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CUTTING EDGE

Cutting Edge: Emergence of CD127\textsuperscript{high} Functionally Competent Memory T Cells Is Compromised by High Viral Loads and Inadequate T Cell Help

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In this report we have inspected whether difficulties in controlling viral infections negatively impacts the generation of CD127\textsuperscript{high} memory T cells. Using both MHC class I and II tetramers we reveal that CD127\textsuperscript{low} T cells are not necessarily rapidly deleted but can persist in a pseudoeffector state in which they display the hallmarks of activated effector cells but are functionally inferior. CD127\textsuperscript{high} cells can, however, emerge if the infection is contained. We also show that in the absence of CD4 T cell help significant populations of CD127\textsuperscript{high} CD8 T cells fail to emerge. Analyses of cytokine-producing activities by both mouse and human CD8 T cells further document that the extended maintenance of T cells in a CD127\textsuperscript{low} state has functional consequences which manifest as an impairment of IL-2 production. The Journal of Immunology, 2005, 174: 5926–5930.

Effective T cell responses are pivotal for controlling many intracellular pathogens and contribute to immunological protection during secondary infections. Although these responses can be highly potent, qualitative differences between Ag-specific T cells have been observed. In the case of chronic viral infections, T cells can emerge which are incapable of elaborating the optimal array of functions necessary for controlling the infection (1–4). MHC class II I-Ab LCMV gp61 tetramers were produced in High Five insect cells using a recombinant baculovirus expression system (15, 16). Cells were stained with I-Ab(gp61) tetramers (8). High Five insect cells using a recombinant baculovirus expression system (15, 16).

Several reports have documented that IL-7 and IL-15 promote the survival and homeostatic proliferation of memory CD8 T cells (7, 8). Expression of the IL-7R α-chain (CD127) has been shown to mark a fraction of activated effector CD8 T cells that are more likely to survive and give rise to robust memory T cells (9–12). In addition, IL-7-dependent signals also support the emergence and survival of memory CD4 T cells (13, 14). Because Ag-specific T cells with reduced effector capacities have been observed in chronically infected hosts, and the absence of CD4 T cell help results in diminished memory CD8 T cell responses, it is of interest to determine whether these disparities are associated with alterations in CD127 expression.

In the present study we have evaluated whether difficulties in controlling viral infections as well as insufficient CD4 T cell help negatively impact the emergence of CD127\textsuperscript{high} T cells. We demonstrate that, in persistently infected hosts, Ag-specific CD127\textsuperscript{low} T cells remain detectable for extended periods but CD127\textsuperscript{high} cells can emerge if the viral load is contained. CD4 T cell help appears to be a critical fate determinant for Ag-specific CD8 T cells as “helpless” CD8 T cells which emerge in the absence of CD4 T cells also retain a CD127\textsuperscript{low} phenotype. We further show that the failure to express high levels of CD127 has functional consequences in both mice and humans which manifest as an inability to produce marked amounts of IL-2 at later timepoints following infection.

Materials and Methods

Mice and virus

C57BL/6J (B6) and C57BL/6-Cd4\textsuperscript{tm1mk} (CD4\textsuperscript{−/−}) mice (The Jackson Laboratory) were bred and maintained in accredited facilities at the University of Alabama at Birmingham. Male and female mice between 6 and 10 wk of age were used. Acute, protracted, and chronic lymphocytic choriomeningitis virus (LCMV) infections were established as previously described (3). For rechallenge studies, LCMV immune B6 mice, which had been infected >80 days previously with 2 × 10\textsuperscript{6} PFU LCMV-Armstrong, were administered 2 × 10\textsuperscript{6} PFU LCMV-clone 13 by i.v. injection.

MHC class I and II tetramer preparation and use

MHC class-I-peptide complexes were prepared and used as previously described (1, 3). MHC class II I-Ab\textsuperscript{gp61} tetramers were prepared in High Five insect cells using a recombinant baculovirus expression system (15, 16). Cells were stained with I-Ab\textsuperscript{gp61} (8 μg/ml) at 37°C for 75 min in R10. Subsequently, anti-CD4-allylphococyanin (clone RM4.5), anti-CD127-FITC (clone A7R34), and anti-CD16/32-biotin (clone 2.4G2) mAbs were added. After 45 min cells were washed and streptavidin-PerCP added. After an additional 30 min, two washes were performed and cells fixed before analysis.

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3 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; SEB, Staphylococcal enterotoxin B; MFI, mean fluorescence intensity.

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Anaysis of murine responses

Freshly explanted spleens were disrupted into single cell suspensions and responses analyzed as previously described (17).

Anaysis of human responses

Blood was obtained from three healthy HIV-seronegative human volunteers and PBMC isolated by standard Histopaque density centrifugation and cryopreserved. Cytokine production by PBMC from human subjects was assessed by standard procedures. Thawed cells (10⁶) were either left untreated or stimulated for 5 h at 37°C in the presence of anti-CD28 and anti-CD49d mAbs (1 μg/ml) together with staphylococcal enterotoxin B (SEB) (1 μg/ml). Monensin was added after the first hour of incubation. Following incubation for an additional 4 h, samples were stained for the expression of CD3, CD8, CD127, IL-2, and IFN-γ.

Flow cytometry

Samples were acquired using either a FACSCalibur or LSRII flow cytometer and data were analyzed with either CellQuest or FlowJo software.

Results and Discussion

Previous reports have shown that CD127high T cells which arise during the effector phase of an immune response acquire the phenotypic and functional traits characteristic of memory T cells (9–12). During the course of infections that are difficult to resolve, virus-specific T cells do emerge but fail to attain the functional attributes which are representative of memory T cells (1–4). We used the LCMV system to address whether the expression of CD127 marked T cells that are maintained regardless of their functional competence or whether functionally compromised T cells which manifest in persistently infected hosts represent CD127low remnants.

The expression of CD127 on virus-specific CD8 T cells was monitored during the course of acute LCMV-Armstrong infection of B6 mice, protracted infection of B6 mice with the clone 13 strain, and during the course of chronic clone 13 infection of CD4+/− mice (Fig. 1). Acute LCMV infection is rapidly controlled and serum viral loads were below the limits of detection (<50 PFU/ml) by 8 days after inoculation (Fig. 1C). Only a fraction (9–15%) of Ag-specific CD8 T cells were CD127high at this timepoint (Fig. 1), but as previously shown, after viral clearance CD127high T cells preferentially survive the contraction phase and dominate the memory pool (9–12).

We next determined whether extended exposure to viral Ags impacts the generation of CD127low T cells. LCMV-clone 13 infection of B6 mice results in a protracted infection which is brought under control over a period of 2–3 mo (2, 3) (Fig. 1C). By 8 days after protracted LCMV infection, the responding antiviral CD8 T cells were predominantly CD127low. Unlike acute LCMV infection during which a transition occurs as CD127high memory T cells emerge, in protractedly infected hosts virus-specific T cells preferentially survive the contraction phase and dominate the memory pool (9–12).

The notion that viral clearance can promote the development of CD127high cells is supported by the patterns of CD127 expression on antiviral T cells at later timepoints. By 260 days postinfection, when protracted LCMV infection was markedly controlled, 78 ± 4% and 40 ± 9% of gp33 and np-396-specific
CD8 T cells were CD127high, respectively (Fig. 1). Thus, CD127high virus-specific T cells can emerge if the high grade infection is brought under control. Improvements in the functional quality of antiviral CD8 T cells following reduction of viral loads has now been demonstrated in several animal models of persistent viral infections as well as in human subjects. In the case of LCMV infection of mice and hepatitis C virus infections of humans at least a partial recovery of functional CD8 T cell activities has been reported as an initially high viral burden is brought under control (3, 18, 19). Decreased expression of CD127 on CD8 T cells has also been reported during HIV infection; however, levels of expression have been shown to increase if successful antiviral therapy is applied (20–23). The observations that CD127high cells can emerge if antigenic stimulation is withdrawn suggest that the maturation of antiviral T cells from a CD127low to CD127high state is associated with an enhancement in their ability to mediate long-term infection control.

Although, as shown above, CD127low CD8 T cells can remain detectable for prolonged periods, constant antigenic stimulation resulting from chronic viral infection is detrimental to the maintenance and function of antiviral T cells (1–4, 24). In CD4−/− mice chronically infected with LCMV-clone 13, the virus-specific CD8 T cells that develop fail to mature into CD127high memory phenotype cells (Fig. 1). By 8 days post-infection all specificities of antiviral CD8 T cells were CD127low, and CD127high cells never became prevalent (Fig. 1). CD127low T cells are, however, not maintained indefinitely in the absence of CD4 T cell help as they succumb to deletion overtime (3–5, 24, and Fig. 1). These data highlight two key points regarding CD127 expression by antiviral CD8 T cells. First, the emergence of CD127high T cells is governed by both the withdrawal of antigenic stimulation and the presence of CD4 T cell help. Second, decreased IL-7 sensitivity due to the absence of receptor expression does not necessarily lead to rapid deletion (19); nevertheless the inability to up-regulate CD127 may result in an attrition of virus-specific T cell responses.

We next addressed whether high viral loads also impeded the emergence of CD127high antiviral CD4 T cells by assessing the expression of CD127 on virus-specific CD4 T cells during the course of acute and protracted infections (Fig. 1, D and E). The dynamics of CD127 expression on LCMV-specific CD4 T cells in acutely infected hosts paralleled that of CD8 T cell responses. By 8 days post-acute infection, expression was low (mean fluorescence intensity (MFI) = 5.4 ± 0.6), however, by 1 mo following infection CD127high virus-specific CD4 T cells predominated and levels of expression remained high (MFI = 19.8 ± 1.8) until the last timepoint checked, at 7.5 mo post-infection. Virus-specific CD4 T cells were also CD127low early following protracted LCMV infection and expression remained low while the virus persisted (Fig. 1, D and E). As the infection was contained CD127high CD4 T cells emerged and by >8 mo post-infection ~65% of virus-specific CD4 T cells were CD127high in protractedly infected hosts. Thus, during protracted LCMV infection the viral load appeared to act as a rheostatic regulator of CD127 expression on both virus-specific CD4 and CD8 T cells, which is consistent with a model that repetitive antigenic stimulation hinders the differentiation of the responding T cells.

We next monitored whether the expression of CD127 on virus-specific CD4 and CD8 T cells changed, as they participated in protective recall responses, to resemble the levels on primary effectors or whether the levels of expression more closely matched that of memory T cells. Rechallenge of LCMV immune mice resulted in downshifts in CD127 expression on both np396- and gp33-specific CD8 T cells (Fig. 2A) as well as on gp61-specific CD4 T cells (Fig. 2B). Thus, after memory is established the expression of CD127 is not permanently set at a high level and interchanges between CD127high and CD127low states can occur which reflect the T cells’ maturation and activation state.

Numerous reports have documented that CD8 T cells, which develop in the absence of CD4 T cell help, exhibit a skewed phenotype, resembling effector rather than central memory T cells and display a diminished capacity to proliferate and function following antigenic re-exposure (6, 17). Strikingly, the presence of CD4 T cells appeared necessary for the rapid emergence of CD127high CD8 T cells. Following acute LCMV infection of CD4−/− mice the majority (56 ± 6% for gp33 and 69 ± 8% for np396) of virus-specific CD8 T cells retained a CD127low phenotype even by 4 mo post-infection (Fig. 3, A and B).

Because “helpless” CD8 T cells show impaired effector activities (6) and these cells remain CD127low for extended periods (Fig. 3, A and B), we compared the cytokine-producing capacity of CD127low and CD127high CD8 T cells (Fig. 3, C and D). At 8 days following acute infection the CD127low CD8 T cells which predominate at this timepoint can produce IFN-γ. In B6 mice the CD127low CD8 T cells are not maintained but the CD127high memory CD8 T cells which emerge over time can produce IFN-γ and a subset of these memory cells also produces IL-2. In acutely infected CD4−/− mice a significant population of CD127high CD8 T cells fails to emerge and the functional capacity of the CD127low CD8 T cells decays over time. Overall, these data illustrate the importance of CD4 T cell help for facilitating the development of functionally robust CD127high CD8 T cells.

**FIGURE 2.** Changes in CD127 expression following viral rechallenge. A, CD127 expression on splenic gp33- or np396-specific CD8 T cells at day 224 post-acute LCMV-Armstrong infection of B6 mice and at 3 and 7 days following rechallenge of immune B6 mice with LCMV-clone 13. B, Using MHC class II tetramers, CD127 expression was assessed on gp61-specific CD8 T cells from LCMV immune mice at day 156 following infection and at 3 and 5 days following rechallenge. Gated tetramer CD8+ or tetramer CD4+ lymphocytes are shown in A and B, respectively. Representative data are shown from two to four mice analyzed at each timepoint.
memory CD8 T cells. In the absence of CD4 T cells the maturation of memory CD8 T cells stalls and CD127low T cells which may survive under these conditions are functionally inferior, exhibiting a marked impairment of IL-2 production.

We next examined whether CD127 expression was similarly associated with IL-2 production by human CD8 T cells. CD127low and CD127high subsets of CD8 T cells from the PBMC of three HIV-seronegative donors were analyzed for their ability to produce IL-2 and IFN-γ following stimulation with SEB. In all samples examined the production of IL-2 was preferentially associated with CD127high CD8 T cells (Fig. 4). This included T cells which coproduced IL-2 and IFN-γ as well as subpopulations that produced only IL-2 (Fig. 4, upper and lower right quadrants). Thus, in both humans as well as mice expression of CD127 marks functionally competent subsets of CD8 T cells.

A consistent theme of the results presented in this study is that CD127high T cells which arise during the effector phase of an antiviral immune response are not necessarily rapidly deleted. The maturation of memory CD8 and CD4 T cells is, however, associated with a CD127high phenotype and the expression of CD127 serves as a predictor of the functional quality of antiviral T cells in both mice and humans. Because the clearance of Ag, in addition to CD4 T cell help, promotes the development of memory CD8 T cells this suggests that responding T cells pass through two checkpoints as they attain the traits of memory T cells. Ag-dependent TCR signaling must be diminished, which is brought about if the overall immune response is sufficient to remove the inducing Ag. Also, a CD4 T cell-dependent licensing occurs that supports the emergence and maintenance of CD127high T cell subsets. If conditions are unfavorable for the progression of memory cell development then CD127low Ag-specific T cells may be retained and adopt a pseudoeffecter phenotype. In the case of chronic viral infections, holding Ag-experienced T cells in a CD127low state may be somewhat advantageous as if viral loads can be reduced within a sufficient timeframe then the maturation of CD127high cells can proceed.

Disclosures

The authors have no financial conflict of interest.

References


