

**Supporting Information:**

- ✚ Table S1. Antibodies used in flow cytometry and definition of T cell subsets.
- ✚ Table S2. Geometric mean and range of multiple cytokine-producing CD4<sup>+</sup> cells.
- ✚ Figure S1. Gating strategies.

**Figure S1. Gating strategies.**

Whole blood cells stimulated with PMA/ionomycin were fixed with 4% paraformaldehyde after lysing the RBCs, and cryopreserved until used. At the time of analysis the cells were thawed and stained with all antibodies in the cocktail (Table S1) except the one that registers in the channel of particular interest [Fluorescence Minus one (FMO) Staining]. The gate drawn for each channel was used to determine the frequency cytokine producing cells in response to antigen stimulation in that channel.

**Table S1**

List of antibodies used in flow cytometry and definition of T helper cell subsets.

Marker	Species	Clone	Fluorochrome	Company
CD3	Mouse	UCHT1	Alexa Fluor 700	eBioscience
CD4	Mouse	RPA-T4	Alexa Fluor 750/PE-Cy5	eBioscience
CD8	Mouse	3B5	Qdot605	Invitrogen
CD25	Mouse	M-A251	APC-Cy7	BD Bioscience
CD127	Mouse	hIL-7R-M21	PE	BD Bioscience
CD152	Mouse	BNI3	APC	BD Bioscience
Foxp3	Rat	PCH101	Alexa Fluor 488	eBioscience
IFN- $\gamma$	Mouse	4S.B3	PE-Cy7	eBioscience
IL-4	Rat	MP4-25D2	FITC	eBioscience
IL-5	Rat	TRFK5	PE	BD Bioscience
IL-10	Rat	JES3-9D7	APC/Pacific Blue	eBioscience
IL-17A	Mouse	eBio64DEC17	PerCp-Cy5.5	eBioscience
TNF- $\alpha$	Mouse	MAb11	Pacific Blue	eBioscience
Th1	CD3 <sup>+</sup> CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> IL-10 <sup>+</sup> TNF- $\alpha$ <sup>+</sup> IL-17A <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> , CD3 <sup>+</sup> CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> IL-10 <sup>+</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> , and CD3 <sup>+</sup> CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> IL-10 <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> ;			
Th2	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-4 <sup>+</sup> IL-5 <sup>+</sup> IL-10 <sup>+</sup> IFN- $\gamma$ <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> , CD3 <sup>+</sup> CD4 <sup>+</sup> IL-4 <sup>+</sup> IL-5 <sup>+</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> , CD3 <sup>+</sup> CD4 <sup>+</sup> IL-4 <sup>+</sup> IL-5 <sup>-</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> , and CD3 <sup>+</sup> CD4 <sup>+</sup> IL-5 <sup>+</sup> IL-4 <sup>-</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup>			
Th17	CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17A <sup>+</sup> TNF- $\alpha$ <sup>+</sup> IL-10 <sup>+</sup> IFN- $\gamma$ <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> , CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17A <sup>+</sup> TNF- $\alpha$ <sup>+</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> , CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17A <sup>+</sup> IL-10 <sup>+</sup> TNF- $\alpha$ <sup>-</sup> IFN- $\gamma$ <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> , and CD3 <sup>+</sup> CD4 <sup>+</sup> IL-17A <sup>+</sup> TNF- $\alpha$ <sup>-</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup>			
aTreg/Tr1	aTreg/Tr1 consisted of CD3 <sup>+</sup> CD4 <sup>+</sup> IL-10 <sup>+</sup> IL-4 <sup>-</sup> IL-5 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> TNF- $\alpha$ <sup>-</sup> IL-17A <sup>-</sup> Foxp3 <sup>+</sup> ;			
nTreg	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup> CD127 <sup>-</sup>			
“TNF- $\alpha$ ”	CD3 <sup>+</sup> CD4 <sup>+</sup> TNF- $\alpha$ <sup>+</sup> IL-10 <sup>+</sup> IFN- $\gamma$ <sup>-</sup> IL-17A <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup> and CD3 <sup>+</sup> CD4 <sup>+</sup> TNF- $\alpha$ <sup>+</sup> IL-10 <sup>-</sup> IFN- $\gamma$ <sup>-</sup> IL-17A <sup>-</sup> IL-5 <sup>-</sup> IL-4 <sup>-</sup>			

Flow Cytometry

Cryopreserved fixed whole blood cells were thawed at room temperature and washed twice with 1X PBS and transferred to 96-wells V-bottom costar plates (Corning). The cells were then incubated with 1X Permeabilization buffer (eBioscience) for 15 minutes at room temperature and washed again with 1X Permeabilization buffer. The cells were then blocked for 15 minutes at room temperature then washed again with 1X Permeabilization buffer. The cells were then incubated with antibodies for the effector and regulatory panels for 30 minutes then washed twice with PBS. After washing, the cells were resuspended in 0.2 ml of PBS and transferred to 96-plug micro tubes (Bio-Rad) and acquired on an LSRII (BD Bioscience).

**Table S2**Median and range of multiple cytokine-producing CD4<sup>+</sup> cells

	<b>Fil-</b>	<b>Fil+</b>	<b>P value</b>
	<b>GM (Range)</b>	<b>GM (Range)</b>	
<b>Lymphocyte count</b>	1.9 x 10 <sup>6</sup> (0.85x10 <sup>6</sup> – 2.5x10 <sup>6</sup> )	1.9 x10 <sup>6</sup> (1.2 x 10 <sup>6</sup> – 3.8 10 <sup>6</sup> )	>0.05
<b>CD4+ count</b>	0.51 x10 <sup>6</sup> (0.7 x10 <sup>5</sup> – 9.2 x 10 <sup>5</sup> )	0.46 x10 <sup>5</sup> (0.09 x10 <sup>5</sup> – 1.16 x10 <sup>6</sup> )	> 0.05
<b>Malaria antigen</b>			
CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup>	1767.0 (322.0 – 5023.0)	481.4 (0.0 – 1631.0)	0.0013
CD4 <sup>+</sup> IL-4 <sup>+</sup>	715.4 (78.5 – 1794.0)	567.3 (19.49 – 5020.0)	>0.05
CD4 <sup>+</sup> IL-5 <sup>+</sup>	5213.0 (113.7 – 72892.0)	3455.0 (53.82 – 21093.0)	>0.05
CD4 <sup>+</sup> IL-10 <sup>+</sup>	884.3 (92.38 – 2929.0)	226.0 (37.12 – 11239.0)	<b>0.04</b>
CD4 <sup>+</sup> IL-17A <sup>+</sup>	1533.0 (437.0 – 6080.0)	949.1 (12.06 – 4474.0)	>0.05
CD4 <sup>+</sup> TNF- $\alpha$ <sup>+</sup>	2064.0 (234.5 – 7458.0)	1055.0 (48.26 – 4078.0)	0.03
<b>Staphylococcus aureus enterotoxin B</b>			
CD4 <sup>+</sup> IFN- $\gamma$ <sup>+</sup>	7241 (629.3 – 21462.0)	9187 (17.63 – 14916)	>0.05
CD4 <sup>+</sup> IL-4 <sup>+</sup>	2816.0 (227.4 – 8511.0)	2725.0 (194.0 – 30680.0)	>0.05
CD4 <sup>+</sup> IL-5 <sup>+</sup>	6817.0 (199.0 – 79534.0)	4810.0 (202.4 – 30371.0)	>0.05
CD4 <sup>+</sup> IL-10 <sup>+</sup>	3229.0 (142.1 – 8900.0)	2185.0 (41.76 – 16259.0)	>0.05
CD4 <sup>+</sup> IL-17A <sup>+</sup>	3229.0 (270.0 – 10376.0)	4079.0 (41.76 – 11130.0)	>0.05
CD4 <sup>+</sup> TNF- $\alpha$ <sup>+</sup>	13763.0 (682.2 – 26837.0)	15185.0 (595.6 – 52628.0)	>0.05

\* P value adjusted for multiple comparisons.

Note: Bold indicates statistically significant

Figure S1

