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Supplementary webappendix

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Web Appendix

Methods

Immunology methods

For cytokine responses, unseparated, heparinised blood was diluted to a final concentration of one-in-four using RPMI medium supplemented with penicillin, streptomycin and glutamine, plated in 96-well plates, and stimulated with crude culture filtrate protein from *Mycobacterium tuberculosis* (cCFP) (5 μg/ml), tetanus toxoid (12 Lf/ml) (Statens Seruminstitut, Denmark) or phytohaemagglutinin (PHA) (10 μg/ml) (Sigma, UK), or left unstimulated. Supernatants were harvested on day six and frozen at -80^{O}C until analysed.

Cytokine concentrations in supernatants were measured by ELISA (BD Pharmingen, Becton Dickinson UK Ltd, UK). Each plate included positive (PHA-stimulated) and negative (unstimulated) control supernatants from a single bulk culture. Test responses were regarded as positive if greater than the mean plus two standard deviations of negative control results for all the assays; based on these control results the cut-off values for positivity were: IFN-γ>73 pg/ml; IL-5>34 pg/ml; IL-13>18 pg/ml; IL-10>48 pg/ml. Values below the cut-off were set to zero. Cytokine production in unstimulated test wells, if higher than these cut-offs, was subtracted from concentrations produced in response to stimulation.

For tetanus toxoid antibody responses, flat bottom 96 well Microlon plates (Greiner Bio-One Ltd, UK) were coated with tetanus toxoid (Staten Seruminstitut) at a concentration of 1.0 Lf /ml in a carbonate-bicarbonate buffer and incubated overnight at 4^{o}C. The plates were washed using phosphate-buffered saline (PBS) with 0.05% Tween 20 (Sigma, UK) and blocked with 1% Marvel in PBS. Blocking buffer was removed and serum samples, diluted 1/4000 for total IgG and IgG4, 1/20 for IgE, were added at 50 μl per well and incubated overnight at 4^{o}C. Plates were washed and then incubated for one hour at room temperature with detection antibody (polyclonal peroxidase-conjugated rabbit anti-human IgG (DAKO, Denmark) for total IgG, or biotinylated mouse anti-human monoclonal antibody 0.5 μg /ml (Becton Dickinson) followed by streptavidin conjugated polyhorseradish peroxidase (Sanquin, Netherlands) for IgG4 and IgE). Plates were developed for 20-30 minutes with o-phenylenediamine dihydrochloride (OPD) substrate (Sigma) and stopped with 2M sulphuric acid. Optical densities were read using test wavelength 490nm and reference wavelength 630nm. Standard curves were included on each plate. For total IgG a standard was obtained from National Institute for Biological Standards and Controls (NIBSC) UK. For IgG4, a standard positive pool was optimized using myeloma immunoglobulins (Sigma; Calbiochem,Germany)). For IgE, human IgE myeloma protein (Calbiochem) was used directly. The ranges of the standard curves were 0.4 to 13 mIU/ml for total IgG, 5 to 313 ng /ml for IgG4, and 6 to 375 ng/ml for IgE.

Virology methods

For detection of HIV-1 proviral DNA in infants at six weeks, DNA was extracted from stored whole blood cell pellets using the Puregene DNA isolation kit (Gentra Systems, Inc., USA). Extracted DNA was amplified by nested PCR of three conserved viral regions, *tat, gp41, and nef* using the Qiagen *Taq* PCR Master Mix Kit (QIAGEN GmbH, Germany). All samples were first attempted on the *tat* and *gp41* PCR; those negative were then attempted on *nef* PCR. Primers were obtained from Oswel DNA Services, UK (Appendix Table 1). For each gene, 1μl of DNA was used as a template. During the primary PCR, product was amplified in 30μl reaction mix including 0.2mM of each of the gene's outer primers. In the secondary PCR, product was amplified from 5ml of 1:20 dilution of primary PCR product in a 30μl reaction mix with 0.2mM each of the gene's inner primers. Both primary and secondary PCR reactions consisted of three initial cycles of denaturation at 94^{o}C for 1 minute, annealing for 1 minute and extension at 72^{o}C for 1 minute. This was followed by 28 cycles of denaturation at 94^{o}C for 30 seconds, annealing for 30 seconds and extension at 72^{o}C for 45 seconds. Annealing temperatures were 55^{o}C (primary and secondary) for *tat*, 56^{o}C (primary) 57^{o}C (secondary) for gp41 and 57^{o}C (primary) 56^{o}C (secondary) for *nef*. For each experiment, there was a final step of extension at 72^{o}C for 5 min and the experiment was held at 4^{o}C. The secondary PCR products were approximately 324bp for *tat*, 783bp for *gp41* and 739bp for *nef*. The success of the PCR was assessed by running 10 μL of the PCR product on 2.5% high-resolution agarose with 4μl ethidium bromide at 100V for 1 hour 15 minutes and examined under ultraviolet transillumination. DNA from a confirmed HIV positive sample was used as positive control and water was used as a negative control.

Table 1. Primers used for HIV-1 proviral DNA-PCR

Gene	Location of primer		sequence
Tat HXB2	5711-5730	outer	5'GGATACYTGGGMAGGAGTTG 3'
	6227 -6207	,,	5'CATTGCCACTGTCTTCTGCTC 3'
	5775-5795	inner	5'CAGAATTGGGTGYCWACATAG 3'
	6137-6116	,,	5'CTATRGTCCACACAACTATTGC 3'
Gp41 (env) HXB2	7932-7952	outer	5'GTCTGGGGCATTAAACAGCTC 3'
	8782-8761	,,	5'CTTTCTAAGCCCTGTCTGATTC 3'
	8004-8032	inner	5'GGAATTTGGGGCTGCTCTGG 3'
	8707-8686	,,	5'CTATCTRTCCMCYCAGCTACTG 3'
Nef HXB2	8513-8533	outer	5'GTGCCTCTTCAGCTACCACCG 3'
	9508-9488	,,	5'AGCATCTGAGGGYTAGCCACT 3'
	8696-8717	inner	5'GKGGAYAGATAGGGYTATAGAA 3'
	9467-9448	,,	5'CRCCTCCCCTGGAAAGTCCCC 3'

Table 2. Effects of maternal treatment with albendazole + praziquantel, albendazole + praziquantel placebo, and praziquantel + albendazole placebo versus placebo only in pregnancy on infant response to BCG, tetanus and measles immunisation

	Cytokine/ Antibody		Double placebo	Albendazole + praziquantel placebo	Praziquantel + albendazole placebo	Albendazole + praziquantel
Cytokine responses						
cCFP	**Interferon-γ**	Geometric mean (pg/ml)[a]	299	331	329	313
		GMR (95% CI)[b]	reference	1.11 (0.82,1.51)	1.10 (0.82,1.49)	1.05 (0.77,1.43)
	IL-5	Geometric mean (pg/ml) [a]	3.9	3.6	3.8	3.4
		GMR (95% CI) [b]	reference	0.92 (0.69,1.24)	0.96 (0.72,1.30)	0.87 (0.65,1.15)
	IL-13	Geometric mean (pg/ml) [a]	17	17	19	17
		GMR (95% CI) [b]	reference	0.97 (0.72,1.32)	1.06 (0.78,1.45)	1.00 (0.73,1.36)
	IL-10	Geometric mean (pg/ml) [a]	98	92	107	89
		GMR (95% CI) [b]	reference	0.93 (0.71,1.22)	1.09 (0.84,1.40)	0.91 (0.69,1.19)
Tetanus toxoid	**Interferon-γ**	Geometric mean (pg/ml) [a]	32	33	41	29
		GMR (95% CI) [b]	reference	1.03 (0.64,1.64)	1.26 (0.79,2.02)	0.88 (0.55,1.40)
	IL-5	Geometric mean (pg/ml) [a]	13	10	12	9
		GMR (95% CI) [b]	reference	0.74 (0.48,1.12)	0.90 (0.58,1.41)	0.72 (0.46,1.11)
	IL-13	Geometric mean (pg/ml) [a]	45	43	37	37
		GMR (95% CI) [b]	reference	0.94 (0.64,1.40)	0.82 (0.55,1.23)	0.82 (0.55,1.20)
	IL-10	Geometric mean (pg/ml) [a]	6.3	4.9	5.6	4.5
		GMR (95% CI) [b]	reference	0.78 (0.54,1.14)	0.89 (0.61,1.32)	0.71 (0.49,1.02)
Antibody levels						
Tetanus toxoid	**Total IgG**	Geometric mean (mIU/ml) [a]	225	138	282	186
		GMR (95% CI) [b]	reference	0.61 (0.31,1.22)	1.26 (0.63,2.53)	0.83 (0.42,1.66)
	IgG4	Geometric mean (ng/ml) [a]	72	70	67	36
		GMR (95% CI) [b]	reference	0.97 (0.39,2.41)	0.93 (0.36,2.36)	0.50 (0.20,1.20)
	IgE	Geometric mean (ng/ml) [a]	1505	1362	1510	1416
		GMR (95% CI) [b]	reference	0.90 (0.67,1.19)	1.00 (0.79,1.27)	0.94 (0.72,1.22)
Measles	**Total IgG**	Geometric mean (mIU/ml) [a]	361	351	342	328
		GMR (95% CI) [b]	reference	0.97 (0.80,1.17)	0.95 (0.78,1.14)	0.91 (0.75,1.09)

[a] geometric mean of response concentration + 1
[b] bias-corrected accelerated confidence intervals computed by bootstrapping

Table 3. Effects of maternal treatment with albendazole + praziquantel, albendazole + praziquantel placebo, and praziquantel + albendazole placebo versus placebo only in pregnancy on incidence of malaria, diarrhoea and pneumonia in infancy

		Double placebo	Albendazole + praziquantel placebo	Praziquantel + albendazole placebo	Albendazole + praziquantel
Number of clinic visits for illness		2509	2435	2525	2676
Malaria	Events (person-years)	223 (533)	214 (537)	223 (530)	217 (544)
	Incidence rate	41.9	39.8	42.1	39.9
	HR (95% CI)	reference	0.95 (0.73,1.23)	1.00 (0.77, 1.30)	0.95 (0.74, 1.23)
	P		0.68	0.98	0.70
Diarrhoea	Events (person-years)	651 (516)	689 (519)	688 (512)	749 (524)
	Incidence rate	126.1	132.8	134.3	143.1
	HR (95% CI)	reference	1.05 (0.92,1.20)	1.06 (0.93,1.22)	1.13 (0.99,1.30)
	P		0.48	0.37	0.07
Pneumonia	Events (person-years)	122 (536)	119 (541)	104 (534)	137 (547)
	Incidence rate	22.8	22.0	19.5	25.1
	HR (95% CI)	reference	0.96 (0.72,1.30)	0.85 (0.62,1.17)	1.10 (0.83, 1.45)
	P		0.81	0.33	0.50

Table 4. Effects of maternal treatment with albendazole + praziquantel, albendazole + praziquantel placebo, and praziquantel + albendazole placebo versus placebo only in pregnancy on vertical HIV transmission among infants of 211 HIV infected women

	Double placebo (n=61)	Albendazole + praziquantel placebo (n=53)	Praziquantel + albendazole placebo (n=43)	Albendazole + praziquantel (n=54)
HIV positive at 6 weeks (%)	14 (23.0%)	11 (20.8%)	8 (18.6%)	6 (11.1%)
OR (95% CI)	reference	0.88 (0.36, 2.15)	0.77 (0.29, 2.03)	0.42 (0.15, 1.18)
P		0.78	0.59	0.10

Table 5. Secondary outcomes: Infant mortality, haemoglobin, growth indices and malaria parasitaemia at one year

		Albendazole	Albendazole placebo	Praziquantel	Praziquantel placebo
Infant mortality	Deaths	38	43	37	44
	Mortality rate per 1000 live births	33.5	37.8	32.7	38.6
	Hazard ratio (95% CI)	0.88 (0.57,1.36)		0.84 (0.54,1.30)	
	P value	0.56		0.44	
Attended clinic for illness at least once during infancy[b]		1048 (90.7%)	1035 (89.3%)	1050 (90.9%)	1033 (89.0%)
Seen at one year[b]		859 (74.3%)	842 (72.6%)	847 (73.3%)	854 (73.6%)
Haemoglobin (107 mv)[a]	Mean (standard deviation)	10.18 (1.40)	10.15 (1.41)	10.17 (1.39)	10.16 (1.42)
	Difference (95% CI)	0.04 (-0.10,0.17)		-0.01 (-0.15,0.13)	
	P value	0.60		0.91	
Weight-for-age z-score (2mv)	Mean (standard deviation)	-0.35 (1.20)	-0.31 (1.17)	-0.37 (1.16)	-0.29 (1.20)
	Difference (95% CI)	-0.04 (-0.15,0.07)		-0.08 (-0.20,0.03)	
	P value	0.47		0.15	
Height-for-age z-score (21 mv)	Mean (standard deviation)	-0.91 (1.23)	-0.82 (1.17)	-0.89 (1.20)	-0.84 (1.20)
	Difference (95% CI)	-0.09 (-0.21,0.02)		-0.04 (-0.16,0.07)	
	P value	0.12		0.46	
Weight-for-height z-score (22 mv)	Mean (standard deviation)	0.12 (1.21)	0.12 (1.19)	0.08 (1.14)	0.15 (1.26)
	Difference (95% CI)	0.00 (-0.12,0.11)		-0.07 (-0.18,0.05)	
	P value	0.98		0.24	
Malaria parasitaemia (110 mv)	Number positive (%)	45 (5.6%)	44 (5.6%)	47 (5.9%)	42 (5.3%)
	Odds ratio (95% CI)	1.00 (0.65,1.54)		1.14 (0.74,1.74)	
	P value	1.00		0.56	

[a] mv: missing values; [b] numbers in parentheses are percentages of live births (excluding younger twins and triplets) for each treatment group