Poster #392

# No Changes in Immune Activation Phenotypes, Response to Antigen, nor CD8+ T-cell Mediated HIV Suppression in Transplant Recipients Receiving Immunosuppression

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## Background

- HIV infection is associated with loss of "naïve" T-cells, activation of "memory" T-cells, increases in soluble activation markers, loss of CD4+ T-cell proliferative responses to recall antigens and T-cell mitogens, and loss of responses to antigen by activated CD8+ T-cells.
- Cyclosporine (CsA) may reduce immune activation-associated HIV pathogenesis when used as post-transplant immunosuppression. If so, such an effect is hypothesized to be beneficial in delaying HIV disease progression.
- Previous studies showed no change in immune activation markers with low dose CsA; however, substantial CD4+ T-cell increases were seen in primary HIV infection treated with CsA, at levels typically used in transplant, plus HAART.
- We evaluated cellular and soluble markers of immune activation, naïve and memory phenotypes, T-cell responses to antigen, and CD8+ T-cell non-cytotoxic anti-HIV response (CNAR) in HIV+ transplant recipients receiving CsA.
- Immunosuppression might inhibit protective immune responses against HIV and co-pathogens; conversely, it might reverse the immunopathology associated with HIV disease.
- We hypothesized that post-transplant immunosuppression would be associated with changes in markers of immune function and activity that are not associated with HIV disease progression and that its use would be safe in HIV-infected transplant recipients.

## Methods

### T-Cell Phenotyping by Flow Cytometry

- Naïve (CD45RA+CD62L+) CD4+ and CD8+ T-cells were analyzed by 4-color flow cytometry on a Beckman Coulter Epics XL flow cytometer, using FITC-conjugated anti-CD45RA, PE-conjugated anti-CD62L, CY5-conjugated anti-CD3, and ECD-conjugated CD4+ or CD8+ cells.
- Memory/effector (CD45RA-CD62L+/CD45RA+/-CD62L-) CD4+ and CD8+ T-cells were analyzed using FITC-conjugated anti-CD45RA, PE-conjugated anti-CD62L, CY5-conjugated anti-CD3, and ECD-conjugated CD4+ or CD8+ cells.
- Activated (CD38+ and/or HLA-DR+) CD4+ and CD8+ T-cells were detected with FITC-conjugated anti-HLA-DR and PE-conjugated anti-CD38 in combination with the CD3+, CD4+ and CD8+ antibodies described above.
- Absolute numbers of each subpopulation were determined by multiplying the fractional percentage of each by the absolute number of CD3+ T cells/mL.

### Soluble Activation Markers

- Neopterin and Beta-2-microglobulin were measured using enzyme immunoassay test kits (ALPCO Diagnostics, Windham, NH) performed according to the manufacturer's instructions.

### Intracellular Cytokine Flow Cytometry (CFC)

- CD4+ and CD8+ T-cell response directed against superantigen Staphylococcal enterotoxin B (SEB) used as a positive control and CMV lysate (sucrose density gradient-purified virus preparation from human CMV strain AD169-infected human foreskin fibroblase cultures —Advanced Biotechnologies, Inc., Columbia, MD). A non-stimulated negative control was used to determine the level of spontaneous activation. Activated cells were fixed and lysed before incubation with FITC-conjugated anti-IFNgamma, PE-conjugated anti-CD69, PECy5-conjugated anti-CD4, and APC-conjugated anti-CD3. The fraction of activated, cytokine-secreting (CD69+IFNgamme+) CD4+ and CD8+ T lymphocytes were determined by flow cytometry on a BD Biosciences FACSCalibur.

### CD8+ T-cell non-cytotoxic anti-HIV response (CNAR)

- Peripheral blood mononuclear cells (PBMC) were isolated by ficoll-hypaque gradient, frozen and stored in liquid nitrogen. CNAR was measured by mixing patient's CD8+ cells with normal CD4+ cells infected with a chemokine insensitive HIV-1 strain (SF33) "in vitro" at a 1:1 cell ratio. HIV replication was measured by reverse transcriptase (RT) activity. Percent suppression was calculated by comparing the average RT in CD4+ cells grown alone with the average RT of the CD8+:CD4+ cell co-culture. Reduction in RT activity >50% is considered positive and over 90% is strongly positive.

## Results

9 of 17 transplanted subjects were studied pre-transplant and at >/= 2 of the following time points: weeks 2, 4, 12, 28 and 52 post-transplant.

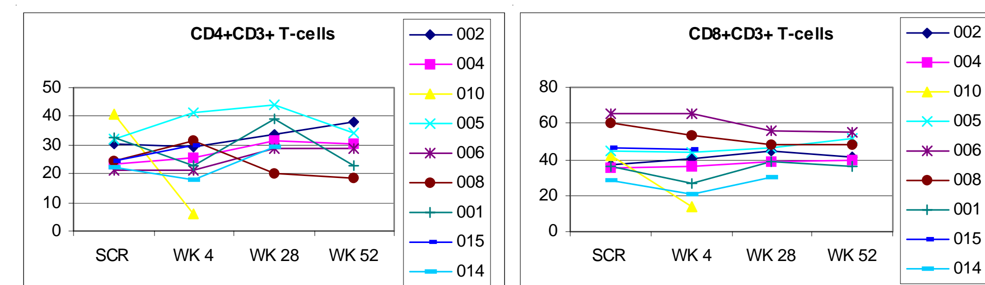
### Kidney and Liver Transplant Recipients

| Organ | ID | Sex | Age | Race | Indication | Donor [1] | Pre-Tx CD4 | Latest CD4 | ARVs | Rejections | Survival Days[2] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| K | 002 | M | 45 | AA | HTN | LR | 366 | 429 | 3TC, ABC, NFV | 2 | 991 |
| K | 004 | M[3] | 53 | AA | HTN/HIVAN | CAD | 334 | 423 | 3TC, ABC, NVP | 1 | 863 |
| K | 010 | M | 45 | C | Diabetes | LR | 480 | 221 | 3TC, ABC, NVP | 1* | 517 |
| K | 005 | M[3] | 38 | C | Diabetes | HR | 407 | 249 | 3TC, ABC, NFV(--> NFP) | 1 | 909 |
| K | 006 | F[3] | 44 | AA | HTN/HIVAN | HR | 422 | 853 | 3TC, ddI ( --> ABC), NVP | 0 | 910 |
| K | 008 | M[3] | 49 | C | HTN/HIVAN | HR | 583 | 130 | 3TC, ddI, NFV, IDV --> 3TC, D4T, NFV, EFV | 1* | 743 |
| L | 001 | M[3] | 15 | L | HCV | LR --> CAD | 973 | 200 | 3TC, D4T, ddI, NFV (intermittent) | 0 | died day 445 |
| L | 015 | M | 54 | A | HBV | CAD | 175 | 330 | 3TC, ABV, NVP, IDV | 0 | 535 |
| L | 014 | M | 48 | C | HBV | LR | 439 | 305 | 3TC, D4T, ddI, SQV, DLV, NVP -> CBV, NFV, NVP | 0 | 627 |

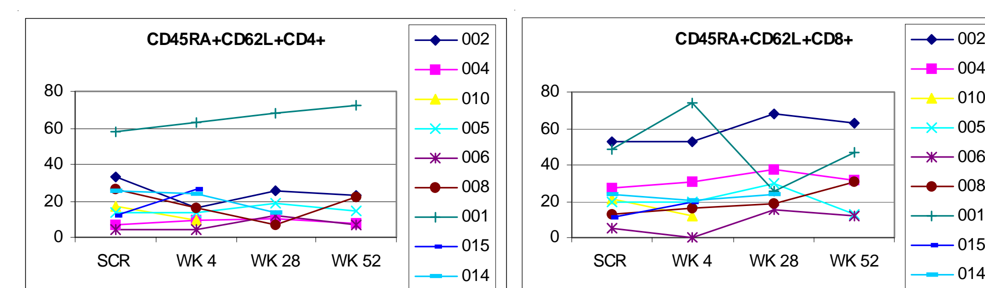
[1]LR=living related; LUR = living unrelated; CAD=cadaveric; HR=high risk cadaver
[2]Survival days as of 1/8/2003
[3] Also Included in CNAR Analysis
*Treated with antibody therapy

### T-Cell Phenotyping by Flow Cytometry

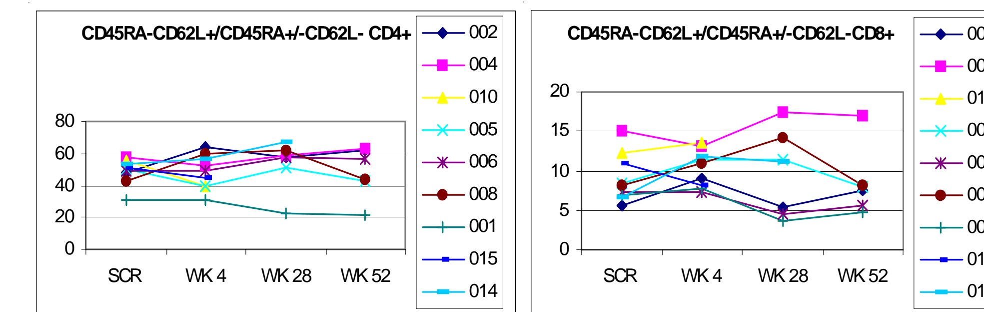
No changes were observed in the percentages of CD4+CD3+ (p=.9) or CD8+CD3+ T-cells (p=.12).



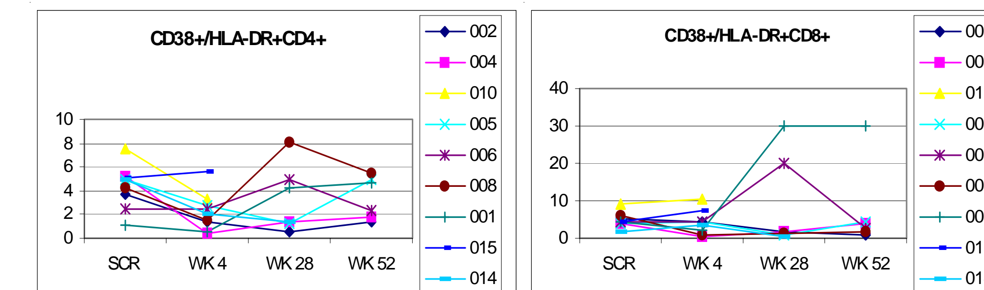

***Naïve phenotype:*** No changes were observed in the percentages of CD45RA+CD62L+ CD4+ (p=.4) and CD8+ T-cells (p=0.3).




***Memory/Effector Phenotype:*** No changes were observed in the percentages of CD45RA- CD62L+/CD45RA+/-CD62L- CD4+ (p=.53) and CD8+ T-cells (p=.17).



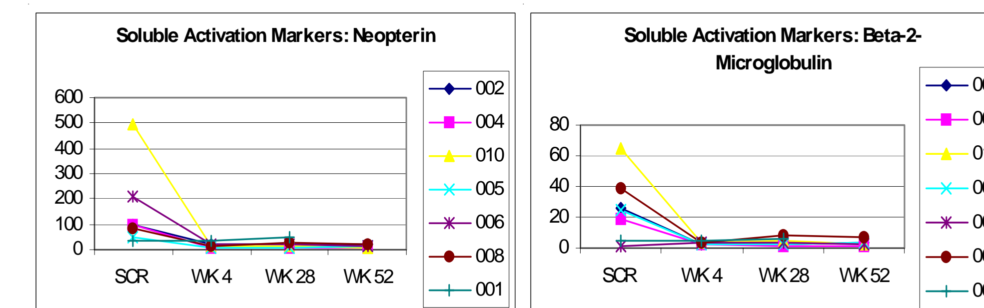

***Cellular Activation Markers:*** No changes were observed in the percentages of CD38+ and/or HLA-DR+ CD4+ and CD8+ T-cells (all p > .4).




At week 4, CD38+/DR+ CD4+ T-cells decreased (p <.001), but this change did not persist.

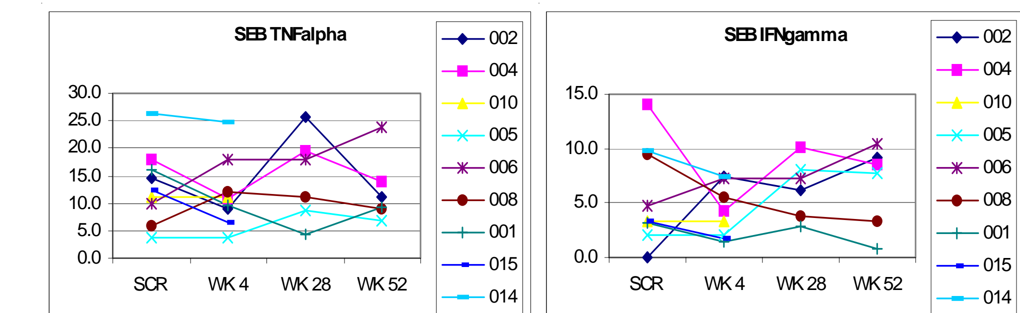
### Soluble Activation Markers

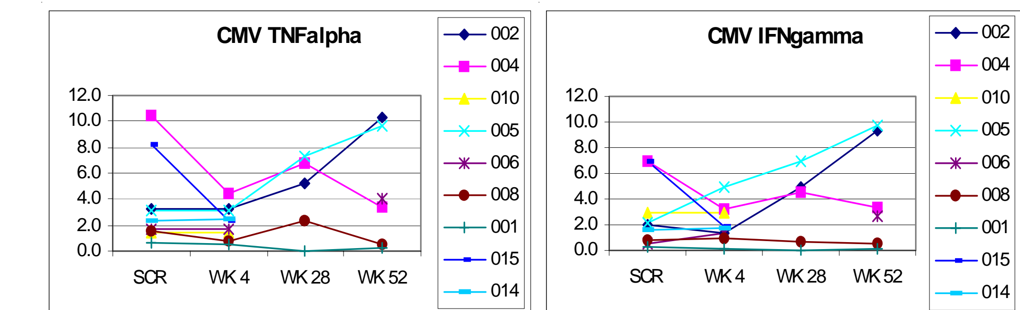
***Neopterin:*** Significant decreases were observed in neopterin (p= .0018) and beta-2-microglobulin (p= .0004) over time.




### Intracellular Cytokine Flow Cytometry (CFC)

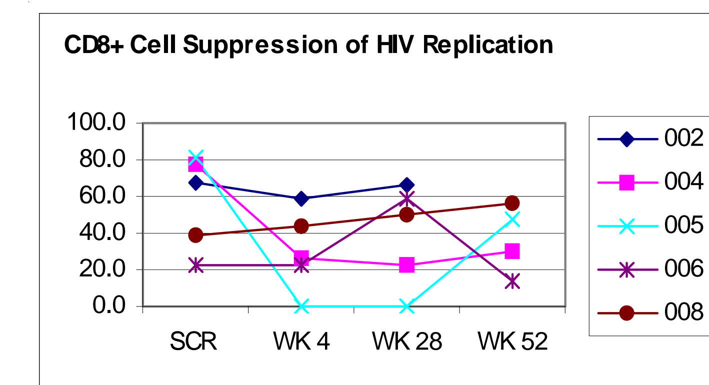
No changes were observed in the ability of CD4+ T-cells to produce TNFalpha or IFNgamma in response to stimulation by SEB or CMV antigens (all p>.5).




### CD8+ T-cell non-cytotoxic anti-HIV response (CNAR)

- 5 subjects who completed week 52 were evaluated for CNAR. There were no consistent patterns of CNAR activity either pre-transplant, or post-transplant compared to pre-transplant.
- The studies were limited by poor cell viability and recovery after cryopreservation.




## Conclusions

- Except for a transient and expected decrease in activated CD4+ T-cells, no statistically significant changes were observed in cellular markers of immune activation or function measured over 1 year in CsA-treated HIV+ transplant recipients.
- Soluble immune activation markers did decrease as expected.
- Clinical outcomes (survival, opportunistic infections, CD4 T-cell counts, HIV RNA levels) in transplant recipients have been excellent.
- In this small group of HIV-infected transplant recipients, cyclosporine use appears to be safe.
- Future research involves the evaluation of these measures in a larger cohort of patients and evaluation of associations between changes in markers and clinical outcome.

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