Supplementary statistical methods

Non-metric multi-dimensional scaling

Data reduction techniques, including non-metric multidimensional scaling (NMS), have been effectively used in a number of previous studies to characterise the key sources of variation in complex immune responses[1-4] and transmission of infectious diseases[5]. To characterise global patterns of the 13 pre- and 6 weeks post-treatment cytokine responses we used NMS, which does not assume linear relationships between variables and uses ranked data thus allowing heterogeneous cytokine data were multiple variables are measured on different scales to be combined in a single analysis[6].

In our study concentrations of all 13 cytokines were square-root(x+1)-transformed prior to analysis since preliminary analyses indicated that square-root(x+1) transformation reduced outlier sensitivity[7] and improved stability of subsequent ordination. Cytokine levels were then ranked and used to calculate a single pre-treatment value and a single post-treatment value (Sorensen dissimilarity) for each participant reflecting how similar/dissimilar their combination of cytokine responses were to those of all other participants (for Sorensen dissimilarity equation refer to reference[6]). Next Sorensen dissimilarity values were plotted relative to 2-dimensional spatial axes by ordination using PC-ORD software[6]. This process is iterative and positions participants relative to one another according to their Sorensen dissimilarity scores so that participants with the most similar cytokine responses are closest together and those with the most dissimilar cytokine responses are furthest apart (the constraints of this process are summarised elsewhere[6]). Thus resulting NMS ordination plots give a visual representation of how similar/dissimilar participants' cytokine profiles (see Figure 3) and the resulting axes represent how these profiles vary between participants. The cytokines accounting for the greatest variation between participants (i.e. correlated with axes

with $r^2>0.4$) were identified by the Pearson's correlation between the original cytokine data and the axes extracted by NMS.

Choice of cut-offs for NMS solutions

In the current study we set a cut-off of $r^2 \geq 0.4$ for a cytokine response to be considered a prominent source of variation between participants, this was chosen because the cytokines that met this cut-off corresponded to the cytokines that differed most significantly in ANOVA analyses (Table 1) thus validating the results of the NMS. NMS ordinations were considered to adequately reflect patterns of cytokine responses if stability≥0.000001 and final stress<20, after a minimum of 100 runs and a maximum of 500 iterations which are standard criteria for NMS solutions[6].

NMS validation

To validate that the patterns of cytokine responses identified by NMS ordinations were stronger than those resulting by chance, all outputs were compared to the outputs of randomised data using Monte-Carlo tests[6]. NMS analysis of cytokine responses to each antigen was run a minimum of 10 times to ensure that the identified patterns did not reflect local minima[6].

Hypothesis testing

To quantify differences between pre- and 6 weeks post-treatment cytokine responses (represented qualitatively by the NMS ordination plots in Figure 3) mean Sorensen dissimilarity values were compared via the non-parametric multiple response permutation procedure (MRPP)[6]. The test statistic (T), p-value and chance-corrected within-group variation (A, a measure of effect size) are reported for all comparisons. MRPP was implemented using PC-ORD software.

Supplementary Table 1. Sources of variation between schistosome-specific cytokine profiles before and 6 weeks after praziquantel treatment

Phenotype	*Cytokine*	*CAP*				*WWH*		*SEA*				*Unstimulated*			
		Axis 1		*Axis 2*		*Axis 1*		*Axis 1*		*Axis 2*		*Axis 1*		*Axis 2*	
		r	r^2	*r*	r^2	*r*	r^2	*r*	r^2	*r*	r^2	*r*	r^2	*r*	r^2
Innate inflammatory	*TNFα*	-0.6	0.3	0.6	0.3	-0.4	0.2	**0.7**	**0.5**	**-0.8**	**0.6**	0.3	0.1	0.0	0.0
	IL-6	**-0.9**	**0.8**	0.4	0.2	**-0.6**	**0.4**	**0.9**	**0.9**	**-0.9**	**0.8**	**0.8**	**0.7**	-0.3	0.1
	IL-8	-0.3	0.1	**-0.9**	**0.8**	0.0	0.0	0.3	0.1	**-0.8**	**0.6**	**0.7**	**0.6**	-0.6	0.3
Th1-type	*IFNγ*	-0.4	0.1	0.5	0.3	-0.4	0.1	**0.7**	**0.5**	-0.6	0.3	-0.1	0.0	0.0	0.0
	IL-2	0.1	0.0	-0.2	0.0	0.0	0.0	-0.1	0.0	-0.1	0.0	0.1	0.0	0.0	0.0
	IL-12p70	-0.5	0.2	0.5	0.2	-0.2	0.0	**0.8**	**0.6**	**-0.8**	**0.6**	-0.1	0.0	0.1	0.0
Th2-type	*IL-4*	0.1	0.0	0.2	0.0	0.0	0.0	0.1	0.0	0	0.0	0.0	0.0	0.0	0.0
	IL-5	0.0	0.0	0.2	0.1	0.1	0.0	-0.1	0.0	0	0.0	0.0	0.0	0.2	0.0
	IL-13	-0.1	0.0	-0.4	0.2	0.1	0.0	0.0	0.0	-0.1	0.0	0.3	0.1	0.6	0.3
Regulatory	*IL-10*	-0.4	0.2	-0.5	0.2	-0.2	0.0	-0.1	0.0	0.1	0.0	0.3	0.1	0.1	0.0
Th17-type	*IL-17A*	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.1	0.0	0.2	0.1
	IL-21	0.0	0.0	-0.7	0.0	0.0	0.0	0.2	0.1	-0.3	0.0	0.5	0.2	**0.7**	**0.6**
	IL-23p19	-0.3	0.1	0.4	0.1	-0.2	0.0	**0.8**	**0.7**	**-0.8**	**0.7**	0.5	0.3	-0.4	0.2
Proportion of variance		0.414		0.545		0.750		0.728		0.256		0.740		0.239	

Axes, reflecting the major sources of variation between participant cytokine responses, were identified by NMS of *S. haematobium* cercariae- (CAP), adult worm- (WWH) and egg (SEA)-specific cytokines present in stimulated and unstimulated (Media) whole blood cultures. The Pearson's correlation coefficient (r), indicating the direction of the association of each cytokine with NMS spatial axes, and coefficient of determination (r^2), indicating the proportion of variance along an axis attributable to each cytokine, is given. Cytokines with an $r^2 \geq 0.4$ are highlighted in bold. The proportion of total variance between participant cytokine responses accounted for by each axis is given below each antigen. Only Axis 1 met the NMS stability criteria (see supplementary statistical methods) for WWH-specific cytokine responses. Corresponding ordination plots for all antigens are shown in Figure 2.

Supplementary Figure Legends

Supplementary Figure 1. Pro-inflammatory cytokine responses to schistosome antigens are not due to endotoxin contamination. Naïve bone marrow-derived endotoxin-sensitive murine dendritic cells were stimulated with 0.1µg/ml lipopolysaccharide (LPS) and 10µg/ml reconstituted *S. haematobium* antigens prepared from whole cercariae (CAP), adult worms (WWH) and eggs (SEA) with (black bars) or without (grey bars) 5µg/ml polymixin B using previously described protocols[8,9]. The reduced levels of pro-inflammatory responses to LPS in polymixin B-treated cultures indicate the efficacy of endotoxin neutralisation. Bars represent the mean of 3 technical replicates and the standard error of the mean is shown.

Supplementary Figure 2. Whole blood cytokine concentrations present in un-stimulated cultures before and 6 weeks after praziquantel treatment. Cytokine responses associated with innate inflammatory- (A), Th1- (B), Th2- (C), Th17- (D) and regulatory- (E) type responses were assayed in unstimulated culture supernatants by ELISA (n=72). Pre-treatment concentrations (ng/ml) are represented by grey triangles and post-treatment cytokine responses are represented by red triangles. Pre- and post-treatment responses were compared by repeated measures ANOVA. Horizontal bars indicate median values. *p   0.05, **p   0.01

Supplementary references

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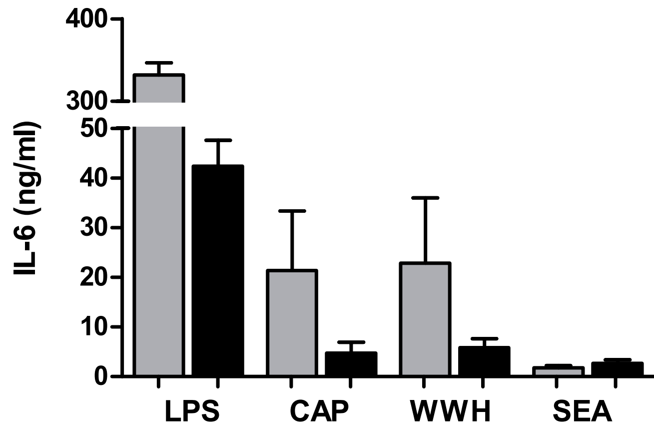

IL-6 (ng/ml)
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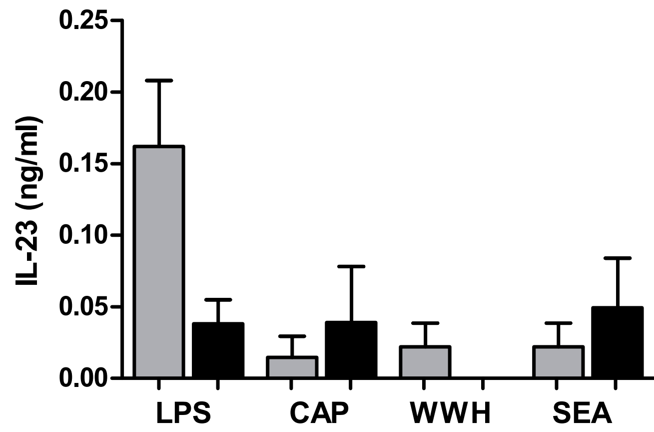
300
50
40
30
20
10
0
LPS
CAP
WWH
SEA



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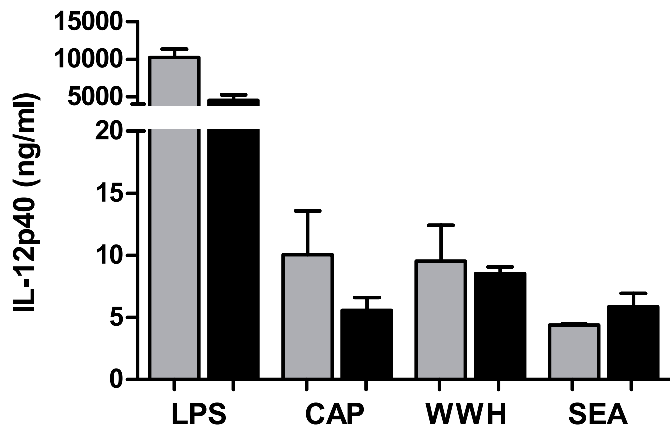
0.20
0.15
0.10
0.05
0.00
LPS
CAP
WWH
SEA



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5000
20
15
10
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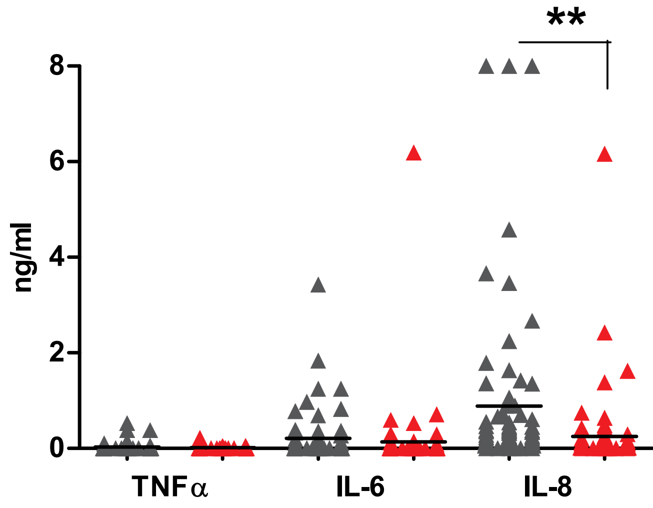
0
LPS
CAP
WWH
SEA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2
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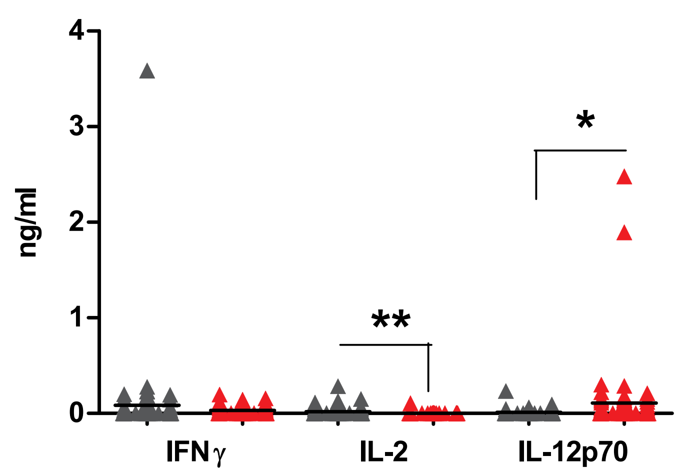
ng/ml
TNFα
IL-6
IL-8


B.
*
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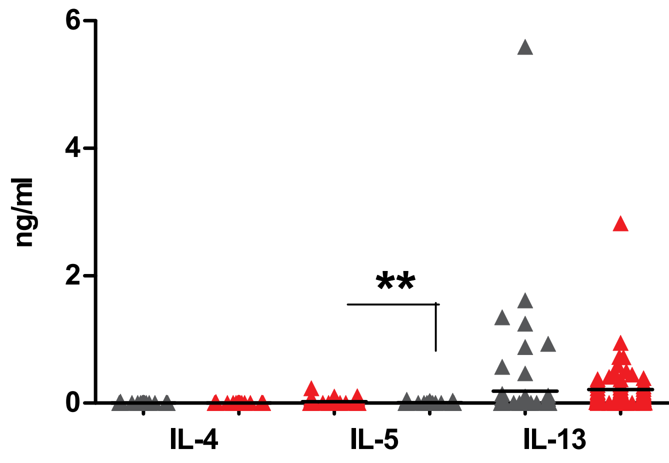
ng/ml
IFNγ
IL-2
IL-12p70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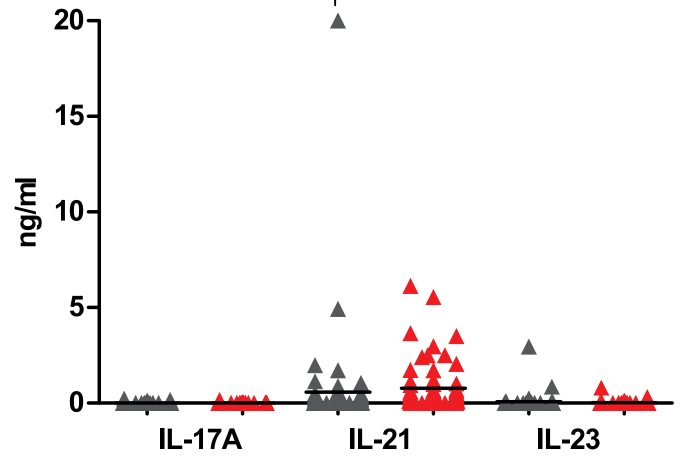
ng/ml
IL-4
IL-5
IL-13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*
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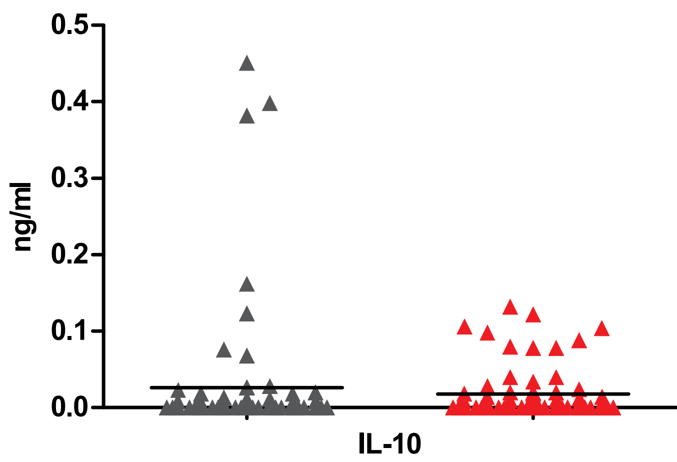
15
10
5
0
ng/ml
IL-17A
IL-21
IL-23


E.
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IL-10