Cellular Immunotherapy of Advanced Human Immunodeficiency Virus Type 1 Infection Using Autologous Lymph Node Lymphocytes: Effects on Chemokine Production

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A pilot study was undertaken in patients with human immunodeficiency virus type 1 (HIV-1) infection to examine the effects of infusing autologous lymph node lymphocytes that had been cultured ex vivo in conditions designed to maximize the specific secretion of HIV-1-suppressive factors, including \( \beta \) chemokines. Ten patients with CD4 cell counts between 119 and 436/\( \mu L \) on antiretroviral drugs received a single infusion of CD4 and CD8 lymph node lymphocytes. There were no serious acute or chronic adverse clinical effects. Increases in serum levels of macrophage inflammatory protein 1/\( \beta \) (MIP-1/\( \beta \)) and increases in the production of MIP-1/\( \beta \) by peripheral blood lymphocytes in response to HIV-1 env were observed. Increases in CD4 and CD8 cell counts and skin test reactivity to recall antigens and decreases in HIV-1 virus load were also observed. This cellular immunotherapy can modulate \( \beta \) chemokine production in patients with advanced HIV-1 infection and may contribute immunorestorative and antiviral activities.

Human immunodeficiency virus type 1 (HIV-1) might be controlled by a cellular immune response that is not dependent on classic cytolytic T lymphocyte (CTL) mechanisms but rather the release of one or more soluble suppressive factors [1]. The \( \beta \) chemokines RANTES, macrophage inflammatory protein (MIP) 1/\( \alpha \), MIP-1/\( \beta \), and macrophage derived chemokine have been identified as HIV-1-suppressive factors [2, 3]. Whether suppressive factors can be practically applied to the therapy of HIV-1 infection is controversial. Chemokines exert proinflammatory effects, and achieving the high, local concentrations of chemokine combinations expected to be necessary for antiviral activity with systemic therapy would be predicted to be toxic. A potentially effective approach would be the infusion of cells that could traffic to reservoirs of HIV-1 activity and release suppressive factors in a regulated, paracrine fashion. We developed a short-term culture method to generate cells from infected lymph nodes that are not cytolytic in vitro but rather respond to HIV-1 by releasing suppressive factors, including MIP-1/\( \alpha \), MIP-1/\( \beta \), and RANTES [4]. Here we report the results of a pilot study to evaluate the feasibility of using these cells therapeutically.

Materials and Methods

Patients. Eligibility criteria for this study included HIV-1 infection documented by ELISA, CD4 cell count >100 but <500/\( \mu L \) within 4 weeks of the time of lymph node excision, palpable peripheral lymph nodes that were considered to be resectable under local anesthesia, Karnofsky performance status >70, absolute neutrophil count >1000/mm\(^3\), hemoglobin >9.5 g/dL, platelet count >75,000/mm\(^3\), creatinine <1.5 mg/dL, bilirubin <1.5 mg/dL, and hepatic transaminases <3 times the upper limit of normal. Exclusion criteria included active opportunistic infection, malignancy except limited Kaposi’s sarcoma, significant autoimmune disease, or concurrent experimental antiretroviral therapy. Subjects had to be receiving approved antiretroviral treatment and Pneumocystis carinii pneumonia prophylaxis, but there could not have been any change in therapy for the 4 weeks prior to enrollment.

Treatment. A clinically palpable lymph node was excised. If examinations for malignancy and microorganisms were negative, lymph node cells were cultured ex vivo. Patients received a single infusion of their cultured cells into a peripheral vein. They were premedicated with acetaminophen and monitored frequently for toxicity, which was graded using World Health Organization Common Toxicity Criteria. Peripheral blood immunophenotyping was performed by the Ohio State University Hospital Cellular Immunology Laboratory. Virus loads were assessed by quantitative polymerase chain reaction (Corning Clinical Laboratories, Pittsburgh).

Cell activation and expansion. Lymph node cells were cultured with anti-CD3 monoclonal antibody and recombinant human interleukin (IL)-2 in serum-free medium for 10 days as described [4]. Cells were harvested, washed, and suspended in 350 mL of 0.9% NaCl plus 1.2% human serum albumin before infusion with a harvester (SteriCell, DuPont, Glenolden, PA). The following were evaluated (as described [4]) to characterize the cells: number and viability, endotoxin activity, morphology, Gram’s stain, cultures for
bacterial and fungal contamination, HIV-1 \textit{gag} mRNA expression, and cell surface marker analysis.

\textit{Chemokine and cytokine production.} Peripheral blood lymphocytes (PBL) were stimulated with irradiated, autologous, Epstein-Barr virus–transformed B cell lines infected with either an empty \textit{Vaccinia} control vector or the \textit{Vaccinia} vPE16 vector, which contains the HIV-1 \textit{env} gene, as described \cite{4}. The vectors were obtained through the National Institutes of Health AIDS Research and Reference Reagent Program (Patricia Earl and Bernard Moss, NIH, Bethesda, MD). Cells were also stimulated with 2 \textmu g/mL phytohemagglutinin as a positive control. ELISA kits were used to quantify MIP-1\textbeta, interferon-\gamma (IFN-\gamma), and IL-5 (R&D Systems, Minneapolis). Assays were conducted in duplicate, and data are presented as mean values.

\textit{Skin testing.} Skin was tested for a delayed-type hypersensitivity response to common recall antigens before and 5 weeks after cell infusion, using the Multitest CMI skin test reaction profile (Connaught Laboratories, Swiftwater, PA) according to the recommendation of the manufacturer.

\section*{Results}

\textit{Patients and lymph nodes.} Lymph nodes were resected from all 14 patients enrolled. All patients had histories of AIDS-defining conditions. At the time of infusion, peripheral blood CD4 cell counts ranged from 119 to 436/\textmu L (mean, 268), and patients had been on unchanged antiretrovirals from 6 weeks to 8 months (median, 5 months). Resections were complicated by seroma formation in 4 patients: 1 underwent incision and drainage, 1 was treated with antibiotics, and in the other 2, seromas resolved without specific therapy. Resections from 12 patients yielded from 0.83 to 8.2 \times 10^8 cells (mean, 3.5 \times 10^8). These lymph nodes were characterized histologically by follicular or interfollicular (or both) hyperplasia. Substantially fewer cells (<2.4 \times 10^7) were obtained from 2 subjects. These lymph nodes were characterized by lymphocyte depletion and plasma cell and eosinophil infiltration. All lymph nodes resected were negative for malignancy and microorganisms.

\textit{Cell activation and expansion.} Cells expanded 2.3- to 200-fold without evidence of microbial contamination. The HIV-1 \textit{gag} mRNA product (reverse transcription–polymerase chain reaction) was undetectable at harvest in 10 of the 14 cultures. The 4 cultures in which \textit{gag} mRNA was detected were characterized by starting cell numbers of \textless 1.5 \times 10^8. The harvested cells were predominantly CD3 T cells (mean, 92%; range, 83%-98%) with a CD4:CD8 ratio of 0.05–1.47 (mean, 0.65; median, 0.53).

\textit{Toxicity.} Cells with undetectable \textit{gag} mRNA were infused. Ten patients received 1.2 to 8.2 \times 10^8 cells (mean, 3.7 \times 10^8). Only 4 reported any adverse reactions. A severe (grade 3) headache was reported in 1 patient, moderate (grade 2) nausea in 1, and mild (grade 1) nausea, arthralgia, and/or fever in 3. These reactions occurred 30 min to 4 h after infusion and resolved without complication with medication. No chronic toxicities were observed. Infectious complications, opportunistic or otherwise, have not been observed up to 25 weeks after infusion.

\textit{Immunologic effects.} Serum MIP-1\textbeta for the treated group increased from 31 ± 13 pg/mL before infusion to 64 ± 20 pg/mL (\textit{P} = .0001, paired \textit{t} test) in 1 day (figure 1A). Changes at 5 and 25 weeks after infusion were not statistically significant. PBL production of MIP-1\textbeta in vitro in response to autologous B cells expressing \textit{env} was examined in 8 patients who were compliant with the necessary phlebotomies (figure 1B). Increases in MIP-1\textbeta were observed at 5 weeks in 5 patients. At 13 or 25 weeks (or both), this response had decreased. Production of IFN-\gamma (>50 pg/mL) by \textit{env}-stimulated PBL was increased in 2 of those 5 patients, but changes in IL-5 production were not observed (data not shown). All cells responded to phytohemagglutinin with an increase in MIP-1\textbeta production.

\begin{figure}[h]
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\caption{Serum levels of macrophage inflammatory protein 1\textbeta (MIP-1\textbeta) before (Pre), 1 day, and 5 and 25 weeks after cell infusion (A) and MIP-1\textbeta production by PBL in response to autologous B cell targets infected with \textit{Vaccinia} expressing HIV-1 \textit{env} (solid lines) or with \textit{Vaccinia} alone (dotted lines) before (Pre) and 5, 13, and 25 weeks after cell infusion (B). Pre values represent mean of at least 2 determinations made within 4 weeks of cell infusion. Symbols refer to individual patients.}
\end{figure}
>200 ng/mL (greater than that of unstimulated cells) and with an increase in IFN-γ production >80 ng/mL. None of the cells responded with significant cytokine production in response to autologous B cells infected with the *Vaccinia* control.

CD4 cell counts increased >100/μL in 4 patients (figure 2); they increased for the group from a mean of 252/μL before infusion to a mean of 342/μL at week 1 (*P* = .02; Wilcoxon sign rank test), 348/μL at week 5 (*P* = .04), 327/μL at week 9 (*P* = .05), 320/μL at week 13 (*P* = .04), 325/μL at week 17 (*P* = .03), and 348/μL at week 21 (*P* = .02). CD8 cell increases of >100/mL were observed in 7 patients; these increases for the group, however, were not statistically significant at specific time points. All patients were poorly reactive on delayed-type hypersensitivity testing before cell infusion; new reactivities to tetanus, streptococcus, candida, trichophyton, and/or proteus developed in 4 of 9 patients tested. The others were anergic to all antigens throughout.

**Virologic effects.** Of 9 patients with measurable virus load before cell infusion, 7 had decreases of >0.5 log during the observation period (figure 2). Decreases the day after cell infusion and decreases to undetectable levels were observed. In 1 patient, virus load increased transiently >0.5 log the day after cell infusion; it was undetectable 5 weeks later.

**Discussion**

The results of this pilot study indicate that cellular immunotherapy can modulate β chemokine production in patients with HIV-1 infection, even in patients with advanced infection. Increases in serum MIP-1β levels and in MIP-1β production developed in 4 of 9 patients tested. The others were anergic to all antigens throughout.

![Figure 2](image-url)  
*Figure 2.* Effect of cell infusion on peripheral blood CD4 cell counts (top) and virus load (bottom). Cell counts and virus loads before cell infusion (Pre) represent mean of at least 2 determinations made within 4 weeks of cell infusion. Limit of detection for virus load is 400 copies/mL. Symbols refer to individual patients.
by PBL in response to HIV-1 env were observed. The results also suggest the possibility that the cell infusions contributed immunologic and virologic effects. Skin test reactivity to recall antigens improved, and increases in CD4 and CD8 cell counts were sustained. HIV-1 virus loads decreased to undetectable levels in individual patients. As all patients were also taking antiretroviral drugs, whether the immunomodulatory and antiviral effects resulted from the cell therapy or the modulation of chemokine production (or both) cannot be concluded and will require further study.

The infused autologous cells were activated and expanded ex vivo to produce HIV-1-suppressive factors and not to mediate CTL activity. A variety of HIV-1-suppressive factors have been identified, including the β chemokines, the CD8 antiviral factor (CAF), and IL-16 [1–3, 5]. The interrelationship and relative roles of these and potentially other suppressive factors in the control of HIV-1 are controversial. In addition to β chemokines, soluble products that demonstrate activity consistent with CAF are produced by the cells generated, as are a variety of other cytokines, including IFN-γ, that could mediate antiviral and immunorestorative effects [4]. Although our culture regimen was not designed to generate cytolytic activity, HIV-1–specific CTL precursors may have been present and played a role in the anti–HIV-1 activity.

A substantial proportion of the cells infused was CD4. We have demonstrated that HIV-1–stimulated chemokine release can be mediated by CD4 cells, and HIV-1–specific CD4 cell responses have recently been reported to be associated with control of viremia [4, 6]. Although activated CD4 cells are the principal targets for HIV-1 and are critical to the progression of the infection, there are theoretical advantages to their infusion in cellular immunotherapy. CD8 cells do not normally make enough IL-2 to support their own expansion and are dependent on IL-2 and possibly other cytokines from CD4 cells. There is evidence from clinical studies that suggests that the infusion of CD4 and CD8 cells is more effective than purified CD8 cells, even when CD8 cells are central to the desired response [7]. Although sample size and study design preclude analysis, substantial differences were not noted between the total number of cells (3.6 vs. 4.1 × 10⁸) or the numbers of CD4 (1.3 vs. 1.4 × 10⁸) or CD8 (2.4 vs. 2.4 × 10⁸) cells infused into patients who did manifest increased MIP-1β production compared with those who did not.

This is the first cellular immunotherapy of HIV-1 infection that used lymph nodes, which are major reservoirs of HIV-1, as a source of cells. Lymph nodes were used because the peripheral blood of patients with advanced HIV-1 infection has not been a good source of HIV-1–suppressive factors [1]. Lymph nodes also contain all the critical elements to expand HIV-1–reactive cells ex vivo, and lymph node cells have a greater spectrum of HIV-1 reactivity and superior trafficking than peripheral blood cells [4, 8]. Cells with reactivities against other pathogens known to compromise HIV-1 infection may also have been generated from the lymph nodes.

Because HIV-1 can escape CTL recognition, it has been difficult to generate effective anti–HIV-1 CTL for cellular immunotherapies [9]. Several approaches of enhancing HIV-1–suppressive factors are under investigation. IL-2 can sustain CAF production, and systemic therapy with IL-2 has been reported to increase the number of CD4 cells [1]. In patients with CD4 cell counts of <200/μL, however, IL-2 therapy is associated with increased viral activation, few immunologic improvements, and substantial toxicity [10]. Our results indicate that a single infusion of activated and expanded autologous lymph node cells into patients on antiretroviral drugs is feasible and safe. Even though a major reservoir of HIV-1 was used as a source of cells and activated CD4 cells were infused, no adverse clinical effects were noted. The infusion of cells as a vehicle for delivering suppressive factors merits further study.

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References