Phenotypic characterisation of virus-specific T cells in treated HIV-1 infection: Profiling total and multimer-specific CD8 T cells

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Introduction

Maturation and activation of T cells is critical for effective immune control of viruses including HIV-1. In patients with established HIV-1 infection, a skewed maturation/differentiation profile has been linked to viral pathogenesis and is not restored by effective combination (c)ART (1, 2). Furthermore, chronic immune activation is one of the strongest predictors of HIV-1disease progression (3). In HIV-1 negative individuals, asymptomatic CMV is associated with higher T-cell activation (4). cART reduces T-cell activation, however little is known about its effect on CD8 T-cell maturation. We evaluated the magnitude and characteristics of both HIV-1- and CMV-specific T-cell responses in order to further elucidate the effect of cART on virus-specific T-cell differentiation and activation.

Methods

• Activation (HLA-DR/CD38) and maturation (CD45RA/CCR7) profiles of total CD8⁺ T cells from 31 cART-treated HIV-1⁺ CMV⁺ co-infected patients (median 420 CD4 T cells/μl blood) and 5 HIV-1 negative healthy controls for comparison, were examined ex-vivo. This involved surface staining of peripheral blood mononuclear cells (PBMC) with monoclonal antibodies: CD3, CD8, CD45RA , CCR7, CD38 and HLA-DR for flow cytometric analysis.

• PBMC of 7 HIV-1⁺ CMV⁺ patients were also stained with HA9-B*3501 pentamer, TM10-B*0702 pentamer (all ProImmune Ltd, Oxford, UK) and TM10-B*0702 dextramer (Immudex, Copenhagen, Denmark) in order to identify antigen-specific CD8⁺ T cells.

• Non-parametric intergroup analysis was performed using Mann-Whitney U test, and paired data was analysed by Wilcoxon signed-rank test, with significance defined as $p<0.05$.

References

1. Champagne *et al.* (2001) *Nature*, **410**,106-111
2. Migueles *et al.* (2009) *J Virol*, **83**,11876-11889
3. Hazenberg *et al.* (2003) *AIDS*, **17**,*1881-1888*
4. Hunt *et al.* (2011) *J Inf Dis*, **203**,1474-1483
5. Champagne *et al.* (2001) *Nature*, **410**,106-111
6. Carrington and Walker (2012) *Annu Rev Med*, **63**, 131-145

Gating strategy — **Differentiation CMV-specific** — **Differentiation HIV-1-specific** — **Activation CMV-specific**

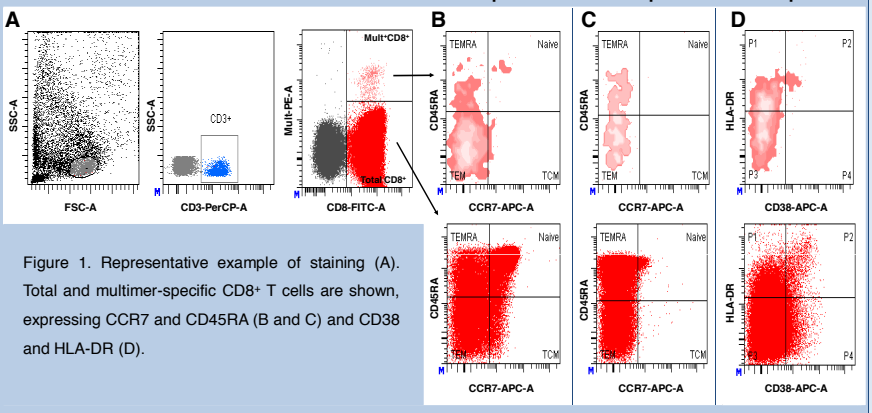


Figure 1. Representative example of staining (A). Total and multimer-specific CD8⁺ T cells are shown, expressing CCR7 and CD45RA (B and C) and CD38 and HLA-DR (D).

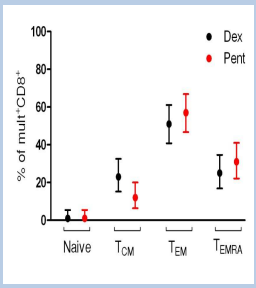
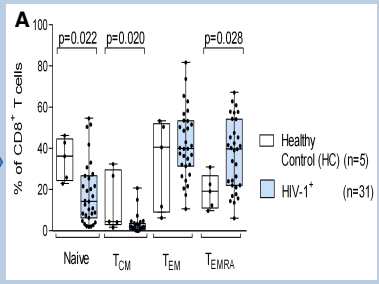
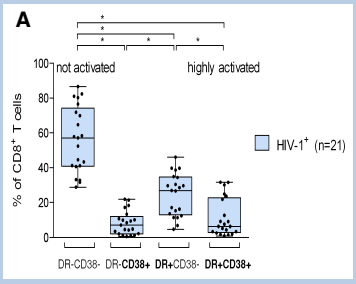


Figure 2. Comparison of the proportion of multimer⁺CD8⁺ memory subsets observed using dextramer and pentamer technologies in parallel. The two technologies were comparable. Symbols represent percentage distribution for each technology and error bars indicate 95% confidence intervals.

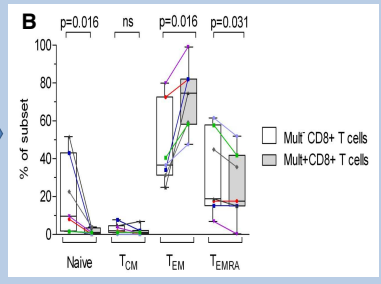
Differentiation — **Activation**

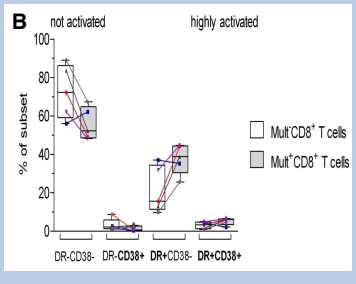
Total CD8⁺ T cells





CMV TM10-specific CD8⁺ T cells





TEM to TEMRA ratio

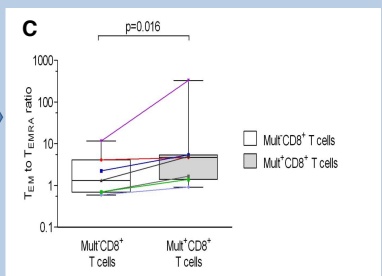


Figure 3. Differentiation profile of total CD8⁺ T cells in HIV-1⁺ patients (A; blue bars) and healthy controls (A; white bars) and mult⁻CD8⁺ T cells (white bars) and mult⁺CD8⁺ T cells (grey bars) in HIV-1⁺ patients (data from B and C). Each coloured line represents one individual.

Figure 4. Activation levels of total CD8⁺ T cells (A), and mult⁻CD8⁺ T cells and mult⁺CD8⁺ T cells (B). Data from HIV-1⁺ patients. Each coloured line represents one individual.

Results

Analysis of multimer-specific CD8⁺ T cells and their differentiation profile (Figure 1 A , B and C) showed that the two multimer technologies were comparable (Figure 2). HIV-1⁺ patients showed a significant reduction in naïve and central memory (T_{CM}) compartments (p=0.022 and 0.020 respectively), and a significant increase in terminally differentiated (T_{EMRA}) subset within total CD8⁺ T cells (p=0.028), compared to healthy controls (Figure 3 A). CMV TM10-specific CD8⁺ T cells had a significantly higher T_{EM}:T_{EMRA} ratio (median= 4.65), compared to the rest of the CD8 T-cell pool (median= 1.32; p=0.016; Figure 3 B and C). The majority of HIV-1⁺ individuals had a low proportion of CD8⁺ T cells that were highly activated (Figure 4 A). There was no significant difference in the activation levels between CMV TM10-specific CD8⁺ T cells and total CD8⁺ T cells (Figure 4 B).

Conclusions

• Maturation profiles of total and multimer-specific CD8⁺ T cells indicate a shift towards the T_{EM} and T_{EMRA} subset.

• Although CMV-specific CD8⁺ T cells have been shown to be predominantly of the T_{EMRA} subset in untreated HIV-1⁺ individuals (5), here we show that in cART-treated HIV-1⁺ patients CMV-specific CD8 T cells are primarily at the effector memory (T_{EM}), stage of differentiation.

• There is no difference in the activation level of CMV TM10-specific CD8⁺ T cells compared to total CD8⁺ T cells , even in the context of cART.

Future Studies

Further studies aim to look at the role of different HLA alleles on the specificity and avidity of T-cell immune responses and how this affects HIV-1 disease progression (6).

Acknowledgments

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