding two weeks;
- current cancer diagnosis or immunosuppressive therapy in the last 6 months;
- any clinically significant abnormalities of the upper respiratory tract;
- any clinically significant acute or chronic respiratory illness;
- any clinically significant bleeding tendency by history;
- hypertension that requires treatment with antihypertensive medications;
- history of angina or other clinically significant cardiac disease;
- any medical condition that in the opinion of the principal investigator is cause for exclusion from the study;
- history of regular use (more than 3 days in 7) of tobacco products within the preceding two weeks;
- history of drug or alcohol abuse in the 6 months preceding the study.

Exclusion criteria at challenge (day 0):

- any upper respiratory infection or allergic rhinitis in the two weeks prior to the challenge;

- any medical condition that in the opinion of the Principal Investigator is cause for exclusion from the study;
- use of any anti-inflammatory (steroids or NSAIDs) or cough/cold or allergy preparation in the two weeks prior to the challenge.

S2. Complete statistical plan

Sample size analysis

Nasal lavage CXCL8 concentrations have been measured by the methods described in this proposal in previously published studies (Barrett *et al.*, 2006; Turner *et al.*, 1998; 1999). Based on these previous studies a sample size of 60 subjects/treatment arm was sufficient to detect approximately a 50% reduction in the change in nasal lavage concentration of CXCL8 in response to rhinovirus infection after probiotic treatment with P_{α}=0.05 (two-sided) and P_{β}=0.2 (one-sided). We planned to enrol up to 80 subjects per arm to assure at least 60 subjects per treatment arm in the analysis cohort.

CXCL8 analyses

The *a priori* planned CXCL8 analysis focused on those volunteers who were infected and completed the study and who were susceptible to RV-A39 by antibody titre and had no virus detected in the nasal lavage on day 0. The CXCL8 data were analysed on the natural logarithmic scale via repeated measure ANCOVA. The ANCOVA response data identified the changes in $\log_e$ CXCL8 from study day 0 to post-rhinovirus-challenge study days 1, 2, 3, 4, and 5. The sources of variation that were examined in the ANCOVA included the study group (i.e. active, and placebo), the CXCL8 assessment study day (1, 2, 3, 4, and 5) and study group by assessment study day interaction. The $\log_e$ CXCL8 data of study day -28 were utilized as a concomitant variable so that all of the between-group comparisons of the changes in $\log_e$ CXCL8 could be standardized to a common day -28 $\log_e$ CXCL8 concentration. An unstructured variance-covariance matrix form was utilized to account for *within-subject* measurement correlation when conducting hypothesis tests and constructing confidence intervals. With regard to hypothesis testing, the pivotal comparison was the between-group difference in the mean change in $\log_e$ CXCL8 response from day 0 to post-challenge study day 3. Comparisons of the mean changes in $\log_e$ CXCL8 from day 0 to post-rhinovirus-challenge study days: 1, 2, 4, and 5, were considered secondary. For the pivotal day 3 comparison a two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion, while for the remaining secondary comparisons, a Bonferonni two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion. Between comparisons will be presented as ratios of geometric means.

Cytokine and chemokine analyses

Day 0, and day 0 to post-rhinovirus-challenge day 4 changes in the cytokine and chemokine concentrations were analysed on the natural logarithmic scale via linear mixed models (LMM). The sources of variation that were examined in the LMM analyses included the study group (i.e. active and placebo) and the assessment study day (i.e. day 0 and day 4), as well as study group

by assessment study day interaction. A *subject-specific* intercept was utilized in LMM as a random effect to account for *within-subject* measurement correlation. A two-sided $P \leq 0.05$ decision rule was utilised as the null hypothesis rejection criterion for the within-group and the between-group comparisons.

Lower respiratory tract inflammation analyses

The eNO longitudinal data were analyzed in exactly the same way as the CXCL8 longitudinal data. With regard to hypothesis testing, the pivotal comparison was the between-group difference in the mean change in $\log_e$ eNO response from day 0 to post-rhinovirus-challenge study day 3. Comparisons of the changes in $\log_e$ eNO from day 0 to post-rhinovirus-challenge study days 1, 2, 4, and 5 were considered secondary. For the pivotal day 3 comparison, a two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion, while for the remain secondary comparisons, a Bonferonni two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion.

Symptom scores analyses

The symptom scores for post-rhinovirus-challenge study days, 1, 2, 3, 4, and 5 were analysed via negative binomial generalized estimating equation (GEE) regression models (Hardin and Hilbe, 2003). The pivotal analysis was with respect to the study day 1, 2, 3, 4, and 5 total symptom scores that were computed by tallying the individual daily symptom scores.

The GEE regression model predictor variables included the study group (treatment, placebo), and the symptom assessment day (1, 2, 3, 4, and 5), as well as study group by symptom assessment day interaction. The day 0 symptom scores were utilized as a concomitant variable so that all of the between-group comparisons could be standardized to a common day 0 symptom score. The variance covariance matrix of each GEE regression model was estimate via the Huber and White sandwich estimator (Huber, 1967; White, 1980). With regard to hypothesis testing, a Bonferonni two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion.

Rhinovirus infection and antibody response analyses

Viral titre load was analysed on the log base 10 scale via repeated measure ANOVA. Study group (treatment, placebo) and study assessment day (1, 2, 3, 4, and 5), as well as study group by study assessment day interactions, were the sources of variability in $\log_{10}$ viral load that were examined. An unstructured variance-covariance matrix form was utilized to account for *within-subject* measurement correlation when conducting hypothesis tests and constructing confidence intervals. With regard to hypothesis testing, a Bonferonni two-sided $P \leq 0.05$ decision rule was established *a priori* as the null hypothesis rejection criterion.

Time to rhinovirus shedding

The 'time to rhinovirus shedding' cumulative distributions were estimated via the Kaplan -Meier estimator, and the log-rank test was utilized to compare the distributions of shedding times between the two study groups (i.e. treatment versus placebo).

References

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