IL-15 induces ID2 to drive differentiation and effector function of CD8 TTM and TEM cells while maintaining TCM pools

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Abstract

Effector memory T cell differentiation is a critical step for the development of effective immunity to viral infections. ID2 has been shown in murine models to play a crucial role in the effector/effector memory differentiation by regulating the expression of anti-apoptotic genes as well as genes involved in cell cycle entry and progression. Here we show that ID2 and its target genes are predominantly expressed in human EM and EMRA and poorly expressed in CM. Silencing of ID2 impacts T cell survival, proliferation and differentiation. ID2 expression is regulated by TCR engagement as well as by IL-15 signaling. IL-15 is the most effective common g-chain cytokine in terms of upregulating ID2 expression and inducing the proliferation and survival of CD8+ EM cells. IL-15 induced proliferation of all memory subsets from healthy subjects while inducing differentiation, GzmB production, and cytotoxic effector function of CD8+ TM and EM cells. Stimulation of CD8+ cells from HIV infected subjects with peptide plus IL-15 induced ID2 expression and differentiation of tetramer+ TM cells, and restored their HIV-specific cytotoxic activity. These results provide the rationale for use of agonists of ID2 such as IL-15 in immunotherapies aimed at HIV eradication.

Introduction

Memory T cells can be found at different stages of differentiation that are distinguished by the expression of several surface markers (CCR7+, CD27+ and CD28+). Among memory CD8+ T cell subsets, central memory T cells (CM) show the greatest memory cell potential in terms of longevity, and proliferative capacity as compared to transitional memory (TM), effector memory T cells (EM) and terminally differentiated effector (EMRA) cells.

CD8+ effector cell development is regulated at the transcriptional level by the coordinated impact of several transcription factors such as T-bet, Blimp-1, inhibitor of DNA binding 2 (Id2) and STAT-4. In contrast, the expression of genes such as FOXO1, TCF7, Eomesodermin (Eomes), BCL-6, Id3 and STAT3 maintains the quiescent long term CM phenotype and prevents the terminal differentiation of these cells to effector T cells.

Id2 belongs to the helix-loop-helix (HLH) protein family that plays a key role in the differentiation of a number of cell lineages. Knockout studies in murine models have demonstrated that CD8+ effector cells from Id2 -/- mice are more susceptible to apoptosis when compared to CD8+ T cells from Id2+/+ wild-type mice. Several reports have now shown that Id2 is required for the development of short-lived effector memory CD8+ T cells after infection.

Effector CD8+ T cell differentiation is impaired in chronic viral infections, such as in HIV and this defect has been associated to failure to control of viral replication and dissemination. In this study we have investigated the role of Id2 in regulating differentiation of memory CD8 + T cells and the impact of gamma-chain(γ-C) cytokines on Id2 control of an effector function that is capable of eliminating virally infected cells.

Id2 is upregulated on effector cells CD8+ T cells.

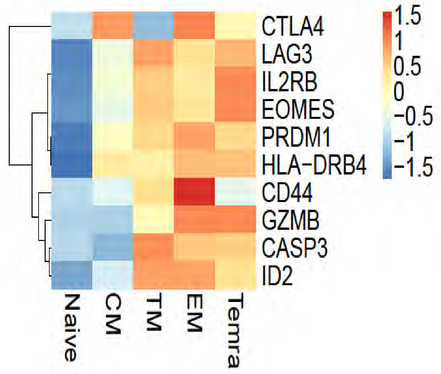


Figure 1. CD8+ T cell subsets were sorted from total PBMCs obtained from healthy volunteers for naïve, CM, TM, EM and EMRA subsets. Gene expression heat map of genes compared across CD8+ naïve and memory T cell subsets (N=5).

TCR triggering leads to Id2 upregulation.

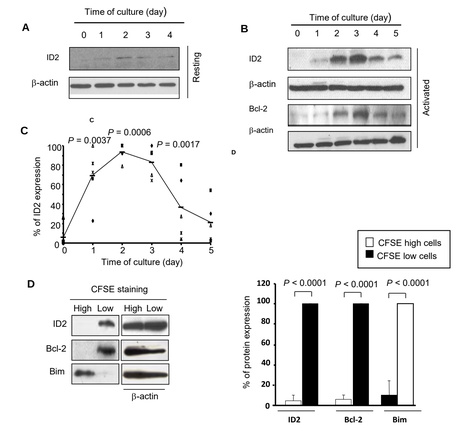


Figure 2. Memory CD8+ T cells were activated or not for 1 to 5 days with anti-CD3 and anti-CD28; **A)**kinetics of ID2 expression on resting or on **B)** activated cells by WB assays and **C)** the graph represents the densitometric quantifications of the ID2. **D)** Memory CD8+ T cells were activated for 3 days and sorted for CFSE low and CFSE high populations; **D)** bands and densit. data of Id2, Bcl-2, Bim and b-actin expression by WB

Id2 shut-down led to significant decrease of Bcl-2 proteins and reduced survival, proliferation and differentiation

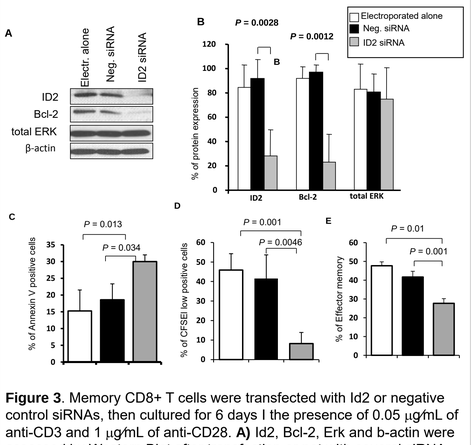


Figure 3. Memory CD8+ T cells were transfected with Id2 or negative control siRNAs, then cultured for 6 days I the presence of 0.05 μg⁄mL of anti-CD3 and 1 μg⁄mL of anti-CD28. **A)** Id2, Bcl-2, Erk and b-actin were assessed by Western Blot after transfection or not with several siRNA. **B)** Densitometric analysis was performed using ImageQuant software (N = 5). Involvement of ID2 in activated and transfected memory CD8+ T-cell populations on different cell properties: **C)** on survival, **D)** on proliferative capacity, and **E)** frequency of effector memory cells (N=5).

IL-15 upregulates ID2 and its target genes involved in differentiation and effector functions in TM an EM subsets

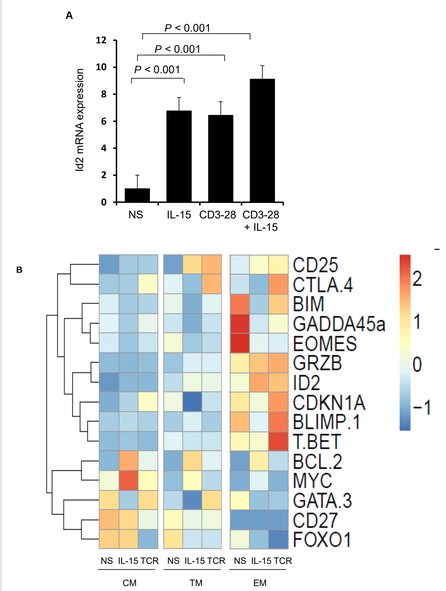


Figure 4. A= Id2 mRNA levels (fold increase) of total memory CD8+ T cells was assessed at 12h in unstimulated cells or after activation in the presence or absence of IL-15 (10ng/ml) (N=3). Sorted CM, TM and EM cells from healthy subjects were treated in the presence or absence of IL-15 and presence or not of anti CD3/CD28 antibodies. **B)**= RTq-PCR gene expression heatmap of z-score transformed gene expression [Avg –Fold Change] across a vector of CM, TM and EM samples with or without IL15 or anti CD3/CD28. Red squares corresponds to expression higher than the mean and blue corresponds to expression below the mean of the gene vectors (N=3).

IL-15 induces proliferation and survival of each memory subsets and differentiation of TM and EM

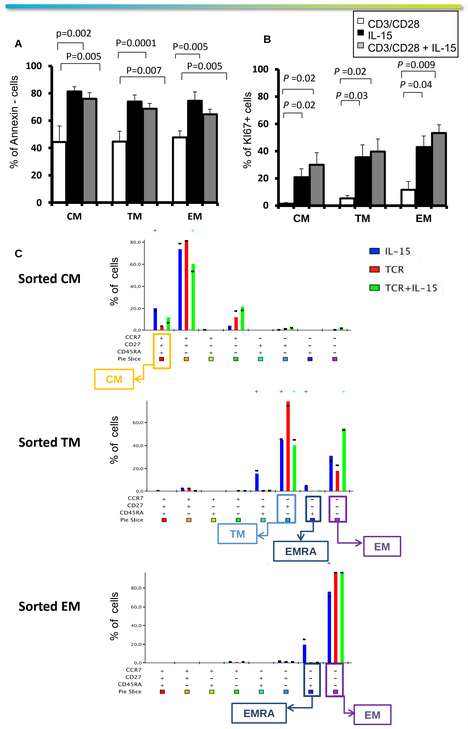


Figure 5. A) Percentage of Annexin V- cells at 6 days of culture of each memory CD8+ T cell subsets; **B)** Percentage of proliferation (Ki67); **C)** phenotype of the cells was measured by CD27, CCR7 and CD45RA expression after 6 days of culture by SPICE analysis (N=6).

IL-15 induces granzyme B production in TM and EM cells and restores HIV-specific CD8+ T cell effector function.

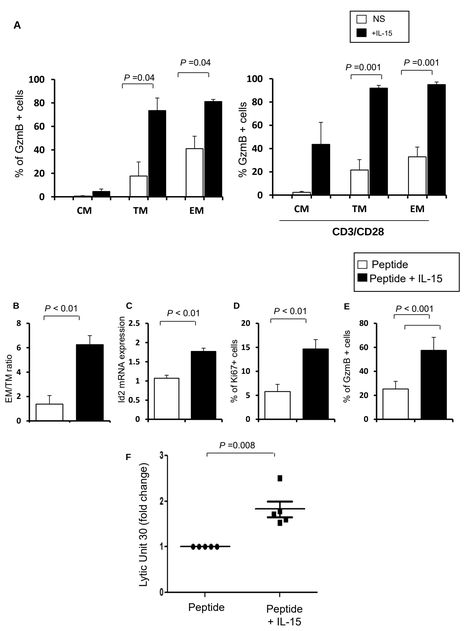
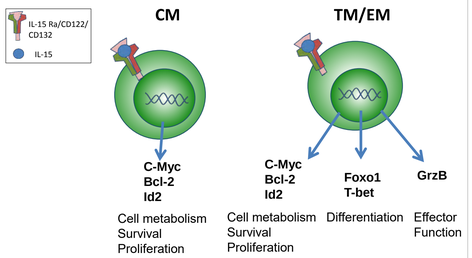


Figure 6. **A)** GzmB production was assessed after 6 days of culture on CM,TM and EM. PBMCs from HIV+ patients were stimulated in presence of specific HIV-peptide and in presence or absence of IL-15 (10ng) (N=6). After 6 days of culture **B)** the EM/TM ratio on tetramer + cells was measured by CD27 and CCR7 expression; **C)** Id2 mRNA levels **D)** Ki67+ cells and **E)** GzmB were assessed on tetramer+ cells by flow cytometry. **F)** Cytolytic activity was measured by flow cytometry on tetramer + cells.

Conclusions



- ID2 and its target genes are more highly expressed in human TM and EM while being poorly expressed in CM.
- Silencing of ID2 impacts on T cell survival, proliferation and differentiation.
- ID2 expression was regulated by TCR but also by IL-15.
- IL-15 induced proliferation of all memory subsets while inducing differentiation, GzmB production, and cytotoxic effector function of CD8+ TM and EM cells.
- Stimulation of HIV-specific CD8+ T cells with peptide plus IL-15 induced ID2 expression and differentiation of tetramer+ TM cells, and restored their HIV-specific cytotoxic activity.

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