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Supplementary information

Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms

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Supplementary information Table 1 | **Clinical cohort and interventional studies assessing links between the microbiota and vaccine responses.**

Vaccine	Samples size (enrolled)	Population	Method used to profile the composition of the microbiota	Study outcomes	Reference
Clinical cohort studies					
ORV	n = 39-154	Ghanaian (n = 39) and Dutch (n = 154) infants	Human Intestinal Tract Chip (HITChip)	The fecal microbiota of vaccine responder infants in Ghana was more similar to that of age-matched Dutch infants (assumed to be responders). Relative abundance of *Bacteroides* and *Prevotella* species correlated with a lack of response to ORV. Relative abundance of *Streptococcus bovis* positively correlated with response to ORV. Enterobacteria-Bacteroides ratio was significantly higher in responders.	[1]
ORV	n = 10 per group	Pakistani vs Dutch infants	Human Intestinal Tract Chip (HITChip)	Increased relative abundance of Gram-negative bacteria notably *Serratia* and *E. coli* in vaccine responders. At a phylum level vaccine responders had significantly higher levels of Firmicutes (*Clostridium* cluster XI and Proteobacteria).	[2]
ORV	n = 170	Indian infants	16S rRNA amplicon sequencing	No significant associations between the composition of the fecal microbiota and responses to ORV were identified.	[3]
ORV	n = 50	Nicaraguan infants	16S rRNA amplicon sequencing	Prior to correction for multiple statistical comparisons the authors found significant	[4]

				associations between the relative abundance of *Enterobacteriaceae* and *Eggerthella* and ORV seroconversion.	
OPV	n = 107	Chinese infants	16S rRNA amplicon sequencing	The relative abundance of Bifidobacteria in the infant fecal microbiota was correlated with increased poliovirus-specific IgA responses. Higher microbiota diversity was associated with poorer responses to vaccination. Relative abundance of *Firmicutes* class *Clostridia* was significantly higher in OPV IgA negative infants.	[5]
OPV	n = 114-704	Indian infants	TaqMan array cards (n=704); 16S rRNA amplicon sequencing (n=114)	No significant differences were found in the relative abundance of specific taxa between responders and non-responders to OPV but higher microbiota diversity was associated with poorer responses to vaccination. Non-polio enteroviruses were significantly associated with reduced OPV seroconversion.	[6]
BCG, OPV, TT, HepB	n = 291	Bangladeshi infants	16S rRNA amplicon sequencing	*Bifidobacterium* relative abundance in early infancy was significantly positively associated with $CD4^+$ T cell response to BCG, TT, and HepB at 15 weeks; $CD4^+$ T cell response to BCG and TT at 2 years; and TT-specific IgG in plasma and OPV-specific IgA in stool at 2 years.	[7]
DTP-HepB-Hib,	n = 29-278	Antibiotic exposed (n=29) and	Not profiled	No significant differences in seroprotection rates or antibody responses to infant	[8]

Polio, PCV13		unexposed (n=87-278) Australian infants enrolled in the MIS-BAIR study.		immunisations in infants exposed to antibiotics at 7 or 13 months of age.	
Interventional clinical studies					
OPV	n = 348-357	Indian infants randomised to receive azithromycin (n=348) or placebo (n=357)	qPCR and TaqMan array cards	Antibiotics did not improve OPV immunogenicity despite reducing biomarkers of environmental enteropathy and the prevalence of pathogenic intestinal bacteria.	[9]
ORV	n = 21 per group	Healthy Dutch adults randomised to receive a broad-spectrum cocktail of antibiotics; vancomycin or no antibiotics.	16S rRNA amplicon sequencing	Antibiotics did not alter absolute anti-ORV IgA titres. An increase in anti-RV IgA boosting was observed in adults treated with vancomycin. An increased ratio of *Enterobacteriaceae* to *Bacteroides* species was associated with enhanced boosting.	[10]
TIV	Cohort 1 n = 11 per group Cohort 2 (with low baseline immunity) n = 17 controls; n = 15 antibiotics exposed.	Healthy American adults randomised to receive a 5-day broad-spectrum cocktail of antibiotics or not.	16S rRNA amplicon sequencing	There was a significant impairment in H1N1-specific neutralization and IgG1 and IgA binding antibody titers in subjects from cohort 2 with low baseline antibody titers against influenza.	[11]

BCG, *Mycobacterium bovis* bacillus Calmette-Guérin; DTP–HepB–Hib, diphtheria tetanus pertussis–hepatitis B–*Haemophilus influenzae* type B; OPV, oral poliovirus vaccine; ORV, oral rotavirus vaccine; PCV, pneumococcal conjugate vaccine; rel., relative; TIV, trivalent influenza vaccine; TT, tetanus toxoid.

Supplementary information Table 2 | **Summary of randomised controlled trials assessing the effects of probiotics on vaccine responses in infants or the elderly**

Probiotic	Age of participants (sample size)	Country	Antibiotic exposure	Effects on vaccine responses	Overall effect	Reference
Lactobacillus rhamnosus strain GG	6–10-week-old infants (n = 135–143 per group)	India	Not recorded	Modest increase in seroconversion rate (+7.5%) in response to ORV in infants receiving the probiotic	+	[12]
Lactobacillus casei strain GG	2–5-month-old infants (n = 30 per group)	Finland	Not recorded	Increased IgM (+11%) and IgA (+19%) seroconversion in response to ORV in the probiotic group	+	[13]
Bifidobacterium breve strain Bbi99, *Propionibacterium freudenreichii* ssp. *shermanii* strain JS, *L. rhamnosus* strain GG and *L. rhamnosus* strain LC705	Neonates (n = 40–47 per group)	Finland	12 antibiotic-exposed infants in probiotic arm	Protective antibody titres to Hib more frequent in the probiotic group (50% compared with 21%); IgG titres to diphtheria and tetanus vaccines comparable	+	[14]
Lactobacillus paracasei ssp. *paracasei* strain F19	4–13-month-old infants (n = 89–90 per group)	Sweden	Antibiotic-exposed infants not excluded	After adjusting for breastfeeding duration, diphtheria toxoid-specific IgG titres were significantly higher in the probiotic group after DTaP–IPV–Hib vaccination	+	[15]
Bifidobacterium longum strain BL999 and *L. rhamnosus* strain LPR	Neonates (n = 28–29 per group)	Singapore	Not recorded	Trend (P=0.07) towards increased HepB virus surface IgG responses in the probiotic group in neonates receiving monovalent doses of HepB vaccine at birth and at 1 month of age and a DTaP–HepB combination vaccine at 6 months	~+	[16]

B. longum strain BB536	Neonates (n = 148–152 per group)	China	Not recorded	No increase in antibody responses to DTP, polio or HepB vaccines in the probiotic group	=	[17]
Bifidobacterium bifidum strain DSMZ20082, *B. longum* strain ATCC157078, *Bifidobacterium infantis* strain ATCC15697 and *Lactobacillus acidophilus* strain ATCC4356	8–10-month-old infants (n = 27–29 per group)	Israel	Not recorded	No significant difference in the number of infants achieving protective titres of antibodies against any of the four MMR vaccine components	=	[18]
L. paracasei strain MCC1849 (heat-killed)	≥65-year-old old adults (n = 22–23 per group)	Japan	Not recorded	No significant differences in antibody responses to TIV in the probiotic group	=	[19]
L. paracasei strain MoLac-1 (heat-killed)	68-83-year-old adults (n = 7–8 per group)	Japan	Not recorded	No increase in antibody or T cell responses to TIV in the probiotic group	=	[20]
L. paracasei strain NCC 2461	≥70-year-old adults (n = 30 per group)	Chile	Antibiotic-exposed adults excluded	No improvement in antibody responses to any serotypes of TIV or PPV23 in the probiotic group.	=	[21]
L. paracasei strain DN-114 001, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*	≥70-year-old adults (n = 109–113 per group)	France	Antibiotic-exposed adults not excluded; no antibiotic use recorded	Significantly higher proportion seroprotected against the influenza virus B strain in the probiotic group following TIV immunisation	+	[22]
Lactobacillus plantarum strain CECT7315/7316	65–85-year-old adults (n = 14–19 per group)	Spain	Antibiotic-exposed adults excluded	Increased TIV-specific IgG and IgA titres and a trend towards increased IgM titre in the probiotic group	+	[23]
B. longum strain BB536	≥65-year-old adults	Japan	2 antibiotic-exposed adults in	No difference in TIV-specific antibody titres or the	=	[24]

	(n = 18–19 per group)		probiotic arm	proportion seroprotected in the probiotic group		
L. casei strain Shirota	≥65-year-old adults (n = 362–375 per group)	Belgium	Antibiotic-exposed adults excluded	No significant difference in mean TIV-specific antibody titre, seroconversion or seroprotection rates in the probiotic group	=	[25]

DTaP, diphtheria, tetanus, acellular pertussis; DTwP, diphtheria, tetanus, whole cell pertussis; HepB, hepatitis B; Hib, *Haemophilus influenzae* type B; IPV, inactivated poliovirus vaccine; MMR, measles, mumps, rubella; ORV, oral rotavirus vaccine; PPV23, 23-valent pneumococcal polysaccharide vaccine; TIV, trivalent influenza vaccine.

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