

Functional Signatures of Antiviral T Cell Immunity as A Measure of Virus Replication Activity and of Virus-Associated Disease

Alexandre Harari, Cristina Cellerai, Miguel Garcia, Pierre-Alexandre Bart and Giuseppe Pantaleo

Laboratoire d'Immunopathologie du SIDA, Service d'Immunologie et Allergie, CHUV, Lausanne



ABSTRACT

Background: Antiviral T-cell responses are functionally and phenotypically heterogeneous. Despite the large number of studies in this field, it has been difficult to correlate different patterns of antiviral T cell responses with virus-associated disease activity.
Methods: PBMC from 25 subjects were stained with CD3, CD8, CCR7, CD45RA, CD27, CD28, CD7, CD127, CD57 or MHC Class I tetramers specific for HIV-1, CMV, EBV and Influenza (Flu). Furthermore, intracellular IFN-γ and IL-2 secretion, proliferation and cytotoxicity measured by the degranulation assay were assessed following stimulation with virus-derived peptides. Flu-infection was considered as a model of virus elimination, CMV and EBV as models of chronic controlled infection and HIV-1 as a model of uncontrolled virus infection.
Results: A large number of CD8 T cell populations, i.e. up to 24 populations, were identified using CCR7 and CD45RA, CD27, CD28, CD7, CD127 or CD57. More importantly, no correlation was found between phenotypically defined CD8 T cell populations and virus replication and virus-associated disease activity. In contrast, when cytokines secretion, proliferation and degranulation/cytotoxicity were evaluated, three functionally distinct CD4 and CD8 T cell populations were identified. In particular, based on IL-2 and IFN-γ secretion, virus elimination was associated with a dominant IL-2 functional signature, virus persistence with control replication with a polyfunctional (IL-2 plus IFN-γ) signature, and virus persistence with non-controlled virus replication with a dominant IFN-γ functional signature. Proliferation capacity was substantially impaired with non-controlled virus replication while the extent of degranulation in CD8 T cells was similar in both controlled and non-controlled virus infections.
Conclusions: These results clearly demonstrate the lack of overlap between the different surface markers commonly used and more importantly that functional and not phenotypic signatures can be a mirror of virus replication activity and a novel tool for monitoring virus-associated disease.

INTRODUCTION AND AIMS

There is an urgent need to identify correlates of protection to evaluate the effectiveness of antiviral agents and, more importantly, of vaccine-induced T cell responses. Despite extensive investigations in this field, very few correlates of protection have been identified. T cell responses can be characterized according to phenotypic markers, i.e. the expression of surface markers, or according to their functional activities. A phenotypic heterogeneity of antigen-specific T cell responses has already been shown and many markers are used to identify memory T cells. Many of these markers are used indiscriminately to identify and characterize naive, effector and memory T cell subsets although the degree of overlap between the different markers has never been extensively investigated. Along the same line, a functional heterogeneity has also been shown and new markers have recently been identified. Of note, the function of virus-specific CD8 T cells against Cytomegalovirus (CMV), Epstein Barr virus (EBV), Influenza (Flu) and HIV-1 was recently analyzed on the basis of the ability of CD8 T cells to secrete IFN-γ and IL-2 and to proliferate (Zimmerli et al. PNAS 2005 and poster #404P). A selective defect of IL-2 secreting CD8 T cells was only found in subjects with progressive HIV-1 infection while the frequency of IFN-γ secreting CD8 T cells was similar within the different virus-specific responses. In this study, we have investigated:
 1) The degree of overlap between different surface markers of CD8 T cell subsets;
 2) The degree of overlap between different surface markers of virus-specific CD8 T cell subsets;
 3) The relevance of the analysis of functional patterns rather than phenotypic patterns.

RESULTS AND DISCUSSION

Degree of overlap between different surface markers of CD8 T cell subsets

Previous studies have shown that several subsets of CD8 T cells can be identified using combinations of different surface markers, basically CD45RA and CCR7 or CD27 and CD28. As shown in Figure 1, four distinct subsets of CD8 T cell were identified using either CD45RA and CCR7 or CD27 and CD28. Based on previous studies, CD45RA+CCR7- CD8 T cells are naive cells, CD45RA+CCR7+ CD8 T cells are T central memory cells, CD45RA-CCR7- CD8 T cells are effector memory cells and CD45RA-CCR7+ CD8 T cells are terminally differentiated T cells. Along the same line, CD28-CD27+CD8 T cells are naive cells or antigen experienced cells in an early stage of differentiation, CD28+CD27+CD8 T cells are intermediate-differentiated and CD28+CD27-CD8 T cells are late-differentiated cells. Of note, CD28-CD27- CD8 T cells, which represent 10-15% of total CD8 T cells (Figure 1), were poorly investigated. We then combined these four markers (i.e. CD45RA, CCR7, CD28 and CD27) to investigate the degree of overlap between each of them on CD8 T cells. As shown in Figure 1, we could identify more than 10 distinct CD8 T cell populations. We then further characterized the different T cell subsets by combining with an additional marker, such as CD7, CD127 or CD57, and observed an even more complex sub-division. Actually, up to 16-24 phenotypically distinct populations were identified (Figure 2). These data suggest that there is a very poor redundancy between each of these markers at

Figure 1

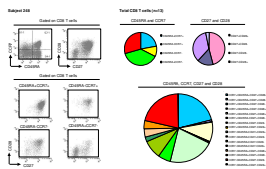
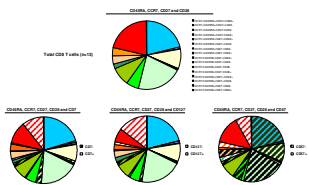


Figure 2



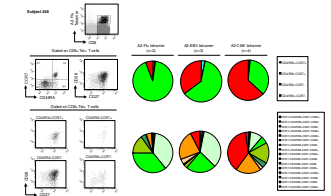
Degree of overlap between different surface markers of virus-specific CD8 T cell subsets

We then performed the same type of analysis on virus-specific CD8 T cells identified by peptide-MHC class I Tetramer complexes. Tetramers were generated for Influenza (Flu), Epstein Barr virus (EBV) and Cytomegalovirus (CMV) peptides. Staining of Tetramer+ CD8 T cells with CD45RA and CCR7 showed that CD45RA-CCR7- and CD45RA+CCR7+ were the dominant populations while CD45RA-CCR7+ and CD45RA-CCR7- were poorly represented (Figure 3). Of note, CD45RA-CCR7- were almost absent in Flu-specific CD8 T cells while it represented the majority of CMV-specific CD8 T cells. These data suggest that the phenotypic heterogeneity is reduced in the context of virus-specific CD8 T cells as compared to total CD8 T cells. Of interest, when we combined these stainings with CD27 and CD28, we observed the same phenomenon as in total CD8 T cell population: most of the subsets, included the ones which were very homogeneous (i.e. the CD45RA-CCR7+), were further sub-divided and we could identify up to 10 phenotypically distinct populations of virus-specific CD8 T cells. These data suggest that even on antigen-experienced CD8 T cells, there is a very poor degree of overlap between each of the above mentioned markers.

Degree of overlap between different surface markers of virus-specific CD8 T cell subsets

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Figure 3



Relevance of the analysis of functional patterns rather than phenotypic patterns

We then characterized the functional profile of virus-specific CD8 T cells on different models of virus infection: Flu-infection was considered as a model of virus elimination, CMV and EBV as models of chronic controlled infection and HIV-1 as a model of uncontrolled virus infection (data on HIV-1 specific CD8 T cell responses are not shown here, please see poster # 404P). For this purpose, we simultaneously evaluated cytokine secretion, proliferation and degranulation. As expected, we observed also some heterogeneity regarding the cytokine secreting CD8 T cell populations. There were populations producing only IFN-γ while others were also able to produce IL-2 (Figures 4 and 5). In this regard, we recently shown (Zimmerli et al. PNAS 2005 and poster # 404P) that CD8 T cell proliferation capacity is associated with the ability to secrete IL-2 following specific stimulation. Furthermore, when we evaluated the expression of CD107a, i.e. the marker of the function of degranulation, we observed a further sub-division of the functionally distinct populations (Figures 4 and 5). Of interest, we observed that about 50% of the virus-specific single IFN-γ secreting and of the IFN-γ/IL-2 secreting CD8 T cell populations were also able to degranulate, i.e. expressed CD107a following specific stimulation (Figures 4 and 5). Unfortunately, degranulation was not assessed on HIV-1-specific CD8 T cells. Finally, we have compared, for each virus-specific CD8 T cell response, i.e. Flu, EBV and CMV, the phenotypic patterns and the functional patterns. As shown in Figure 6, the high level of heterogeneity observed with the different surface markers, i.e. more than 10 distinct populations with poor or unknown relevance, was contrasting with the identification of the four functionally distinct cell subsets with known functions.

Figure 4

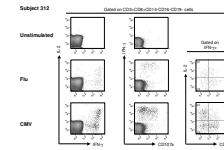
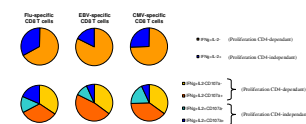


Figure 5



CONCLUSIONS

A large number of CD8 T cell populations, i.e. up to 24 populations, were identified using CCR7, CD45RA, CD27, CD28, CD7, CD127 or CD57. Furthermore, a large phenotypic heterogeneity was also observed for virus-specific CD8 T cells. More importantly, no correlation was found between phenotypically defined CD8 T cell populations and virus replication and virus-associated disease activity. In contrast, when cytokines secretion, proliferation and degranulation/cytotoxicity were evaluated, four functionally distinct CD8 T cell populations were identified. In particular, based on IL-2 and IFN-γ secretion, virus elimination and virus persistence with controlled virus replication were associated with a polyfunctional (IL-2 plus IFN-γ) signature, and virus persistence with non-controlled virus replication with a dominant IFN-γ functional signature. Proliferation capacity was substantially impaired with non-controlled virus replication while the extent of degranulation in CD8 T cells was similar in both controlled and non-controlled virus infections. These results clearly demonstrate the lack of overlap between the different surface markers commonly used and more importantly that functional and not phenotypic signatures can be a mirror of virus replication activity and a novel tool for monitoring virus-associated disease.

Figure 6

