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TH1 and TH2 Cytokine mRNA and Protein Levels in Human Immunodeficiency Virus (HIV)-Seropositive and HIV-Seronegative Youths

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The roles of cytokines in the progression of human immunodeficiency virus (HIV)-associated disease are controversial. The patterns of innate cytokine production have been postulated to shift from TH1- to TH2-type cytokines with the progression of HIV-associated disease. Although there have been studies of cytokines in children and adults, no data are available on cytokine production in healthy or HIV-infected adolescents. We analyzed and characterized cytokine mRNA and protein levels for gamma interferon, interleukin 2 (IL-2), IL-4, and tumor necrosis factor alpha and protein levels of IL-6 in both stimulated and unstimulated peripheral blood mononuclear cells obtained from a large longitudinal, observational cohort study of HIV-seropositive and -seronegative adolescents. We correlated cytokine results with viral load and CD4$^+$-T-cell counts as critical markers of disease progression in HIV-infected adolescents. These data were used to examine hypotheses related to the TH1-to-TH2 cytokine shift in a sample of HIV-infected adolescents. Five hundred twenty subjects participating in the REACH (Reaching for Excellence in Adolescent Care and Health) Project of the Adolescent Medicine HIV/AIDS Research Network contributed blood samples. Samples selected for the cross-sectional data set analyzed had to meet selection criteria developed to minimize the potential confounding effects of acute intercurrent illnesses or infections, recent vaccination for hepatitis, and altered hormone status and to optimize congruence of cytokine measurements with assays of viral load and CD4$^+$-T-cell counts. Group differences in the proportions of subjects with detectable levels of each cytokine marker were compared. In the subset of subjects with detectable cytokine values, differences in detected values were compared across subgroups defined by HIV serostatus and among HIV-seropositive subjects by three viral load classifications. The study sample was 65% HIV seropositive, 71% African-American, and 75% female with a mean age of 17.4 years. HIV-seropositive subjects were relatively healthy with mean and median CD4$^+$-T-cell counts of 534 and 499 cells/mm^3, respectively. Only 8.1% of subjects had CD4$^+$-T-cell counts below 200 cells/mm^3, and 25% had viral loads that were below the threshold of detection (<400 copies/ml). Detailed analyses of these data indicate that there were no differences in cytokines detected in HIV-seropositive and HIV-seronegative adolescents, and there was no apparent relationship between the cytokine measurements and the viral load or CD4$^+$-T-cell categorization, the parameters selected as markers of HIV-associated disease status. These adolescents, including the HIV-seropositive subjects, were relatively healthy, and the HIV-infected subjects were at an early stage in the course of their HIV-associated disease. On the basis of our data, we conclude that, early in the course of HIV-associated disease in adolescents, there are no detectable shifts from TH1 to TH2 cytokine production.

The roles of cytokines in the progression of human immunodeficiency virus (HIV)-associated disease are controversial. The patterns of innate cytokine production have been postulated to shift in the course of HIV infection (4). This shift has been described as a change from an environment characterized predominantly by T-helper type 1 (TH1) cytokines, associated with cell-mediated immune responses, to an environment in which TH2 cytokines, known to enhance humoral immune responses, are more prevalent. TH cells are functionally heterogeneous with distinct cytokine patterns. TH1 cells produce interleukin 2 (IL-2) and interferon gamma (IFN-γ), while TH2 cells produce IL-4, IL-5, IL-6, and IL-10. Prior to differentiation, the majority of T cells produce both TH1- and TH2-type cytokines and are designated TH0 cells (12). Generally, TH2 cytokines inhibit TH1 cytokines. IL-12 is a distinct cytokine that is important in the regulation of TH1 and TH2 cytokines.

In children and adults, higher IL-4 and lower IL-2 levels have been associated with HIV infection (4, 5, 11, 20). In addition, deficient IL-2 production in children has been associated with decreased T-cell responses to viral antigens and decreased cytotoxic T-cell lymphocyte activity (9). Low circu-

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lating levels of the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) correlated with lower viral load and slower progression of HIV-associated disease (19). The circulating levels of TNF-α protein and mRNA were higher in HIV-infected children than in healthy control children, an observation that has also been reported for adults (13). In contrast, other studies have found elevated levels of TH1 and TH2 cytokines in HIV-infected persons compared to those in uninfected controls. These cytokine levels did not change with disease progression (8). Graziosi et al. (8) found that both IFN-γ and IL-10 were produced in large amounts by the $CD8^+$ T cells of HIV-infected individuals and that these levels remained relatively high throughout the course of HIV infection. One possible reason why the reported data have remained difficult to interpret is the variation in laboratory techniques across studies. In general, the in vitro use of mitogens and antigens for stimulation of peripheral blood mononuclear cells (PBMCs) has been required in order to detect the low levels of cytokines (3). The use of different antigens and mitogens to stimulate the PBMCs contributes to the difficulty of comparing results across studies (3). Altfeld et al. found that competitive reverse transcriptase PCR was a more sensitive measure of cytokine production than protein enzyme-linked immunosorbent assay (ELISA) and allowed detection of cytokines in unstimulated PBMCs (1). Elevated IL-4/IFN-γ ratios were associated with higher viral loads and reduced $CD4^+$-T-cell counts in this study (1).

Although there have been studies of cytokines in children and adults, there are no data available on cytokine production, stimulated or unstimulated, for healthy or HIV-infected adolescents. Our objectives were to analyze and characterize mRNA and cytokine protein levels of IFN-γ, IL-2, IL-4, IL-6, and tumor necrosis factor alpha (TNF-α) in both unstimulated and stimulated PBMCs obtained from a large cohort of HIV-seropositive and -seronegative adolescents and to correlate the cytokine results with HIV type 1 (HIV-1) RNA levels and $CD4^+$-T-cell counts, critical markers of disease progression in HIV-seropositive adolescents. We examined the following hypotheses: (i) there would be a shift from a TH1 response to a TH2 response in the presence of HIV infection: (ii) this pattern would be seen most clearly in subjects with severe HIV-associated disease, as measured by either $CD4^+$-T-cell count or HIV load: and (iii) subjects with HIV infection who were in relatively good health would not differ significantly from high-risk HIV-seronegative subjects.

MATERIALS AND METHODS

Study population. This cross-sectional analysis reports on data collected from the REACH (Reaching for Excellence in Adolescent Care and Health) Project of the Adolescent Medicine HIV/AIDS Research Network. A detailed description of the REACH cohort has been published previously (14). Briefly, the REACH Project was a longitudinal observational study of HIV-seropositive and at-risk HIV-seronegative adolescents, who were 12 to 19 years old at the beginning of the study and who were recruited from 15 clinical sites throughout the United States. To be eligible for enrollment, HIV-seropositive subjects had to acquire HIV infection through risk behaviors such as sexual and/or intravenous drug-taking activities. Individuals infected through vertical transmission, contaminated blood products, or early childhood sexual abuse were not enrolled. HIV-seronegative subjects had similar risk behavior profiles and no chronic illness. Accrual to this longitudinal study, at a 2:1 ratio of HIV-infected/uninfected subjects, began in February 1996 and ended in November 1999. A total of 550 adolescents were enrolled. The measures reported in this analysis are components of the REACH study protocol that was approved by the institutional review board at each of the participating sites. All subjects were informed of study requirements, and written informed consent was obtained. This report covers data collected between March 1996 and March 2001.

Schedule of assessments of HIV-seropositive and -seronegative subjects. HIV-seropositive subjects were examined every 3 months. Blood was drawn for $CD4^+$- and $CD8^+$-T-cell counts at every visit. Blood for cytokine measurements and an expanded immunophenotyping panel was drawn every 6 months. HIV-seronegative subjects had blood drawn every 6 months for $CD4^+$- and $CD8^+$-T-cell counts, cytokine measurements, and a detailed immunophenotyping panel (6, 7).

Laboratory methods. (i) Stimulation. PBMCs were prepared using Ficoll-Paque (Amersham Pharmacia Biotech, Arlington Heights, Ill.) and resuspended in RPMI 1640 medium (Gibco BRL, Grand Island, N.Y.) with 10% fetal bovine serum. A total of 2×10^6 cells in 1 ml of medium was incubated for 24 h for ELISA, and 10^6 cells were incubated for 24 h for RNA analysis. Each intracellular cytokine assay used a total of $l0^6$ cells in 1 ml of medium. Phorbol myristate acetate (PMA) (Sigma, St. Louis, Mo.) was used at a final concentration of 100 ng/ml for ELISA and RNA analysis, previously demonstrated to provide optimal conditions for the assay (18).

(ii) Cytokine detection. Cell culture supernatants were harvested, frozen, and stored at −70°C. ELISAs for IL-2, IL-4, IL-6, TNF-α, and IFN-γ were performed in triplicate wells according to the manufacturer's instructions (Endogen, Woburn, Mass.). Published studies have shown that ELISAs are generally reproducible, with a typical interassay variation of approximately 50% (2). The lower limit of detection for ELISA is 3 to 8 pg/ml (18).

RNA was prepared using TriReagent (Molecular Research Center, Cincinnati, Ohio), and cDNA was prepared by reverse transcription of RNA by avian myeloblastosis virus reverse transcriptase using oligo(dT) primers (Boehringer Mannheim, Indianapolis, Ind.). Each amplification used cDNA equivalent to 5×10^4 cells. Mimics and primers for IL-2, IL-4, TNF-α, and IFN-γ were obtained from Clontech (Palo Alto, Calif.) and used in a standard dilution series with 40 cycles of amplification according to the manufacturer's suggested protocol. Quantitation was performed by comparison of cDNA target and mimic amplimers as recommended by the manufacturer. The lower limit of detection was 0.00005 amol/10^4 cells.

(iii) HIV load. Quantitative plasma HIV-1 RNA was assessed in a central laboratory with frozen specimens using either NASBA or NucliSens assay (Organon Teknika, Durham, N.C.).

Statistical methods. Eligibility criteria for the data set analyzed. All subjects in the REACH cohort who had cytokine analysis performed at least once during the study were potentially eligible for inclusion in this analysis. One set of cytokine protein measurements and one set of cytokine mRNA measurements were selected per subject. Subjects who had only protein data or only mRNA data were included in the analysis for those measures that were available. We also required that a detailed lymphocyte immunophenotyping panel (6, 7) and HIV load (for HIV-seropositive subjects) be available for each visit that was selected for analysis.

All cytokines were not available and analyzed for every subject; therefore, the number of subjects included in the analysis for each cytokine varied.

Comparison of cytokine protein markers in groups of subjects. Ten cytokine protein markers were examined in the analysis: unstimulated and stimulated IL-2, IFN-γ, IL-4, IL-6, and TNF-α. Eight cytokine mRNA markers were examined: unstimulated and stimulated IL-2, IFN-γ, IL-4, and TNF-α. Because there were a large number of values that were below the limit of detection for most of these markers, the data were analyzed in two ways. First, groups were compared for differences in the proportion of subjects who had detectable values for each marker. Second, among those subjects who had detectable values, groups were compared for differences in the distributions of the values.

HIV-seropositive subjects were divided into three subgroups based on HIV load at the time of the selected visit: undetectable (<400 copies/ml), detectable but ≤10,000 copies per ml, and >10,000 copies per ml. For each cytokine marker, five group comparisons were made as follows: (i) all HIV-seronegative subjects were compared to all HIV-seropositive subjects, (ii) HIV-seronegative subjects were compared to HIV-seropositive subjects with viral loads of >10,000 copies/ml, (iii) HIV-seropositive subjects with undetectable viral loads were compared to HIV-seropositive subjects with viral loads of >10,000, (iv) a trend analysis was performed to examine differences across the three HIV-seropositive subgroups, and (v) a trend analysis was performed to examine differences across the HIV-seronegative group and the three HIV-seropositive subgroups.

A secondary analysis was performed in the same manner, but subjects were divided into three subgroups based on $CD4^+$-T-cell counts at the time of the selected visit: <200, 200 to 499, and ≥500 cells/mm^3.

For two-group comparisons of the proportions of subjects with detectable

TABLE 1. Descriptive profile of the study population (n = 520)

Demographic or clinical characteristic	No. of subjects (%)[a]	
	HIV seropositive (n = 337)	HIV seronegative (n = 183)
Sex		
Female	251 (74.5)	140 (76.5)
Male	86 (25.5)	43 (23.5)
Race		
African-American	249 (74.0)	118 (64.5)
Other	64 (19.1)	44 (24.0)
Caucasian	23 (6.9)	21 (11.5)
Unknown	1	
Mean age ± SD (yr)	17.6 ± 1.4	17.1 ± 1.2
Plasma viral load (median no. of copies/ml)	7,800 (range, 80–700,000)	
<400	84 (25.0)	
400–10,000	129 (38.2)	
>10,000	124 (36.8)	
$CD4^+$ T-cell count (median no. of cells/mm^3)	499 (range, 10–1,801)	
<200	27 (8.1)	
200–499	139 (41.9)	
>500	166 (50.0)	
Unknown	5	

[a] Percentages are based on known data.

responses, chi-square or Fisher's exact tests were used, depending on cell size. For three- and four-group comparisons of the proportions with detectable responses, the Cochran-Armitage trend test was used. For differences in distributions of detectable values, the nonparametric Kruskal-Wallis rank sum test was used.

RESULTS

Of the 550 subjects in the REACH Project that were enrolled in this study, two subjects were excluded from this analysis because they seroconverted during the study. Of the remaining 548 subjects, 520 were included in the analysis; 515 (94%) for cytokine protein analysis and 395 (72%) for mRNA analysis.

Clinical and demographic characteristics of the 520 eligible subjects are shown in Table 1. Sixty-five percent of the subjects (n = 337) were HIV seropositive. Approximately 71% (n = 367) were African American, and 75% (n = 391) were female. The mean age of the study cohort was 17.4 years. The mean and median $CD4^+$-T-cell counts were 534 and 499 cells/mm^3, respectively, for HIV-seropositive subjects. Most of the HIV-seropositive subjects were relatively healthy; only 8.1% had $CD4^+$-T-cell counts below 200 cells/mm^3. Approximately 25% of the HIV-seropositive subjects had viral loads below the level of detection (defined as <400 copies/ml). Mean and median viral load values for subjects with viral loads equal to or above 400 copies/ml (n = 253) were 39,382 and 10,000 copies/ml, respectively. Approximately half of the HIV-seropositive subjects were taking antiretroviral medication at the sampling time point selected for inclusion in the data set analyzed: 49% were on combination antiretroviral therapy, and 3% were on monotherapy. The remaining HIV-seropositive subjects were not taking any antiretroviral medication at the sampling time point selected for inclusion in the data set analyzed.

A summary of the percentages of subjects with detectable responses for each of the 18 cytokine markers is shown in Table 2. In general, unstimulated IL-4 was the least frequently detected cytokine (both protein and mRNA), with 12.6 to 24% of subjects having detectable IL-4 protein or mRNA. As expected, stimulated samples consistently showed a higher proportion with detectable markers compared to unstimulated samples. However, stimulation with PMA had only a limited effect on responses.

A summary of the medians and interquartile ranges for the cytokine markers by HIV serostatus groups and by HIV load subgroups is shown in Table 3. Data for each marker are based only on values contributed by subjects with detectable responses for that marker.

Overall, 90 different group comparisons of the percentages of subjects with detectable responses were performed in the initial exploratory analysis. Only four of these group comparisons achieved a P value of ≤0.05, unadjusted for multiple comparisons. Another 90 comparisons were made of the distribution of detectable responses, and only three of these achieved a P value of ≤0.05, unadjusted for multiple comparisons. Of the seven comparisons with a P value of ≤0.05, five were in the direction opposite that of the hypothesized shift from a TH1-to-TH2 cytokine response to HIV-associated disease. Results were similar when analyses were repeated using $CD4^+$-T-cell counts (data not shown). No patterns consistent with a TH1-to-TH2 cytokine shift were detected.

DISCUSSION

We measured stimulated and unstimulated mRNA and protein levels for IFN-γ, IL-2, IL-4, IL-6, and TNF-α in samples derived from a large cohort of HIV-seropositive and -seronegative adolescents. Detailed analyses of these data indicate that there were no differences detected between HIV-seropositive and HIV-seronegative adolescents and that there was no apparent relationship between these measurements and viral loads or $CD4^+$-T-cell counts, parameters selected as markers of HIV-associated disease status. In the data set examined, the hypothesis of a shift from TH1 to TH2 cytokines is not supported. The observed associations were not consistent with any of the following hypothesized relationships: (i) that there would be a shift from a TH1 response to a TH2 response in the presence of HIV infection; (ii) that this pattern would be seen most clearly in subjects with severe HIV-associated disease, as measured by either $CD4^+$-T-cell count or HIV load; and (iii) that subjects with HIV infection who were in relatively good health would not differ significantly from high-risk HIV-seronegative subjects.

The cohort contributing the samples used in this study was comprised primarily of relatively healthy adolescents, including those with HIV infection, compared to the HIV-infected subjects used in studies that reported a cytokine shift. For example, the recent reports of Altfeld et al. (1) and Rizzardi et al. (13) included patients with AIDS-defining illnesses and advanced HIV-associated disease. We did not observe any evi-

TABLE 2. Percentages of subjects with detectable cytokine markers

Marker	Subjects with detectable cytokine markers[a]									
	HIV seronegative		HIV seropositive							
			All		VL <400		VL 400–10,000		VL >10,000	
	n	% Detectable	*n*	% Detectable	*n*	% Detectable	*n*	% Detectable	*n*	% Detectable
Unstimulated protein										
IL-2	136	40.4	235	40.0	47	48.9	106	42.5	82	31.7
IFN-γ	142	36.6	244	33.2	51	31.4	108	34.3	85	32.9
IL-4	142	19.0	248	15.7	52	23.1	111	12.6	85	15.3
IL-6	143	66.4	233	65.7	49	69.4	105	60.0	79	70.9
TNF-α[b]	142	35.2	240	46.7	49	53.1	110	46.4	81	43.2
Stimulated protein										
IL-2[c]	181	48.1	326	50.6	63	63.5	145	46.2	118	49.2
IFN-γ	176	83.5	324	80.3	64	84.4	145	79.3	115	79.1
IL-4	177	24.9	323	19.8	63	23.8	145	20.0	115	17.4
IL-6	180	94.4	328	92.7	63	92.1	146	93.2	119	92.4
TNF-α	178	83.2	326	84.7	63	79.4	146	86.3	117	85.5
Unstimulated mRNA										
IL-2	136	14.7	250	15.2	50	10.0	108	18.5	92	14.1
IFN-γ	136	25.0	252	27.8	50	34.0	110	29.1	92	22.8
IL-4[d]	136	24.3	252	17.5	50	24.0	110	17.3	92	14.1
TNF-α	136	32.4	252	34.5	50	36.0	110	37.3	92	30.4
Stimulated mRNA										
IL-2	114	53.5	195	48.2	46	47.8	83	48.2	66	48.5
IFN-γ	121	52.9	201	62.2	46	58.7	84	61.9	71	64.8
IL-4	119	32.8	201	32.8	46	37.0	84	28.6	71	35.2
TNF-α	121	43.8	201	49.8	46	56.5	84	48.8	71	46.5

[a] The number (*n*) and percentage of subjects with detectable cytokine markers are shown for HIV-seronegative and HIV-seropositive subjects. All HIV-seropositive subjects and HIV-seropositive subjects with viral loads (VL) of <400, 400 to 10,000, and >10,000 copies/ml were studied.
[b] The values (percentage of subjects) for HIV-negative and all HIV-positive groups and the values for HIV-negative subjects and HIV-positive subjects with viral loads of <400 copies/ml were significantly different ($P = 0.03$ by the chi-square test).
[c] The values (percentage of subjects) for HIV-negative subjects and HIV-positive subjects with no detectable virus were significantly different ($P = 0.03$ by the chi-square test).
[d] $P = 0.04$ by the Cochran-Armitage test for trends across all four groups.

dence of a cytokine shift in our study, even in comparisons of HIV-seronegative subjects and HIV-seropositive subjects with viral loads of >10,000 copies/ml.

On the basis of our data, we conclude that, early in the course of HIV-associated disease in adolescents, there are no detectable shifts in cytokine production. Previously published immunologic studies conducted on the REACH cohort have indicated that these adolescents were relatively early in their course of HIV infection (6, 16). Among the HIV-seropositive youths, 50% had $CD4^+$-T-cell counts of greater than 500 cells/mm^3, 63% had plasma viral loads of less than 10,000 copies per ml, and 89% had intact delayed-type hypersensitivity responses (anergy responses) (15). In addition, the immune systems of HIV-infected adolescents in this cohort are functioning at a higher level than those of HIV-infected children or adults (7). A unique observation of the REACH cohort is the finding of increased naive $CD8^+$-T-cell numbers at all $CD4^+$-T-cell strata in comparison to adults (7). These findings are consistent with the concept that HIV-infected adolescents may have the potential to repopulate naive T lymphocytes (7). High numbers of Ki-67 proliferating cell populations in both the $CD4^+$- and $CD8^+$-T-cell subsets have also been observed (17).

One of the technical limitations of our data, a limitation shared by others working in this area, is the use of bulk PMA or phytohemagglutinin (PHA) stimulation assays. During the course of these investigations, we measured cytokine secretion and intracellular protein expression in mRNA in resting and stimulated PBMCs in a group of subjects in order to examine the effects of various stimuli (18). The limitations of bulk (PMA or PHA) stimulation of PBMCs relates to the existing cellular makeup of the PBMC population (18). Ledru et al. observed a significant alteration in the proportions of T cells producing type 1 cytokines (specifically CD45RA and CD45RO subsets), rather than a cytokine shift (10). In this analysis, we did not control for intersample variation in the cellular makeup.

The cross-sectional sampling approach used to develop the data set analyzed also may have influenced our results. This approach was selected in order to make optimal use of the available cohort. Since only a small subset of the REACH cohort developed progression to an AIDS-defining condition, future longitudinal analyses of cytokine profiles in sequential samples from this subset may be more informative.

In summary, our data do not support the hypothesized TH1-to-TH2 shift with advancing HIV-associated disease. This finding is most likely related to both the HIV-1-associated disease stage and to the relatively well-preserved state of the immune system in the cohort of adolescents in this study. Technical limitations may also have contributed to our inability to detect a shift in cytokine expression. Nevertheless, failure to identify

TABLE 3. Medians and interquartile ranges for cytokine measures in subjects with detectable cytokine responses

Marker	Subjects with detectable cytokine responses														
	HIV seronegative			HIV seropositive											
				All			VL <400			VL 400–10,000			VL >10,000		
	n	Median	Q_{25}–Q_{75}	n	Median	Q_{25}–Q_{75}	n	Median	Q_{25}–Q_{75}	n	Median	Q_{25}–Q_{75}	n	Median	Q_{25}–Q_{75}
Unstimulated protein															
IL-2[b]	55	44.0	16–103	94	44.4	15–139	23	41.3	15–118	45	68.0	21–221	26	39.4	6–51
IFN-γ	52	22.0	6–69	81	17.0	6–54	16	18.5	5–72	37	25.0	9–142	28	13.5	6–22
IL-4	27	32.0	6–74	39	22.0	6–75	12	36.4	8–80	14	11.0	5–83	13	48.0	12–61
IL-6	95	55.0	19–149	153	69.0	20–281	34	32.5	17–142	63	87.0	14–317	56	85.2	27–268
TNF-α	50	24.2	15–96	112	27.2	12–93	26	27.2	14–53	51	22.0	7–102	35	39.0	17–92
Stimulated protein															
IL-2	87	54.0	30–197	165	49.0	21–171	40	42.1	23–176	67	52.0	20–171	58	50.7	20–193
IFN-γ	147	178.0	39–739	260	266.0	60–661	54	358.0	77–644	115	205	39–771	91	276.0	66–592
IL-4	44	8.5	4–38	64	9.5	4–64	15	9.0	6–65	29	12.0	4–26	20	6.0	4–69
IL-6	170	301.6	69–598	304	333.5	74–656	58	276.1	83–799	136	344.0	56–638	110	352.0	93–621
TNF-α[c]	148	367.6	184–725	276	238.0	93–599	50	343.0	99–847	126	222.5	70–554	100	246.5	100–618
Unstimulated mRNA															
IL-2	20	4.0	2–4	38	2.0	2–4	5	2.0	2–4	20	2.0	2–4	13	2.0	2–4
IFN-γ	34	3.0	2–8	70	4.0	1–10	17	6.0	2–20	32	4.0	1–10	21	4.0	2–8
IL-4	33	2.0	1–2	44	1.8	1–2	12	1.5	1–2	19	2.0	1–4	13	1.0	0.2–2
TNF-α	44	4.0	2–20	87	4.0	2–20	18	5.0	2–20	41	3.2	2–40	28	6.0	2–20
Stimulated mRNA															
IL-2	61	10.0	4–40	94	10.0	4–40	22	20.0	4–20	40	10.0	3–20	32	20.0	4–60
IFN-γ	64	200.0	40–1,000	125	200.0	40–600	27	200.0	60–1,000	52	200.0	33–400	46	250.0	200–600
IL-4	39	2.0	1–4	66	2.5	1–6	17	3.0	2–6	24	2.0	0.3–4	25	4.0	2–8
TNF-α	53	40.0	20–400	100	100.0	20–400	26	150.0	20–400	41	40.0	20–400	33	100.0	24–400

[a] The number (n) of subjects with detectable cytokine responses and median and interquartile range values for cytokine protein and mRNA levels are shown for HIV-seronegative and HIV-seropositive subjects. All HIV-seropositive subjects and HIV-seropositive subjects with viral loads (VL) of <400, 400 to 10,000, and >10,000 copies/ml were studied. Values for protein are in picograms per milliliter. Values for mRNA are in attomoles per 10^4 cells. Interquartile ranges are from Q_{25} (first quartile [25th percentile]) to Q_{75} (third quartile [75th percentile]).

[b] The values for the three HIV-positive groups with viral loads of <400, 400 to 10,000, and >10,000 copies/ml were significantly different ($P = 0.04$ by Kruskal-Wallis rank sum test).

[c] The values for the HIV-seronegative and HIV-seropositive (all) subjects ($P = 0.003$) and for all four groups ($P = 0.01$) were significantly different by the Kruskal-Wallis rank sum test.

a TH1-to-TH2 cytokine shift in those adolescents with the most advanced HIV-associated disease suggests strongly that this model may not be appropriate for understanding progression of HIV-associated disease in adolescents.

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