

PREMIERE ISSUE

[www.bioprocessingjournal.com](http://www.bioprocessingjournal.com)

# BioProcessing

Technical Developments and Opportunities

A publication of  
*The Williamsburg BioProcessing Foundation*

Vol. I No. I

March 2002

# Ex Vivo Activation and Adoptive Transfer of T Cells for Immune Augmentation

BY BRUCE L. LEVINE

*Portions of this article were presented at the Williamsburg BioProcessing Foundation, Cell & Tissue BioProcessing Conference, Williamsburg, VA, Nov. 2001.*

**W**e have developed a procedure for large-scale enrichment, growth and harvesting of T cells suitable for adoptive immunotherapy. In two recently completed clinical trials, we investigated the feasibility of immune reconstitution in patients with HIV infection, or with relapsed/refractory Non-Hodgkin's Lymphoma (NHL) following infusions of autologous activated CD4+ T cells or CD4+/CD8+ T cells. Autologous T cells were activated via CD3/CD28 stimulation, ex vivo, and were then reinfused.

In the first trial, eight patients with intermediate-stage HIV-1 infection were given infusions of up to  $3 \times 10^{10}$  activated CD4 cells. No significant clinical toxicity was noted. A dose-dependent increase in CD4 counts and in the CD4/CD8 ratio was observed. The response was specific because the size of other lymphocyte subsets did not change. Sustained increases were noted in vivo of lymphocyte proliferation to alloantigen, and in the percent of cytokine-secreting T cells, suggesting improved lymphocyte effector function. Furthermore, this approach may lead to increased resistance to HIV, as we noted

a decrease in the expression of the HIV co-receptor CCR5 in vitro and in vivo.

In the second trial, anti-CD3/CD28 ex vivo expanded and costimulated T cells were administered as a therapeutic adjunct to patients with relapsed/refractory NHL following the use of High Dose Chemotherapy (HDC) and CD34+ selected Peripheral Blood Progenitor Cells (PBPC). For 13 patients, the median CD4/CD8 ratio increased in vitro from 1.2 to 1.6. The culture procedure successfully expanded human T cells, ex vivo, including CD4+ T cells from patients with NHL, and reversed the in vitro cytokine response of impaired T cells.

These Phase I trials demonstrated that adoptive transfer of costimulated T cells is feasible in heavily pretreated patients with NHL and in intermediate stage HIV+ patients, while exhibiting minimal to moderate infusional toxicity. A second generation of trials is now underway to test the effectiveness of costimulated polyclonal T cell therapy for HIV and various cancers.

## Background

Evidence indicates that the activation of pathogen-specific and tumor-specific T lymphocytes is central to an effective immune response to HIV and various cancers.

T lymphocytes recognize cell associated antigen presented in the context of MHC Class I (CD8 cells) or Class II (CD4 cells). Immune responses coordinated by T lymphocytes are initiated by processed antigen fragments in the context of MHC binding to the T Cell Receptor (TCR). Coupled with a supplementary signal, this binding process

is the primary means by which activation can progress. TCR stimulation is antigen (Ag) specific and is frequently referred to as signal 1, while costimulation is Ag non-specific and is commonly referred to as signal 2.

Stimulation of CD4+ T cells, via Ag in the context of MHC Class II, leads to T cell proliferation and clonal expansion. Then, released cytokines can induce the activation of not just other T cells, but also B cells and cells of the macrophage/monocyte lineage. Thus, the activation of T cells is central to the mobilization of an effective immune response, because T cells can exert regulatory influence over other cells of the immune system.

The "second signal" referred to above appears to be an important control mechanism in the immune system. This supplementary signal can be delivered by the engagement of cell surface costimulatory receptors, such as CD28, whereas in memory or effector cells, cytokines such as IL-2 may be sufficient.<sup>1</sup> CTLA-4 is a receptor structurally related to CD28 and is upregulated on activated cells. Recent evidence indicates that CD28 and CTLA-4 have opposing effects on T cell activation, where CTLA-4 binds with greater affinity to CD80 and CD86 on antigen presenting cells than does CD28, and serves to inhibit or down-regulate a response.<sup>2</sup>

Thus, the activation state of a T cell and the regulation of an immune response may be determined by the relative strength of the signal delivered by either CD28 or CTLA-4, which is further dependent on the absolute amount and relative levels of the costimulatory ligands CD80 and CD86.

*Bruce Levine, Ph.D. is research assistant professor, dept. of pathology and laboratory medicine and the Leonard and Madlyn Abramson Family Cancer Research Institute at the University of Pennsylvania Cancer Center, Philadelphia, PA 19104, Levinebl@mail.med.upenn.edu.*

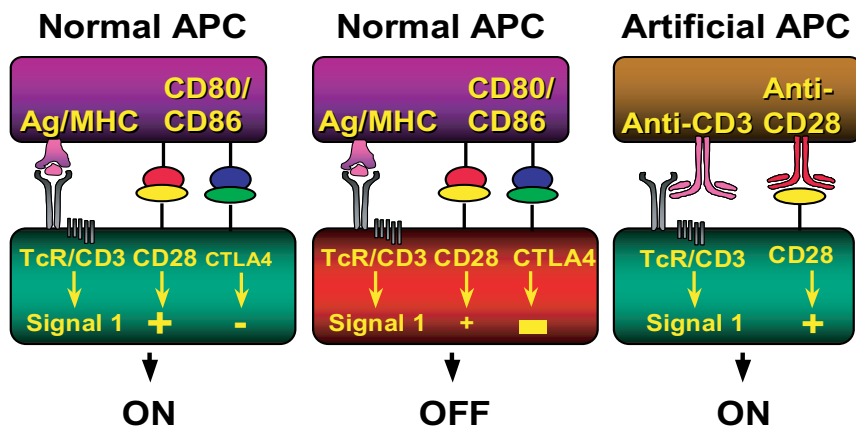


Figure 1. The 3 signal model of T lymphocyte activation. Antigen/MHC interactions with T cells generate signal 1. In resting T cells (left), there is a much higher level of CD28 present than CTLA4 (CD152), so that a positive signal (signal 2) is delivered through CD28. In activated T cells (Center), CTLA4 is upregulated and because of its higher affinity for CD80 and CD86, may deliver a negative signal (signal 3) to the T cell. The negative regulatory effects of CTLA4 may be overcome by constructing an artificial APC (right) using Mab directed against CD3 and CD28, and thus bypassing negative signals delivered by CTLA4.

An artificial antigen presenting cell may be constructed by coating beads with a monoclonal antibody (Mab) directed against CD3 and CD28, and thereby bypass the negative signal delivered by CTLA-4 (See Figure 1). Using beads coated with antibodies to CD3 and CD28 in small-scale culture systems, we have noted efficient long-term polyclonal proliferation of CD4+ T cells. This method does not require the use of feeder cell layers or the addition of exogenous cytokines.<sup>3,4</sup>

Furthermore, activation of CD4+ cells by CD3/CD28 costimulation specifically induces a potent anti-HIV effect that permits the culture of cells from HIV+ patients.<sup>5</sup> As a result, addition of anti-retroviral drugs to growth media is not routinely required. This effect is specific for macrophage-tropic HIV-1 and is due to a marked down-regulation of the HIV-1 fusion cofactor CCR5,<sup>6</sup> plus an enhanced production of the  $\beta$ -chemokines RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$ <sup>7</sup> which are the natural ligands for CCR5.<sup>8,9</sup>

Adoptive immunotherapy of malignancy or infection involves the ex vivo manipulation of immune cells, which is then followed by reinfusion in order to augment the immune response and reconstitute the immune system. There are several rationales for immunotherapy using CD4+ T cells in the treat-

ment of HIV. While there has been dramatic evidence of the power of protease inhibitors and multi-drug cocktails in lowering viral burden, there is an incomplete restoration of CD4 cell levels.<sup>10-12</sup> In addition, there are recent reports of increasing HIV resistance to these drug cocktails.<sup>13,14</sup>

CD4 immunotherapy, or gene therapy, could potentially be useful in repairing or maintaining the immune memory that is important for prevention of opportunistic infections and AIDS-associated malignancies. An association between increased CD4+ response to HIV-1 p24 and a decreased plasma viral load has been noted.<sup>15</sup> While studies using CD8+ T cell immunotherapy have generally proven to be safe and feasible, the defect in CD4 cell "help" in HIV<sup>16</sup> may lead to rapid CTL exhaustion of any infused CD8+ T cells. It therefore seems likely that augmenting the T helper and CTL responses in the treatment of HIV and other viral infections is required for an effective immune response, and that it is able to control, if not eliminate, viral replication.<sup>17,18</sup>

Like HIV, many tumors have developed immune escape mechanisms. These include down-regulation of MHC, low levels or absence of costimulatory molecules, and secreted factors that are immunosuppressive.<sup>19</sup> The

major rationale for the use of T cells in the adoptive immunotherapy of cancer is that cytotoxic lymphocytes have the capacity to specifically kill tumor cells. Since CTLs are predominantly CD8+, infusion of a CD8+ cell population (90-100%) was thought to be beneficial.

However, it has been noted that reduced CD4/CD8 ratios of TILs correlated with increasing tumor bulk and lymph node metastases in patients with cervical carcinoma,<sup>20</sup> plus an inadequate Ag-specific CD4+ T cell response. The implications are that previous attempts at immunotherapy may have failed due to limiting T cell help or sub-optimal activation. This has led to the suggestion that improved methods of T cell ex vivo manipulation can provide in vivo immune augmentation in the settings of HIV infection and cancer.

## Methods

### HIV Trial

Written, informed consent was obtained in accordance with institutional review board guidelines. CD4+ T cells were isolated by ficoll (Lymphocyte Separation Medium, BioWhittaker, Walkersville, MD) gradient centrifugation of leukopacks obtained by apheresis of HIV+ donors. This step was followed by a 90-minute incubation at 4° C with anti-CD8 and anti-CD20, each added at 1 $\mu$ g of mAb per 1 x 10<sup>6</sup> target cells. GMP-grade CD8 (M-T415, IgG1) and CD20 (L-3b3, IgG1) Mab were used to remove CD8+ cells and B cells respectively (Nexell, Irvine, CA—formerly Baxter Immunotherapy).

The percent of target cells was determined from the laboratory phenotyping data of the patient. CD3 (UCHT1, IgG1), CD4 (T4, IgG1), CD8 (T8, IgG1) and CD19 (89B, IgG1) (all from Coulter) were used for phenotyping patient peripheral blood. Excess Mab was washed from the cells, and sheep anti-mouse Ig coated paramagnetic beads (Nexell) were added at 3 beads per target cell and then incubated at 4° C for 90 minutes. The non-adherent CD4+ enriched T cells were then washed and counted on a Coulter Multisizer IIe (Coulter, Miami, FL).

Cells were cultured in X-VIVO 15 medium (BioWhittaker, Walkersville,

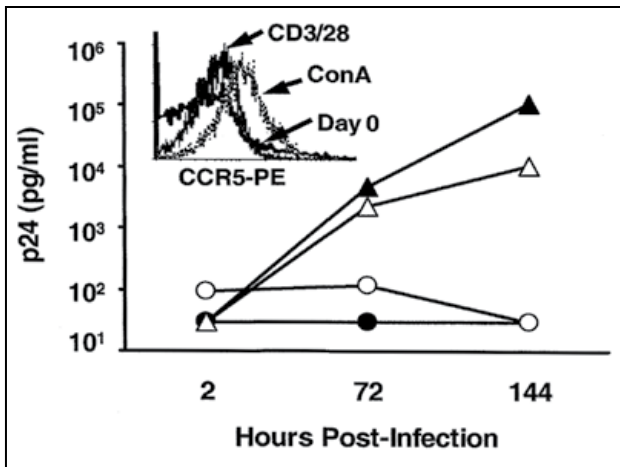


Figure 2. Resistance of CD3/CD28 stimulated cells to HIV infection. Cryopreserved CD4 cells from patient 1 were cultured as described in Methods. The cells were infected with CCR5-tropic HIV<sub>US1</sub> or with the autologous HIV isolate on Day 9. Culture supernatants were analyzed for p24<sub>gag</sub> Ag by ELISA 2 hrs, 3 days and 6 days post infection (Day 9, 12, and 15 post-stimulation). Cells stimulated with CD3/CD28 beads and infected with HIV<sub>US1</sub> (●), stimulated with CD3/CD28 beads and infected with autologous isolate HIV (○), stimulated with ConA/IL-2 and infected with HIV<sub>US1</sub> (G), stimulated with ConA/IL-2 and infected with autologous isolate HIV (△). (inset): expression of CCR5 on the CD4 cells.

MD), which was supplemented with 10% Normal Human Serum (NABI, Miami, FL), 2mM L-glutamine (BioWhittaker), 20mM HEPES (BioWhittaker) and 20U/ml rhIL-2 (Chiron, Emeryville, CA). Anti-CD3 Mab OKT3 (Ortho, Raritan, NJ) and anti-CD28 Mab 9.3 were mixed and bound to magnetic beads (Tosylactivated M-450; Dynal). Dynal beads were added to the T cells, at 3 beads per cell, and then seeded with media into gas permeable bags (Baxter Lifecell®).

Once cells were transferred to gas permeable bags, the cells were maintained in a closed system throughout the culture. The culture was sampled with the aid of a Sterile Connect Device (Terumo, Elkton, MD), and fresh media was added via a Solution Transfer Pump (Baxter) according to the manufacturer's instructions. The cell cultures were counted and monitored for cell size and volume using a Coulter Multisizer IIe (Coulter, Miami, FL). After 12-19 days in culture, the cells were reinfused back into the patient.

The first phase, a dose escalation study, consisted of three infusions:  $3 \times 10^9$ ,  $1 \times 10^{10}$ , and  $3 \times 10^{10}$  CD4+ T cells given at six week intervals. This was followed by a maintenance phase of up to seven infusions with an average of  $5 \times 10^9$  cells given every eight weeks.

Viral load determinations in patient serum were performed with the Roche-Amplicor Assay. Using appropriate dilutions of culture supernatants, p24 was measured using a Coulter (Kendall, FL) ELISA kit. A quantitative PCR assay was used to measure HIV-1 proviral DNA from culture-derived cell pellets. The assay is sensitive to about 10 copies per  $10^5$

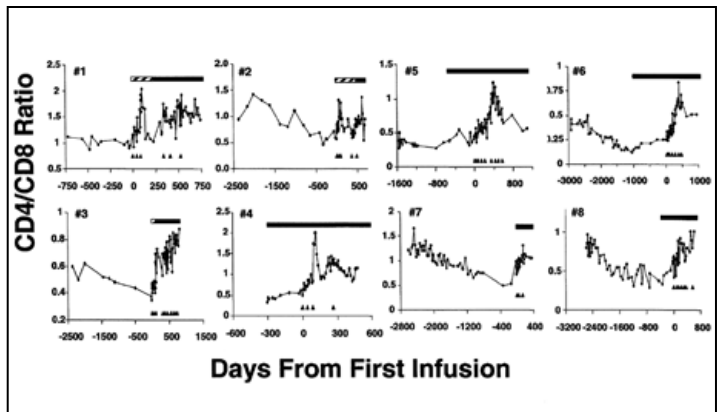


Figure 3. Effects of multiple costimulated CD4 T cell adoptive transfers and HAART on CD4/CD8 ratio in HIV patients. Shown on the y-axis is the CD4/CD8 ratio and on the x-axis, the days before and after the first infusion (Day 0) for all subjects. Each triangle represents an infusion. The bar in the upper right of each graph represents the subject drug regimen: AZT monotherapy is depicted as a hatched bar and HAART is depicted as a solid bar. Note that the X-axis scale changes on Day 0 in order to display the available baseline ratios.

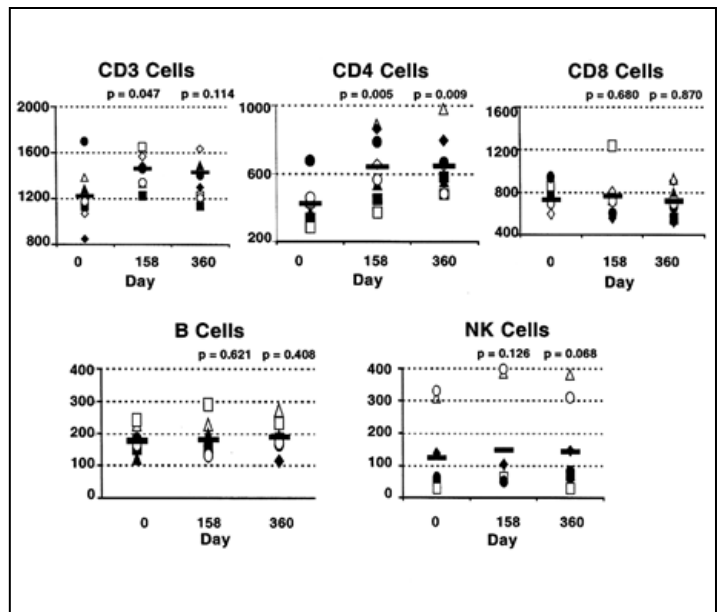


Figure 4. Changes in lymphocyte counts in HIV patients after activated CD4 cell infusions. Results are shown for Study Day 0, 158, and 360; day 158 followed the initial 3-infusion dose escalation while day 360 followed the maintenance phase of the protocol (Fig. 1). The mean of all 8 study subjects is shown as a solid bar while each subject is depicted by a symbol as follows: #1, ◆; #2, ◇; #3, ▲; #4, △; #5, ■; #6, □; #7, ●; #8, ○. The P value (paired student's t-test) for differences between Study Day 0 and Study Day 158 or Study Day 0 and Study Day 360 are shown.

cells. The amplified products were detected by liquid hybridization with end-labelled oligonucleotide probes, followed by gel electrophoresis. PCR products were quantitated as described<sup>5</sup> using a Molecular Dynamics phosphorimager.

### Lymphoma Trial

Written, informed consent was obtained in accordance with institutional review board guidelines. Eligibility criteria

included biopsy-proven NHL (may include low, intermediate or high grade), which had been refractory to a doxorubicin-based regimen, or was a relapsed disease from such a regimen. Patients with T cell NHL were specifically excluded. Patients underwent a steady-state apheresis procedure prior to receiving mobilization chemotherapy. This product served as the source of cells for the CD3+/CD28+ T cell expansion.

Mobilized leukapheresis commenced when 1) circulating CD34+ cells were >5/ $\mu$ l, 2) WBC were >5,000/ $\mu$ l, and 3) platelets were >30,000/ $\mu$ l without transfusion support. For this study, "mobilization" refers to growth factor induced mobilization of CD34+ cells from bone marrow to the peripheral circulation. A minimum of  $4.0 \times 10^6$  CD34+ cells/kg were required to proceed to CD34+ selection. Failure to meet the collection endpoints after 6 daily leukapheresis procedures disqualified the patient from the protocol.

CD34+ selection of peripheral blood mononuclear cells was performed with a Baxter Isolex<sup>®</sup> 300i. For high dose chemotherapy, patients received BEAC (BCNU, Etoposide, Cytarabine, Cyclophosphamide). GM-CSF (Leukine, Immunex, Seattle, WA) was given beginning on day +1 until neutrophils were >5,000 cells/ $\mu$ l.

Ex vivo costimulation and expansion of CD3+/CD28+ T Lymphocytes was performed as described above, except for the T cell enrichment of the pheresis product via CD8+ and CD20+ cell depletion. Instead, for the NHL trial, the washed peripheral blood mononuclear cell (PBMC) product was enriched for lymphocytes using magnetic bead depletion of monocytes in a closed system.

The total dose of reinfused T cells was based on the number of CD3+ cells, as determined by the total cell count with flow cytometry and anti-CD3. After the 14 days of expansion, the CD3+ cells constituted >95% of the total cells in the culture. The expanded T cell product was harvested, washed, and resuspended in 200-500 mL of Plasmalyte A containing 1% HSA. This was a dose escalation study, and ex vivo expanded T cells were infused at the fol-

lowing doses 13-14 days after PBSC: Dose Level 1:  $2 \times 10^9$  CD3+ T cells, Dose Level 2:  $5 \times 10^9$  CD3+ T cells, Dose Level 3:  $1 \times 10^{10}$  CD3+ T cells.

## Results

### Adoptive transfers of activated CD4+ T cells in HIV

Initially, three drug naïve patients with intermediate-stage HIV infection (CD4 count 350-500 per ml) participated in a dose escalation study to test the safety and feasibility of costimulated CD4+ T cell infusions (described in Methods). Since large numbers of essentially pure activated-CD4 cells had not been administered previously, the principal safety issues included the possible induction of autoimmune disorders and/or increased viral replication.

Secondary objectives were to examine the effects of these infusions on circulating CD4 cell numbers, immune function, and viral load.<sup>21</sup> Cells were obtained by apheresis, enriched for CD4 cells via negative selection, and cultured with anti-CD3/anti-CD28 monoclonal antibody-coated beads as described.<sup>22</sup> On the day of harvest, these cultures ranged from 93% to 100% CD3+CD4+ (mean 97.2% + 0.7 SE; n=9). Supernatant collected during the culture and one day prior to harvest was assayed for HIV-1 p24 by ELISA. All cultures showed a decline in p24 antigen levels during culture (peak 280 + 50 pg/ml; harvest 26 + 3 pg/ml).

Analysis of the cell pellets showed that the number of cells containing HIV-1 gag DNA also declined in all cultures. In many instances, p24 and/or gag DNA content decreased to below the detection limits of 10 pg/ml and 10 copies per  $10^5$  cells, respectively. During preclinical studies, this in vitro decline of viral load had been shown in small-scale cultures of cells from HIV infected donors.<sup>5-7</sup>

Analysis of CD4 cells immediately following ex vivo culture showed that these cells were spontaneously secreting high levels of cytokines and beta chemokines.<sup>21</sup> This was important in that beta chemokines are the natural ligand of the HIV co-receptor CCR5 and could serve to block HIV entry.

To determine the resistance of the

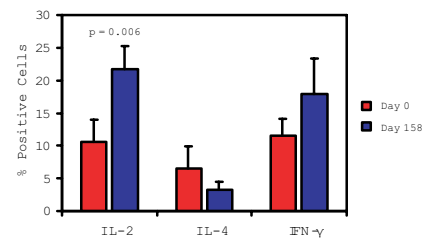


Figure 5. Intracellular IL-2, IL-4 and IFN- $\gamma$  production in HIV+ patient cells before and after 3 CD4+ T cell infusions. Peripheral blood CD4 cells obtained at baseline (open bar) and on study day 158 (solid bar) were examined for intracellular cytokine staining as described in Methods. The mean percent of CD4+ cytokine positive cells is shown for all 8 subjects. There was a significant rise in the percentage of CD4+ cells induced to express IL-2 (P = 0.006, student's t-test).

infused cells to HIV infection, the CD4 starting cell population of patient 1 was stimulated with anti-CD3/CD28 or with ConA and IL-2. The cells were harvested and infected with either the CCR5 tropic strain US1, or an autologous HIV isolate. p24 was collected from the culture supernatants, post infection. The anti-CD3/28 culture process induced high-level resistance to infection with the exogenous CCR5 strain, as well as the autologous HIV isolates. The resistance was specific, because high-level viral replication to both strains of HIV was detected in the ConA + IL-2 stimulated cells (Figure 2). In addition, culture in anti-CD3/28 induced complete downregulation of CCR5, while CCR5 was easily detected on the cells cultured in ConA (Figure 2, inset).

We observed a dose dependent increase in CD4 counts following the infusions. The kinetics of the increase in CD4 counts was notable, as there was often a period of a week, or more, before maximal CD4 counts were reached. This is consistent with the possibility that the infused cells either continued to proliferate for some period of time following infusion, or that the infused cells induced the proliferation of bystander T cells. The CD4 counts at 1 and 4 weeks following the first three infusions for all 8 patients are shown in Table 1. There was a significant increase in the mean

CD4 count for the group, particularly following the third CD4 cell infusion.

Based on these results, we used an intermediate CD4 dose, between dose levels 2 and 3, for the maintenance phase of the protocol. Patients were carefully and continuously monitored for changes in viral load (see Methods), and there were no infusion-related sustained increases or bursts in viral load observed following the CD4+ T cell infusions in any patient. The absence of bursts in plasma viral load following infusions was encouraging in this regard, and suggests that the cells remained resistant to reinfection for some period of time, in vivo. No severe adverse effects and no significant clinical or laboratory toxicities were observed.

### Effects of multiple infusions of activated CD4+ T cells on CD4/CD8 ratio in HIV

We next tested the effects of repeated CD4 cell infusions to determine patient safety and tolerance, and to see whether sustained increases in CD4 counts could be achieved. The ability to give multiple infusions of activated CD4 cells also permitted us to assess whether changes in the homeostasis of CD4 and CD8 counts would occur. With the advent of combination antiretroviral therapy (HAART), the protocol was amended and the suppression of HIV replication was documented for all subsequently enrolled subjects before they were given infusions. In the amended protocol, subjects with CD4 counts greater than 200 were given infusions of  $\sim 5 \times 10^9$  cells at approximately 8 week intervals.

In Figure 3, the CD4/CD8 ratio for all eight subjects is shown before and after CD4+ T cell infusions. A total of 51 infusions were administered. In all subjects, a progressive decline in the CD4/CD8 ratio, consistent with the previously described natural history of HIV, was documented prior to the initiation of CD4 infusions.

All subjects showed a remarkable increase in the CD4/CD8 ratio following the initiation of CD4 cell infusions. This increase was durable, as it was maintained for months following the

cessation of infusions. In patients 1, 2, and 3, zidovudine was started concomitant with the CD4 cell infusions. The magnitude of the increase in CD4 counts seen in the first 160 days for subjects 1 and 2 was much greater than previously reported for patients beginning AZT monotherapy.<sup>23</sup> Subjects 4 to 8 were on HAART therapy prior to the adoptive cell transfers, and inspection of the results suggests that the increased CD4/CD8 ratios were independent of HAART. For example, some subjects were on HAART therapy for many months (subject 4), and in some cases years (subjects 5, 6, and 8) prior to the infusions.

Each subject experienced further increases in CD4/CD8 ratio that coincided temporally with the initiation of the activated CD4 cell infusions. The changes in CD4 counts were specific, as the numbers of CD8+ T cells, B cells, and NK cells remained stable for at least a year (Figure 4). Estimating that the number of CD4+ T cells in the healthy adult is about  $5 \times 10^{11}$  (ref.<sup>24</sup>), most of the subjects received approximately 10% of the total body mass of cells during the protocol. The specificity of the increase in CD4 cells and the sustained duration of the increase indicate that these changes do not reflect redistribution of CD4 T cells from lymphoid tissues into the peripheral blood.

### Immune function and CCR5 expression in vivo after activated CD4 cell infusions in HIV

To assess whether the adoptive transfers had an effect on T cell effector functions, we first examined the intracellular production of cytokines at the single cell level following pharmacologic stimulation with phorbol ester and calcium ionophore (Figure 5). Peripheral blood CD4 cells from all eight subjects were obtained at baseline and after three CD4 infusions. The fraction of CD4 cells staining positive for IL-2, IL-4 and IFN- $\gamma$  secretion was determined, and we found there was a significant increase in the percentage of stimulated cells producing IL-2 ( $P=0.006$ ). On the day of harvest, the CD4+ cells spontaneously secreted high levels of cytokines, and four hours following infusion of the

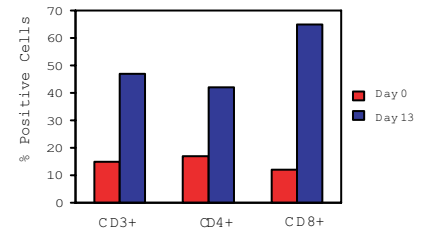


Figure 6a. Intracellular IFN- $\gamma$  production in NHL patient cells before and after in vitro stimulation with anti-CD3/anti-CD28 beads. T cells were examined for intracellular cytokine staining as described in Methods. The mean percent of IFN- $\gamma$  positive cells is shown, gated on CD3+, CD3+CD4+, or CD3+CD8+ cells.

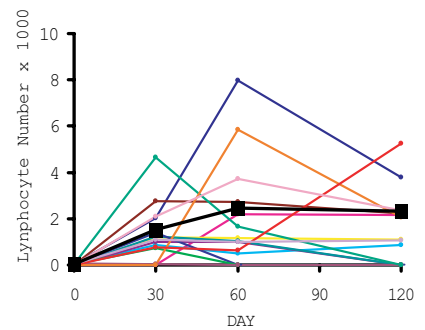


Figure 6b. Kinetics of lymphocyte reconstitution in NHL patients following Peripheral Blood Stem Cell autologous transplant and infusion of CD3/CD28 stimulated T cells. Results are shown for 15 patients who received T cells. Mean lymphocyte counts = black line, square symbols.

cells, there was an increase in systemic GM-CSF ( $P=0.02$ ) and IFN- $\gamma$  ( $P < 0.01$ ) levels noted in the subjects.<sup>21</sup>

Following CD4 cell reconstitution, we tested the mixed leukocyte reaction (MLR) as another measure of immune function, since patients with HIV infection have been shown to have impaired responses to alloantigens.<sup>25</sup> Seven of eight subjects developed an increased allo-MLR (baseline, 1210 cpm  $\pm$  390 sem; day 158, 3840 cpm  $\pm$  1060,  $P = 0.04$ ). Modestly increased proliferative responses were noted to the recall antigen, tetanus toxoid, in 4 of the 5 subjects tested (data not shown). Thus, activated CD4 cell infusions were associated with augmented IL-2 secretion, increased circulating levels of IFN- $\gamma$  and GM-CSF, and

increased proliferation to alloantigens.

We sought further evidence that the infused CD4 cells affected in vivo immune homeostasis by measuring HIV coreceptor CCR5 levels. We have previously shown that CCR5 surface expression and mRNA levels are dramatically downregulated in vitro following CD3/CD28 costimulation.<sup>6,7</sup>

In order to test the hypothesis that activated CD4+ T cell infusions would lead to decreased levels of CCR5 expression in vivo, CCR5 expression on CD4+ lymphocytes was determined in the subjects at baseline and on study day 158. Each of the subjects showed a substantial decrease in the percentage of CD4+CCR5+ cells ( $P=0.004$ ).<sup>21</sup> This result suggests that an HIV resistant state was conferred on the cellular immune system, which is consistent with a prolonged survival of the infused cells.

### Autologous Adoptive Transfer in Lymphoma

We explored the safety and feasibility of administering escalating doses of anti-CD3/CD28 ex vivo expanded and costimulated T cells as a therapeutic adjunct to patients who had experienced relapsed/refractory NHL following HDC and CD34+ selected PBPC.

Seventeen patients with a median of three prior chemotherapy regimens received cyclophosphamide (CY) and VP16, followed by GM-CSF plus G-CSF for mobilization. Immediately after leukapheresis, the PBSCs were CD34+ selected using the Baxter Isolex 300i

and then cryopreserved. Autologous peripheral blood mononuclear cells were collected by leukapheresis prior to mobilization, and stimulated using Dynal® magnetic beads coated with anti-CD3 and anti-CD28 (as described in Methods).

On day 14 after PBSC transplant, the magnetic beads were removed from the costimulated CD3+ cells and the cells were infused at the following doses: Dose Level 1:  $2 \times 10^9$  CD3+ T cells, Dose Level 2:  $5 \times 10^9$  CD3+ T cells, Dose Level 3:  $1 \times 10^{10}$  CD3+ T cells.

In vitro, a median 5.3 fold CD3+ T cell expansion was observed. For 13 cell cultures, the median CD4:CD8 ratio increased from 1.2 (pre-expansion) to 1.6 (post-expansion). The culture procedure reversed impaired cytokine responsiveness in T cells, in vitro, as evidenced by restored production of Th1 cytokines after expansion (Figure 6A).

Dose-dependent infusional toxicities were observed in 2 patients at dose level 3 were the only patients who experienced infusion-related toxicities that included transient fever, dyspnea, rigors and pulmonary edema. Maximal responses included 5 patients with complete remission, 7 patients with partial remission, and 5 patients with stable disease.

At a median follow-up of 474 days (range=105-1097), 5 patients are alive with stable or relapsed disease, and 3 patients remain in complete remission. Figure 6B shows the kinetics of lymphocyte reconstitution following costimulated T cell infusion in these patients. The levels of reconstitution are mark-

edly faster than previously reported T cell regeneration in adults without activated T cell infusion.<sup>26</sup>

### Summary and Implications

We have reported progress on the development of a potential new form of transfusion therapy for HIV and cancer. In HIV, we have engineered T cells to mimic a natural genetic lesion in the HIV co-receptor CCR5, which renders some people resistant to HIV infection. We recently published the first report of autologous transfers of pure CD4 T cells in 8 HIV+ study subjects.<sup>21</sup>

Safety was demonstrated in that HIV viral load did not increase in the study subjects. The CD4/CD8 ratios of most study subjects rose to the normal range following activated CD4 T cell infusions. This result was unexpected, given that only about 10% of the normal adult CD4 T cell number was infused. The cultured cells were resistant to reinfection with a lab strain of HIV and the study subject's own HIV strain.

A second clinical trial with HIV+ patients involved the reinfusion of CD3/CD28 stimulated T cells transduced via a retrovirus with a chimeric receptor. Results from this trial show that the transduced T cells persist in lymph nodes and blood for up to a year.<sup>27-28</sup> There was also evidence of T cell trafficking to rectal mucosa. Further, an adoptive transfer study in macaques demonstrated that CD3/CD28 stimulated PBMCs were able to effectively suppress SIV. In vivo, there was an increased anti-SIV CTL activity.<sup>29</sup>

Together, these studies point to the promise of costimulated T cells in adoptive immunotherapy for HIV. The recently completed NHL Phase I trial demonstrates that anti-CD3/CD28 costimulation: 1) is feasible in heavily pretreated patients with advanced NHL, 2) successfully expands human T cells, ex vivo, including CD4+ cells, 3) reverses cytokine activation deficits, in vitro, and 4) has minimal to moderate infusional toxicity of the costimulated T cells.

Intact CD4 T cell function appears to be critical for control of many chronic viral infections and cancers. Potential therapeutic approaches to augment HIV-specific CD4 T cells in people with chronic HIV infection can

**Table 1.<sup>30</sup> CD4 Counts following CD4 T cell infusions<sup>a</sup>**

Patient	Baseline	Infusion #1		Infusion #2		Infusion #3	
	Day 0	Day 7	Day 28	Day 7	Day 28	Day 7	Day 28
1	447	562	426	433	737	886	778
2	409	576	459	699	688	707	622
3	367	505	477	417	442	562	333
4	434	463	441	571	682	1282	1178
5	335	408	425	408	345	416	611
6	308	330	340	264	301	399	308
7	555	886	881	832	948	722	632
8	462	600	520	541	570	806	764
Mean (SEM)	415 (28)	541** (59)	496 (58)	521* (64)	589** (77)	723** (101)	653* (97)

<sup>a</sup>Patient CD4 counts at baseline, 1 and 4 weeks following the first three infusions.

\* $P < 0.05$  vs. baseline; \*\* $P < 0.01$  vs. baseline (student's t- test)

be envisioned using adoptive transfers of engineered T cells. In cancer, the goal is to use activated T cells to more quickly reconstitute the immune system of patients who have received chemotherapy or radiation, and to augment the immune response to any residual cancer cells.

CD3/CD28 stimulated T cells are also easily transduced with retroviral and lentiviral vectors. Clinical trials planned, and in progress, are using T cells as carriers to deliver potentially therapeutic payloads of genes. In this sense, T cells are superior drug delivery devices as they traffic to sites of infection and can persist, in vivo, for years. Along with our collaborators, our laboratory is now working on T cell cocktails of Ag-specific or enriched T cells, or T cells with a particular functional phenotype (Th1 vs Th2) that can be used as transfusion therapy for a wide variety of diseases.

## Acknowledgements

This work was the product of the supreme efforts of many, including: Dr. Naomi Aronson, Peggy Bennett, Dr. Wendy Bernstein, Dr. Richard Carroll, Julio Cotte, Nancy Craighead, Hannah Flaks, Tara Francomano, Brian Gregson, Brian Hudson, M.J. Humphries, Dr. Carl June (Program Director), Daniel Kim, David Kim, Dr. Silvia Kim, Dr. Alan Landay, Dr. Ginna Laport, Dr. David Liebowitz, Gil McCrary, Mary O'Connell, Stephen Perfetto, Dr. James Riley, David Ritchey, Dr. Katia Schlienger, Carolyn Small, Dr. Carolyn Steffens, Jennifer Tench, and Zoe Zheng. I would like to especially recognize the commitment of the study participants.

## References

1. Lenschow, D. J., T. L. Walunas, and J. A. Bluestone. 1996. CD28/B7 system of T cell costimulation. *Annu. Rev.Immunol* 14:233-258.
2. Thompson, C. B. and J. P. Allison. 1997. The emerging role of CTLA-4 as an immune attenuator. *Immunity* 7:445-450.
3. Levine, B. L., W. B. Bernstein, M. Connors, N. Craighead, T. Lindsten, C. B. Thompson, and C. H. June. 1997. Effects of CD28 costimulation on long-term proliferation of CD4+ T cells in the absence of exogenous feeder cells. *Journal of Immunology* 159:5921-5930.
4. Levine, B. L., Y. Ueda, N. Craighead, M. L. Huang, and C. H. June. 1995. CD28 ligands CD80 (B7-1) and CD86 (B7-2) induce long-term autocrine growth of CD4+ T cells and induce similar patterns of cytokine secretion in vitro. *Int.Immunol.* 7:891-904.
5. Levine, B. L., J. D. Mosca, J. L. Riley, R. G. Carroll, M. T. Vahey, L. L. Jagodzinski, K. F. Wagner, D. L. Mayers, D.

- S. Burke, O. S. Weislow, D. C. St.Louis, and C. H. June. 1996. Antiviral effect and ex vivo CD4+ T cell proliferation in HIV- positive patients as a result of CD28 costimulation. *Science* 272:1939-1943.
6. Carroll, R. G., J. L. Riley, B. L. Levine, Y. Feng, S. Kaushal, D. W. Ritchey, W. Bernstein, O. S. Weislow, C. R. Brown, E. A. Berger, C. H. June, and D. C. St.Louis. 1997. Differential regulation of HIV-1 fusion cofactor expression by CD28 costimulation of CD4+ T cells. *Science* 276:273-276.
7. Riley, J. L., R. G. Carroll, B. L. Levine, W. Bernstein, D. C. St.Louis, O. S. Weislow, and C. H. June. 1997. Intrinsic resistance to T cell infection with HIV type 1 induced by CD28 costimulation. *J.Immunol.* 158:5545-5553.
8. Rollins, B. J. 1997. Chemokines. *Blood* 90:909-928.
9. Cocchi, F., A. L. DeVico, A. Garzino-Demo, S. K. Arya, R. C. Gallo, and P. Lusso. 1995. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 270:1811-1815.
10. Autran, B., G. Carcelain, T. S. Li, C. Blanc, D. Mathez, R. Tubiana, C. Katlama, P. Debre, and J. Liebowitch. 1997. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 277:112-116.
11. Gulick, R. M., J. W. Mellors, D. Havlir, J. J. Eron, C. Gonzalez, D. McMahon, D. D. Richman, F. T. Valentine, L. Jonas, A. Meibohm, E. A. Emini, and J. A. Chodakewitz. 1997. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N.Engl.J.Med.* 337:734-739.
12. Hammer, S. M., K. E. Squires, M. D. Hughes, J. M. Grimes, L. M. Demeter, J. S. Currier, J. J. Eron, Jr., J. E. Feinberg, H. H. Balfour, Jr., L. R. Deyton, J. A. Chodakewitz, and M. A. Fischl. 1997. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. *N.Engl.J.Med.* 337:725-733.
13. Tozser, J. 2001. HIV inhibitors: problems and reality. *Ann.N.Y.Acad.Sci.* 946:145-159.
14. Richman, D. D., Bozette, S., Morton, S., Chien, S., Wrin, T., Dawson, K., and Hellmann, N. The Prevalence of Antiretroviral Drug Resistance in the US. Abstract of the 41st Annual Interscience Conference on Anti-Microbial Agents and Chemotherapy . 2001.
15. 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.
16. Fauci, A. S. 1996. Host factors and the pathogenesis of HIV-induced diseases. *Nature* 384:529-534.
17. Kalams, S. A. and B. D. Walker. 1998. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. *J.Exp.Med.* 188:2199-2204.
18. Greenberg, P. D. and S. R. Riddell. 1999. Deficient cellular immunity—finding and fixing the defects. *Science* 285:546-551.
19. Marincola, F. M., E. M. Jaffee, D. J. Hicklin, and S. Ferrone. 2000. Escape of human solid tumors from T-cell recognition: molecular mechanisms and func-

- tional significance. *Adv.Immunol* 74:181-273.
20. Sheu, B. C., S. M. Hsu, H. N. Ho, R. H. Lin, P. L. Torng, and S. C. Huang. 1999. Reversed CD4/CD8 ratios of tumor-infiltrating lymphocytes are correlated with the progression of human cervical carcinoma. *Cancer* 86:1537-1543.
21. Levine, B. L., W. B. Bernstein, N. E. Aronson, K. Schlienger, J. Cotte, S. Perfetto, M. J. Humphries, S. Ratto-Kim, D. L. Bircx, C. Steffens, A. Landay, R. G. Carroll, and C. H. June. 2002. Adoptive transfer of costimulated CD4+ T cells induces expansion of peripheral T cells and decreased CCR5 expression in HIV infection. *Nat.Med.* 8:47-53.
22. Levine, B. L., J. Cotte, C. C. Small, R. G. Carroll, J. L. Riley, W. B. Bernstein, D. Van Epps, R. A. Hardwick, and C. H. June. 1998. Large scale production of CD4+ T cells from HIV-1 infected donors following CD3/CD28 costimulation. *Journal of Hematotherapy* 7:437-448.
23. Fischl, M. A., D. D. Richman, N. Hansen, A. C. Collier, J. T. Carey, M. F. Para, W. D. Hardy, R. Dolin, W. G. Powderly, and J. D. Allan. 1990. The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection. A double-blind, placebo-controlled trial. The AIDS Clinical Trials Group. *Ann.Intern. Med.* 112:727-737.
24. Roederer, M. 1995. T-cell dynamics of immunodeficiency. *Nature Medicine* 1:621-627.
25. Clerici, M., N. I. Stocks, R. A. Zajac, R. N. Boswell, D. R. Lucey, C. S. Via, and G. M. Shearer. 1989. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J.Clin.Invest* 84:1892-1899.
26. Mackall, C. L., F. T. Hakim, and R. E. Gress. 1997. Restoration of T-cell homeostasis after T-cell depletion. *Semin.Immunol.* 9:339-346.
27. Mitsuyasu, R. T., P. A. Anton, S. G. Deeks, D. T. Scadden, E. Connick, M. T. Downs, A. Bakker, M. R. Roberts, C. H. June, S. Jalali, A. A. Lin, R. Pennathur-Das, and K. M. Hege. 2000. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. *Blood* 96:785-793.
28. Walker, R. E., C. M. Bechtel, V. Natarajan, M. Baseler, K. M. Hege, J. A. Metcalf, R. Stevens, A. Hazen, R. M. Blaese, C. C. Chen, S. F. Leitman, J. Palensky, J. Wittes, R. T. Davey, Jr., J. Fallow, M. A. Polis, J. A. Kovacs, D. F. Broad, B. L. Levine, M. R. Roberts, H. Masur, and H. C. Lane. 2000. Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection. *Blood* 96:467-474.
29. Villinger, F., G. T. Brice, A. E. Mayne, P. Bostik, K. Mori, C. H. June, and A. A. Ansari. 2002. Adoptive transfer of simian immunodeficiency virus (SIV) naive autologous CD4(+) cells to macaques chronically infected with SIV is sufficient to induce long-term non-progressor status. *Blood* 99:590-599.
30. Table 1 and Figs 2-5 were adapted from reference 21.