Interleukin-7-Dependent Production of RANTES That Correlates with Human Immunodeficiency Virus Disease Progression

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There is a relationship between CD4-T-cell number and circulating interleukin 7 (IL-7) levels in human immunodeficiency virus (HIV)-positive individuals. Here, we show that IL-7 induced a dose-dependent production of CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES) in peripheral blood mononuclear cells (PBMC), ex vivo tonsil lymphoid tissue of HIV⁻ individuals, and PBMC from HIV⁺ individuals, suggesting that IL-7 may regulate β -chemokine production in vivo. In a cross-sectional study of HIV⁺ individuals ($n = 130$), a weak but significant correlation between IL-7 and RANTES was noted ($r = 0.379$; $P < 0.001$). Remarkably, the correlation between IL-7 and RANTES increased to an r value of 0.798 ($P < 0.001$) if individuals with low CD4 cell counts (<200 cells/ μ l) were excluded from the analysis. Our results suggest that there is a relationship between IL-7 and the production of RANTES both in vitro and in vivo that is lost in immune-compromised patients (CD4 count of <200 cells/ μ l) but that could be restored by antiretroviral therapy. Unlike the case for IL-7, high levels of RANTES suggest an intermediate stage of HIV disease progression.

In human immunodeficiency virus type 1 (HIV-1) infection, host factors play a central role in the evolution of the disease (2, 9). Genetic factors may protect or reduce the rate of disease progression (20), and a number of soluble factors have been linked to immune-controlled and noncytolytic antiviral response of CD8⁺ T cells (42) that may correlate with disease progression (3).

β -chemokines released by activated CD8 T cells have been shown to be responsible for soluble suppressor activity against HIV-1 strains. CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES) are potent in vitro inhibitors of HIV replication at an early step of the virus life cycle (6). RANTES, MIP-1 α , and MIP-1 β bind to chemokine receptor CCR5, which is required for entry by macrophage-tropic strains (R5 strains) (E. A. Berger, R. W. Doms, E. M. Fenyo, B. T. Korber, D. R. Littman, J. P. Moore, Q. J. Sattentau, H. Schuitemaker, J. Sodroski, and R. A. Weiss, *Nature* **391**:240, 1998). T-cell-tropic HIV-1 strains use another chemokine receptor, CXCR4. The chemokine CXCL12 (SDF-1) (4) blocks the replication of virus isolates that use this receptor (X4 strains) (2, 10) and may prevent the emergence of X4 strains in vivo (22). Overproduction of β -chemokines has been associated with resistance to infection with R5 HIV-1 in vitro (28), protection of HIV-exposed uninfected individuals (14, 45), slow disease progression (32, 44), and asymptomatic status of HIV⁺ individuals (7, 15, 29). Nevertheless, clarification of the exact role of β -chemokines in the course of HIV infection and disease progression has remained elusive. The search for a correlation between chemokine levels in plasma or serum and the protection from HIV infection or progression to AIDS has been at-

tempted by a number of groups, but most of them have failed (5, 24, 26, 40, 43, 46). HIV-1 infection or accessory HIV gene products may induce the production of RANTES, MIP-1 α , and MIP-1 β by HIV-1-infected macrophages, triggering both chemotaxis and activation of resting T lymphocytes and permitting productive HIV-1 infection (37). Furthermore, RANTES has been reported to enhance HIV replication (16, 39), and there is data suggesting that an elevation of RANTES in serum predicts a rapid progression of the disease (31), supporting the idea that β -chemokine production could, in fact, promote HIV-1 infection and propagation. Thus, conclusive evidence of the relevance of β -chemokine-mediated anti-HIV activity in vivo is still unclear.

Interleukin 7 (IL-7) is a critical homeostatic cytokine for T-cell development (11, 30, 35, 38). HIV-1-seropositive individuals show elevated levels of IL-7 in plasma that are inversely correlated with CD4⁺-T-cell number and positively correlated with increased HIV-1 viral load (VL) (27). We have recently demonstrated a possible association between IL-7 levels in plasma and the evolution of X4 HIV-1 in vivo (23). Besides increasing the number of target cells for HIV, IL-7 influence in the disease progression may be due to its capacity to upregulate CXCR4 expression on mature CD4⁺ T cells (21, 23, 36), suggesting that IL-7 also potentially modulates mature T-cell function. In fact, IL-7 may costimulate T-cell activation, induce a weak type 1 T-cell differentiation, block T-cell apoptosis, and increase the activity of CD8⁺ cytotoxic T-lymphocytes (CTL) (reviewed in reference 12).

In this work, we have studied the effect of IL-7 on the in vitro production of β -chemokines and its correlation with RANTES in plasma of HIV⁺ individuals. We demonstrate for the first time a tight correlation between IL-7 and RANTES that is lost in immune-compromised patients.

IL-7 stimulates the production RANTES, MIP-1 α , and

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MIP- β . We first evaluated the production of β -chemokines by peripheral blood mononuclear cells (PBMC) obtained from HIV⁻ or HIV⁺ donors after 6 days of incubation with IL-7 and IL-2. PBMC and plasma samples were obtained as described previously (23) and evaluated for IL-7 receptor expression by flow cytometry. A mean of $84\% \pm 6\%$ of CD4⁺ and $61\% \pm 19\%$ of CD8⁺ cells from HIV⁻ donors expressed the IL-7 receptor ($n = 5$). The PBMC were cultured in RPMI medium without stimulation (those of HIV⁺ individuals) or supplemented with phytohemagglutinin (PHA) (4 μ g/ml) and IL-2 (6 U/ml) (those of HIV⁻ individuals) at a concentration of 10^6 cells per ml. After 2 days, cells were washed and cultured in RPMI medium with or without IL-2 (10 U/ml) and with various IL-7 concentrations. After 6 days of stimulation, 50 μ l of the culture was collected to analyze expression of various receptors, and supernatants were collected and stored frozen at -80°C until use.

Cells from HIV-negative donors, without previous stimulation, did not respond to IL-7 stimuli (data not shown). As shown in Fig. 1A, increasing concentrations of IL-7 induced a dose-dependent increase in the concentrations of RANTES, MIP-1 α , and MIP-1 β in the supernatant of PHA-activated cells that reached statistical significance ($P < 0.05$) at 500 ng of IL-7/ml. CD4 and CD8 T cells were separated by negative selection (following the manufacturer's guidelines of StemSep Columns for Gravity Feed; [diameter, 0.3 inches]; StemCell Technologies, Barcelona, Spain). Similar to PBMC, purified CD4⁺ or CD8⁺ cells responded to IL-7 by producing RANTES (data not shown), confirming that both T-cell subsets are a source of β -chemokines. (6, 7, 28, 34). Conversely, and unlike ADP, a known agonist of platelet aggregation and RANTES release, IL-7 did not induce the production of RANTES by platelets isolated by centrifugation of platelet-rich plasma following a protocol described previously (19).

IL-7 did not induce a significant increase in T-cell number at the concentrations tested compared to results for cells that were not stimulated with IL-7, suggesting an effect of cell activation without T-cell proliferation. In fact, IL-7 induced a dose-dependent increase in CXCR4 expression and a modest but clear down regulation of CCR5 (up to 33% decrease by 500 ng of IL-7/ml) in PBMC from healthy donors. Thus, costimulation of PBMC with IL-7 of activated cells induced β -chemokine production.

In order to clarify the effect of IL-7, we used a well-defined model system (17, 18), based on human tonsil tissue blocks cultured ex vivo, that does not require exogenous stimulation or activation, avoiding the use of PHA or IL-2. As observed with PBMC, IL-7 induced an upregulation (up to threefold) of RANTES, MIP-1 α (up to sixfold), and MIP-1 β (up to 7-fold) production in tonsil lymphoid tissue (Fig. 1B). Similarly, in PBMC from HIV-infected individuals that do not require costimulation with IL-2 or PHA stimulation to produce RANTES (1), IL-7 induced the production of RANTES >20-fold (at 100 ng of IL-7/ml), MIP-1 α (>20-fold), or MIP-1 β (>5-fold) compared to the untreated (control) cells (Fig. 1C). However, the effect of IL-7 in β -chemokine production was not reflected in the level of CCR5 expression (data not shown). Thus, we demonstrate that IL-7 may costimulate with IL-2 (in HIV⁻ PBMC) or directly stimulate (in lymphoid tissue) T cells that respond with the production of β -chemokines. Our obser-

FIG. 1. IL-7-stimulated β -chemokine production. PHA- and IL-2-stimulated PBMC from HIV⁻ donors ($n = 5$) (A), tonsil lymphoid tissue ex vivo ($n = 2$) (B) without PHA or IL-2, or PBMC from HIV⁺ individuals ($n = 3$) without PHA or IL-2 (C) were stimulated with various concentrations of IL-7. After 6 days in culture, RANTES (white bars), MIP-1 α (grey bars), or MIP-1 β (black bars) was evaluated by ELISA of cell supernatants. Results represent the mean $\pm$ standard deviation.

vation that IL-7 enhanced the production of β -chemokines in PBMC from HIV⁺ individuals without the need of exogenous stimulation suggested that IL-7 may influence chemokine levels in vivo.

IL-7 in plasma correlates with RANTES. A cross-sectional study was done to evaluate the relationship between IL-7 and RANTES in HIV⁺ individuals. Plasma from 130 HIV individuals was isolated after centrifugation of blood samples at 1,400 rpm for 10 min in a Centra-GP8 IEC centrifuge (Huco, Barcelona, Spain) and was immediately cryopreserved and stored at -80°C until use. Plasma IL-7 levels were determined by an ultrasensitive commercial enzyme-linked immunosorbent as-

TABLE 1. Immunological, virological, and epidemiological variables of a cohort of HIV⁺ individuals

Variable	Value for group ^a		
	HIV+ patients	HIV+ patients with CD4 cell count of <200	HIV+ patients with CD4 cell count of >200
<i>n</i> ^b	130	64	66
VL (log ₁₀)	5.1 ± 5.47	5.3 ± 5.59	4.6 ± 4.9
Amt of:			
CD4 (cells/μl)	247 ± 221	71 ± 58	400 ± 192
CD8 (cells/μl)	776 ± 551	433 ± 357	1,011 ± 622
RANTES (ng/ml)	27 ± 20	26 ± 18	26.5 ± 17
IL-7 (pg/ml)	10 ± 7	13 ± 7	6.5 ± 4
Gender (% male/% female)	82/18	88/12	75/25
Time since HIV diagnosis (yr)	4 ± 3	5 ± 3	4 ± 3
% with risk factor ^c			
IVDU	25	27	23
Homosexual	39	40	42
Heterosexual	14	19	21
CBP	14	11	3
IND	8	3	11

^a Some values are shown as mean ± standard deviation.

^b *n*, number of patients.

^c IVDU, intravenous drug user; CBP, contaminated blood product; IND, indeterminate.

say (ELISA) (Quantikine HS Human IL-7 Immunoassay; R&D Systems, Minneapolis, Minn.) following the manufacturer's instructions. RANTES levels were measured by a commercial ELISA (Endogen, Barcelona, Spain). Patient samples used for cross-sectional analysis were those corresponding to the basal sample prior to initiation of an antiretroviral treatment clinical study. The immunological, virological, and epidemiological variables of the complete cohort are shown in Table 1. HIV⁺ individuals could be stratified into groups of low (<200 cells/μl) and high (>200 cells/μl) CD4-T-cell count. There were no significant differences in the ratio of male/female individuals, time since HIV diagnosis, or risk factor between the two groups, suggesting that these variables did not influence the CD4- or CD8-T-cell count, VL, or IL-7 or RANTES level. Conversely, the groups of low and high CD4-T-cell count had significantly ($P < 0.001$) different IL-7 levels in plasma. Similarly, mean CD8-T-cell numbers and mean HIV VL were significantly ($P < 0.001$) different between the two groups.

Despite increased levels of RANTES in plasma from HIV patients (25), members of our group and others have not been able to detect a correlation between β-chemokine levels and CD4-T-cell count (1, 7). Similarly, there were no significant differences between RANTES levels in plasma of low- or high-CD4-T-cell-count groups (Table 1). Conversely, there was a weak but significant correlation ($r = 0.379$; $P < 0.001$) between IL-7 and RANTES levels in plasma of HIV⁺ individuals (Fig. 2A). This correlation increased to an r value of 0.798 and P value of <0.001 when only individuals with a CD4 count of >200 cells/μl ($n = 66$) were included in the analysis, suggesting a tight control between IL-7 and RANTES in those patients that were not immune compromised (Fig. 2B).

HIV⁺ individuals could be stratified in three categories according to IL-7 and RANTES in plasma: IL-7, <7 pg/ml/RANTES, <25 ng/ml (Low/Low); IL-7, >7 pg/ml/RANTES, >25 ng/ml (High/High); and IL-7, >7 pg/ml/RANTES, <25 ng/ml (High/Low) (Fig. 2A and C). Only three individuals could be grouped in the Low/High (IL-7 < 7 pg/ml/RANTES > 25 ng/ml) group. The immunological and virological char-

acteristics of each group are shown in Table 2, and the distribution, range, and median CD4 and CD8 values are shown in Fig. 2C. The mean CD4-T-cell count was significantly ($P < 0.05$) higher in the Low/Low group, followed by the High/High group and High/Low group. The mean CD8-T-cell count was statistically different between the Low/Low and High/Low groups and between the High/High and High/Low groups. Taken together, these results suggest an association between RANTES and IL-7 levels and the CD4 and CD8 cell count in which immune-competent individuals have low IL-7 and low RANTES, immune-deficient patients have high IL-7 and low RANTES, and an intermediate stage of disease is characterized by high IL-7 and high RANTES.

HIV-negative individuals have low or undetectable levels of IL-7 (23), and the level of RANTES is normally low (1, 7). Conversely, HIV⁺ individuals have significantly higher levels of RANTES than uninfected individuals (data not shown) (25). IL-7 has been shown to increase T-cell number, presumably through activation and proliferation of early T-cell subsets. In HIV infection this increased T-cell number is masked by the rapid destruction of T cells and increased HIV replication (8, 13, 27). As more cells become activated because of HIV infection, more RANTES is produced. Thus, the increased RANTES correlates with increased IL-7. As disease progresses, IL-7 inversely correlates with CD4 cell count (23, 27). T cells are an important source of RANTES and other β-chemokines (6, 41, 45); thus, a drop in the level of CD4⁺ and CD8⁺ T cells should be tied to a drop in RANTES levels. However, as shown in Fig. 2, the significant change in CD4 cell count between the Low/Low group and the High/High group is not yet followed by a significant change in CD8 T cells. RANTES levels drop only when a dramatic change in T-cell count (both CD4s and CD8s) occurs; that is, RANTES drops after exhaustion of the immune system when IL-7 production cannot compensate for the depletion of T cells. Therefore, low T-cell count and immunodeficiency are marked by high and low levels of IL-7 and RANTES, respectively.

RANTES measurements are known to be problematic be-

FIG. 2. Correlation between IL-7 and RANTES levels in plasma of HIV⁺ individuals. (A) Linear correlation ($r = 0.374$; $P < 0.001$) between IL-7 and RANTES of 130 HIV⁺ individuals. HIV⁺ individuals may be separated into four separate groups according to low (<7 pg/ml) and high (>7 pg/ml) levels of IL-7 (vertical cutoff line) and low (<25,000 pg/ml) and high (>25,000 pg/ml) levels of RANTES (horizontal cutoff line). Colors depict individuals in the Low/Low (green), Low/High (pink), High/High (blue), and High/Low (red) groups for IL-7 and RANTES levels, respectively. (B) Correlation between IL-7 and RANTES ($r = 0.798$; $P < 0.001$) of HIV⁺ individuals with CD4 cell counts of >200 cells/μl. (C) CD4-T-cell (white bars) and CD8-T-cell (grey bars) values for each of the four groups. Results are depicted in box plot diagrams, where the box represents the 25th and 75th quartile and the line represents the median value. Bars indicate 5th and 95th percentiles, and circles depict atypical values. P values shown represent significant differences (Student's t test) between cell subsets of the corresponding groups. The mean $\pm$ standard deviation values are shown in Table 2.

cause platelets are a rich source of RANTES (19) that could be released upon platelet activation in the glass tube when blood is drawn. However, there is only a weak correlation between RANTES levels and platelet count for individual patients (31), and disease progression is marked by chronic thrombocytopenia. It is unlikely that platelet-derived RANTES was released only in a proportion of individuals with high IL-7, since plasma was systematically collected for all individuals following the same procedure. Nevertheless, we cannot exclude that the correlation between IL-7 and RANTES may also reflect platelet activation or another cell source of RANTES in HIV⁺ individuals.

The correlation between IL-7 and RANTES is maintained in

patients in HAART. A second cohort of HIV⁺ individuals was used to confirm the relationship between IL-7 and RANTES. Plasma samples ($n = 36$) from a subset of HIV⁺ individuals were used to measure IL-7 and RANTES in plasma. These patients were enrolled in our HIV clinic and have been efficiently treated with highly active antiretroviral therapy (HAART) for two or more years that includes at least three reverse transcriptase inhibitors (RTIs) or two RTIs plus a protease inhibitor (PI). The following patients were included in this cohort: 21 males and 9 females, who acquired HIV through sexual intercourse (63%), intravenous-drug use (27%), or an indeterminate route of transmission (10%). After treatment the mean CD4-T-cell count increased from $373 \pm$

TABLE 2. Immunological and virological variables for HIV⁺ individuals stratified according to IL-7 and RANTES levels in plasma

Variable	Value for group ^a			
	Low/Low ^b	High/High ^c	High/Low ^d	Low/High ^e
<i>n</i>	42	49	36	3
VL (log ₁₀)	4.6 ± 5	5.1 ± 5.5	5.5 ± 5.6	4.0 ± 3.9
Amt of:				
CD4 (cells/μl)	338 ± 192	215 ± 201	100 ± 139	423 ± 132
CD8 (cells/μl)	802 ± 553	777 ± 652	332 ± 254	692 ± 260
RANTES (ng/ml)	12.8 ± 6	46 ± 17	15 ± 7	38 ± 9
IL-7 (pg/ml)	4 ± 1	14 ± 6	14 ± 6	5 ± 1

^a Mean ± standard deviation values are shown.

^b <7 pg of IL-7/ml, <25 ng of RANTES/ml.

^c >7 pg of IL-7/ml, >25 ng of RANTES/ml.

^d >7 pg of IL-7/ml, <25 ng of RANTES/ml.

^e <7 pg of IL-7/ml, >25 ng of RANTES/ml.

183 to 515 ± 327 cells/μl, the mean VL decreased 1.1 log₁₀ RNA copies/ml (from 59,542 ± 178,712 to 4,743 ± 12,470 copies/ml), and a positive correlation ($r = 0.73$; $P < 0.001$) between IL-7 and RANTES was found (Fig. 3A). Thus, a strong correlation was found between IL-7 and RANTES in

patients with a CD4 cell count of >200 cells/μl as a consequence of HAART.

Longitudinal changes in IL-7 and RANTES were further studied in a cohort of HIV⁺ individuals enrolled in a structured treatment interruption (33) study consisting of optimized

FIG. 3. IL-7 and RANTES in patients under HAART. (A) Linear correlation ($r = 0.74$; $P < 0.001$) between IL-7 and RANTES in plasma of HIV⁺ individuals after 2 years of HAART. The vertical and horizontal lines show the IL-7 and RANTES cutoff values, respectively. (B) Plasma samples from patients in a treatment interruption study were collected before and after interruption of treatment, and RANTES and IL-7 were measured by ELISA. The figure represents the correlation ($r = 0.77$; $P < 0.01$) between the inpatient change in RANTES and IL-7 in 19 of 24 patients evaluated. (C, D, and E) Plasma samples from three representative patients were collected before and after interruption of treatment and after reinitiation of HAART. IL-7 (filled line) and RANTES (dotted line) were measured by ELISA. HIV VL in RNA copies/milliliter is shown for each time period.

antiretroviral therapy (at least two RTIs with or without a PI) that led to a VL of <400 copies/ml and CD4 cell counts of >500 cells/ μ l. Patients discontinued treatment until the VL increased up to 100,000 copies/ml or the CD4 cell count decreased to <350 cells/ μ l. Plasma samples from each patient were collected before and after interruption of treatment and evaluated for IL-7 and RANTES, and the inpatient change in both parameters were calculated. An increase or decrease in IL-7 correlated with an increase or decrease in RANTES in 19 of 24 patients (Fig. 3B). A strong correlation ($r = 0.77$; $P < 0.01$) between the change in IL-7 and the change in RANTES was observed in this cohort, indicating that a strong inpatient decrease or increase in IL-7 was associated to a strong decrease or increase in RANTES. Variations in the VL as a consequence of treatment interruption or reinitiation of HAART showed that a change in IL-7 resulted in a change in RANTES (Fig. 3C and D show results for two representative patients). Conversely, no change in the IL-7 level resulted in no change in the RANTES level (Fig. 3E). Of note, RANTES levels could increase before a detectable change in IL-7 (Fig. 3C). A prospective study of individuals with low CD4 cell counts that improve their immune status after HAART should formally address whether changes in β -chemokines levels are a consequence of changes in IL-7 as suggested by our in vitro studies. Nevertheless, a longitudinal study of HIV⁺ individuals for whom HAART is interrupted suggests that HAART will affect IL-7 and RANTES production in a similar fashion.

Our results could explain, in part, the discrepancies observed in relationship to β -chemokine production and disease progression. Previous works have not taken into account an analysis that includes cytokines such as IL-7 that, as shown here, may directly alter the production of RANTES by T cells from uninfected or infected individuals. A beneficial effect on T-cell number could be associated to β -chemokines, i.e., RANTES, if individuals with similar IL-7 levels are compared. Conversely, RANTES levels could be associated to disease progression if distinct IL-7 groups are compared. Our results suggest the possibility that individuals with high RANTES levels but low IL-7 levels are indeed protected from infection. Genetic, virological, or immunological factors besides those discussed herein could influence RANTES production and disease progression.

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REFERENCES

- Barretina, J., J. Blanco, A. Gutierrez, L. Puig, C. Altisent, T. Espanol, I. Caragol, B. Clotet, and J. A. Este. 2000. Evaluation of the putative role of C-C chemokines as protective factors of HIV-1 infection in seronegative hemophiliacs exposed to contaminated hemoderivatives. *Transfusion* **40**: 461-467.
- Berger, E. A., P. M. Murphy, and J. M. Farber. 1999. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.* **17**:657-700.
- Blackbourn, D. J., C. E. Mackewicz, E. Barker, T. K. Hunt, B. Herndier, A. T. Haase, and J. A. Levy. 1996. Suppression of HIV replication by lymphoid tissue CD8⁺ cells correlates with the clinical state of HIV-infected individuals. *Proc. Natl. Acad. Sci. USA* **93**:13125-13130.
- Bleul, C. C., M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, and T. A. Springer. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382**:829-833.
- Clerici, M., C. Balotta, D. Trabattini, L. Papagno, S. Ruzzante, S. Rusconi, M. L. Fusi, M. C. Colombo, and M. Galli. 1996. Chemokine production in HIV-seropositive long-term asymptomatic individuals. *AIDS* **10**:1432-1433.
- Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and P. Lusso. 1995. Identification of RANTES, MIP-1 α , and MIP-1 β as the major HIV-suppressive factors produced by CD8⁺ T cells. *Science* **270**: 1811-1815.
- Cocchi, F., A. L. DeVico, R. Yarchoan, R. Redfield, F. Cleghorn, W. A. Blattner, A. Garzino-Demo, S. Colombini-Hatch, D. Margolis, and R. C. Gallo. 2000. Higher macrophage inflammatory protein (MIP)-1 α and MIP-1 β levels from CD8⁺ T cells are associated with asymptomatic HIV-1 infection. *Proc. Natl. Acad. Sci. USA* **97**:13812-13817.
- Douek, D. C., J. M. Brechnley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto, J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A. Oxenius, D. A. Price, M. Connors, and R. A. Koup. 2002. HIV preferentially infects HIV-specific CD4⁺ T cells. *Nature* **417**:95-98.
- Fauci, A. S. 1996. Host factors and the pathogenesis of HIV-induced disease. *Nature* **384**:529-534.
- Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**:872-877.
- Fry, T. J., E. Connick, J. Falloon, M. M. Lederman, D. J. Liewehr, J. Spritzler, S. M. Steinberg, L. V. Wood, R. Yarchoan, J. Zuckerman, A. Landay, and C. L. Mackall. 2001. A potential role for interleukin-7 in T-cell homeostasis. *Blood* **97**:2983-2990.
- Fry, T. J., and C. L. Mackall. 2002. Interleukin-7: from bench to clinic. *Blood* **99**:3892-3904.
- Fry, T. J., M. Moniuszko, S. Creekmore, S. J. Donohue, D. C. Douek, S. Giardina, T. T. Hecht, B. J. Hill, K. Komschlies, J. Tomaszewski, G. Franchini, and C. L. Mackall. IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected non-human primates. *Blood*, in press.
- Furci, L., G. Scarlatti, S. Burastero, G. Tambussi, C. Colognesi, C. Quillent, R. Longhi, P. Loverro, B. Borronovo, D. Gaffi, E. Carrow, M. Malnati, P. Lusso, A. G. Siccardi, A. Lazzarin, and A. Beretta. 1997. Antigen-driven C-C chemokine-mediated HIV-1 suppression by CD4(+) T cells from exposed uninfected individuals expressing the wild-type CCR-5 allele. *J. Exp. Med.* **186**:455-460.
- Garzino-Demo, A., R. B. Moss, J. B. Margolick, F. Cleghorn, A. Sill, W. A. Blattner, F. Cocchi, D. J. Carlo, A. L. DeVico, and R. C. Gallo. 1999. Spontaneous and antigen-induced production of HIV-inhibitory β -chemokines are associated with AIDS-free status. *Proc. Natl. Acad. Sci. USA* **96**:11986-11991.
- Gordon, C. J., M. A. Muesing, A. E. Proudfoot, C. A. Power, J. P. Moore, and A. Trkola. 1999. Enhancement of human immunodeficiency virus type 1 infection by the CC-chemokine RANTES is independent of the mechanism of virus-cell fusion. *J. Virol.* **73**:684-694.
- Grivel, J. C., Y. Ito, G. Faga, F. Santoro, F. Shaheen, M. S. Malnati, W. Fitzgerald, P. Lusso, and L. Margolis. 2001. Suppression of CCR5- but not CXCR4-tropic HIV-1 in lymphoid tissue by human herpesvirus 6. *Nat. Med.* **7**:1232-1235.
- Grivel, J. C., and L. B. Margolis. 1999. CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. *Nat. Med.* **5**:344-346.
- Holme, P. A., F. Muller, N. O. Solum, F. Brosstad, S. S. Froland, and P. Aukrust. 1998. Enhanced activation of platelets with abnormal release of RANTES in human immunodeficiency virus type 1 infection. *FASEB J.* **12**:79-89.
- Ioannidis, J. P., P. S. Rosenberg, J. J. Goedert, L. J. Ashton, T. L. Benfield, S. P. Buchbinder, R. A. Coutinho, J. Eugen-Olsen, T. Gallart, T. L. Katzenstein, L. G. Kostrikis, H. Kuipers, L. G. Louie, S. A. Mallal, J. B. Margolick, O. P. Martinez, L. Meyer, N. L. Michael, E. Operskalski, G. Pantaleo, G. P. Rizzardi, H. Schuitemaker, H. W. Sheppard, G. J. Stewart, I. D. Theodorou, H. Ullum, E. Vicenzi, D. Vlahov, D. Wilkinson, C. Workman, J. F. Zagury, and T. R. O'Brien. 2001. Effects of CCR5-Delta32, CCR2-64I, and SDF-1 3'A alleles on HIV-1 disease progression: an international meta-analysis of individual-patient data. *Ann. Intern. Med.* **135**:782-795.
- Jourdan, P., J. P. Vendrell, M. F. Huguet, M. Segondy, J. Bousquet, J. Pene, and H. Yssel. 2000. Cytokines and cell surface molecules independently induce CXCR4 expression on CD4⁺ CCR7⁺ human memory T cells. *J. Immunol.* **165**:716-724.
- Llano, A., J. Barretina, J. Blanco, A. Gutierrez, B. Clotet, and J. A. Este. 2001. Stromal-cell-derived factor 1 prevents the emergence of the syncytium-inducing phenotype of HIV-1 in vivo. *AIDS* **15**:1890-1892.
- Llano, A., J. Barretina, A. Gutierrez, J. Blanco, C. Cabrera, B. Clotet, and J. A. Este. 2001. Interleukin-7 in plasma correlates with CD4 T-cell depletion and may be associated with emergence of syncytium-inducing variants in human immunodeficiency virus type 1-positive individuals. *J. Virol.* **75**:10319-10325.

24. Mackewicz, C. E., E. Barker, G. Greco, G. Reyes-Teran, and J. A. Levy. 1997. Do beta-chemokines have clinical relevance in HIV infection? *J. Clin. Invest.* **100**:921–930.
25. Malnati, M. S., G. Tambussi, E. Clerici, S. Polo, M. Algeri, V. Nardese, L. Furci, A. Lazzarin, and P. Lusso. 1997. Increased plasma levels of the C-C chemokine RANTES in patients with primary HIV-1 infection. *J. Biol. Regul. Homeost. Agents* **11**:40–42.
26. McKenzie, S. W., G. Dallalio, M. North, P. Frame, and R. T. Means, Jr. 1996. Serum chemokine levels in patients with non-progressing HIV infection. *AIDS* **10**:F29–F33.
27. Napolitano, L. A., R. M. Grant, S. G. Deeks, D. Schmidt, S. C. De Rosa, L. A. Herzenberg, B. G. Herndier, J. Andersson, and J. M. McCune. 2001. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat. Med.* **7**:73–79.
28. Paxton, W. A., S. R. Martin, D. Tse, T. R. O'Brien, J. Skurnick, N. L. VanDevanter, N. Padian, J. F. Braun, D. P. Kotler, S. M. Wolinsky, and R. A. Koup. 1996. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposure. *Nat. Med.* **2**:412–417.
29. Paxton, W. A., A. U. Neumann, S. Kang, L. Deutch, R. C. Brown, R. A. Koup, and S. M. Wolinsky. 2001. RANTES production from CD4⁺ lymphocytes correlates with host genotype and rates of human immunodeficiency virus type 1 disease progression. *J. Infect. Dis.* **183**:1678–1681.
30. Plum, J., M. De Smedt, G. Leclercq, B. Verhasselt, and B. Vandekerckhove. 1996. Interleukin-7 is a critical growth factor in early human T-cell development. *Blood* **88**:4239–4245.
31. Polo, S., F. Veglia, M. S. Malnati, C. Gobbi, P. Farci, R. Raiteri, A. Sinicco, and P. Lusso. 1999. Longitudinal analysis of serum chemokine levels in the course of HIV-1 infection. *AIDS* **13**:447–454.
32. Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. D. Walker. 1997. Vigorous HIV-1-specific CD4⁺ T cell responses associated with control of viremia. *Science* **278**:1447–1450.
33. Ruiz, L., G. Carcelain, J. Martinez-Picado, S. Frost, S. Marfil, R. Paredes, J. Romeu, E. Ferrer, K. Morales-Lopetegui, B. Autran, and B. Clotet. 2001. HIV dynamics and T-cell immunity after three structured treatment interruptions in chronic HIV-1 infection. *AIDS* **15**:F19–F27.
34. Saha, K., G. Bentsman, L. Chess, and D. J. Volsky. 1998. Endogenous production of beta-chemokines by CD4⁺, but not CD8⁺, T-cell clones correlates with the clinical state of human immunodeficiency virus type 1 (HIV-1)-infected individuals and may be responsible for blocking infection with non-syncytium-inducing HIV-1 in vitro. *J. Virol.* **72**:876–881.
35. Schluns, K. S., W. C. Kieper, S. C. Jameson, and L. Lefrancois. 2000. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. *Nat. Immunol.* **1**:426–432.
36. Steffens, C. M., E. Z. Managlia, A. Landay, and L. Al-Harthi. 2002. Interleukin-7-treated naive T cells can be productively infected by T-cell-adapted and primary isolates of human immunodeficiency virus 1. *Blood* **99**:3310–3318.
37. Swingle, S., A. Mann, J. Jacque, B. Brichacek, V. G. Sasseville, K. Williams, A. A. Lackner, E. N. Janoff, R. Wang, D. Fisher, and M. Stevenson. 1999. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat. Med.* **5**:997–1103.
38. Tan, J. T., E. Dudl, E. LeRoy, R. Murray, J. Sprent, K. I. Weinberg, and C. D. Surh. 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl. Acad. Sci. USA* **98**:8732–8737.
39. Trkola, A., C. Gordon, J. Matthews, E. Maxwell, T. Ketas, L. Czaplewski, A. E. Proudfoot, and J. P. Moore. 1999. The CC-chemokine RANTES increases the attachment of human immunodeficiency virus type 1 to target cells via glycosaminoglycans and also activates a signal transduction pathway that enhances viral infectivity. *J. Virol.* **73**:6370–6379.
40. Vicenzi, E., P. Bagnarelli, E. Santagostino, S. Ghezzi, M. Alfano, M. S. Sinnone, G. Fabio, L. Turchetto, G. Moretti, A. Lazzarin, A. Mantovani, P. M. Mannucci, M. Clementi, A. Gringeri, and G. Poli. 1997. Hemophilia and nonprogressing human immunodeficiency virus type 1 infection. *Blood* **89**:191–200.
41. Wagner, L., O. O. Yang, E. A. Garcia-Zepeda, Y. Ge, S. A. Kalams, B. D. Walker, M. S. Pasternack, and A. D. Luster. 1998. Beta-chemokines are released from HIV-1-specific cytolytic T-cell granules complexed to proteoglycans. *Nature* **391**:908–911.
42. Walker, C. M., D. J. Moody, D. P. Stites, and J. A. Levy. 1986. CD8⁺ lymphocytes can control HIV infection in vitro by suppressing virus replication. *Science* **234**:1563–1566.
43. Weiss, L., A. Si-Mohamed, P. Giral, P. Castiel, A. Ledur, C. Blondin, M. D. Kazatchkine, and N. Haeflner-Cavaillon. 1997. Plasma levels of monocyte chemoattractant protein-1 but not those of macrophage inhibitory protein-1alpha and RANTES correlate with virus load in human immunodeficiency virus infection. *J. Infect. Dis.* **176**:1621–1624.
44. Zagury, D., A. Lachgar, V. Chams, L. S. Fall, J. Bernard, J. F. Zagury, B. Bizzini, A. Gringeri, E. Santagostino, J. Rappaport, M. Feldman, A. Burny, and R. C. Gallo. 1998. Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS. *Proc. Natl. Acad. Sci. USA* **95**:3851–3856.
45. Zagury, D., A. Lachgar, V. Chams, L. S. Fall, J. Bernard, J. F. Zagury, B. Bizzini, A. Gringeri, E. Santagostino, J. Rappaport, M. Feldman, S. J. O'Brien, A. Burny, and R. C. Gallo. 1998. C-C chemokines, pivotal in protection against HIV type 1 infection. *Proc. Natl. Acad. Sci. USA* **95**:3857–3861.
46. Zanussi, S., M. D'Andrea, C. Simonelli, U. Tirelli, and P. De Paoli. 1996. Serum levels of RANTES and MIP-1 alpha in HIV-positive long-term survivors and progressor patients. *AIDS* **10**:1431–1432.