Origin and Fate of Lymphocytic Choriomeningitis Virus-Specific CD8⁺ T Cells Coexpressing the Inhibitory NK Cell Receptor Ly49G2

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CD8+ T cells that coexpress the inhibitory NK cell receptor, Ly49G2 (G2), are present in immunologically naive C57BL/6 mice but display Ags found on memory T cells. To assess how G2+CD8+ cells relate to bona fide memory cells, we examined the origin and fate of lymphocytic choriomeningitis virus (LCMV)-induced G2+CD8+ cells. During early (day 4) acute LCMV infection, both G2+ and G2-CD8+ T cell subsets underwent an attrition in number and displayed an activation (CD69hiB220loCD62Llo) phenotype. By day 8, both subsets synthesized IFN-γ in response to immunodominant LCMV peptides, though the expansion of G2+ cells was less than that of G2- cells. Adoptive transfer experiments with purified G2- or G2+CD8+ cells from naive mice indicated that the LCMV-specific G2+ subset was derived from a pre-existing G2- population and not generated from G2- cells responding to LCMV infection. Their participation in the LCMV-specific T cell response increased with age, reflecting an increase in the size of the pre-existing G2+ pool. Following establishment of stable LCMV memory, the proportion of CD8+ cells coexpressing G2 was reduced in comparison to naive controls, presumably due to displacement by G2- LCMV-specific memory cells. LCMV-specific G2+ cells were present in the memory pool, but at low frequencies, and they did not exhibit the typical phenotypic changes of reactivation during secondary challenge. We suggest that G2+CD8+ cells represent a cell lineage distinct from bona fide memory T cells, but that they can participate in an acute virus-specific T cell response. The Journal of Immunology, 2004, 173: 478–484.

The question as to how CD8+ memory T cells survive this programmed contraction still remains largely unresolved, but it is thought that delivery of too strong an antigenic stimulus might induce death pathways in CD8+ effector T cells, driving them into activation-induced cell death (AICD; reviewed in Ref. 9). Ly49+CD8+ T cells have a memory cell phenotype (2), including expression of high levels of CD44, Ly6C, and the common IL-2Rα/β chain for IL-2 and IL-15 (CD122). This observation has given rise to the hypothesis that iNKR may provide CD8+ T cells with a selective advantage during the formation of T cell memory by establishing the threshold where engagement of the TCR with its peptide/MHC class I ligand induces signaling through the TCR. In this regard, inhibitory signals transduced through iNKR during the AICD phase of the CTL response may be important in protecting CD8+ T cells from apoptosis. Interaction of the iNKR, Ly49A, with its H-2Dα ligand protected T cell hybridoma clones from apoptosis induced by anti-CD3 mAbs or cognate Ag (10). In another study, Ugolini et al. (11) reported that CD8+ T cells from mice transgenic for the inhibitory receptor KIR2DL3 and its cognate MHC class I ligand exhibit decreased AICD. Interestingly, the vast majority of KIR+ T cells are CD28− (3), a phenotype associated with acquisition of resistance to AICD by human CD8+ T cells (12).

The origin of iNKR+CD8+ T cell remains uncertain. The increasing presence of Ly49+CD8+ T cells in aging immunologically naive mice raises questions concerning whether they are a bona fide foreign Ag-experienced memory population or another cell type. For example, CD8+ T cells that have undergone homoeostatic proliferation possess an activation marker profile similar to that of bona fide memory cells (13). In this study, we have examined whether expression of the iNKR Ly49G2 (G2) is acquired on G2- cells during the course of a virus-specific T cell response and becomes a marker for bona fide virus-specific memory T cells, as might be predicted from the aforementioned hypothesis.
contrary, we report that the G2⁺CD8⁺ subset induced by lymphocytic choriomeningitis virus (LCMV) infection expands from a pre-existing G2⁺ population present in immunologically naive mice, undergoes attrition after participating in the primary response, makes only a minor contribution to the LCMV-specific memory pool, and responds poorly during secondary challenge.

Materials and Methods

Infection of mice

Male C57BL/6 (B6), B6.PL Thy1a/Cy (Thy1.1⁺), B6.129P2 Tcrbtml/Thy1.2 (53-2.1) were purchased from The Jackson Laboratory (Bar Harbor, ME) at 5–6 wk of age and maintained under specific pathogen-free conditions within the Department of Animal Medicine at the University of Massachusetts Medical School (Worcester, MA). For generation of acute virus-specific T cell responses, mice were infected i.p. with 5 × 10⁴ PFU of the Armstrong strain of LCMV (14). Mice are considered LCMV immune after 6 wk of infection, but in our experiments, mice were housed for at least 6 mo after primary infection before sacrifice.

Flow cytometry

Single cell suspensions were prepared from spleens, and erythrocytes were removed by lysis using a 0.84% NH₄Cl solution. Following preincubation with 1 μl of the unlabeled mAb 2.4G2 in 96-well plates containing 100 μl of FACS buffer (HBSS, 2% FCS, 0.1% NaN₃), cells were stained (30 min, 4°C) with combinations of fluorescently labeled mAbs specific for CD8α (53-6.7), CD44 (IM7), G2 (4D11), CD122 (TM-B1), Ly6C (AL21), TCRβ (H57-597), CD69 (H1.2F3), L-selectin (CD62L) (MEL-14), CD43 activation-associated glycoform (1B11), Thy1.1 (OX-7), and Thy1.2 (53-2.1). All mAbs were purchased from BD Pharmingen (San Diego, CA). Freshly stained samples were analyzed using a FACS caliber and CellQuest software (BD Pharmingen).

Intracellular IFN-γ Staining

LCMV-specific memory CD8⁺ T cells were detected by measuring intracellular IFN-γ in response to immunodominant LCMV peptides using the Cytofix/Cytoperm Plus kit (with GolgiPlug; BD Pharmingen), as described previously (15). Briefly, 1–2 × 10⁶ cells were incubated in 96-well plates (5 h, 37°C) with 5 μM synthetic peptide, 10 U/ml human rIL-2 (BD Pharmingen), and 0.2 μl of GolgiPlug. Cells were then washed in FACS buffer, blocked with Fc Block (BD Biosciences), and incubated (30 min, 4°C) with combinations of fluorescently labeled mAbs. Subsequent fixation and permeabilization of the cells was performed to allow intracellular access to the anti-IFN-γ mAb (XMG1.2; BD Pharmingen). Freshly stained samples were analyzed using a FACS caliber and CellQuest software (BD Pharmingen).

Adoptive transfer of FACS-purified CD8⁺ T cell subsets

Single cell suspensions were prepared from the spleens of naive mice, preincubated with Fc Block (2.4G2), and then stained (30 min, 4°C) with combinations of fluorescently labeled mAbs specific for CD8α, G2, and CD44. Purified (>95%) populations of CD8⁺CD44low, CD8⁺G2⁺, or CD8⁺G2⁻ cells were sorted using a FACStar plus and CellQuest software (BD Pharmingen). A total of 1–2 × 10⁶ purified CFSE-labeled cells were adoptively transferred into αβKO or C57BL/6 mice by tail vein injection. Mice were infected with LCMV 1 day later and harvested on day 8 postinfection (p.i.). Donor CD8⁺ T cell populations were traced by the use of mAbs specific for congenic (Thy) markers.

Statistical analyses

Results are expressed as arithmetic mean ± SEM. An unpaired Student’s t test was used for data analysis where indicated. All the data presented are calculated from individual mice and each figure is representative of at least five separate experiments.

Results

G2⁺CD8⁺ T cells exhibit a memory phenotype in naive mice

CD44 is rapidly up-regulated during T cell activation and can serve as a marker of previous Ag experience. In naive C57BL/6 mice, CD8⁺ T cells coexpressing the G2 receptor do so in conjunction with other high levels of CD44 (Fig. 1A) (2, 8). Compared with other CD44highCD8⁺ T cells, the proportion of G2⁺ cells expressing two other memory-associated Ags, CD122 and Ly6C, was higher, and their expression of the TCR β-chain was lower (Fig. 1B). This low level of TCR suggests prior stimulation through this receptor, but few G2⁺ or G2⁻CD8⁺ T cells from naive mice coexpressed the very early activation marker, CD69, or CD44 (Fig. 1C) (previously (15)). Brieﬂy, 1–2 × 10⁶ purified CFSE-labeled cells were adoptively transferred into αβKO or C57BL/6 mice by tail vein injection. Mice were infected with LCMV 1 day later and harvested on day 8 postinfection (p.i.). Donor CD8⁺ T cell populations were traced by the use of mAbs specific for congenic (Thy) markers.

FIGURE 1. G2⁺CD8⁺ T cells from naive C57BL/6 mice possess a memory phenotype. A, CD8⁺ T lymphocytes from the spleens of 35-wk-old naive mice were electronically gated by FACS for analysis of CD44 expression. Subsets of CD44highCD8⁺ cells defined by the absence or presence of G2 were assessed for mean levels of CD44 expression (MFI) as indicated on the dot plot analysis of a representative animal. B, The proportion of cells expressing CD122 and Ly6C within G2⁺ and CD44highCD8⁺ subsets was subsequently compared, as were their intensities of staining for the TCR β-chain. Results are the mean of six to nine individual mice from three separate experiments.
significant contributor to the memory pool after the response subsides.

Early activation and attrition of both G2⁺ and G2⁻ CD8⁺ subsets during acute LCMV infection

Previous studies in our laboratory have described an early apoptosis-driven reduction in CD8⁺ T cell number during LCMV infection, occurring at the peak of the IFN-αβ response and persisting for several days (17). Because CD44[^high]CD8⁺ T cells were especially sensitive to this attrition, we evaluated whether G2⁺ and G2⁻ CD44[^high] subsets behaved similarly during early LCMV infection. Cell numbers in both populations were similarly and significantly reduced on day 4 of LCMV infection compared with the equivalent populations in naive controls (Fig. 3; t test, p < 0.0001). At the same time, there was a dramatic increase in the proportion of both G2⁺ and G2⁻ CD44[^high] cells expressing CD69 and IB11 (Fig. 2A; t test, p < 0.0001), particularly within the latter subset. Serving as a further indication that both subsets were activated by day 4 of LCMV infection, there was an accompanying down-regulation of CD62L expression on G2⁺ and G2⁻ CD44[^high] cells (Fig. 2B). There was no evidence of LCMV-specific production of IFN-γ by either subset at this time point, as this is before LCMV-specific clones become a significant proportion of the CD8⁺ T cell population (data not shown). Thus, as with G2⁻ CD44[^high]CD8⁺ T cells, a large fraction of the G2⁺ subset is lost during early LCMV infection, with a substantial proportion of the surviving cells exhibiting a phenotype that indicates recent activation.

![FIGURE 2. Activation marker expression on G2⁺ CD8⁺ T cells during acute LCMV infection. Splenocytes were isolated from mice at various time points after LCMV infection, and the CD8⁺ population was electronically gated into distinct subsets on the basis of their expression of G2 and/or CD44. The proportion of each of these subsets coexpressing CD69 (A), or the CD43 activation-associated glycoform recognized by the mAb 1B11, or CD62L (B) was determined by FACS analysis. Results are the mean of six individual mice from two separate experiments.](http://www.jimmunol.org/)

![FIGURE 3. Changes in CD8⁺ T cell subset number during acute LCMV infection. G2⁺ CD44[^high], G2⁻ CD44[^high], and total CD8⁺ T cells were enumerated from the spleens of mice at various days after LCMV infection, including immune mice (24 wk p.i.), and from young (naive 11-wk-old) and old (naive 35-wk-old) uninfected controls. Results are the average of eight to nine individual mice from three separate experiments.](http://www.jimmunol.org/)

**The peak of the CTL response to LCMV infection encompasses an expanded functional population of virus-specific G2⁺ CD8⁺ T cells**

The early LCMV-induced decline in CD8⁺ T cell number is followed by a dramatic proliferation of virus-specific CD8⁺ T cell precursors into a greatly expanded CD44[^high] CTL subset that peaks about day 8 p.i. (18). Fig. 3 shows an ~6.8-fold increase in total splenic CD8⁺ number between days 0 and 8 of LCMV infection. This encompassed a 22-fold increase in the size of the G2⁺ CD44[^high] compartment, but only a moderate 3.3-fold increase in the size of the G2⁻ CD44[^high] subset (Fig. 3). Accordingly, the percentage of G2⁺ cells within the total CD8⁺ population on day 8 was approximately half of that found in naive mice. The proportion of both G2⁺ and G2⁻ CD44[^high] cells in the spleen that were 1B11[^high] or CD62L[^low] further increased between days 4 and 8 of LCMV infection, indicating a continued activation of both subsets based on phenotypic criteria (Fig. 2, A and B).

Substantial numbers of LCMV-specific cells were found in both G2⁺ and G2⁻ CD8⁺ populations by 8 days p.i. (Fig. 4). However, a significantly lower percentage of splenic G2⁺ CD8⁺ T cells (19 ± 3.1%) secreted IFN-γ in response to the two most immunodominant LCMV peptides, gp33 (33–41) and nucleoprotein (NP)396 (396–404), than did the G2⁻ CD44[^high] subset (33 ± 7.9%).
4.3%; t test, p < 0.005). This difference was particularly apparent for the NP396 epitope (Fig. 4A). A similar pattern of LCMV-specific IFN-γ production was observed for G2+ and G2− CD44high subsets from the peritoneal cavities of LCMV-infected mice on day 8 (Fig. 4B). Most nonanergized, virus-specific CD8+ T cells will synthesize IFN-γ in vitro when their TCR are cross-linked with anti-CD3. Mirroring the smaller LCMV epitope-specific response, the percentage of splenic G2+CD8+ T cells producing IFN-γ in response to anti-CD3 on day 8 of LCMV infection (16 ± 1.4) was significantly less than for the G2− CD44high subset (48 ± 1.8; t test, p < 0.0001). Hence, the G2+ subset is capable of expansion, activation, and cytokine secretion in response to acute LCMV infection, but the total LCMV-specific response is substantially lower than for the much larger G2− subset (Figs. 4 and 5). These data parallel earlier studies comparing the cytotoxic activity of splenic G2+ and G2−CD8+ T cell subsets during LCMV infection (8).

**Origin and stability of LCMV-specific G2+CD8+ T cells**

We questioned whether LCMV-specific G2− and G2+CD8+ T cells represented two separate lineages or whether the phenotypes interconverted during the response to viral challenge. To address this issue, CD44low donor CD8+ T cells specific for the gp33 epitope of LCMV were sorted by flow cytometry from naive TCR-transgenic mice (Thy1.2+), CFSE-labeled, and then adoptively transferred into naive C57BL/6 hosts (Thy1.1+). After 8 days of LCMV infection and multiple rounds of division (i.e., CFSElow), these CD44low donor cells had converted into a CD44high population capable of secreting IFN-γ in response to their cognate gp33 Ag, but significantly, did not contain a G2− subset (Fig. 6A, and data not shown). In a similar experiment, purified G2− donor CD8+ T cells from naive nontransgenic C57BL/6 mice remained negative for G2 expression 8 days after LCMV infection of their T cell-deficient αβγKO hosts (data not shown). Thus, the G2−CD8+ T cells participating in the immune response to LCMV were not derived from G2− splenocytes.

To determine whether LCMV-induced G2+CD8+ T cells were derived from G2− cells existing before infection, G2+ and G2− cells were sorted from the CD8+ compartments of naive Thy1.1 or Thy1.2 congenic C57BL/6 mice, respectively, and combined before adoptive transfer into αβγ T cell KO hosts. Both the Thy1.1 G2− and Thy1.1 G2+ CD8+ subsets preserved their unique pattern of G2 expression on day 8 of the response to LCMV infection in these mice (Fig. 6B). Therefore, our data indicate that G2 expression occurs on a distinct pre-existing subset of CD8+ T cells that can mount a response to LCMV in parallel with the larger G2− population, and that, in contrast to some other NK cell Ags such as NK1.1 (19) and KLRG1 (20), is not newly expressed on LCMV-specific G2− cells responding to the infection. The data also illustrate the stability of G2 expression, or lack thereof, on CD8+ T cells.

**The LCMV-specific memory pool contains a small number of G2+CD8+ cells**

We questioned whether the expression of G2 provided any survival advantage to LCMV-specific CD8+ T cells and facilitated enrichment of this population in the LCMV-specific bona fide memory T cell population. A programmed contraction of Ag-specific CTL follows the peak of the CD8+ T cell response to virus, restoring total CD8+ T cell numbers to preinfection levels while permitting a small proportion to persist as long-lived memory cells (9). The most extensive attrition of CD8+ T cells following

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**FIGURE 5.** Kinetics of the G2+ CTL subset response to LCMV. A, Total LCMV-specific cell number was determined at several time points after LCMV infection. This was calculated by multiplying the size of the splenic G2− and G2+CD44high populations by the proportion of cells within them that were capable of producing IFN-γ to the two LCMV epitopes examined (i.e., gp33 and NP396). Results shown are the mean of six individual mice taken from two separate experiments. The average ratio of LCMV-specific cells that were G2− compared with G2+ at each of the time points examined is indicated on the plot. B, Flow cytometry plot showing peptide-induced IFN-γ production by G2+CD8+ T cells in LCMV memory mouse 24 wk p.i. The proportion of cells secreting IFN-γ within G2− and G2+CD44high/CD8+ subsets at this time point is indicated on the dot plot analysis of a representative animal. Results are the mean of six individual mice from two independent experiments.

**FIGURE 6.** Primary LCMV infection does not alter G2 expression on CD8+ T cell subsets. G2− and G2+CD8+ T cell subsets were sorted from gp33-specific TCR-transgenic mice (Thy1.2+) and adoptively transferred into naive C57BL/6 hosts (Thy1.1+) (A), or sorted from Thy1.1 and Thy1.2 congenic C57BL/6 mice and adoptively transferred into αβγKO hosts (B). Spleens were harvested 8 days after LCMV infection of host mice, and donor cells within the expanded CD8+ T cell population were electronically gated by FACS and assessed for G2 expression. The percentage of each donor CD8+ T cell population expressing G2 pre- and post-LCMV infection is indicated on the dot plot analyses of a representative animal.
LCMV-induced proliferation occurred between days 8 and 16, during which the ratio of LCMV-specific cells in the spleen that were G2⁺ vs G2⁻ increased from ~1:160 to 1:60 (Fig. 5A). This might mean that the G2⁻ cells are slightly more resistant to the apoptosis or less likely to disseminate during the contraction phase of the response. This coincided with some changes in surface Ag profile, including a significant decline in expression levels of G2 on G2⁺CD8⁺ T cells (mean fluorescence intensity (MFI) = 984 ± 81 vs 666 ± 29; t test, p < 0.001) and a more rapid reacquisition of CD62L than on G2⁻ cells (Fig. 3B). This suggests that G2 expression is associated with a more rapid return of CD8⁺ T cells to a resting state. However, down-regulation of another activation marker, IB11, occurred at similar rates on G2⁺ and G2⁻ subsets of CD44highCD8⁺ T cells over the same time frame (Fig. 2A). In the resting immune state, LCMV-specific cells could be detected in the G2⁺CD8⁺ subset, but they represented a much lower percentage of this pool than did LCMV-specific cells in the G2⁻ subset (Fig. 5B) and contributed only a small fraction to the total LCMV-specific cell number present in the memory compartment (Fig. 5A).

**Secondary infection fails to normally activate LCMV-specific memory cells within the G2⁺ subset**

We questioned whether G2⁺CD8⁺ T cells from LCMV-immune mice harbored a G2⁺ memory cell population capable of responding normally to secondary challenge. On day 5 of a secondary LCMV challenge, LCMV-specific memory cells from both G2⁺ and G2⁻ CD44high subsets underwent modest (~1.9-fold) expansions in number, compared with unchallenged LCMV-immune mice (data not shown). As in unchallenged immune mice (Fig. 5B), the percentage of LCMV-specific cells within the G2⁺CD8⁺ T cell subset (4.9 ± 0.7) continued to lag behind that of the G2⁻ subset (19 ± 0.6) during the recall response on day 5. Accompanying the G2⁺ CD44high subset at this time was a change in surface Ag profiles characteristic of activation, including significant increases in the percentage of cells expressing IB11 (Fig. 7; t test, p < 0.02) and the cytotoxic enzyme granzyme B (from 3.3 ± 0.3 to 65 ± 3.6; t test, p < 0.03), and a decrease in the percentage of CD62Lhigh cells (Fig. 7; t test, p < 0.03). In contrast, these phenotypic changes did not occur in the G2⁻ subset following secondary LCMV challenge, as expression of these markers was essentially unchanged (t test, p > 0.05) at day 5 and out until at least day 8 p.i. (Fig. 7, and data not shown). That the G2⁺ subset of age-matched mice had increased expression of IB11 (Fig. 3) and granzyme B (from 2 ± 0.5 to 73 ± 2.3) and decreased CD62L expression (Fig. 3) on day 5 of a primary LCMV infection indicates that this is not an age-related defect of this population. Thus, although the LCMV-specific memory cells that express G2 appear to respond to secondary challenge by increasing in number and continuing to produce IFN-γ in response to LCMV epitopes, they do not show the usual antigenic properties of having been activated.

**A history of LCMV infection reduces the G2⁺ CD8⁺ population in older mice**

Some 6 mo after infection, the size of the total CD8⁺ T cell population in LCMV-immune mice (9.1 × 10⁶) was similar to that in naive mice at the time of challenge (i.e., 11 wk; 9.1 × 10⁶), but contained a 50% greater number of G2⁺ CD44high cells (Fig. 3; t test, p < 0.02). In contrast, LCMV infection actually abrogated the normal age-related increase in numbers of G2⁺ CD44highCD8⁺ T cells witnessed in age-matched naive mice (i.e., 35 wk; t test, p < 0.002), such that numbers of G2⁺ cells in the older LCMV-immune mice (2.6 × 10⁵) were similar (t test, p > 0.05) to those present in naive mice at the time of challenge (Fig. 3; 2.6 × 10⁵).

In older mice, the enhanced G2⁺ subset is associated with a higher proportion of G2⁺ cells during a primary LCMV infection

The size of the G2⁺ subset in 35 wk-old naive mice (5.7 × 10⁵) was greater than twice as large as that from 11-wk-old mice (Fig. 3). During an acute LCMV response, the older CD8⁺ compartment gave rise to a virus-specific population with an increased percentage of G2 expression, such that the frequency of G2⁺ cells within the total LCMV-specific population present on day 8 of acute infection increased from ~1:180 (0.6%) in young mice to 1:40 (2.5%) in aged mice (Fig. 8). These data are consistent with the LCMV-specific G2⁺ subset response emanating from a pre-existing population of G2⁺ cells. Also of note, as shown by others (21), is that the magnitude of the total LCMV-specific T cell response in older mice was much reduced in comparison to younger mice (Fig. 8).

![FIGURE 7.](http://www.jimmunol.org/) Activation marker expression on G2⁺CD8⁺ T cells from LCMV-immune mice during a secondary LCMV infection. Splenocytes were isolated from mice that had either been unchallenged for 24 wk after LCMV infection or infected with a second dose of LCMV 5 days earlier. The CD8⁺ T cell populations from each group were electronically gated into distinct subsets on the basis of their expression of G2 and/or CD44, and assessed for coexpression of CD62L or the CD43 activation-associated glycoform recognized by the mAb IB11. The mean results of six individual mice taken from two separate experiments are indicated on the dot plot analysis of a representative animal.

![FIGURE 8.](http://www.jimmunol.org/) G2⁺CD8⁺ T cells contribute more extensively to the LCMV-specific response in old mice. Total LCMV-specific cell number (i.e., size of the gp33/NP396-specific IFN-γ⁺ population) was calculated for the splenic G2⁺ and G2⁻CD8⁺ T cell subsets from young (naive 11-wk-old) and old (naive 35-wk-old) mice 8 days after LCMV infection. Results shown are the mean of six to nine individual mice taken from three separate experiments. The average ratio of LCMV-specific cells that were G2⁺ compared with G2⁻ in each of the age groups examined is indicated on the plot.
Discussion

This study demonstrates that G2⁺CD8⁺ T cells participating in the virus-specific response are derived from a memory-like CD44<sup>high</sup>CD8⁺ subset existing before infection and not from G2⁻ cells induced to express G2 during infection. These G2⁺CD44<sup>high</sup> cells have antigenic and functional properties distinct from other CD44<sup>high</sup>CD8⁺ T cells and may not represent a bona fide memory cell population. Interestingly, the size of the overall G2⁺CD8⁺ T cell compartment did not increase following the resolution of LCMV infection. Some LCMV-specific G2⁺ cells can be detected in the memory state after LCMV infection, but they respond abnormally on secondary challenge by failing to develop an activated phenotype. These data argue that G2⁺CD8⁺ T cells are a unique lineage of cells that can participate in a virus-specific response, but that lack the properties of bona fide memory cells and are not enriched after infection in the memory state.

Like the greater CD8⁺ T cell population, the G2⁺ subset exhibited phenotypic changes characteristic of acute activation and produced IFN-γ in response to the major immunodominant peptides of LCMV during a primary infection. However, proliferation of G2⁺ cells during LCMV infection was relatively limited, and they consequently made only a small contribution to the total CTL response. The expression of iNKR has previously been shown, in the presence of identifiable class I ligands, to impair a number of T cell responses (5–8). Accordingly, CD8⁺ T cells with transgenic expression of Ly49A, an inhibitory receptor that shares the same H-2D<sup>b</sup> ligand as G2, were impaired in their capacity to proliferate in response to LCMV infection in H2<sup>d</sup> but not H2<sup>b</sup> mice (22). Hence, in our experiments with H2<sup>b</sup> mice, which similarly lacked a recognized ligand for G2, it is unlikely that the attenuation of virus-induced proliferative responses resulted from delivery of a G2 transduced inhibitory signal to CD8⁺ T cells. Instead, expression of Ly49 receptors may simply be associated with CD8⁺ T cells that have an impaired capacity to proliferate due to other factors.

The coexpression of G2 and other iNKR is also associated with CD8⁺ T cells that exhibit antigenic characteristics of memory cells, even in previously unchallenged mice. This has led to the speculation that these receptors facilitate the escape of effector CD8⁺ T cells from the programmed contraction at the end of the CTL response. Current models of memory formation propose that protection of effector CD8⁺ T cells from apoptosis depends upon their exposure to an activation signal of limited strength. In this regard, the slightly increased frequency of G2 expression among LCMV-specific cells during the decline phase of the CTL response, when most effector CD8⁺ T cells are undergoing apoptosis, seems consistent with several observations suggesting that TCR transduced signaling of G2⁺CD8⁺ T cells was limited during LCMV infection. First, the level of KIR expression on human CD8⁺ T cells has been shown to decline in the absence of TCR engagement (23), and the levels of G2 expression decreased substantially during the decline phase of the CTL response to LCMV (i.e., days 8–16). Second, the G2⁺ subset demonstrated a more rapid reacquisition of CD62L than did G2⁻ CD44<sup>high</sup> cells during the decline phase of the CTL response to LCMV. Studies using gp33-specific transgenic CD8⁺ T cells have demonstrated that high levels of CD62L expression are linked with a reduced level of cytotoxicity (24). The rate that effector cells became CD62L<sup>high</sup> was determined early in the immune response, and correlated with the dose of Ag to which they were exposed. As such, T cells exposed to lower doses of Ag became CD62L<sup>high</sup> more rapidly than those exposed to high doses (25).

Nevertheless, there are a number of features of G2⁺CD8⁺ T cells that argue against them being typical memory cells. First, before infection they express different levels of memory marker Agrs than the G2⁺CD44<sup>high</sup> subset (Fig. 1). Second, the G2⁺ cells present at the peak of the CTL response to LCMV are the progeny of a G2⁻ subset that exists before infection (Fig. 6). Third, the G2⁻ subset does not harbor a substantial memory population following resolution of LCMV infection, as most LCMV-specific cells in immune mice reside in the G2⁺CD44<sup>high</sup> compartment (Fig. 5). Fourth, the proportion of G2⁺ cells within the LCMV-specific memory compartment does not increase during a recall response, indicating that these cells have no advantage in responding to a secondary encounter with Ag. Fifth, unlike G2⁻ LCMV-specific memory cells, secondary challenge does not elicit the characteristic phenotypic changes that indicate activation within the G2⁻ subset. This includes the preservation of CD62L expression by G2⁺CD8⁺ T cells (Fig. 7), which is normally lost on memory cells responding to Ag (25). Sixth, while bona fide memory CD8⁺ T cells increase in number following LCMV infection and are subsequently maintained at stable levels by their slow homeostatic division over time (26, 27), the background proliferation of Ly49⁺CD8⁺ T cells in naive mice (2) is associated with an increase in size of this population over time, but this is, paradoxically, nullified by LCMV infection (Fig. 3), perhaps due to competition from the accumulated G2⁻ LCMV-specific memory CD8⁺ T cells.

If G2⁺CD8⁺ cells are not a bona fide memory population, why do they exhibit a “memory-like” phenotype? In a lymphopenic environment, naive CD8⁺ T cells become responsive to self-MHC/peptide ligands and undergo division in an attempt to redress the shortfall. At the same time, they up-regulate many of the same memory markers coexpressed on Ly49⁺CD8⁺ T cells, such as CD44, Ly6C, and CD122 (13). We have previously proposed that homeostatic reconstitution of an early lymphopenia, similar to that occurring during the first days of LCMV infection, accounts for the subsequent division of CD44<sup>high</sup>CD8⁺ T cells induced by poly(I:C) treatment (17, 28). Hence, it is interesting that, compared with untreated mice, the incorporation of BrdU by Ly49⁺CD8⁺ T cells is elevated following poly(I:C) treatment (2). Perhaps not surprisingly, we have observed that poly(I:C)-induced division within the CD8⁺ T cell compartment is associated with an increase in the percentage of G2⁺ cells (our unpublished observations), but a decrease in the frequency of bona fide virus-specific memory cells (28). Expression of inhibitory Ly49 receptors may act to suppress the potentially deleterious consequences of responding to a self-MHC/peptide ligand, during homeostatic CD8⁺ T cell responses to signals directing reconstitution of a “hole” in this immune compartment. However, expression of Ly49 receptors was not observed on CD44<sup>high</sup>CD8⁺ T cells arising following the transfer of naive donor CD8⁺ T cells into lymphopenic hosts (29). Nevertheless, a recent paper describes the induction of Ly49A expression upon CD8⁺ T cells arising from a CD8⁺ population in mice dual-transgenic for the LCMV-derived gp33 peptide and a TCR specific for gp33 (30). Up-regulation of Ly49A expression on these self-reactive CD8⁺ T cells substantially reduced their activation upon encounter with their cognate self-Ag. This suggests that stimulation with self-Ag induces the expression of iNKR on CD8⁺ T cells as a possible mechanism for maintaining peripheral self-tolerance. Our studies indicate that a possible downside of having to restrain the activity of memory-like CD8⁺ T cells arising from self-MHC/peptide-driven homeostatic responses may be a reduced capacity to proliferate during subsequent foreign Ag-driven immune events.
In conclusion, rather than providing an advantage for entry into the memory pool of immune mice and therefore specifying a bona fide memory population, we feel that the expression of G2 and associated “memory” markers on CD8+ T cells from naïve mice reflects the prior division of these cells in a normal, self-MHC/peptide-induced, homeostatic response. The pre-existing Ly49^CD8+ T cells are able to participate in subsequent foreign Ag-induced responses mounted by the host, but these are diminished in comparison to other CD8+ T cells and are only minor contributors to the subsequent bona fide memory pool.

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References