HIV-1 Integration Sites in Macrophages and CD4⁺ T Cells are Distinct

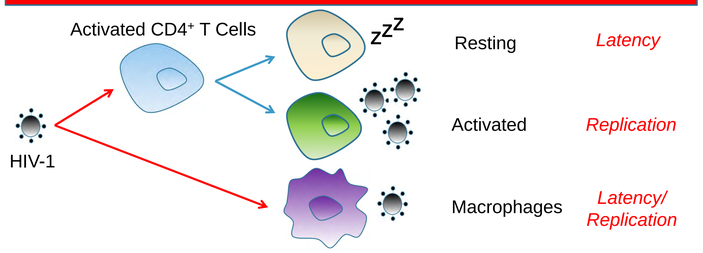
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BACKGROUND



- The integration sites (IS) of HIV-1 provirus in the human genome have a major impact on its fate, *i.e.* replication or latency.
- Despite macrophages being an important cellular target of HIV-1, IS analysis of macrophages has been relatively under-examined.
- We compared and characterized 1,484 HIV-1 IS in treated HIV-1+ patients´ monocyte-derived macrophages (MDMs) and activated CD4⁺ T cells infected *ex vivo* with the patients´ autologous primary HIV-1 isolates or clonal strains within the same experimental settings.

METHODS

Patients (Zurich Primary HIV Infection Study)
(≥ 2 years of ART)
↓
Peripheral Whole Blood
↓
CD8⁺ T Cells Depletion
↓
MACS
↓
CD14⁺ Monocytes — CD4⁺ T Cells
Differentiation — *Activation*
MDMs — Activated CD4⁺ T Cells
↓
Infection with Patients´ Autologous HIV-1 or Clonal Strains
↓
Genomic DNA Extraction and Amplification of Integration Sites
(Non-Restrictive Linear Amplification-Mediated PCR)
↓
Illumina MiSeq DNA Sequencing
↓
Filtering and Trimming of Sequencing Reads
↓
Mapping to the Human Genome &
Clustering of Integration Sites with DAVID

DAVID: Database for Annotation, Visualization and Integrated Discovery

RESULTS

Figure 1: HIV-1 favours integration into transcription units, especially introns, regardless of cell type.

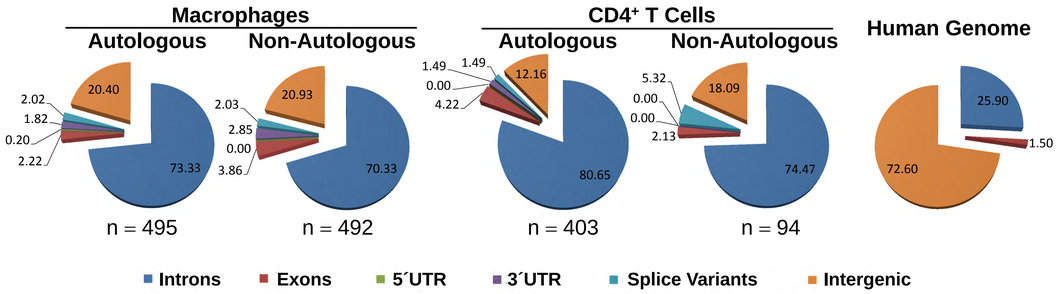


Figure 2: A weakly conserved palindromic sequence and an AT-rich region are observed upstream of the HIV-1 provirus.

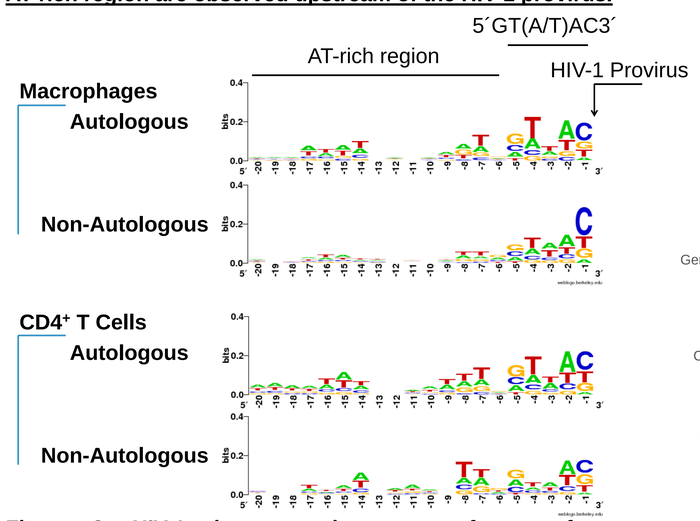


Figure 3: HIV-1 does not have a preference for any transcription orientation relative to the gene in which it is integrated.

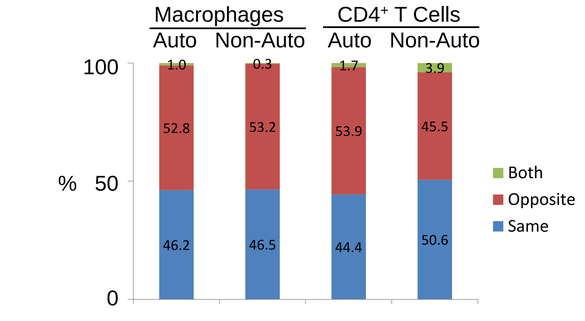
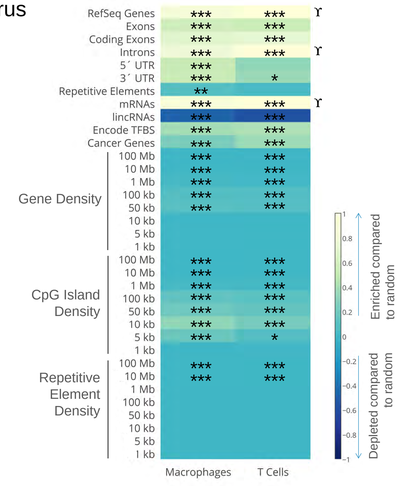


Figure 4: Similar genetic features between HIV-1 IS in macrophages and CD4⁺ T cells.



Introns are slightly less favoured by HIV-1 in macrophages compared to CD4⁺ T cells. There was no significant difference between autologous and non-autologous HIV-1 in both cell types. ***p<0.0001, **p<0.01 and *p<0.05 compared to random. ϒ Macrophages p<0.001 and OR<-0.2 compared to CD4⁺ T cells.

Figure 5: HIV-1 favours integration into gene-dense regions.

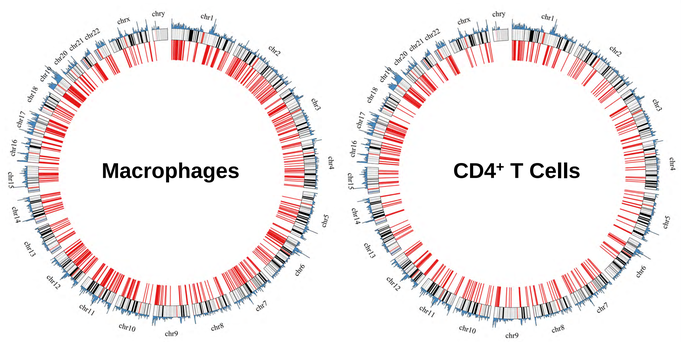
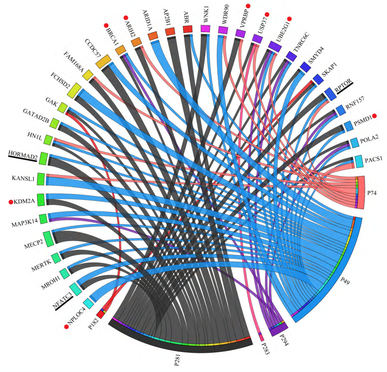


Figure 6: Macrophages have a substantially higher percentage of cluster overlaps compared to between cell types and between CD4⁺ T cells.

Cell Type – HIV-1	Top 40 DAVID Clusters Overlap (%)	Cell Type – HIV-1
Macrophages - Autologous	47.5	Macrophages – Non-Autologous
	32.5	CD4⁺ T cells – Autologous
	37.5	CD4⁺ T cells – Non-Autologous
Macrophages – Non-Autologous	37.5	CD4⁺ T cells – Autologous
	40.0	CD4⁺ T cells – Non-Autologous
CD4⁺ T cells – Autologous	32.5	CD4⁺ T cells – Non-Autologous

Intragenic HIV-1 IS were analysed with DAVID Functional Annotation Clustering at high stringency.

Figure 7: HIV-1 Hotspots



A total of 33 genes in which ≥3 unique HIV-1 IS were found in different *ex vivo* infected patient samples and/or cell types are shown. Red dots indicate genes that formed an enriched cluster (p<0.0001) associated with protein catabolic processes with DAVID Functional Annotation Clustering analysis. Comparison with the studies of Wagner *et al.* (2014) and Maldarelli *et al.* (2014) showed that 3 hotspots (underlined) were found in clonally expanded CD4⁺ T cells *in vivo*.

CONCLUSIONS

- HIV-1 has specific requirements for integration into the human genome as evidenced by the overall similar genetic surroundings of HIV-1 provirus in macrophages and CD4⁺ T.
- The repertoire of genes accessible for HIV-1 integration appear to be more restricted in macrophages, and they are substantially distinct from those of CD4⁺ T cells.
- Nonetheless, macrophages and CD4⁺ T cells share some common hotspots for HIV-1 integration.

ACKNOWLEDGEMENTS: We would like to thank all our colleagues from the Infectious Diseases Department of University Hospital Zurich and Institute of Medical Virology of University of Zurich, and all the patients from the Zurich Primary HIV Infection Study.