

Relationship between Perforin Expression and Degranulation Activity in Virus-specific CD8 T-Cells

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ABSTRACT

Background: Perforin expression and degranulation activity, as measured by CD107a mobilization, are currently used to define cytotoxic memory CD8 T cells. Limited information is available on the relationship between these markers.

Methods: A variety of virus-specific CD8 T cell responses including CMV (n=14), EBV (n=7), Flu (n=5) and HIV-1 from both progressors and LTNP (n=11) were identified using peptide-MHC tetramer complexes and analyzed for proliferation capacity, expression of perforin and CD127 (i.e. IL-7R α) and CD107a mobilization. CCR7 and CD45RA were used to distinguish T cell populations at different stages of differentiation.

Results: Combined expression of perforin and CD127 revealed the existence of 3 populations of virus-specific CD8 T cells: CD127⁺perforin⁻ cells that represented the large majority (90%) of Flu-specific CD8 T cells; CD127⁺perforin⁻ cells that were the majority (64%) of EBV-specific CD8 T cells; and CD127⁺perforin⁻ cells that contained 43% of CMV-specific CD8 T cells. HIV-1-specific CD8 T cells from progressors were mostly CD127⁺perforin⁻ cells while LTNP had significantly more HIV-1-specific CD127⁺ CD8 T cells (21 vs 7%, P<0.01). Progressors had more perforin⁺ cells than LTNP (10 vs 4%). Combined staining with CCR7 and CD45RA showed that CD127⁺perforin⁻, CD127⁺perforin⁻ and CD127⁺perforin⁻ were CD45RA⁺CCR7⁻, CD45RA⁺CCR7⁺ and CD45RA⁻CCR7⁺ CD8 T cells, respectively. Ag-specific proliferating CD8 T cells were contained within the CD127⁺perforin⁻ cells population. Comparison of perforin expression in tetramer⁺ CD8 T cells and CD107a mobilization following Ag stimulation for the different viruses showed a discrepancy between these two markers. In particular, high levels of CD107a mobilization were observed despite the absence of perforin expression. Of interest, 7 days after *in vitro* Ag-specific stimulation, there was a substantial increase in the CD127⁺perforin⁻ cell subset in all virus-specific CD8 T cell responses: Flu: from 2 to 42%; EBV: from 3 to 45%; CMV: from 43 to 60%; HIV: from 10 to 25%; (all P<0.05).

Conclusions: These data indicate that perforin and CD107a mobilization represent independent markers of CD8 T cells with potential cytotoxic capacity. In addition, perforin expression but not CD107a mobilization correlated with the stage of differentiation of memory CD8 T cells.

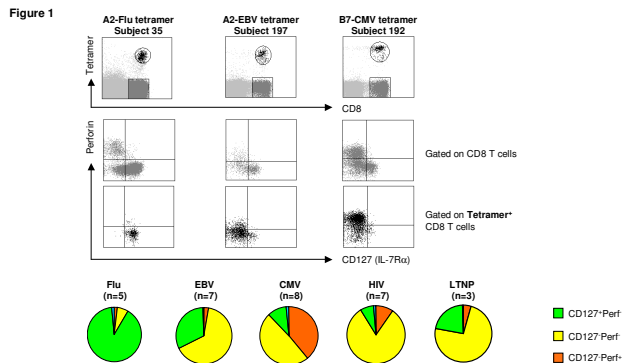
INTRODUCTION AND GOAL

A key effector function of CD8 T cells is their cytolytic activity toward infected cells. Perforin expression and degranulation activity, as measured by CD107a mobilization, are currently used to define cytotoxic antigen-experienced CD8 T cells but limited information is available on the relationship between these markers. Furthermore, a recent study performed in mice has shown that the selective expression of the interleukin-7 receptor alpha (IL-7R α , CD127) identifies effector CD8 T cells that give rise to long-lived Ag-specific T-cells.

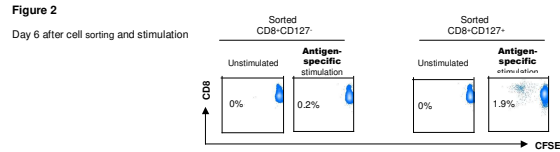
In this study, we have compared perforin expression and degranulation activity in a large number of virus infections including Influenza (Flu), CMV, EBV and HIV-1 in both progressive and non-progressive (i.e. in LTNP) infections. In addition, we have investigated the relevance of CD127 expression on virus-specific CD8 T cells in human and, in particular, the ability of this marker to identify cells with proliferative capacity.

RESULTS

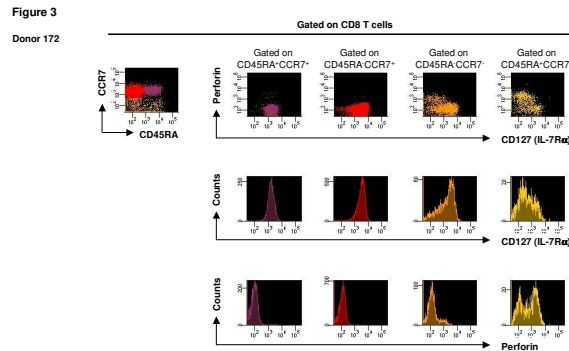
Combined expression of perforin and CD127 revealed the existence of 3 populations of virus-specific CD8 T cells: CD127⁺perforin⁻ cells that represented the large majority (90%) of Flu-specific CD8 T cells; CD127⁺perforin⁻ cells that were the majority (64%) of EBV-specific CD8 T cells; and CD127⁺perforin⁻ cells that contained 43% of CMV-specific CD8 T cells. HIV-1-specific CD8 T cells from progressors were mostly CD127⁺perforin⁻ cells while LTNP had significantly more HIV-1-specific CD127⁺ CD8 T cells (21 vs 7%, P<0.01) (Fig. 1).



We have then evaluated the ability of CD127 to identify virus-specific CD8 T cells endowed with proliferation capacity. For this purpose, CD127⁺ and CD127⁻ CD8 T cells were sorted and stimulated. Of interest, only CD127⁺ sorted cells showed significant proliferation (one representative example is shown in Fig. 2) demonstrating that Ag-specific proliferating CD8 T cells were contained within the CD127⁺ cells population.

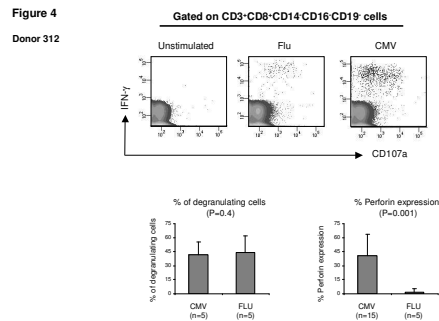


We have then evaluated the differentiation/maturation state of the three different virus-specific CD8 T cell populations identified by CD127 and perforin. For this purpose, we have combined the staining with CCR7 and CD45RA. This showed that CD127⁺perforin⁻, CD127⁺perforin⁻ and CD127⁺perforin⁻ were CD45RA⁺CCR7⁻, CD45RA⁺CCR7⁺ and CD45RA⁻CCR7⁺ CD8 T cells, respectively (Fig. 3)

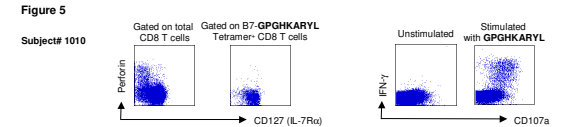


Degranulation following Ag stimulation was then evaluated for the different viruses and showed that, in contrast to perforin expression, high levels of CD107a mobilization were observed in situations, e.g. Flu infection, where perforin expression was lacking (Fig. 4).

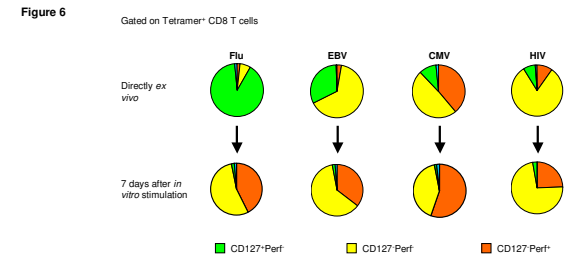
The direct comparison of perforin expression in tetramer⁺ CD8 T cells and CD107a mobilization following stimulation showed a discrepancy between these two markers. In particular, no difference was observed between Flu- and CMV-specific CD8 T cells with regards to the percentage of degranulating cells (P=0.4) while the percentage of perforin expression was significantly different between these two responses (P=0.01) (Fig. 4).



In addition, the discrepancy between degranulation activity and perforin expression was also confirmed within the same individual (Fig. 5). This subject, i.e. patient 1010, has a documented HIV-1 infection since about 5 years (March 1999). He was treated with antiviral therapy at the time of primary infection and remained on therapy for 18 months. He interrupted therapy spontaneously in December 2000. During the last 4 years he constantly had levels of viremia < 50 HIV-1 RNA copies/ml and CD4 T cell count in the range of 1400 cells/ml. Patient 1010 had a dominant B7-restricted gp response to GP98KARVL (Fig. 5).



Finally, we have investigated whether following prolonged stimulation (7 days after *in vitro* Ag-specific stimulation), there was a substantial increase in the level of perforin expression. Of interest, we observed a significant increase in the CD127⁺perforin⁻ cell subset in all virus-specific CD8 T cell responses: Flu: from 2 to 42%; EBV: from 3 to 45%; CMV: from 43 to 60%; HIV: from 10 to 25%; (all P<0.05) (Fig. 6).



CONCLUSIONS

In conclusion, we have demonstrated a discrepancy between the degranulation activity, as measured by the CD107a mobilization following stimulation, and the expression of perforin measured on Tetramer-positive CD8 T cells. Furthermore, we have shown that combined staining of perforin and CD127 identified three subsets of virus-specific CD8 T cells: a) CD127⁺perforin⁻ cells that represented the large majority (90%) of Flu-specific CD8 T cells; b) CD127⁺perforin⁻ cells that were the majority (64%) of EBV-specific CD8 T cells; and c) CD127⁺perforin⁻ cells that contained 43% of CMV-specific CD8 T cells. Furthermore, we have shown that virus-specific proliferating CD8 T cells were contained within the CD127⁺ cells population

In the situation of virus clearance, i.e. Flu infection, the majority of the virus-specific CD8 T cells lacked the expression of perforin but were almost all (90%) CD127⁺. Of note, after 7 days of culture, Flu-specific CD8 T cells were 42% perforin⁺.

In contrast, in the model of virus persistence and high viral load, i.e. HIV-1 infection, the majority of the cells expressed perforin but lack the proliferative capacity. Finally, in the model of protracted and chronic virus infection and low viral load, i.e. EBV infection or non-progressive HIV-1 infection (LTNP), the majority of the cells lacked perforin expression *ex vivo* but significantly increased perforin expression after *in vitro* stimulation.

Overall, these data suggest that proliferation and cytotoxicity represent independent functions and that the balance between proliferation and cytotoxicity is influenced by the virus load.