1. ABSTRACT

Follicular Helper T Cells Serve as the Major CD4 T-Cell Compartment for HIV-1 Infection, Replication and Production

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Background: Lymphoid organs are the primary anatomical compartments for both the generation of the immune response and for HIV replication and spreading. T follicular helper cells (Tfh) reside within the germinal centers (GCs) and are specialized in providing B-cell help, express the transcription factor BCL-6, the chemokine receptor CXCR5, ICOS and PD-1. Recently, studies have shown an expansion of Tfh cells in HIV and Simian Immunodeficiency Virus (SIV) infection and that Tfh are susceptible to HIV infection. However, limited data are available on the HIV infection of Tfh cells and their role as potential reservoirs for HIV.

Methods: We have investigated the distribution of HIV-specific and HIV-infected CD4 T-cells within different populations of memory CD4 T-cells isolated from lymph nodes of 23 subjects with chronic HIV infection with CD4 T-cell count >400 per mm3 and plasma HIV RNA levels >5000 copies per mL, from 14 subjects with undetectable plasma viremia (<20 HIV RNA copies per mL) after 72 weeks of ART, from 3 subjects with nonprogressive HIV disease, i.e. long-term non progressors (LTNP) and low plasma HIV viremia levels and from 13 HIV negative subjects.

Results: Four memory CD4 T-cell populations were identified on the basis of the expression of CXCR5 and PD-1: CXCR5+PD-1-, CXCR5+PD-1+, CXCR5-PD-1- and CXCR5-PD-1+. CD4 T cells. On the basis of Bl-6 expression, IL-21 production and functional properties (stimulating B cells production from germinal center B cells), CXCR5+PD-1+ CD4 T-cell population was considered to correspond to the Tfh-cell population. We showed that: 1) Tfh and CXCR5+PD-1+ CD4 T-cell populations produced significantly (P=0.0007) higher levels of IL-21 as compared to CXCR5-PD-1- and CXCR5-PD-1+ CD4 T cells, 2) CXCR5-PD-1+ CD4 Tfh T-cell population produced significantly (P=0.001) higher levels of HIV as compared to the three other memory CD4 T-cell populations, 3) Tfh and CXCR5+PD-1+ CD4 T-cell populations are enriched in HIV-specific CD4 T-cells (P=0.03), 4) Tfh cell population contained the highest percentage (5%) of CD4 T cells harboring HIV DNA, 5) Tfh cells were the most efficient CD4 T-cell population in supporting HIV production when isolated from subjects with high HIV viremia (>10000 HIV RNA copies) and low HIV viremia (<3000 HIV RNA copies) and 6) the only percentage of Tfh cells correlated with the levels of plasma viremia (R=0.815, P=0.002).

Conclusion: These results demonstrate that the Tfh cells serve as the major CD4 T-cell compartment for HIV infection, replication and production.

2. MATERIALS & METHODS

Phenotypic and functional analyses of CD4+ T cell populations including cytokine production and proliferation of lymph node mononuclear cells from chronically HIV infected viremic subjects prior to and after ART and from healthy subjects were assessed using flow cytometry. Cells were stained with a panel of antibodies including CD45RA, CD3, CD4, CD8, CXCR5, PD-1, ICOS and BCL-6 and Ki-67 for phenotypic analysis. Cytokine (IL-2, IL-21, IFN-γ and TGF-β) production was determined using intracellular cytokine staining and cell proliferation using the CFSE flow cytometry assay. Identification of HIV-specific CD4 T-cells was performed using peptide pools derived from different HIV proteins. Phenotypic analysis of B cells was performed by flow cytometry using CD20, CD27, CD80 and I-A antibodies. T cell help to B cells was determined in T-B cocultures evaluating the induction of Ig production. HIV DNA within different cell populations was assessed using a Real Time PCR assay targeting the gag gene and HIV production using an in vitro virus isolation assay. The efficiency in supporting HIV replication and production was evaluated after performing virus isolation or HIV infection in vitro. Determination of cell proliferation, quantification of HIV DNA, HIV production and induction of Ig production was assessed on sorted purified cell populations.

3. RESULTS

Figure 1. Phenotype of CD4 T cell subsets in lymph nodes of untreated HIV-infected individuals.

Figure 2. Relative frequency of CD4 T cell subsets in lymph nodes of healthy versus HIV-infected individuals.

Figure 3. Correlation of Tfh cells frequencies and GC-B cell frequencies and HIV plasma viremia.

Figure 4. Frequencies of B cell populations in lymph nodes of HIV-negative, viremic untreated and treated HIV-infected subjects.

Figure 5. Enrichment of HIV infected cells within the Tfh cell subset.

Figure 6. Enrichment of HIV infected cells within the Tfh cell subset.

Figure 7. HIV replication and production by the different lymph node memory CD4 T cells subsets.

4. CONCLUSIONS

Our findings show that Tfh cells:

1. are defined by a high expression level of CXCR5 and PD-1 as such Bl-6 and ICOS,
2. support the production of Igs from GC-B cells,
3. are expanded during the viremic phase,
4. are enriched in HIV-specific CD4 T-cells,
5. are enriched in HIV infected cells during the viremic phase of HIV infection,
6. are the most efficient cells in supporting virus replication and production.

Taken together, these results show that Tfh cells are a cellular compartment of HIV infection, replication and production.

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