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Effects of human interleukin 7 on HIV-1 replication in monocyte-derived human macrophages

Haihan Song, Emi E. Nakayama and Tatsuo Shioda

Interleukin 7 (IL-7) contributes to development and proliferation of T cells. We investigated the effect of IL-7 on HIV-1 infected monocyte-derived human macrophages. IL-7 treatment of macrophages at a concentration of 10 ng/ml reduced replication of the R5 HIV-1 strain by approximately 50%. Meanwhile, HIV-1-infected macrophages themselves could excrete approximately 20% more IL-7 than uninfected macrophages. These results suggest that IL-7 could be used as a therapeutic modality to recover CD4 T cells.

Macrophages and CD4 T cells are the predominant cell types for HIV-1 infection [1,2]. Macrophages can be infected by HIV-1 in many tissues, including the brain, lung and lymph nodes [3]. Unlike infected CD4 T cells, which have a short half-life of 1–1.5 days, macrophages are quite resistant to the cytopathic effect of the virus, and may thus provide a reservoir for persistent infection and virus dissemination. It is known that cytokines play an important role in HIV-1 infection. Interleukin-7 (IL-7) is a cytokine produced by stromal cells of the thymus and bone marrow [4–6]. It has the capacity to induce growth of immature B lymphocytes [7], and contributes to development and proliferation of T cells [8–11]. With respect to HIV-1 infection, there is a reverse correlation between CD4 T cell number and IL-7 serum levels in HIV-1-infected patients [12–14]. After patients started to receive antiretroviral drugs, the elevated IL-7 in the serum decreased to normal levels [12]. On the other hand, IL-7 increases HIV-1 replication in thymic organ cultures [15–17] and induces latent HIV-1 in resting CD4 T cells [18]. However, effects of IL-7 on HIV-1 replication in macrophages remain unclear. In the present study, we evaluated levels of HIV-1 proliferation in monocyte-derived human macrophages treated with or without exogenous IL-7.

Peripheral blood mononuclear cells (PBMC) from blood buffy coats of healthy donors were isolated by centrifugation through Ficoll-Hypaque and plated on a 24-well MULTIWELL^{TM} PRIMARIA^{TM} plate (Becton Dickinson, Franklin Lakes, New Jersey, USA) with RPMI 1640 supplemented with 10% fetal calf serum (FCS). After incubation at 37°C for 1 day, the floating cells were removed by washing the plate with phosphate-buffered saline four times and the adherent cells were incubated at 37°C for 11 days with 0.5 ml of RPMI 1640 supplemented with 10% FCS plus 100 ng/ml of granulocyte macrophage colony stimulating factor (GM–CSF).

To determine whether or not differentiated macrophages express the IL-7 receptor, we analysed total RNA extracted from macrophages. PBMC stimulated with phytohaemagglutinin (PHA) and 10 ng/ml of IL-2 for 3 days and monkey kidney CV-1 cells served as positive and negative controls, respectively. RNA was reverse-transcribed into cDNA, and levels of IL-7 receptor cDNA were measured by an ABI 7500 Real-time PCR System. As shown in Fig. 1a, IL-7 receptor mRNA in macrophages was clearly detected. It was also detected in PHA and IL-2-stimulated PBMC. Levels of IL-7 receptor mRNA in macrophages were approximately one-tenth of those in PHA and IL-2 stimulated PBMC, while CV-1 cells were totally negative for expression of the IL-7 receptor.

Macrophages were infected with 6.5 ng of p24 of HIV-1 SF162 strain for 2 h on day 12. Cells were then incubated with 1 ml of RPMI 1640 supplemented with 10% FCS plus 100 ng/ml of GM–CSF and 0, 3 or 10 ng/ml of IL-7 (Techne, Minneapolis, Minnesota, USA). Infected macrophages were fed on days 3, 6, 9 and 12 (after infection) by exchanging one-half of the culture supernatant with fresh media containing the same amount of cytokines. Levels of HIV-1 p24 antigen in culture supernatants on days 1, 3, 6, 9 and 12 were measured by using an HIV-1 P24 Antigen ELISA Kit (Fig. 1b). The p24 antigen level in the culture supernatant of macrophages treated with 3 ng/ml of IL-7 was apparently less than that of macrophages without IL-7 treatment (Fig. 1b). This difference was evident on day 3 and became greater with time. On day 12, the p24 level of macrophages treated with 3 ng/ml of IL-7 was 2220 pg/ml while that of untreated macrophages was 3542 pg/ml. That is, the suppression with 3 ng/ml of IL-7 on HIV-1 replication was 37.4% on day 12. Treatment of macrophages with 10 ng/ml of IL-7 showed a greater suppressive effect on HIV-1 replication (Fig. 1b). On day 12, the p24 level of macrophages treated with 10 ng/ml of IL-7 was 1760 pg/ml. That is, suppression with 10 ng/ml of IL-7 on HIV-1 replication was 50.3% on day 12.

Monocyte-derived human macrophages were also infected with the Z strain of the Sendai virus. Hemagglutination assay (HA) titres were measured on days 1, 3 and 6 after infection. As shown in Fig. 1c, there

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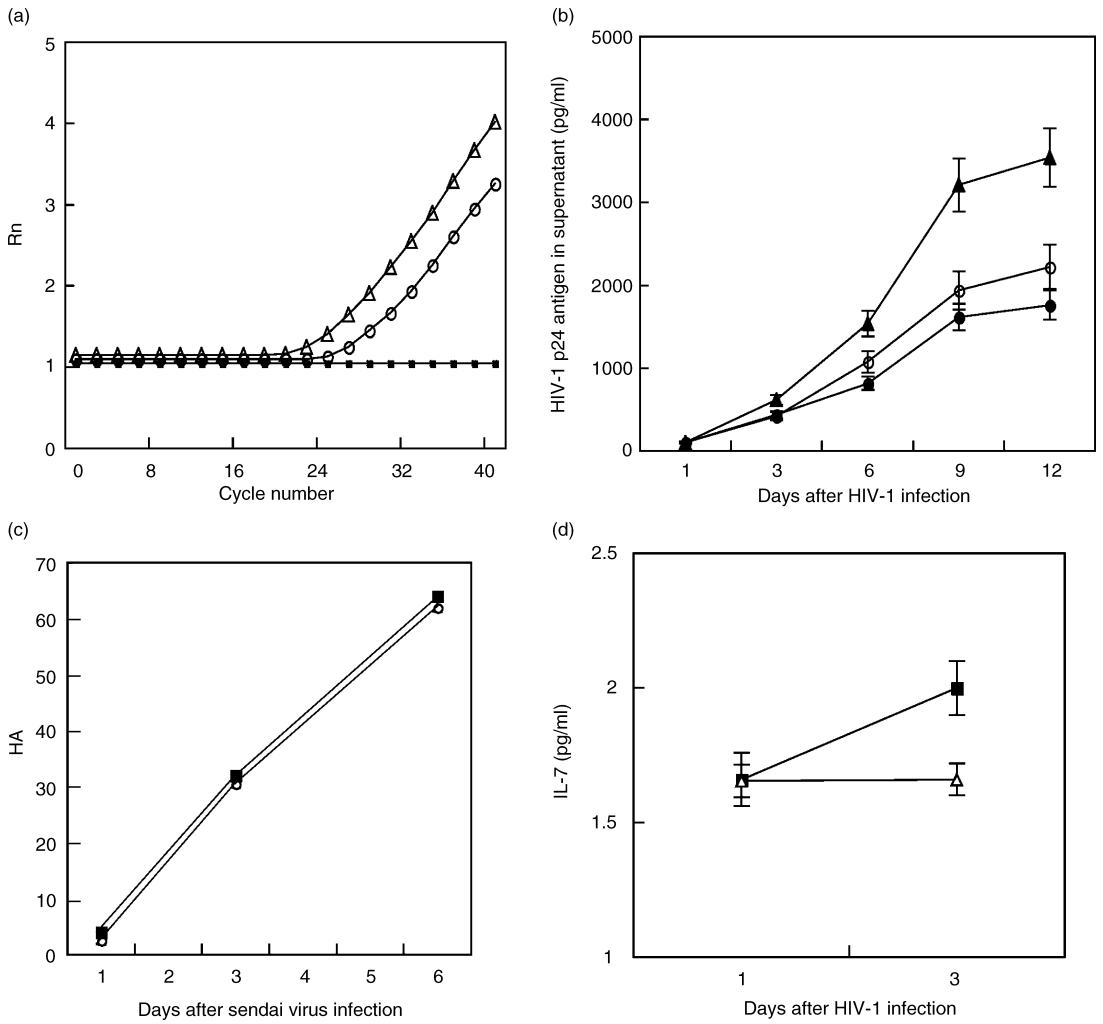

Fig. 1. (a) Expression of IL-7 receptor mRNA in macrophages measured by real-time monitoring of fluorescence signals. IL-7 receptor mRNA in macrophages (open circles), PHA-stimulated PBMC (open triangles) as the positive control and CV-1 cells (closed squares) as the negative control are shown. Rn is the ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intensity of the passive reference dye. Data shown are representative of two independent experiments with similar results. (b) The HIV-1 p24 antigen in the supernatant of HIV-1 infected macrophages treated with 0 (closed triangles), 3 (open circles) or 10 ng/ml (closed circles) of IL-7 was measured on days 1, 3, 6, 9 and 12 after HIV-1 infection. Data show the mean of duplicate samples from the same blood donor with bars indicating the actual fluctuation between two individual measurements. Data shown are the representative of three independent experiments with similar results. (c) Growth of the Sendai virus in monocyte-derived macrophages treated with 10 ng/ml of IL-7 (open circles) or untreated with IL-7 (closed squares). HA titres in culture supernatants were measured on days 1, 3 and 6 after infection. Data shown are representative of two independent experiments with similar results. (d) Levels of IL-7 in the supernatants of HIV-1 infected macrophages (closed squares) or uninfected macrophages (open triangles) were measured on days 1 and 3 after HIV-1 infection. Data show the mean of duplicate samples from the same blood donor with bars indicating the actual fluctuation between two individual measurements. Data shown are representative of two independent experiments with similar results.

was no difference in HA titres between IL-7-treated and untreated macrophages, indicating no apparent effect of IL-7 on Sendai virus replication in macrophages.

Levels of IL-7 in supernatants of macrophages were measured on days 1 and 3 after HIV-1 infection (Fig. 1d). On day 1, there was no difference in levels of IL-7 between HIV-1 infected and uninfected macrophages. On day 3, IL-7 levels in HIV-1-infected macrophages slightly increased, while those in uninfected macrophages did not.

The mechanisms of the suppressive effect of IL-7 on R5 HIV-1 replication in macrophages are still not clear. A previous study showed that IL-7 treatment for PBMC enhanced excretion of β chemokines including MIP-1β, which can suppress R5 HIV-1 replication [19]. We pretreated macrophages with 10 ng/ml of IL-7 for 1 day,

and then inoculated them with HIV-1. However, we failed to detect any further suppression of HIV-1 replication compared with macrophages treated with IL-7 only after HIV-1 infection (data not shown). Therefore, it is unlikely that IL-7 could suppress HIV-1 replication by upregulation of MIP-1β. It is possible that IL-7 could affect HIV-1 replication after HIV-1 enters macrophages.

We have shown that HIV-1-infected macrophages could excrete more IL-7 than uninfected ones. Therefore, it is likely that IL-7 is a self-defence system for macrophages against HIV-1 infection. Furthermore, our finding that IL-7 could moderately suppress HIV-1 replication in macrophages suggested the advantage of IL-7 as an immune modulator which could be used to recover CD4 T-cell numbers in HIV-1-infected individuals.

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Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Correspondence to T. Shioda, Department of Viral Infections, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita-shi, Osaka 565-0871, Japan.
Tel: +81 6 6879 8346; fax: +81 6 6879 8347;
e-mail: shioda@biken.osaka-u.ac.jp

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Interferon-γ decreases replication of primary R5 HIV-1 isolates in thymocytes

Livia Pedroza-Martins[a], W. John Boscardin[b], Deborah J. Anisman-Posner[a], Beverly N. Redsar[a], Yvonne J. Bryson[c,d], Otto O. Yang[a,e,d] and Christel H. Uittenbogaart[a,c,d,f]

As interferon-γ (IFN-γ) is produced at lower levels by neonatal than adult T cells, we determined whether IFN-γ influences HIV-1 replication in thymocytes. IFN-γ significantly decreased replication of R5 but not X4 viruses, and reduced depletion of $CD3^{hi}CD27$ (mature) thymocytes, the preferential targets for R5 HIV-1. Thus infection and

depletion of functionally mature thymocytes that can produce endogenous IFN-γ may mutually contribute to HIV-1 replication in the thymus and to reduced T-cell output.

Interferon-γ (IFN-γ), a cytokine produced by activated T lymphocytes in response to antigen, plays an important role in adaptive and innate immune responses to viral infections (reviewed in [1]). Because T cells are generated in the thymus, which is also an important site of HIV-1 infection, the inter-relationships of IFN-γ production, viral replication, and T-cell development likely are important in HIV-1 immunopathogenesis. IFN-γ mRNA is expressed during thymocyte maturation, within both positively-selected $CD3^{hi}CD4CD8$ thymocytes and mature $CD3^{hi}CD4$ and $CD3^{hi}CD8$ thymocytes [2]. This suggests that IFN-γ may have an important protective role against viral infections in the thymus, and/or contribute to T-cell development. Supporting this concept, neonates are more susceptible to viral infections, and their lymphocytes produce lower levels of IFN-γ than adults [3–6].

Although CCR5 expression is low on most thymocytes [7–9], it supports R5 viral infection of specific thymocyte subsets [10]. We have observed that mature $CD3^{hi}CD27$ thymocytes are efficient targets for R5 HIV-1 infection *in vitro* [10] and that preferential entry and replication of R5 HIV-1 takes place in $CD3^{hi}CD27$ thymocytes *in vivo* in the SCID-hu model (Gurney and Uittenbogaart,

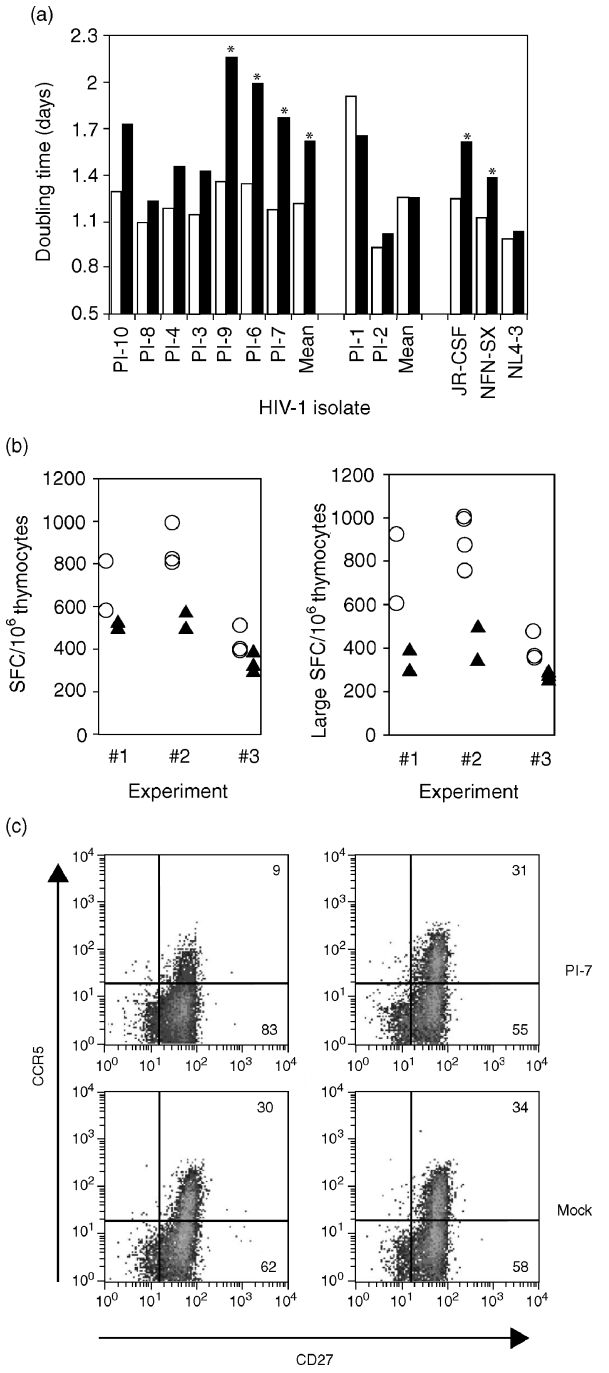

Fig. 1. (a) To test the effect of exogenous IFN-γ on HIV-1 replication, longitudinal increase in p24 was modeled linearly on the logarithmic scale using mixed effect linear models to account for the nesting of measurements within each viral isolate and isolates within thymic tissue [13]. Estimated slopes on the logarithmic scale were converted to doubling times for ease of interpretation. IFN-γ (500 U/ml) decreased HIV-1 replication in all seven R5 primary isolates (doubling time increased by 34% on average, $P < 0.0001$) and in the two laboratory strains JR-CSF (doubling time increased by 29%, $P < 0.0001$) and NFN-SX (23% increase, $P = 0.002$). Doubling times were not significantly affected in the two X4 primary isolates (although there was a very limited amount of data available to examine this question) nor in the NL4-3 laboratory strain (6% increase, $P = 0.18$). (b) To measure endogenous IFN-γ production thymocytes were infected *in vitro* with R5 isolate JR-CSF or mock-infected with the supernatant from the same cells (CD4 T-cell pool) used to generate the virus stock. The cells were cultured at 1×10^7 cells/ml in serum-free medium with interleukin (IL)-2 plus IL-4. Two to three weeks postinfection, thymocytes were counted and $2.5–3 \times 10^5$ thymocytes were added to each well of a 96-well plate coated with antibody to IFN-γ. Thymocytes were cultured for 24 h in replicate wells in the presence or absence of stimuli (CD2/CD2R plus CD28). The number of spots in unstimulated cells was less than 20 spot forming cells (SFC)/1×10^6 thymocytes. The numbers of SFC/1×10^6 thymocytes was significantly lower in JR-CSF than in mock infected thymocytes ($P = 0.002$). In addition the number of large SFC was significantly lower in JR-CSF than in mock infected thymocytes ($P < 0.0001$). (c) To determine the effect of IFN-γ on depletion of CCR5CD27 thymocytes, thymocytes were infected with HIV-1 R5 isolates or mock infected and cultured in the presence or absence of IFN-γ (500 U/ml). Thymocytes were cell surface phenotyped, apoptotic and dead cells were excluded from the analysis by 7-amino-actinomycin D staining. Expression of CCR5-phycoerythrin (PE) and CD27-PE is shown. Appropriate isotype control antibodies were used in parallel.

unpublished data). Maturing and functionally competent thymocytes, such as those at the $CD3^{hi}CD27+CD1-$ stage of development [11] are also particularly susceptible to R5 HIV-1 infection. As these also are the cells that can produce IFN-γ, HIV-1-induced perturbation of IFN-γ production could play a role in potentiating viral replication in the thymus.

To assess these issues, thymocytes were infected with HIV-1 pediatric primary isolates (with parallel mock-infected controls), and viral replication in the presence or absence of exogenous IFN-γ was monitored by supernatant p24 quantitation. Addition of exogenous IFN-γ (500 U/ml) slowed viral replication significantly in thymocytes infected with the seven primary R5 isolates (Fig. 1a; mean p24 doubling time 1.21 days without versus 1.62 days with IFN-γ, $P < 0.0001$). In contrast, the two X4 primary isolates were unaffected by IFN-γ (1.25 days with or without IFN-γ), although one of these X4 isolates (PI-2) appeared to be inhibited by IFN-γ (Fig. 1a). However, the small number of X4 primary isolates does not allow for definitive conclusions about a role for co-receptor phenotype.

To further examine the potential role of co-receptor expression on the impact of IFN-γ, laboratory strains differing in the V3 region of the *env* gene were compared in parallel (Fig. 1a). Replication of the X4 molecular clone NL4-3 in thymocytes was not significantly decreased ($P = 0.18$) by exogenous IFN-γ. In contrast, the R5 clone JR-CSF was significantly suppressed (p24 doubling time 1.24 days versus 1.61 days, $P< 0.0001$), as well as the R5 HIV-1 strain NFN-SX, which differs from NL4-3 only in the Env V3 region [12] (doubling time 1.12 versus 1.38 days, $P = 0.002$). These results indicate that viral sensitivity to IFN-γ may be related to viral tropism, as the broader distribution of CXCR4 in the thymus may allow X4 infection of thymocyte subsets that do not respond to IFN-γ.

The impact of IFN-γ on depletion of thymocyte subsets by HIV-1 infection was assessed by comparing cells cultured without or with IFN-γ after infection. We previously reported [10] that R5 HIV-1 primary isolates predominantly replicate in more mature thymocytes ($CD27CD3^{hi}$), depleting the CCR5CD27 subset. Across all R5 pediatric HIV-1 isolates, the percentage of CCR5CD4 thymocytes was higher in the presence of added IFN-γ (mean $2 \pm 1\%$ without versus $16 \pm 14\%$ with IFN-γ, $P = 0.06$, analysed by mixed effects inference [13]). Specific R5 primary HIV-1 isolates, obtained from infants with varying rates of disease progression, responded similarly to IFN-γ and similar results were obtained for JR-CSF and NFN-SX, while IFN-γ had no effect on the subset distributions of thymocytes infected with X4 viruses (data not shown). In mock-infected cultures, IFN-γ had no apparent effect on the percentage of CCR5CD4 thymocytes (mean $15 \pm 12\%$ versus $13 \pm 14\%$, $P = 0.9$). Furthermore, in thymocytes infected with R5 viruses, IFN-γ reduced the viral-induced loss of CD4 and CD4CD8 thymocytes rather than increasing the CCR5CD4 subset, although the differences did not reach statistical significance (CD4CD8 thymocytes $33 \pm 13\%$ without versus $39 \pm 15\%$ with IFN-γ, $P = 0.3$; CD4+CD8− thymocytes $9 \pm 3\%$ versus $13 \pm 5\%$, $P= 0.1$). IFN-γ did not affect the CD4/CD8 subset distribution of mock-infected thymocytes, indicating lack of a direct cytokine effect. Thus, IFN-γ helped preserve the CD4 thymocyte population by reducing depletion of CCR5CD4 thymocytes after exposure to R5 primary HIV-1 isolates.

The thymocytes depleted by R5 HIV-1 are more mature cells that are capable of producing IFN-γ. Quantitative ELISpot assays [14,15] were utilized to examine IFN-γ production by thymocytes 14–18 days post-infection with JR-CSF or a pediatric R5 isolate, in response to CD2/CD2R-stimulation (Fig. 1b). JR-CSF infection resulted in fewer spot forming units/10^6 cells and less IFN-γ per cell (decreased spot size) than mock-infected thymocytes 2–3 weeks post-infection. Similar data were obtained after infection with an R5 primary HIV-1 isolate (data not shown). Moreover, we have observed that the few freshly isolated thymocytes (approximately 0.1%) that produce IFN-γ after CD2/CD2R/CD28 stimulation are 100% CD3CD27 and > 50% CD3CD1 (by intracellular IFN-γ staining and cell surface co-staining; data not shown). Interestingly, exogenous IFN-γ also prevented reduction of the CD3CD27CCR5 thymocyte subset (Fig. 1c), which is preferentially depleted after infection with R5 HIV-1 pediatric isolates [10]. Thus HIV-1 exposure and/or infection reduced both the number of thymocytes capable of producing IFN-γ as well as the cytokine produced per cell, suggesting both quantitative and qualitative impairment of thymocytes after exposure to HIV-1.

In conclusion, we found that IFN-γ mediates antiviral activity against R5 HIV-1 in thymocytes, and that R5 HIV-1 has a propensity to selectively deplete the more mature thymocytes that produce IFN-γ. These findings suggest a mechanism contributing to HIV-1 persistence in the thymus, through HIV-1 targeting and depletion of cells that are important for producing IFN-γ. Because these are also the more mature thymocytes that are destined for export to the periphery, this may have additional implications in the immunopathogenesis of T cell depletion in HIV-1 infection.

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Department of [a]Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, California, USA; [b]Department of Biostatistics, University of California, Los Angeles, California, USA; [c]Department of Pediatrics, University of California, Los Angeles, California, USA; [d]UCLA AIDS Institute, University of California, Los Angeles, California, USA; [e]Department of Medicine, University of California, Los Angeles, California, USA; and [f]Jonsson Comprehensive Cancer Center, University of California, Los Angeles, California, USA.

Correspondence to C.H. Uittenbogaart, Department of Microbiology, Immunology, and Molecular Genetics, UCLA School of Medicine, 10833 Le Conte Ave, Los Angeles, CA 90095-1747, USA.
Tel: +1???; fax: +1???; e-mail: uittenbo@ucla.edu

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HIV, syphilis, herpes simplex virus 2, and behavioral surveillance among conflict-affected populations in Yei and Rumbek, southern Sudan

Reinhard Kaiser[a], Tekleab Kedamo[d], Judith Lane[e], George Kessia[f], Robert Downing[b], Thomas Handzel[a], Elizabeth Marum[c], Peter Salama[a], Jonathan Mermin[b], William Brady[a] and Paul Spiegel[a]

Little is known about the HIV epidemic in conflict-affected southern Sudan. During 2002–2003, we conducted behavioral and biological surveillance surveys and sequential sampling in antenatal clinics in Yei, Western Equatoria, and Rumbek, Bar-el-Ghazal. HIV prevalence among individuals aged 15–49 years ranged between 0.4% in Rumbek town and 4.4% in Yei town, and among pregnant women between 0.8 and 3.0%, respectively. After the recent peace agreement, targeted prevention programmes are urgently needed to prevent further spread.

In January 2005, Sudan's government and the Sudanese Peoples' Liberation Movement signed a peace agreement [1], ending a 21-year-old civil war that resulted in approximately 4 million internally displaced persons and 500 000 refugees (not including the Darfur crisis) [2]. Little is known about the HIV epidemic in southern Sudan. In November 2002 and April 2003, we conducted linked behavioral and biological surveillance surveys and sequential sampling in antenatal clinics in Yei, Western Equatoria, and Rumbek, Bar-el-Ghazal.

We conducted two-stage household cluster surveys in Yei town, the surrounding 20 km outside of Yei town and Rumbek town. In addition to the population-based sample, we surveyed sequential samples of pregnant women at antenatal clinics in Yei and Rumbek town.

Blood specimens were transported to a field laboratory and tested for HIV-1/2 antibodies using two parallel rapid tests: Determine (Abbott, USA) and Unigold (Trinity Biotech, Ireland). Discordant test results were retested using Hemastrip (Saliva Diagnostics, USA) as a tiebreaker.

Syphilis testing was performed using Determine Syphilis TP (Abbott Laboratories, USA) for screening and quantitative rapid plasma reagin and *Treponema pallidum* hemagglutination assays for confirmation. All HIV-positive specimens and 20% of HIV-negative specimens were retested at reference laboratories in Uganda and Kenya using enzyme immunoassay algorithms. A subsample from Yei was also tested for herpes simplex virus 2 by the Centers for Disease Control and Prevention (CDC) laboratory in Atlanta, using glycoprotein G-based immunoblot assays.

Participants did not receive their HIV result, but were offered free voluntary HIV counseling and testing and education material. Participants with positive syphilis results and their partners were offered treatment.

The overall sample in Yei and Rumbek comprised 965 households with at least one eligible individual who agreed to participate in the survey. A total of 2443 (89.5%) of 2731 eligible individuals 15–49 years old present at the time of the survey agreed to participate in the interview, and 2020 (74.0%) agreed to have blood drawn. Approximately half of the respondents in Yei were internally displaced, and approximately one third in Yei reported that they had left Sudan as refugees; both types of displacement were considerably lower in Rumbek. The survey sample was also characterized by relatively high proportions of polygamous marriage and low literacy, especially in women and soldiers.

HIV prevalence ranged between 0.4% in Rumbek town and 4.4% in Yei town (Table 1). In Yei town, HIV was two times more common among women than men, and four times more common among women aged 15–24 years than in men of this age (Table 1). Five to 10% of men reported abnormal urethral discharge, and 4–16% of participants reported genital ulcer symptoms during the past year.

HIV prevalence in the sequential sample of pregnant women ranged between 0.8% [3/392, 95% confidence interval (CI) 0–1.6%] in Rumbek town and 3.0% (7/233, 95% CI 0.8–5.2%) in Yei town.

The median age at first sex was 17.5 years for men in Yei town and 16.5 years for women in areas outside Yei town, which is lower than the median of 18.2 and 17.5 years, respectively, reported from other sub-Saharan countries [3]. Condom use during high-risk sex (defined as sexual intercourse with someone other than a regular partner in the past 12 months) ranged from 0 to 8.3% among women and 17.6 to 38.7% among men, which was overall lower than the median of 21 and 38%, respectively, reported in other sub-Saharan countries [3].

In a multivariate model adjusting for survey area, age, and literacy, women who were widowed, separated or divorced were more likely to have HIV than were women in married, monogamous relationships [adjusted odds ratio (OR) 7.7, 95% CI 2.3–25.4, $P = 0.0008$], and women who self-reported genital ulcers in the past year were more likely to have HIV than were those who did not (OR 2.3, CI 0.9–5.8, $P = 0.0759$). We found no association between displacement status and HIV prevalence.

Table 1. HIV, syphilis and herpes simplex virus-2 prevalence rates, southern Sudan prevalence and behavioral surveillance surveys, 2002–2003.

Indicator	Yei total	Yei outside town	Yei town	Rumbek town
Numerator/denominator %, 95% CI				
HIV total	30/1054	3/438	27/616	4/966
	2.8, 1.6–4.0	0.7, 0–1.8	4.4, 2.9–5.9	0.4, 0–0.8
HIV, by sex and age (years)				
Men total	7/404	1/186	6/218	0/344
	1.7, 0.2–3.2	0.5, 0–1.8	2.8, 0–5.6	
15–24	1/136	0/54	1/82	0/137
	0.7, 0–2.3		1.2, 0–4.0	
25–39	5/206	0/91	5/115	0/151
	2.4, 0–5.0		4.3, 0–9.4	
40–49	1/62	1/41	0/21	0/55
	1.6, 0–5.0	2.4, 0–7.9		
Women total	23/650	2/252	21/398	4/622
	3.5, 2.0–5.1	0.8, 0–1.9	5.3, 3.2–7.3	0.6, 0–1.3
15–24	12/301	2/97	10/204	2/295
	4.0, 1.3–6.7	2.1, 0–4.9	4.9, 1.0–8.8	0.7, 0–1.6
25–39	9/290	0/123	9/167	2/280
	3.1, 0.8–5.5		5.4, 1.4–9.4	0.7, 0–1.7
40–49	2/54	0/31	2/23	0/39
	3.7, 0–9.2		8.7, 0–23.3	
Syphilis total	7/1054	1/438	6/616	27/966
	0.7, 0.1–1.2	0.2, 0–0.7	1.0, 0–1.9	2.8, 1.4–4.2
Herpes simplex virus 2 total	18/329	6/133	12/196	Not assessed
	5.5, 3.2–7.7	4.5, 0.3–8.7	6.1, 3.2–9.0	

CI, Confidence interval.

Although representing a limited area, our survey findings allow some pertinent observations about the HIV epidemic in southern Sudan. Given that the prevalence rates among adults aged 15–49 years and pregnant women were close to or above 1%, HIV appeared to be in the process of spreading into the generalized population. However, rates may still be lower than in surrounding countries, which may be related to low rates when the war started. Findings also indicated urban–rural variation and possibly lower rates with increasing distance from the Ugandan border; Yei and Rumbek towns are located on the same main road from Arua, Uganda, into southern Sudan, but Rumbek is approximately 500 km further from the Ugandan border. Our finding that women aged 15–24 years were more likely than men to be HIV infected is similar to other African countries, in which women have earlier ages of sexual debut than men, and are likely to be younger than their partners [4]. Syphilis and herpes simplex virus 2 prevalence rates were relatively low, which contrasted with the substantial proportion of participants reporting genital ulcer symptoms, but may have contributed to limited HIV acquisition and transmission during the years of conflict. Low median age at first sex and low condom use during high-risk sex were the main behaviors of concern.

A major limitation of the surveys was the limited participation. Among individuals present during the time of interview, 74% agreed to have blood drawn for HIV and syphilis testing. Although participation was comparable with other population-based surveys [5], selection bias could not be ruled out.

The reduced mobility and accessibility of populations in prolonged conflict settings has been considered a protective factor against the spread of HIV [6], whereas increased mobility is considered a risk factor for the spread of HIV during peacetime [7,8]. Given the relatively low HIV prevalence rates found in our surveys, southern Sudan, after decades of conflict that may now be turning into peace, has an important opportunity to prevent a potential rapid increase as the country opens up to reconstruction and development [9]. The main priorities should include targeted HIV prevention programmes and behavioral and sentinel surveillance systems in the towns close to the borders and along the major roads into southern Sudan.

[a]*Centers for Disease Control and Prevention, Atlanta, GA, USA;* [b]*Centers for Disease Control and Prevention, Entebbe, Uganda;* [c]*Centers for Disease Control and Prevention, Nairobi, Kenya;* [d]*American Refugee Committee, Kampala, Uganda;* [e]*International Rescue Committee, Nairobi, Kenya; and* [f]*AIDS Information Centre, Kampala, Uganda.*

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Lipodystrophy is an independent predictor of hypertriglyceridemia during pregnancy in HIV-infected women

Marco Floridia[a], Giovanni Guaraldi[b], Enrica Tamburrini[c], Cecilia Tibaldi[d], Anna Bucceri[e], Gianfranco Anzidei[f], Alessandra Meloni[g], Antonella Vimercati[h], Cosimo Polizzi[a], Ernesto Renato Dalle Nogare[i], Salvatore Alberico[j] and Marina Ravizza[k]

Lipid values were measured during pregnancy in HIV-infected, treatment-experienced women. A previous history of lipodystrophy was associated with significantly higher triglyceride values at all pregnancy trimesters. In multivariate analyses lipodystrophy independently increased the risk of hypertriglyceridemia by threefold at the first trimester, and by eightfold at the second and third trimesters. Protease inhibitor treatment was also independently associated with hypertriglyceridemia.

Lipodystrophic changes are commonly observed in individuals with HIV [1]. Universal clinical definitions are lacking, and most of the currently accepted grading systems for severity rely on a visual approach to body changes [1,2]. Antiretroviral treatment is considered to play an important role, and HIV protease inhibitors (PI) and the reverse transcriptase inhibitor stavudine were more consistently associated with body changes in different

studies [3–5]. Women with HIV may develop a variety of morphological changes, with an heterogeneous array of manifestations [6]. Although pregnancy is associated with important changes in lipid profile, even in women with HIV [7], no information is available on serum lipid changes during pregnancy in women with a previous history of lipodystrophy. We investigated this issue using data from the National Program on Surveillance on Antiretroviral Treatment in Pregnancy, an ongoing national surveillance study established in Italy in 2001 [8].

We considered for analysis only treatment-experienced women with information available on the presence or absence of a previous history of lipodystrophy. As a result of the body changes observed in pregnancy, the presence of lipodystrophy was referred to in the past as a history of lipodystrophy before pregnancy (at any time). Lipodystrophy was defined as the presence of moderate or severe thinning of the arms or legs (peripheral wasting), an increase in abdomen or breasts, or loss in buttocks (central adiposity), or both (combined form). Grading for the severity of lipodystrophy was as follows: mild (noticeable only if specifically looked for, no change in clothing fit), moderate (easily noted by patient or physician, clothing has become loose), severe (obvious to the casual observer, has required a change in clothing size). For the purpose of this analysis, a previous history of lipodystrophy was defined by moderate or severe disease only; women with mild disease were not included.

Fasting lipid values were collected at routine clinical visits during pregnancy at the participating centres. Differences in quantitative measures were analysed by the *t*-test or the Mann–Whitney U test, as appropriate, and categorical variables were compared using the Pearson χ^2 test.

Antiretroviral treatment at each trimester was categorized according to PI and the use or non-use of stavudine at the time of blood sampling. Hyperlipidemia was defined by triglyceride values higher than 200 mg/dl or cholesterol values higher than 240 mg/dl, according to the National Cholesterol Education Program Adult Treatment Panel III thresholds [9]. These thresholds were also used as outcome measures in univariate and multivariate logistic regression analyses. Univariate analyses considered the following independent variables collected at study entry: previous history of lipodystrophy, age (< 32 versus ≥ 32 years) and body mass index (BMI), plus the following variables registered at the time of blood sampling: use or non-use of PI, use or non-use of stavudine, CD4 cell count (< 250 versus $\geq 250/mm^3$) and HIV-1-RNA values (< 1000 versus ≥ 1000 copies/ml).

Multivariate analysis considered only the covariates that showed a significance level below 0.15 in univariate analysis. All the analyses were performed using the SPSS software, version 11.0.1, 2001 (SPSS Inc., Chicago, Illinois, USA).

As of 31 August 2005, information on a previous history of lipodystrophy was available for 261 pregnant women. Overall, 14% (37/261) of them had a history of lipodystrophy at any time before pregnancy. Information on the type of lipodystrophy was available for 31 (84%). Sixteen (52%) had peripheral wasting, 14 (45%) a combined form, and only one woman (3%) had a pure central adiposity form. Women with and without a previous history of lipodystrophy were similar in terms of CD4 cell count, HIV-RNA, BMI, route of transmission, nationality, Centers for Disease Control and Prevention clinical status, treatment status at conception, and indication for antiretroviral treatment in pregnancy. Compared with women without a history of lipodystrophy, women with lipodystrophy were on average 2.5 years older (35.3 versus 32.8 years, $P < 0.001$) and had a significantly longer history of antiretroviral treatment (340.3 versus 218.3 weeks; $P < 0.001$).

A total of 149 women (57%) had at least one serum triglyceride or cholesterol measurement available during pregnancy. At all trimesters, women with a previous history of lipodystrophy had significantly higher triglyceride values compared with women without such a history (mean differences 55.4, 102.2 and 72.3 mg/dl; Table 1).

The effect of lipodystrophy on total cholesterol was not significant, although lipodystrophic women showed slightly higher values at all trimesters (Table 1). No differences in values of HDL or LDL-cholesterol at each trimester were observed between the two groups (data not shown).

Univariate logistic regression analyses showed that treatment with PI at the moment of blood sampling and a previous history of lipodystrophy were associated at all trimesters with hypertriglyceridemia (all *P* values between < 0.001 and 0.13). Other factors related to hypertriglyceridemia at single trimesters (*P* values between 0.015 and 0.095) were age and HIV-RNA (at second trimester) and BMI and CD4 cell count (at third trimester). In multivariate logistic regression analyses based on the above covariates, after adjusting for the use of PI and other potential co-factors, lipodystrophy remained an independent predictor at all trimesters (with borderline significance during the first), with a threefold increase in the risk of hypertriglyceridemia at the first trimester [adjusted odds ratio (AOR) 3.58; 95% confidence interval (CI) 0.95–13.5], and an eightfold increase at both the second (AOR 8.34; 95% CI 2.6–26.7) and third (AOR 8.89; 95% CI 2.27–29.0) trimesters. Independently, PI use was also a strong predictive factor (Table 1).

No consistent association was found between hypercholesterolemia and a history of lipodystrophy, and no differences in triglyceride values were found at any

Table 1. Triglyceride and cholesterol levels during pregnancy according to lipodystrophy status and adjusted odds ratios for hypertriglyceridemia in multivariate analyses at each pregnancy trimester.

Trimester	*N*	Variable	Presence of lipodystrophy		Difference (mg/dl) 95% CI for the difference	*P* value
			No	Yes		
I	144	Triglycerides (mg/dl)	113.4	168.8	55.4 (7.0–103.8)	0.026
II	140	Triglycerides (mg/dl)	176.8	279.0	102.2 (49.0–155.4)	0.001
III	128	Triglycerides (mg/dl)	266.3	338.7	72.3 (20.2–124.5)	0.007
I	149	Total cholesterol (mg/dl)	176.9	189.9	13.0 (−3.6–29.7)	0.124
II	141	Total cholesterol (mg/dl)	205.2	226.2	21.0 (−3.2–45.2)	0.088
III	122	Total cholesterol (mg/dl)	237.8	248.7	10.9 (−19.8–41.6)	0.484

Trimester	Variable	AOR for triglyceride levels > 200 mg/dl	AOR, 95% CI	*P* value
I	History of lipodystrophy	3.58	0.95–13.5	0.06
	Use of protease inhibitors	9.09	2.2–36.6	0.002
II	Age > 32 years	1.93	0.76–4.90	0.165
	Use of protease inhibitors	4.15	1.74–9.87	0.001
	History of lipodystrophy	8.34	2.6–26.7	< 0.001
	HIV-RNA < 1000 copies/ml	3.36	1.25–9.03	0.016
III	CD4 cell count < 250 cells/μl	0.7	0.22–2.24	0.54
	History of lipodystrophy	8.89	2.27–29.0	< 0.001
	Use of protease inhibitors	2.68	1.04–6.89	0.04
	Body mass index	1.03	0.85–1.24	0.774

AOR, Adjusted odds ratio; CI, confidence interval. Lipid values are reported as means in mg/dl. Comparisons between lipid values according to lipodystrophy status were made using the *t*-test for independent samples.

trimester between women with the atrophic and combined forms of the syndrome (data not shown).

Our results indicate that the causes of hypertriglyceridemia during pregnancy are multifactorial. We also confirm the dyslipidemic role of PI in pregnancy, and show for the first time that moderate to severe lipodystrophy before pregnancy is associated with significantly higher triglyceride levels during all trimesters of pregnancy. The less consistent association with low RNA values (possibly reflecting higher levels of adherence) should be interpreted cautiously. More data are also needed in order to apply the general conclusions of this study to women with pure central adiposity.

Pregnancy, with its complex hormonal changes, may facilitate the expression of metabolic changes in susceptible women. Our data point to the need to explore this issue further and identify potential mechanisms and genetic or biochemical markers, which could be helpful in identifying women at risk and preventing clinical and metabolic abnormalities.

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[a]Department of Drug Research and Evaluation, Istituto Superiore di Sanità (ISS), Rome; [b]Department of Medical Specialities, Infectious Diseases Clinic, University of Modena and Reggio Emilia, Modena; [c]Department of Infectious Diseases, Catholic University, Rome; [d]University of Turin, Dept. Obstet. Gynecol., and A.O. OIRM S. Anna, Turin; [e]II Dept. Obstet. Gynecol., Clinica L. Mangiagalli, University of Milan; [f]INMI L. Spallanzani, Rome; [g]University of Cagliari, Dept. Obstet. Gynecol. S. Giovanni di Dio Hospital, Cagliari; [h]University of Bari, Dept. Obstet. Gynecol., and Policlinic Hospital, Bari; [i]Infectious Diseases Unit, Hospital Casa del Sole, Palermo; [j]Dept. Obstet. Gynecol., Policlinic Hospital, Trieste; and [k]University of Milan, Dept. Obstet. Gynecol., and S. Paolo Hospital, Milan.

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Appendix

The Italian Group on Surveillance on Antiretroviral Treatment in Pregnancy

Project coordinators: M. Floridia, M. Ravizza, E. Tamburini.

Participants: M. Ravizza, E. Tamburini, P. Ortolani, F. Mori, C. Monticelli, E.R. dalle Nogare, G. Sterrantino, M. Meli, M. Mazzetti, B. Borchi, F. Vichi, E. Pinter, E. Anzalone, L. Roberti, L. Pietrogiacomi, A. Carocci, E. Grilli, A. Maccabruni, A. Moretti, G. Natalini, G. Guaraldi, C. Vanzini, F. Sabbatini, G. Nardini, A. Zoncada, A. Degli Antoni, A. Molinari, P. Rogasi, M.P. Crisalli, E. Chiesa, G. Placido, M. Dalessandro, A. Vivarelli, P. Castelli, F. Savalli, V. Portelli, S. Alberico, M. Bernardon, A. Meloni, A. Citernesi, M.F. Ravagni Probizer, A. Vimercati, B. Guerra, M. Sansone, C. Tibaldi, A. Calcagno, S. Marini, G. Masuelli, L. Di Lenardo, E. Ferrazzi, V. Conserva, T. Brambilla, E. Rubino, A. Bucceri, R. Matrone, G. Scaravelli, G. Anzidei, R. Cavallini, C. Fundarò, O. Genovese, C. Cafforio, C. Pinnetti, G. Liuzzi, V. Tozzi, P. Massetti, M. Anceschi, A.M. Casadei, F. Montella, A.F. Cavaliere, M. Finelli, C. Riva, M. Cellini, V. Venturi, M. Lanari, S. Garetto, G. Castelli Gattinara, L. Mangiarotti, M. Ierardi, S. Foina, B. Salerio, S. Dalzero, A. Mattei, C. Polizzi, E. Germinario, M.F. Pirillo, R. Amici, C.M. Galluzzo, M. Floridia.

Advisory Board: A. Cerioli, M. De Martino, P. Mastroiacovo, M. Moroni, F. Parazzini, G. Pardi, E. Tamburini, S. Vella.

SIGO-HIV Group National Coordinators: E. Ferrazzi, P. Martinelli.