

## **SUPPLEMENTARY DATA**

### **Clinical tests**

Semen was collected as described previously (Gianella et al., 2012). From blood, CD4+ T-lymphocyte subsets were measured by flow-cytometry (LabCorp) and HIV RNA was quantified (Amplicor HIV Monitor Test, Roche Molecular Systems Inc.). The studies were conducted with appropriate written subject consent and were approved by the Human Research Protections Program at the University of California San Diego, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, and the University of Southern California.

### **Multiplex-bead-array assay for cytokines/chemokines quantification**

We measured the concentrations of 31 cytokines/chemokines involved in different immunological functions:

- (i) Mediators of innate immunity, inflammation and chemotaxis (interleukin [IL]-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-17, IL-18, IL-22, IL-33, IL-8 (CXCL8), MIG (CXCL9), IFN-inducible-protein [IP]-10 (CXCL10), I-TAC (CXCL11), TNF- $\alpha$ , lactoferrin, (monocyte-chemotactic-protein [MCP]-1 (CCL2), macrophage-inflammatory-protein [MIP]-1 $\alpha$  (CCL3), MIP-1 $\beta$  (CCL4), regulated-on-activation-normally-T-cell-expressed-and-secreted [RANTES, CCL5], eotaxin (CCL11), MIP-3 $\alpha$  (CCL20), GRO- $\alpha$  (CXCL1)).
- (ii) Mediator of hematopoiesis (macrophage-colony-stimulating-factor [M-CSF] and granulocyte-macrophage-colony-stimulating factor [GM-CSF]).
- (iii) Anti-inflammatory cytokine (IL-10, IL-13, transforming-growth-factor [TGF]- $\beta$ ).

(iv) Mediators of lymphocytes activation, proliferation, and differentiation (IL-2, IL-7, IL-12, IL-15, IL-16, CCL3, CCL4, CCL5, CCL20, IFN- $\gamma$ ).

In consideration of their pleotropic effects, CCL3, CCL4, CCL5, CCL20 were included in two different functional groups, both as mediators of chemotaxis and as mediators of activation, proliferation, and differentiation of lymphocytes. All standards, capture and detection antibodies were purchased from R&D (Minneapolis, MN) with the exception of IL-10 (BioLegend, San Diego, CA), IL-17A and IL-22 (Peprotech, Rocky Hill, NJ), IL-18 (eBioscience, San Diego, CA), IL-33 and M-CSF (R&D Systems, Minneapolis, MN), and lactoferrin (Abcam, Cambridge, UK).

Values that were below the lower limit of detection (LLD) were reported as the midpoint between zero and the LLD (LLDs in pg/mL): IL-1 $\alpha$  (1.00), IL-1 $\beta$  (1.00), IL-2 (2.60), IL-6 (2.60), IL-7 (2.60), IL-10 (16.40), IL-12 (9.20), IL-13 (41.00), IL-15 (9.80), IL-16 (3.00), IL-17A (4.00), IL-18 (17.4), IL-22 (4.00), IL-33 (5.20), CXCL1 (5.20), CXCL8 (1.00), CXCL9 (5.20), CXCL10 (5.20), CXCL11 (5.00), CCL2 (2.60), CCL3 (2.00), CCL4 (2.60), CCL5 (2.60), CCL11 (5.20), CCL20 (16.40), M-CSF (5.20), GM-CSF (6.60), IFN- $\gamma$  (6.40), TNF- $\alpha$  (1.00), TGF- $\beta$  (9.80), lactoferrin (131.00).

#### **REFERENCES:**

Gianella S, Strain MC, Rought SE, et al. Associations between virologic and immunologic dynamics in blood and in the male genital tract. *J Virol* 2012; 86:1307-15.

<b>Class</b>	<b>Definitions for stage of HIV-1 infection</b>
A1.0	If there is a first positive RNA <sup>†</sup> and negative enzyme immunoassay (EIA) within 7 days of the first positive RNA, and no prior within 7 days of the first positive RNA, and no prior positive/indeterminate western blot (WB), then EDI = first positive RNA date – 11 days. (~Fiebig Stages I-II)
A2.0	If there is an indeterminate WB within 7 days of the first positive RNA, then EDI = first positive RNA date – 20 days. (~Fiebig Stages III-IV)
A3.0	If the last negative EIA or negative/indeterminate WB occurred ≤ 30 days before the first positive WB (with associated positive RNA), then EDI = midpoint of the positive WB date and the negative EIA or negative/indeterminate WB date (earlier of two) – 19 days. (~Fiebig Stage IV)
A3.1	If the first positive WB p31/32 band is absent, then EDI = first positive WB date – 89 days. (~Fiebig Stage V)
E1.0A	If there is a detuned EIA (dtEIA) consistent with infection of ~3 mo within 30 days of the first positive WB and CD4 count > 200 or CD4% > 14 within 30 days of the first positive WB, then EDI = first dtEIA date – 70 days. (Fiebig VI)
E1.0B	If there is a dtEIA consistent with infection of ~3-6 mo within 30 days of the first positive WB and CD4 count > 200 or CD4% > 14), then EDI = first dtEIA date – 133 days. (Fiebig VI)
E1.0C	If there is a dtEIA consistent with infection of ~6-12 mo within 30 days of the first positive WB and CD4 count > 200 or CD4% > 14, then EDI = first dtEIA date – 170 days. (Fiebig VI)
E2.0	If there is a first positive WB and a negative EIA within 365 days participant enrollment (Day 0), then EDI = midpoint between the last negative EIA and Day 0. (Fiebig VI)

**Supplemental Table 1:** Brief Summary of the Algorithm for Computing the Estimated Date of HIV-1 Infection.

Each rule applied sequentially until EDI criteria satisfied.

† Positive RNA was defined as a NAT/viral load exceeding the detectable level for a given assay.

	HIV- (controls) (N=40)	Early HIV+ART- (N=42)	Late HIV+ART- (N=74)	Late HIV+ART+ (N=96)
<b>Mediators of Innate Immunity and Inflammation</b>				
IL-1 $\alpha$	0.50 (0.50-0.56)	0.50 (0.50-1.13)	0.50 (0.50-0.50)	0.50 (0.50-0.50)
IL-1 $\beta$	0.60 (0.50-1.94)	1.14 (0.60-2.05)	0.88 (0.50-2.10)	0.50 (0.50-0.80)
IL-6	1.30 (1.30-7.41)	1.30 (1.30-1.30)	1.30 (1.30-1.30)	1.30 (1.30-1.30)
IL-17	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)
IL-18	9.80 (9.80-9.80)	47.9 (15.7-81.2)	45.1 (15.7-82.7)	22.0 (9.8-65.6)
IL-22	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)
IL-33	2.60 (2.60-2.60)	2.60 (2.60-2.60)	2.60 (2.60-2.60)	2.60 (2.60-2.60)
IL-8	0.66 (0.50-1.28)	0.73 (0.50-1.20)	0.69 (0.50-0.1.24)	0.50 (0.50-0.50)
MIG	47.7 (33.2-66.0)	429.4 (150.2-606.2)	180.5 (113.7-328.8)	93.6 (61.6-191.2)
IP-10	6.42 (2.60-24.83)	150.6(75.2-229.1)	108.2 (69.0-204.6)	103.7 (66.4-184.0)
I-TAC	2.50 (2.50-2.50)	23.2 (12.1-51.6)	21.2 (8.1-45.0)	18.9 (9.3-41.0)
TNF- $\alpha$	0.50 (0.50-0.50)	2.02 (0.67-3.91)	1.03 (0.50-3.54)	1.40 (0.80-2.45)
Lactoferrin	220.1 (220.1-727.8)	535.5 (65.5-2050)	297.2 (65.5-1477)	65.5 (65.5-65.5)
<b>Mediator of Haematopoiesis</b>				
M-CSF	2.60 (2.60-2.60)	2.60 (2.60-2.60)	2.60 (2.60-2.60)	2.60 (2.60-2.60)
GM-CSF	3.30 (3.30-20.25)	3.30 (3.30-16.2)	3.30 (3.30-18.6)	3.30 (3.30-3.30)
<b>Mediators of Chemotaxis</b>				
MCP-1	31.6 (22.0-55.3)	76.1 (53.9-101.9)	73.4 (50.9-98.5)	129.4 (64.2-199.1)
MIP-1 $\alpha$	1.00 (1.00-1.00)	22.3 (4.64-34.1)	4.64 (1.00-22.32)	1.00 (1.00-6.85)
MIP-1 $\beta$	16.0 (12.3-25.3)	17.5 (12.9-24.3)	21.2 (16.0-27.8)	17.7 (13.1-26.7)
RANTES	829.2 (437.2-1546)	1544 (1091-1950)	1351 (931.0-1906)	1138 (558-1540)
Eotaxin	231.6 (163.2-390.7)	364.2 (269.8-470.3)	343.0 (255.1-501.0)	582.8 (369.9-751.2)
MIP-3 $\alpha$	8.20 (8.20-8.20)	8.20 (8.20-8.20)	8.20 (8.20-8.20)	17.7 (13.1-26.7)
<b>Anti-inflammatory Cytokine</b>				
IL-10	52.3 (14.1-110.6)	67.6 (48.2-115.9)	48.2 (32.4-134.6)	8.20 (8.20-189.2)
IL-13	20.5 (20.5-20.5)	20.5 (20.5-20.5)	20.5 (20.5-20.5)	20.5 (20.5-20.5)
TGF- $\beta$	4.90 (4.90-28.75)	4.90 (4.90-4.90)	4.90 (4.90-4.90)	4.90 (4.90-4.90)
<b>Mediators of Activation, Proliferation, and Differentiation of Lymphocytes</b>				
IL-2	7.18 (1.30-27.31)	19.7 (2.35-42.1)	3.03 (1.30-38.6)	1.30 (1.30-6.10)
IL-7	1.30 (1.30-1.30)	1.30 (1.30-1.30)	1.30 (1.30-1.30)	1.30 (1.30-1.30)
IL-12	4.60 (4.60-382.9)	17.8 (4.60-487.2)	4.60 (4.60-112.0)	4.60 (4.60-81.1)
IL-15	72.3 (50.1-126.3)	64.5 (21-119)	71.5 (19.2-122.4)	11.4 (4.90-36.75)
IL-16	372.1 (267.9-566.4)	584.0 (455.0-957.5)	486.4 (342.2-929.8)	157.1 (97.2-217.2)
IFN- $\gamma$	3.20 (3.20-35.36)	3.20 (3.20-5.19)	3.20 (3.20-3.20)	3.20 (3.20-3.20)
GRO- $\alpha$	10.71 (2.60-39.85)	15.6 (8.41-34.5)	11.9 (7.64-34.1)	2.60 (2.60-6.40)

**Supplemental Table 2:** Comparison of cytokine/chemokine levels (pg/mL) in blood plasma between four groups, namely HIV-uninfected individuals, ART-naïve early HIV-infected individuals, ART-naïve individuals in a later stage of HIV infection and HIV-infected individuals on long-term ART and with undetectable HIV RNA levels in blood. Median and interquartile range (IQR) is shown for each cytokine/chemokine.

	HIV- (N=40)	Early HIV+ART- (N=42)	Late HIV+ART- (N=74)	late HIV+ART+ (N=96)
<b>Mediators of Innate Immunity and Inflammation</b>				
IL-1 $\alpha$	69.7 (37.5-187.8)	24.3 (9.50-55.2)	31.9 (16.8-94.7)	35.2 (18.74-93.17)
IL-1 $\beta$	14.2 (5.85-40.6)	4.65 (1.70-7.90)	6.40 (3.70-19.3)	6.32 (2.88-23.2)
IL-6	44.0 (9.11-130.6)	17.6 (1.30-71.9)	18.7 (6.90-74.7)	100.9 (36.4-213.9)
IL-17	2.00 (2.00-2.00)	2.00 (2.00-4.00)	2.00 (2.00-7.20)	2.00 (2.00-2.00)
IL-18	9.80 (8.72-9.80)	9.80 (9.80-9.80)	9.80 (9.80-16.3)	9.80 (9.80-9.80)
IL-22	2.00 (2.00-8.84)	10.4 (2.00-62.3)	8.80 (2.70-38.0)	85.5 (47.6-123.8)
IL-33	8.43 (2.60-31.9)	2.60 (2.60-2.60)	2.60 (2.60-2.60)	2.60 (2.60-14.6)
IL-8	1246 (604.2-2699)	1013 (402.8-1656)	1042 (625.1-2065)	2489 (1457-3810)
MIG	83'842 (43213-227489)	282'681 (55519-588993)	174'658 (84368-380731)	27'735 (14908-77210)
IP-10	59'410 (45343-87928)	70'231 (27106-147542)	55'877 (34635-109084)	25'896 (18376-52453)
I-TAC	5659 (4093-9083)	12936 (4396-36536)	12231 (6642-25651)	7607 (3459-17852)
TNF- $\alpha$	1.58 (0.50-4.30)	2.35 (0.50-5.40)	4.25 (0.50-8.10)	0.50 (0.50-2.88)
Lactoferrin	2037 (65.5-5617)	2036 (93.6-3032)	1448 (547.2-2481)	3731 (2361-7354)
<b>Mediator of Haematopoiesis</b>				
M-CSF	552.1 (350.0-1061)	767.4 (366.8-1676)	552.9 (265.4-987.0)	746.8 (509.3-987.1)
GM-CSF	3.30 (3.30-13.26)	3.30 (3.30-3.30)	3.30 (3.30-3.30)	27.1 (8.1-51.0)
<b>Mediators of Chemotaxis</b>				
MCP-1	1779 (860.2-3301)	1529 (568.5-2465)	1223 (611.6-2225)	2243 (1089-4597)
MIP-1 $\alpha$	1.00 (1.00-1.00)	22.3 (140.2-357.8)	4.64 (1.00-22.32)	1.00 (1.00-6.85)
MIP-1 $\beta$	96.8 (45.5-241.1)	59.0 (30.2-90.6)	79.3 (34.0-163.7)	363.0 (201.0-643.8)
RANTES	220.1 (147.2-474.2)	321.8 (122.4-528.1)	254.6 (159.4-516.5)	473.3 (318.6-801.4)
Eotaxin	170.2 (82.8-272.1)	362.9 (205.9-512.2)	190.5 (116.1-367.7)	431.6 (276.5-614.1)
MIP-3 $\alpha$	2785 (2174-5820)	3447 (2474-5904)	3501 (2565-6193)	5176 (3482-8014)
<b>Anti-inflammatory Cytokine</b>				
IL-10	8.20 (8.20-150.0)	8.20 (8.20-8.20)	8.20 (8.20-219.0)	8.20 (8.20-189.2)
IL-13	20.5 (20.5-20.5)	20.5 (20.5-182.9)	20.5(20.5-20.5)	20.5 (20.5-104.0)
TGF- $\beta$	2192 (1579-4077)	1000 (432.3-1910)	1759 (794.1-3014)	487.8 (209.5-1279)
<b>Mediators of Activation, Proliferation, and Differentiation of Lymphocytes</b>				
IL-2	38.9 (26.7-59.2)	99.9 (41.6-176.2)	74.0 (33.3-117.0)	114.4 (85.5-159.9)
IL-7	1451 (1092-1866)	2796 (1463-4294)	2039 (1583-3387)	1932 (1382-2768)
IL-12	4.60 (4.60-4.60)	4.60 (4.60-95.8)	41.5 (4.60-112.0)	4.60 (4.60-4.60)
IL-15	49.1 (50.1-126.3)	54.6 (4.90-171.4)	88.0 (4.90-165.6)	239.7 (167.8-329.4)
IL-16	372.1 (20.8-222.8)	22.9 (1.50-81.2)	41.0 (10.3-231.2)	328.3 (210.3-809.4)
IFN- $\gamma$	3.20 (3.20-16.8)	149.1 (3.20-287.4)	106.6 (3.20-214.2)	93.0 (36.6-159.6)
GRO- $\alpha$	5035 (3216-11485)	3184 (1310-10049)	4518 (2153-8516)	7620 (4677-13738)

**Supplemental Table 3:** Comparison of cytokine/chemokine levels (pg/mL) in seminal plasma between four groups, namely HIV-uninfected individuals, ART-naïve early HIV-infected individuals, ART-naïve individuals in a later stage of HIV infection and HIV-infected individuals on long-term ART and with undetectable HIV RNA levels in blood. Median and interquartile range (IQR) is shown for each cytokine/chemokine.