Knockdown of CCR5 expression in antigen-specific cytotoxic CD4 T cells

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Introduction

- Approximately half of human memory CD4 T cells are CCR5+.
- Therefore they are susceptible to HIV-1 infection.
- Long-term non-progressors (LTNP), and especially Elite Controllers (EC), have large populations of HIV-specific CD4 T cells.
- In one EC subject, 5% of CD4 were HIV-specific CD4 T cells.
- These cells were CCR5+ cytotoxic T lymphocytes (CTL) effector CD4 T cells, but they were highly proliferative.
- LTNP may also have HIV-specific CD8 T cells, but so can progressors.
- HIV appears to be able to relatively easily escape CD8 responses.
- Except CD8 responses in the context of certain HLA Class I alleles (B27 and B57) that target highly conserved epitopes that can only be changed with significant fitness cost.
- Similarly, HIV appears to be able to relatively easily escape neutralizing antibody responses.
- Can we make HIV-resistant, Gag-specific CD4 CTL in large numbers for cell therapy?
- Can we combine gene therapy with cell therapy, using shRNA to knockdown CCR5 in antigen-specific CD4 T cells?

Aims

- We investigated whether we can isolate viable antigen-specific CD4 T cells using the OX40 assay to transduce them with shRNA for CCR5, then expand them in vitro.
- We began this work with antigen-specific CD4 T cells in PMBC samples from healthy adult controls.

Methods

- 30 to 40 x 10^6 PBMC from healthy HIV-uninfected adults were incubated for 48hr with recall antigens, either CMV lysate for CMV seropositive, or tetanus toxoid (TT) for vaccinated individuals.
- Antigen-specific CD4 T cells were identified by up-regulation of both CD25 and CD134, referred to as OX40+ cells (see Fig 2).
- Antigen-specific OX40+ cells were then cell sorted on a FACSria.
- Importantly, we cell sorted CD25+CD134+ cells that were negative for CD39, to avoid antigen-specific Tregs, and were rested overnight in IL-2 containing medium, prior to transduction with lentivirus.
- VSV-G pseudotyped lentivirus containing shRNA specific for CCR5 (under control of the H1 promoter) and e-GFP (under the control of the ubiquitin C promoter) was spinoculated onto a RetroNectin-coated 96-well plate.
- 10,000 to 30,000 antigen-specific OX40+ (CD39 negative) CD4 T cells were incubated with spinocultured lentivirus for 3 days with IL-2.
- Transduction efficiency was checked by fluorescence microscopy using GE Cytell Cell Imaging System.
- Cells were transferred to cultures with IL-2 and feeder cells (either purified autologous B cells, or irradiated autologous PBMC, or with autologous monocyte-derived dendritic cells (moDC)).
- Cells were grown for a further 4-6 weeks, with additional IL-2 every three days and feeder cells and antigen every 2 weeks.
- GFP+ cells were purified by cell sorting, typically at 3-4 weeks.
- GFP+ cells were further phenotyped for CCR5 expression, as well as for the presence of cytotoxic effector molecules, Granzymes A and B.

Results: continued

Transduction and expansion of CMV-specific OX40+ cells

(a) Subject C031
Day 3
Day 20
Post GFP+ sort
(b) Subject C002
Day 3
Day 20
Post GFP+ sort
Day 35

Fig. 3 Representative transductions, expansion and purification of GFP+ CMV-specific OX40+ CD4 T cells. At day 3, transduction efficiency was typically 5-10% of CD4 T cells

Fig. 4 – Expansion of CMV-specific GFP+ OX40+ CD4 T cells for 3 different donors

Fig. 5 – Representative flow plots showing that GFP+ CD4 T cells (prior to cell sorting to purify GFP+ cells) are negative for CCR5 expression, compared to untransduced GFP-negative cells

Conclusions

- The results show that it is possible to isolate viable antigen-specific CD4 T cells and genetically modify them to knockdown CCR5.
- The expanded GFP+ CD4 T cells were CCR5-negative and had a cytotoxic T lymphocyte phenotype.
- The best feeder cells were monocyte-derived DC, but the transduction efficiency and cultures need to be improved to produce more cells.
- Experiments are currently underway to confirm resistance to HIV infection in vitro.
- It appears to be feasible to produce autologous HIV-resistant cytotoxic CD4 T cells that are HIV Gag-specific which may help control viral replication in the absence of ART.
- Similarly, it may be also possible to produce HIV-resistant CD4 T cells specific for other antigens, such as to boost immunity to TB infection, if immune reconstitution after ART is not sufficient.

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