Supplementary flow cytometric methods (MiFlowCyt data)

MiFlowCyt for manuscript: Altered innate immune ontogeny in HIV-exposed uninfected infants.

MIFlowCyt standard compliant information for submitted flow cytometric data.

1. Experiment overview.

1.1. Purpose:

The purpose of this experiment was to compare and contrast the changing responses of monocytes, B cells (by negative gating), plasmacytoid dendritic cells, and myeloid dendritic cells to TLR stimulation from 2 weeks to 12 months of life in HIV-exposed but uninfected (HEU) and HIV-unexposed (UE) South African infants. This was done in whole blood to reduce possible processing artifact. We hypothesized that–given the known period of marked clinical susceptibility of HEU infants relative to UE infants, that differences in innate immune responses would be observed during this same period of development (0-6 months of life). This knowledge will help us understand the etiology of increased HEU susceptibility to infectious morbidity and mortality in the initial months of life.

1.2. Keywords:

HIV exposed but uninfected, HIV exposed, HEU, immune ontogeny, immune development

1.3. Organization:

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1.4. Primary Contact:

    1.4.1. P.I. Dr. Tobias Kollmann tkollm@mac.com

    1.4.2. Graduate Student. Mr. Brian Reikie bareikie@ucalgary.ca

1.5. Date: Experiments were set up from June 2009 to June 2010 and stained from August 2010 February 2011.

1.6. Conclusions: HEU (versus UE) infants exhibit a PRR ligand specific, pro-inflammatory innate cellular immune response in an age dependent manner.

1.7. Quality Control Measures: Unstimulated controls were set up for each condition tested. Single stain controls were set up by staining 3 ul of Anti-Mouse Ig CompBeads (BD #552843) and 3 ul of anti-FBS negative control beads (included with BD #552843) with 3ul of each antibody used.

2. Flow Sample/Specimen Description

2.1. Sample/Specimen Material

    2.1.1. Biological Samples:

        2.1.1.1. Biological Sample Name: Whole blood obtained by peripheral

blood venipuncture.

2.1.1.2.Biological Sample Source: Healthy human peripheral blood; obtained and processed within < 4h from.

2.1.1.2.1. Biological Sample Source Organism:

2.1.1.2.1.1. Taxonomy: Kingdom Animalia; Subkingdom Metazoa; Phylum Chordata; Subphylum Vertebrata; Superclass Tetrapoda; Class Mammalia Subclass Theria; Infraclass Eutheria; Order Primates; Suborder Anthropoidea; Family Hominidae; Subfamily Homininae; Tribe Hominini Genus Homo Subspecies sapiens

2.1.1.2.1.2. Age: 2 weeks to 12 months of age

2.1.1.2.1.3. Gender: Male and Female

2.1.1.2.1.4. Phenotype: healthy (none)

2.1.1.2.1.5. Genotype: not applicable

2.1.1.2.1.6. Treatment: Whole blood diluted 1:1 in RPMI.

2.1.2. Environmental Samples: not applicable

2.1.3. Control Sample Description: Single stain controls were set up by staining 3 ul of Anti-Mouse Ig CompBeads (BD #552843) and 3 ul of anti-FBS negative control beads (included with BD #552843) with 3ul of each antibody used.

2.1.4 Sample Treatment Description: Cells were plated in a 96 well plate and cultured for a total of 6 hrs. Cells were stimulated with either nothing, or PGNSA (TLR2, NOD1/2, InVivogen), PAM3CSK4 (TLR2/1, EMC microcollections); poly I:C (TLR3, Amersham); 0111:B4 LPS (TLR4, InVivogen); R848 (TLR7/8, InVivogen); CpGA (TLR9, Coley). After culture, cells were treated with a final concentration of 2mM EDTA for 15 min at 37°C, then centrifuged @400g for 5min @22°C and resuspended in 100ul of 1x BD FACS Lysing solution (BD 349202) for 10 minutes at room temperature before being frozen at -80 ^{o}C.

3. Fluorescence Reagent Description (clone) [dilution]:

	Characteristic Being Measured	Antibody Name *Clone Name*	Vendor cat# *dilution used*
VIOLET			
Pacific Blue	Intracellular Protein	IL12p40/70 *(eBio: C8.6)*	eBio#577129 *1:100*
RED			
APC	Cell Surface Protein	CD11c (5HCL3)	BD#340714 *1:50*
APC-Cy7	Intracellular Protein	IL6 *(AS12)*	BD #custom *1:100*
Alexa 700	Intracellular Protein	TNFa *(Mab11)*	BD#557996 *1:100*
BLUE			
FITC/OG	Intracellular Protein	IFNa *(A11)*	Antigenix#MC100133 *1:100*
PerCPCy5.5	Cell Surface Protein	MHCII *TU36*	BD#custom *1:100*
PE-Cy7	Cell Surface Protein	CD14 (M5E2)	BD #557742 *1:50*
PE	Cell Surface	CD123 (6H6)	eBio #121239

	Protein		

Instrument Details:

3.1. Manufacturer: BD Biosciences
3.2. Model: BD FACSAria Flow 3 Laser, Blue/Red/Violet serial # P22300055
3.3. Instrument Configuration and Settings: All lasers, filters and mirrors were manufactured by BD Biosciences. The machine has not been altered.

> **3.3.1. Light Sources:** The light path, filters and detectors are described below in Table 2. The lasers are listed in the order the cells pass through them. The detectors and filters are listed in the order the light hits them, with the exception of FSC which is measured from light that passes through the cell/bead while all the other 488 detectors detect light that has been scattered 90 $_o$, in the order listed. For example, for blue laser detector A light passes through or is reflected off of filter 1, 735 LP, then the light passes through filter 2, 780/60 BP, then it hits the PMT detector. Light that is reflected off the long pass goes to detector B and so on. For parameters used in this experiment, it is indicated whether Area (-A), Height (-H) or Width (-W) was used.

Abbreviations: PMT = photomultiplier tube; PD = photodiode; BP = band pass filter, first number is center of interval, second number is the width of the interval; LP = long pass filter, lets light waves through that have a longer wavelength than the number specified. All LP filters are dichroic and reflect at an angle of incidence at 11.25$_o$
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Laser	Detector Name (Type)	Filter 1 (LP)	Filter 2 (BP)	Parameter detected	Detector voltage	Amplification Type
Blue Laser (488 nm)	SSC (PD)	na	488/10 BP	SSC-A	440	LINEAR
	488 A (PMT)	735 LP	780/60 BP	PE-Cy7-A	605	LOG
	488 B (PMT)	655 LP	695/40 BP	PerCP-Cy5.5-A	585	LOG
	488 C (PMT)	595 LP	610/20 BP	PE-TexRed	na	
	488 D (PMT)	556 LP	575/26 BP	PE	498	LOG
	488 E (PMT)	502 LP	530/30 BP	FITC	480	LOG
Violet Laser (407 nm)	407 A (PMT)	502 LP	530/30 BP	Alexa-430-A	na	
	407 B (PMT)	blank	450/40 BP	Pacific Blue-A	530	LOG
Red Laser (633 nm)	633 A (PMT)	755 LP	780/60 BP	APC-Cy7-A	605	LOG
	633 B (PMT)	685 LP	720/40 BP	Alexa700-A	515	LOG
	633 C (PMT)	blank	660/20 BP	APC-A	492	LOG

4. Data Analysis

4.1. FCS Data File: For raw data please contact Dr. Tobias Kollmann;

      4.1.1. Total Count of Events: Recorded within individual FCS files, as keyword $TOT, 300,000.
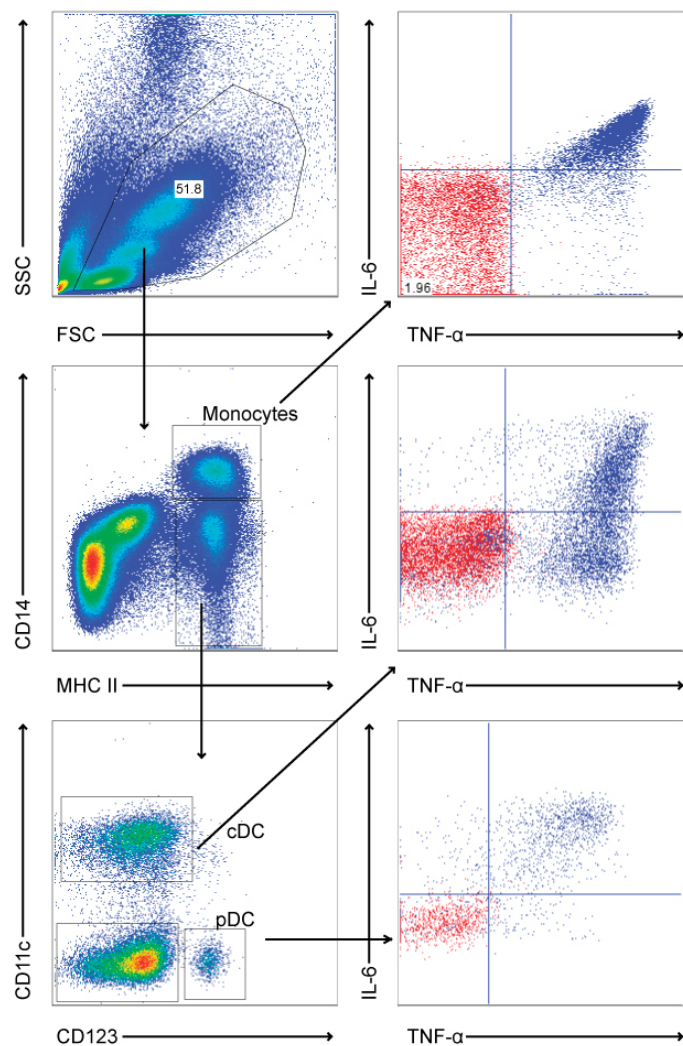
4.2. Compensation Description:
Compensation was done in FlowJo using BDCompBeads as single stain controls. A representative compensation matrix for one of the samples is shown below

	FITC-A	PE-A	PerCP-Cy5-5-A	PE-Cy7-A	APC-A	APC-Cy7-A	Pacific Blue-A	Alex 700-A
FITC-A		29.11	1.847	0.2719	0	0.1527	0.6106	0.4128
PE-A	0.6607		6.615	1.07	-0.249	0.3758	0.3544	0.6247
PerCP-Cy5-5-A	0.1683	0.2436		33.73	2.026	11.66	1.175	30.06
PE-Cy7-A	0.1653	1.982	0.3268		0	15.32	0.1029	0.5033
APC-A	0.1415	0.1788	1.576	0.4223		20.5	0.9705	65.71
APC-Cy7-A	0.08948	0.119	0.3078	2.183	12.56		0.3553	13.26
Pacific Blue-A	0.1281	0.1833	0	0	0.05744	-0.172		0.2704
Alex 700-A	0.1859	0.208	1.44	0.9827	0.2883	29.7	0.662	

4.3. Gating (Data Filtering) Description:

		Gate Statistics (% Parent Gate)	
Gate Description:	Qualitative Description of the Subpopulation	Unstim (red)	R848 stim (blue)
Live Cells	High cell density excluding lower left corner population	61.0	62.9
Monocytes	CD14 high, MCHII high	8.63	4.61
Other MHCII+ cells	MHCII high, CD14 mid to low	6.24	7.02
Myeloid Dendritic Cells (mDCs)	MHCII high, CD11c high, CD123 low	41.8	30.7
Plasmacytoid Dendritic Cells (pDCs)	MHCII high, CD11c low, CD123 high	2.76	2.62
Monocyte TNF+ IL-6	"Monocyte" TNFa high, IL-6 low	0.145	27.5
Monocyte TNF+ IL-6+	"Monocyte" TNFa high, IL-6 high	0.0104	65.6
Monocyte TNF- IL-6+	"Monocyte" TNFa low, IL-6 high	0.28	0.306
Monocyte TNF- IL-6	"Monocyte" TNFa low, IL-6 low	99.5	6.65
Monocyte TNF+ IL-12	"Monocyte" TNFa high, IL-12 low	0.149	64.3
Monocyte TNF+ IL-12+	"Monocyte" TNFa high, IL-12 high	0.00346	28.7
Monocyte TNF- IL-12+	"Monocyte" TNFa low, IL-12 high	0.0588	0.174
Monocyte TNF- IL-12	"Monocyte" TNFa low, IL-12 low	99.8	6.78
Monocyte TNF+ IFNa	"Monocyte" TNFa high, IFNa low	0.0934	92.1
Monocyte TNF+ IFNa+	"Monocyte" TNFa high, IFNa high	0.0554	0.917
Monocyte TNF- IFNa+	"Monocyte" TNFa low, IFNa high	0.166	0.535
Monocyte TNF- IFNa	"Monocyte" TNFa low, IFNa low	99.7	6.38
mDC TNF+ IL-6	"mDC" TNFa high, IL-6 low	0.194	40.1
mDC TNF+ IL-6+	"mDC" TNFa high, IL-6 high	0	52.7
mDC TNF- IL-6+	"mDC" TNFa low, IL-6 high	0.343	0.446
mDC TNF- IL-6	"mDC" TNFa low, IL-6 low	99.5	6.79
mDC TNF+ IL-12	"mDC" TNFa high, IL-12 low	0.194	43.0
mDC TNF+ IL-12+	"mDC" TNFa high, IL-12 high	0	49.8
mDC TNF- IL-12+	"mDC" TNFa low, IL-12 high	0.0915	2.9
mDC TNF- IL-12	"mDC" TNFa low, IL-12 low	99.7	4.34
mDC TNF+ IFNa	"mDC" TNFa high, IFNa low	0.194	92.7
mDC TNF+ IFNa+	"mDC" TNFa high, IFNa high	0	0.0297
mDC TNF- IFNa+	"mDC" TNFa low, IFNa high	0	0.0149
mDC TNF- IFNa	"mDC" TNFa low, IFNa low	99.8	7.19
pDC TNF+ IL-6	"pDC" TNFa high, IL-6 low	0	70.0
pDC TNF+ IL-6+	"pDC" TNFa high, IL-6 high	0	5.23
pDC TNF- IL-6+	"pDC" TNFa low, IL-6 high	0	0
pDC TNF- IL-6	"pDC" TNFa low, IL-6 low	100	25.1
pDC TNF+ IL-12	"pDC" TNFa high, IL-12 low	0	73.7
pDC TNF+ IL-12+	"pDC" TNFa high, IL-12 high	0	1.57
pDC TNF- IL-12+	"pDC" TNFa low, IL-12 high	0	0.174
pDC TNF- IL-12	"pDC" TNFa low, IL-12 low	100	24.9
pDC TNF+ IFNa	"pDC" TNFa high, IFNa low	0	5.92
pDC TNF+ IFNa+	"pDC" TNFa high, IFNa high	0	69.2
pDC TNF- IFNa+	"pDC" TNFa low, IFNa high	0	15.0
pDC TNF- IFNa	"pDC" TNFa low, IFNa low	100	10.1

4.3.1. Example of gating strategy:

 4.4. Data Transformation Description: Data was transformed using FlowJo's "Define BiExponential Transformation" function using the above mentioned compensation matrix, with an additional negative display size set at 0.5 and Positive Decades of "log" Display set at 5.
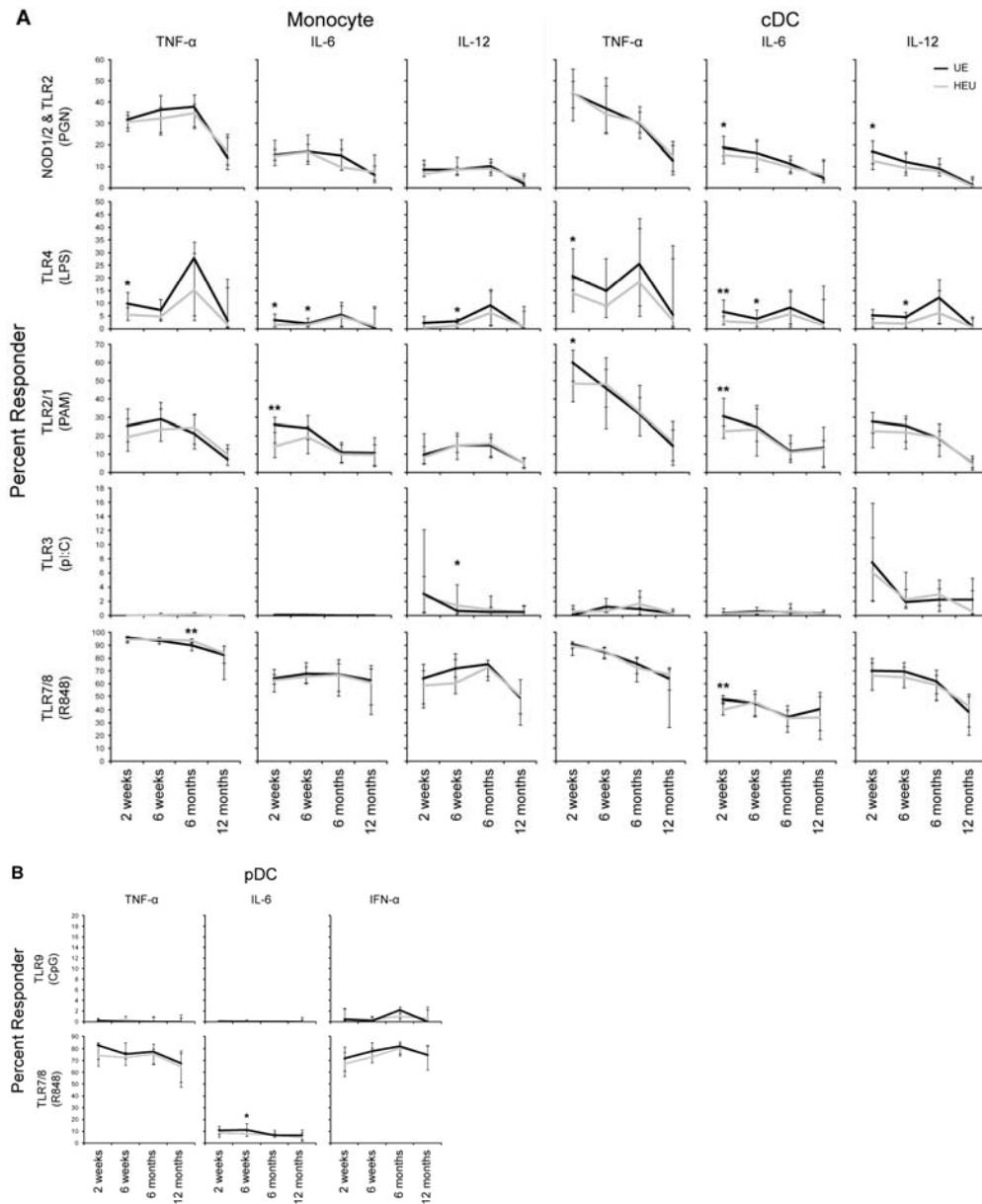
Supplemental Figure S1

Figure S1. Elevated proportion of antigen presenting cells responsive to PAMP stimulation in HIV-exposed uninfected versus HIV-unexposed infants. Shown are the proportions of cytokine producing cells for HEU (black) and UE (grey) subjects. The cytokines detected were TNF-α, IL-6 or IL-12/23p40 in A) monocytes and cDC, and B) TNF-α, IL-6 or IFN-α in pDC. Y-axis represents total percentage of cytokine-producing cells; error bars indicate interquartile range. Figure 2 was generated by subtracting the median UE response from the median HEU response for each time point as shown. Mann-Whitney test was used to compare HEU and UE response at each time point and differences are signified by * (p<0.05) and ** (p<0.01). Unstimulated samples (near 0% of cytokine producing cells) were subtracted from stimulated samples.
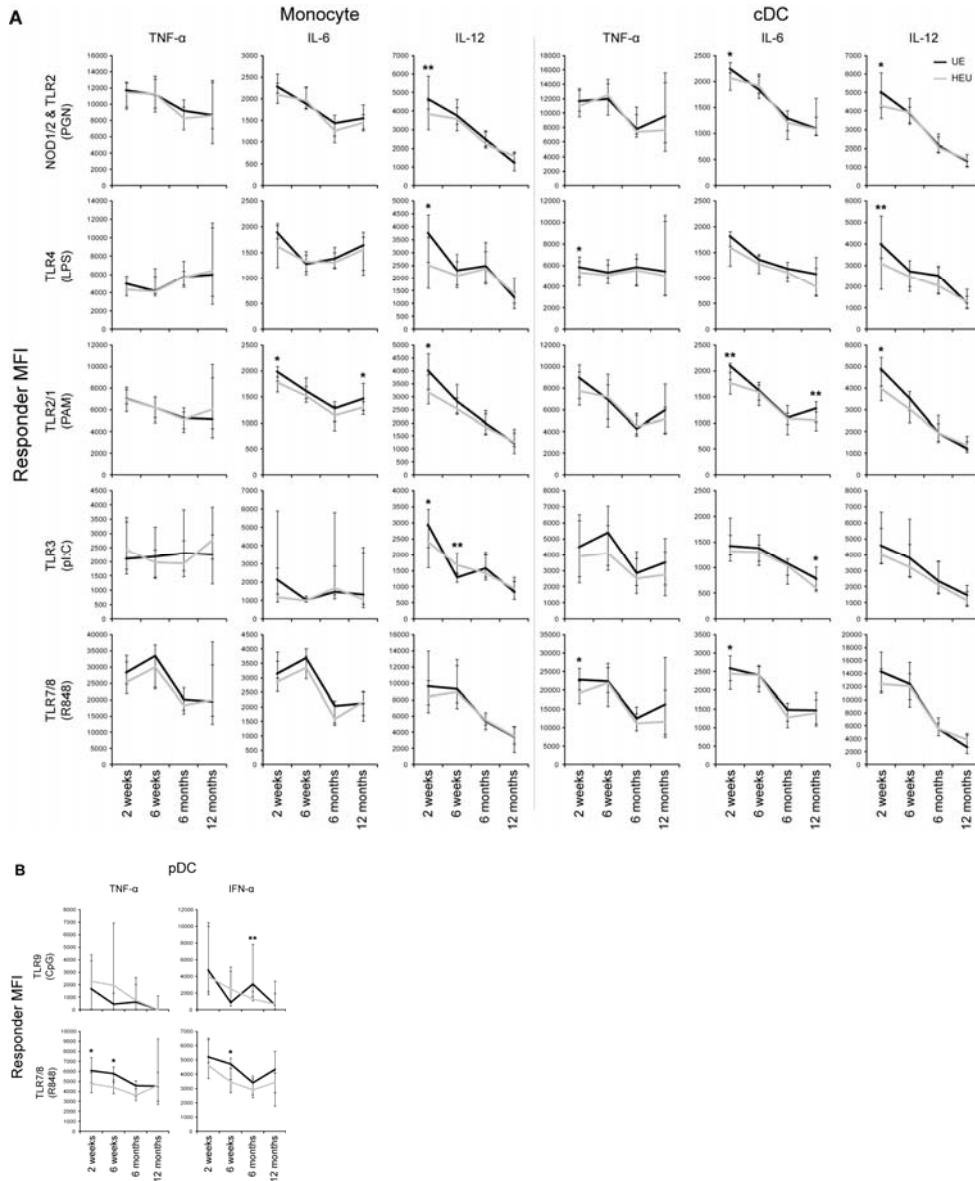
Figure S2. HIV-exposed uninfected infant antigen presenting cells produce more cytokine than in HIV-unexposed infant antigen presenting cells after PAMP stimulation. Shown are the same samples as in Figure 2, but with mean fluorescent intensity (MFI) graphed for HEU (black) and UE (grey) in the respective samples for A) monocytes and cDC, for which we measured TNF-α, IL-6 and IL-12/23p40, and B) pDC, with TNF-α, IL-6 and IFN-α measured. Medians for each population's MFI (y-axis) are derived from FlowJo software; error bars indicate interquartile range. Figure 3 was generated by subtracting the median UE response from the median HEU response. Differences in MFI between HEU and UE groups as detected by Mann-Whitney test are indicated by * (p<0.05) and ** (p<0.01).
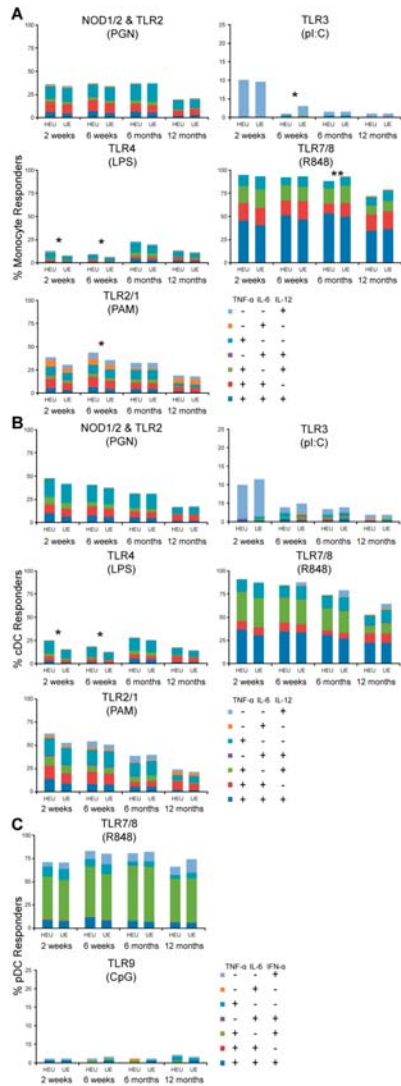
Figure S3. HIV-exposed uninfected infant poly- and mono-functional antigen presenting cells respond stronger than HIV-unexposed infants, but few differences detected in total fraction of responder cells. Whole blood samples stimulated with the indicated TLR ligands were followed at 2 weeks, 6 weeks, 6 months and 12 months of life. Multiparameter flow cytometry was used to detect production of TNF-α, IL-6 and IL-12/23p40 in A) monocytes, and B) cDC; C) TNF-α, IL-6 and IFN-α was detected in pDC. Total percentage of cytokine-producing cells is represented as total bar height, with differences measured by Student's t-test indicated by * ($p < 0.05$) or ** ($p < 0.01$). Color-coded segments allow differentiation of monocytes producing various combinations of the 3 cytokines analyzed. Cytokine profile – color combinations are indicated in the key in identical order from top to bottom as shown in bar graphs. Unstimulated samples, with near 0% of cytokine producing cells, were subtracted from stimulated samples.

Supplemental Table S1

Monocytes

		2 week	6 week	6 month	12 month
TNF-α	PGN	0.570	0.546	0.902	0.601
	LPS	**0.034**	0.064	0.496	0.626
	PAM	0.096	0.194	0.789	0.913
	pIC	0.174	0.134	0.081	0.445
	R848	0.522	0.770	*0.008*	0.652
IL-6	PGN	0.194	0.453	0.522	0.324
	LPS	**0.013**	**0.048**	0.577	0.761
	PAM	**0.006**	0.096	0.757	0.893
	pIC	0.630	0.849	0.448	0.472
	R848	0.249	0.634	0.877	0.706
IL-12	PGN	0.256	0.170	0.773	0.458
	LPS	0.067	**0.007**	0.584	0.961
	PAM	0.469	0.227	0.820	0.913
	pIC	0.726	*0.011*	0.676	0.537
	R848	0.826	0.187	0.781	0.855

cDC

		2 week	6 week	6 month	12 month
TNF-α	PGN	0.153	0.534	0.951	0.817
	LPS	**0.011**	0.055	0.536	0.864
	PAM	**0.023**	0.956	0.635	0.893
	pIC	0.110	0.262	0.118	0.680
	R848	0.055	0.898	0.635	0.733
IL-6	PGN	**0.044**	0.534	0.509	0.817
	LPS	**0.002**	**0.035**	0.516	0.990
	PAM	**0.004**	0.701	0.926	0.724
	pIC	0.755	0.755	0.230	0.732
	R848	**0.005**	0.770	0.409	0.884
IL-12	PGN	**0.032**	0.234	0.353	0.779
	LPS	0.065	**0.019**	0.458	0.951
	PAM	0.059	0.595	1.000	0.894
	pIC	0.814	0.528	0.216	0.741
	R848	0.400	0.595	0.445	0.635

pDC

		2 week	6 week	6 month	12 month
TNF-α	R848	0.153	0.087	0.789	0.817
	CpG	0.426	0.280	0.601	0.483
IL-6	R848	0.107	**0.028**	0.427	0.942
	CpG	0.398	0.099	0.491	0.461
IFN-α	R848	0.714	0.138	0.734	0.894
	CpG	0.909	0.177	0.248	0.476

Table S1. Stronger pro-inflammatory antigen presenting cell responses in HIV-exposed uninfected infants versus HIV-unexposed infants. Shown are the p-values from Mann-Whitney test of the same samples shown in Figure 2. Significantly different responses (p<0.05) are highlighted in blue when HEU > UE, and in yellow when HEU < UE.

Monocytes

		2 weeks	6 weeks	6 months	12 months
TNF-α	PGN	0.680	0.833	0.375	0.825
	LPS	0.184	0.652	0.967	0.611
	PAM	0.522	0.804	0.975	0.907
	pIC	0.977	0.942	0.556	0.328
	R848	0.050	0.422	0.665	0.990
IL-6	PGN	0.051	0.855	0.265	0.246
	LPS	0.405	0.683	0.796	0.136
	PAM	0.023	0.204	0.256	0.018
	pIC	0.053	0.087	0.248	0.197
	R848	0.076	0.360	0.223	0.787
IL-12	PGN	0.008	0.833	0.934	0.192
	LPS	0.010	0.642	0.837	0.171
	PAM	0.016	0.286	0.570	0.969
	pIC	0.034	0.005	0.522	0.611
	R848	0.143	0.704	0.726	0.473

cDC

		2 weeks	6 weeks	6 months	12 months
TNF-α	PGN	0.400	0.622	0.375	0.648
	LPS	0.044	0.583	0.288	0.686
	PAM	0.115	0.672	0.726	0.611
	pIC	0.401	0.988	1.000	0.506
	R848	0.028	0.490	0.375	0.667
IL-6	PGN	0.038	0.662	0.063	0.419
	LPS	0.052	0.360	0.409	0.171
	PAM	0.008	0.715	0.127	0.005
	pIC	0.078	0.609	0.483	0.020
	R848	0.017	0.735	0.124	0.629
IL-12	PGN	0.028	0.833	0.885	0.735
	LPS	0.004	0.622	1.000	0.764
	PAM	0.017	0.214	0.606	0.303
	pIC	0.604	0.965	0.853	0.348
	R848	0.138	0.683	0.563	0.442

pDC

		2 weeks	6 weeks	6 months	12 months
TNF-α	CpG	0.665	0.095	0.641	0.290
	R848	0.035	0.010	0.055	0.825
IFN-α	CpG	0.578	0.403	0.006	0.883
	R848	0.111	0.014	0.536	0.540

Table S2. More cytokine detected on a per-cell basis in stimulated HIV-exposed uninfected infant antigen presenting cells compared to HIV-unexposed antigen presenting cells. Shown are p-values from Mann-Whitney test of the same samples shown in Figure 2. Significant differences between groups (p<0.05) are highlighted in blue when HEU > UE, and in yellow when HEU < UE.

Monocytes

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	PGN	0.3835	0.6652	0.6887	0.1155
	LPS	0.2146	0.7026	0.0986	0.1204
	PAM	0.0099	0.0631	0.1202	0.9908
	pIC	0.718	0.623	0.0676	0.3639
	R848	0.2489	0.1828	0.0279	0.2366
IL-6	PGN	0.6791	0.5135	0.2695	0.0912
	LPS	0.1192	0.7535	0.0909	0.3011
	PAM	0.0006	0.2663	0.0769	0.8182
	pIC	0.7359	0.2978	0.5342	0.5395
	R848	0.1072	0.7665	0.7619	0.3065
IL-12	PGN	0.465	0.6529	0.1682	0.0643
	LPS	0.3368	0.44	0.1067	0.2852
	PAM	0.8533	0.6776	0.1871	0.8813
	pIC	0.8699	0.0461	0.6846	0.6678
	R848	0.8396	0.7665	0.4756	0.6051

cDC

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	PGN	0.5672	0.1565	0.8374	0.2853
	LPS	0.5091	0.8453	0.4401	0.3225
	PAM	0.3373	0.9932	0.5126	0.6875
	pIC	0.1706	0.8473	0.0796	0.4075
	R848	0.5913	0.9391	0.8835	0.5502
IL-6	PGN	0.6662	0.1126	0.5775	0.3065
	LPS	0.2347	0.9797	0.3583	0.1573
	PAM	0.037	0.5245	0.4756	0.6541
	pIC	0.2594	0.3315	0.214	0.5263
	R848	0.0927	0.225	0.807	0.5656
IL-12	PGN	0.6035	0.085	0.2695	0.0289
	LPS	0.7576	0.9662	0.2145	0.6945
	PAM	0.7051	0.2591	0.8527	0.9359
	pIC	0.8628	0.0833	0.237	0.8624
	R848	0.6283	0.05	0.4878	0.352

pDC

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	R848	0.3646	0.786	0.9766	0.8093
	CpG	0.7539	0.5395	0.273	0.7947
IL-6	R848	0.3286	0.0946	0.6321	0.5734
	CpG	0.3335	0.1707	0.5535	0.9096
IFN-α	R848	0.7985	0.1376	0.7397	0.9542
	CpG	0.7631	0.2147	0.1201	0.4414

Table S3. Similar antigen presenting cell responses in infants with African and Mixed race backgrounds. Whole blood from infants of African and Mixed racial background was stimulated with the indicated TLR ligands at 2 weeks, 6 weeks, 6 months and 12 months of life. Multiparameter flow cytometry was used to detect production of TNF-α, IL-6 or IL-12/23p40 in monocytes and cDC, and TNF-α, IL-6 or IFN-α in pDC. Shown are p-values from Mann-Whitney test. Significant differences between groups (p<0.05) are highlighted when African > Mixed, and in yellow when Mixed > African.

Monocytes

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	PGN	0.4189	0.0527	0.1122	0.7045
	LPS	0.1739	0.022	0.1691	0.9137
	PAM	0.5056	0.0833	0.4685	0.1714
	pIC	0.1628	0.9755	0.3267	0.8496
	R848	0.0046	0.1676	0.254	0.8496
IL-6	PGN	0.1959	0.0167	0.1691	0.4482
	LPS	0.3765	0.0527	0.6467	0.3038
	PAM	0.6481	0.3042	0.2864	0.7865
	pIC	0.5166	0.0446	0.0861	0.7761
	R848	0.1483	0.0782	0.332	0.1902
IL-12	PGN	0.3227	0.0886	0.2367	0.0527
	LPS	0.9621	0.8602	0.0901	0.4811
	PAM	0.4027	0.3475	0.0606	0.4811
	pIC	0.4915	0.0594	0.5434	0.3569
	R848	0.27	0.1129	0.0397	0.1843

cDC

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	PGN	0.1565	0.0116	0.1328	0.8496
	LPS	0.4876	0.0069	0.0606	0.6648
	PAM	0.151	0.0345	0.5721	0.11
	pIC	0.3067	0.8421	0.3011	0.2847
	R848	0.0637	0.0886	0.4491	0.6259
IL-6	PGN	0.1959	0.0161	0.0305	0.3431
	LPS	0.5944	0.1268	0.1222	0.4322
	PAM	0.4189	0.0459	0.075	0.839
	pIC	0.8059	0.5706	0.2816	0.6746
	R848	0.2272	0.071	0.3373	0.5695
IL-12	PGN	0.1679	0.0161	0.5019	0.2178
	LPS	0.3274	0.4995	0.0942	0.8284
	PAM	0.2129	0.0687	0.2453	0.9352
	pIC	1	0.679	0.424	0.8285
	R848	0.464	0.0063	0.2367	0.1672

pDC

		2 wk.	6 wk.	6 mo.	12 mo.
TNF-α	R848	0.1305	0.7027	0.332	0.4645
	CpG	0.2344	0.4951	0.2538	0.783
IFN-α	R848	0.0254	0.3628	0.5019	0.8074
	CpG	0.5423	0.5693	0.0046	0.3523

Table S4. More cytokine detected on a per-cell basis in stimulated antigen presenting cells in African infants compared to infants with mixed racial backgrounds. Whole blood from infants of African and Mixed racial background was stimulated with the indicated TLR ligands at 2 weeks, 6 weeks, 6 months and 12 months of life. Multiparameter flow cytometry was used to detect production of TNF-α, IL-6 or IL-12/23p40 in monocytes and cDC, and TNF-α or IFN-α in pDC. Shown are p-values from Mann-Whitney test. Significant differences between groups (p<0.05) are highlighted when African > Mixed, and in yellow when Mixed > African.