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The Rapidity with Which Virus-Specific CD8$^+$ T Cells Initiate IFN-γ Synthesis Increases Markedly over the Course of Infection and Correlates with Immunodominance[1]

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Primary CD8$^+$ T cell responses play a major role in controlling infection by many viruses, and CD8$^+$ memory T cells can confer immunity to virus challenge. In this study we report that for many epitope-specific CD8$^+$ T cell populations, the regulation of an important effector molecule, IFN-γ, changes dramatically over the course of infection. During the acute phase of infection, many CD8$^+$ T cells exhibit a significant lag before producing IFN-γ in response to Ag contact; in contrast, the onset of IFN-γ production by memory cells of the same epitope specificity is markedly accelerated. The biological consequences of this improved responsiveness are manifold. Moreover, during the acute phase of the CD8$^+$ T cell response when immunodominance is being established, there is a strong correlation ($p = 0.0002$) between the abundance of each epitope-specific T cell population and the rapidity with which it initiates IFN-γ synthesis. Previous studies have indicated that IFN-γ plays a critical role in determining the immunodominance hierarchy of an on-going T cell response, and in this report we present evidence for an underlying mechanism: we propose that the CD8$^+$ T cells that most rapidly initiate IFN-γ production may be at a selective advantage, permitting them to dominate the developing T cell response. *The Journal of Immunology,* **2004, 173: 456–462.**

Adaptive immune responses are central to the control and eradication of viruses. Abs inactivate viruses in fluid phase, thereby diminishing the number of host cells that become infected, and CD8$^+$ T lymphocytes exert their effector functions on infected cells, limiting virus propagation and thus reducing the quantity of virus shed into the extracellular milieu. In this way, Abs and CD8$^+$ T cells play complementary roles, each easing the biological load on the other (reviewed in Refs. 1 and 2). The beneficial effects of adaptive immune responses are exerted not only during primary infection (i.e., during the host's first encounter with a virus), but also in response to secondary challenge. In the latter case, pre-existing virus-specific adaptive responses (Abs and memory T cells) can rapidly combat the virus challenge, usually preventing disease. The enhanced protection afforded by secondary immune responses may be explained by both quantitative and qualitative considerations. The responses are more abundant: anamnestic Ab responses usually rise more quickly and to a higher peak, than primary responses, and memory T cells can quickly expand upon Ag contact, allowing the number of virus-specific cells early in secondary infection to rapidly outstrip the numbers available soon after a primary virus infection. However, qualitative considerations, too, are important. The marker phenotypes (3–5), anatomical distribution, and effector functions of memory cells are areas of active investigation (6, 7) and are somewhat controversial. However, there is no doubt that certain B and T cell effector functions improve with time. B cells undergo somatic hypermutation, leading to the cell surface expression of higher affinity Abs, and such cells are selectively expanded after Ag exposure. T cell effector functions also change over the course of infection in at least two ways. First, the pattern of cytokines that they produce upon Ag contact changes. Almost 100% of memory cells produce both IFN-γ and TNF after Ag contact, but during a primary response, a substantial proportion (~30–90%) of IFN-γ^+ cells fails to synthesize TNF (8–11). Second, although the structural avidity of T cells increases only slightly over the course of infection (12, 13), the functional avidity of CD8$^+$ T cells improves dramatically. The cells become better at responding to low levels of Ag (14, 15), and the triggering of cytokine synthesis by cells harvested 4 days after infection requires ~80-fold more Ag than is needed to trigger cytokine production by more mature cells (15). Both these changes in effector function appear to be hard-wired, being maintained in memory cells for at least 1 year after resolving an acute viral infection.

In this report we describe a third way in which the effector functions of CD8$^+$ memory cells differ from those of primary cells. Memory cells initiate IFN-γ synthesis more rapidly after Ag contact, thus conferring upon the host the capacity to more speedily combat virus infection. Furthermore, we report that for each epitope-specific T cell population, the speed of onset of IFN-γ synthesis shows a strong correlation with the position of the cells in the immunodominance hierarchy. Many factors (T cell repertoire, Ag processing/presentation, etc.) (reviewed in Ref. 16) are involved in determining immunodominance, and our findings indicate that the speed with which a CD8$^+$ T cell responds to Ag contact also plays a pivotal role in regulating this important biological phenomenon.

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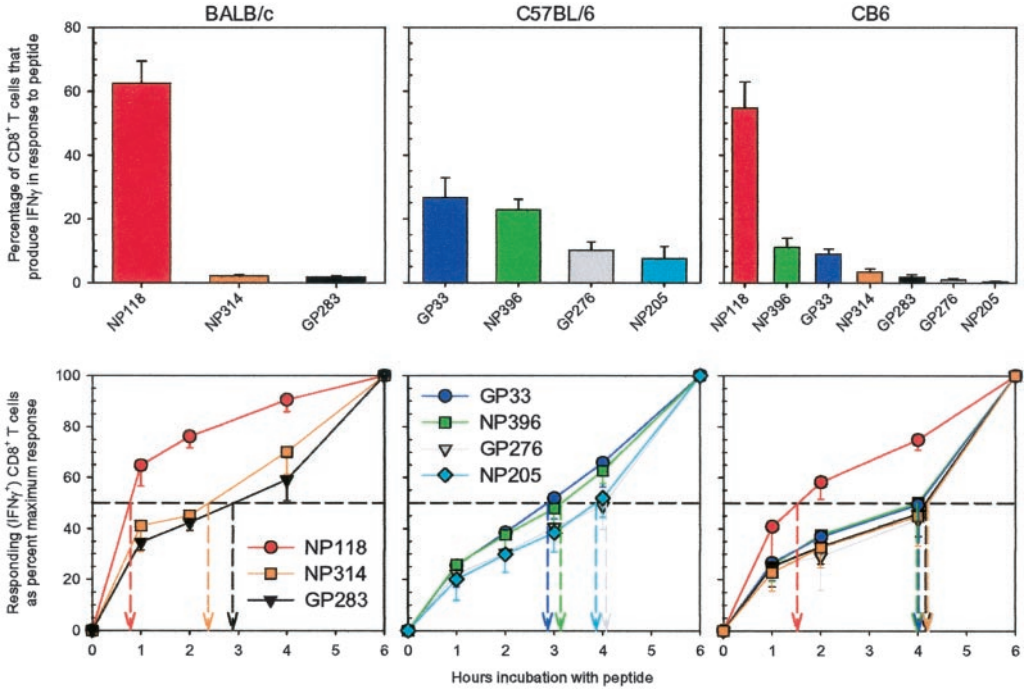

FIGURE 1. Response rates of various epitope-specific $CD8^+$ T cell populations at 8 days postinfection. Mice of the indicated strains were infected with LCMV, and 8 days later, their epitope-specific $CD8^+$ T cell responses were measured by ICCS as described. The data represent results from four to eight mice; the arithmetic means are shown, and the error bars represent 1 SD. *Upper panels*, Epitope-specific responses as percentages of total splenic $CD8^+$ T cells after subtraction of background as described in *Materials and Methods*. *Lower panels*, IFN-γ production after 1, 2, 4, or 6 h of peptide stimulation, shown as a proportion of the number of cells responding at 6 h. For each epitope-specific population, a drop-arrow indicates the OR 1/2.

Materials and Methods

Virus and mice

The Armstrong strain of lymphocytic choriomeningitis virus (LCMV)[4] was used throughout the study. BALB/cByJ, C57BL/6, and CB6 mice (F_1 cross of male C57BL/6 and female BALB/cByJ) were bred at The Scripps Research Institute breeding facility. Naive mice (6–12 wk of age) were infected with LCMV (2×10^5 PFU i.p.) and were killed at the indicated time points for analysis of $CD8^+$ T cell responses. Secondary challenge of LCMV-immune mice was conducted by injecting 2×10^6 PFU of LCMV i.p., and the $CD8^+$ T cell responses were analyzed 4 days later. All animal experiments were performed in compliance with the institutional animal care and use committee at The Scripps Research Institute.

Peptides

Peptides representing the various LCMV epitopes were purchased from PeptidoGenic Research (Livermore, CA) as HPLC-purified (>95% pure) material, and stock solutions were prepared in water or in 10% DMSO/water.

Intracellular cytokine staining (ICCS) and flow cytometry

Spleen cells from LCMV-immune mice were cultured in 96-well, U-bottom plates (2×10^6 cells/well) in complete RPMI 1640 (RPMI 1640 with 5% FBS, 20 mM HEPES, 2 mM L-glutamine, 1 U/ml penicillin G, and 100 μg/ml streptomycin) at 37°C in 5% CO_2 for 1–6 h in the presence of the indicated peptides (at a final concentration of 10^{-6} M). Brefeldin A (5 μg/ml; Sigma-Aldrich, St. Louis, MO) was present throughout the incubation. At the indicated time points, cells were harvested, washed with 2% FBS-PBS, and stored on ice. Cells were stained overnight at 4°C with anti-CD8 Ab (clone CT-CD8a; Caltag, Burlingame, CA), washed, and fixed in PBS containing 2% formaldehyde. After fixation, the cells were permeabilized and stained for cytokines according to the manufacturer's directions (BD Pharmingen, San Diego, CA). Samples were resuspended in PBS containing 2% formaldehyde and acquired on a FACScan flow cytometer (100,000–300,000 events acquired/sample). Data were analyzed with CellQuest software (BD Biosciences, San Jose, CA). For each epitope-specific $CD8^+$ T cell population, the epitope-specific responses to peptide stimulation were calculated by subtracting the percentage of cells that scored positive for IFN-γ production in the absence of peptide. Each experimental group comprised four mice, and the data presented are representative of experiments that were repeated three to eight times.

Statistical analysis

The statistical significance of on-rate kinetics vs immunodominance was determined by linear regression analysis using Excel software (Microsoft, Seattle, WA). A value of $p \leq 0.05$ was considered statistically significant.

Results

During the acute phase of the immune response to virus infection, the speed at which IFN-γ synthesis is initiated (the on-rate) varies depending on the epitope specificity of the cells

The primary $CD8^+$ T cell response to LCMV infection peaks ~8 days after infection and is composed of several populations of cells, each of a different epitope specificity. There are three main epitope-specific populations in BALB/c mice (specific for epitopes nucleoprotein 118 (NP118), NP314, and gp283) and four in C57BL/6 mice (epitopes gp33, NP396, gp276, and NP205); and all seven epitopes are recognized by CB6 mice (the F_1 progeny of a BALB/c×C57BL/6 cross). To determine how quickly each of the various epitope-specific primary populations could initiate IFN-γ synthesis in response to Ag contact (their on-rate), the proportion of cells synthesizing IFN-γ was evaluated after 1, 2, 4, and 6 h of peptide exposure. The response at 6 h was defined as 100%, the prior responses were plotted as a fraction of that maximum response, and the time taken for 50% of cells to respond to Ag (the on-rate for 50% of the epitope-specific population (OR 1/2)) was identified for each population (Fig. 1). The responses detected after 6 h of peptide stimulation (Fig. 1, *upper row*) accurately reflected

[4] Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; ICCS, intracellular cytokine staining; NP, nucleoprotein; OR 1/2, the time taken for 50% of an epitope-specific population to initiate IFN-γ production.

the published immunodominance hierarchies for the BALB/c and C57BL/6 haplotypes (10, 17, 18): in BALB/c mice (H-2^{dd}) the overwhelming majority of cells responded to NP118, and in C57BL/6 mice (H-2^{bb}) the dominance hierarchy was less marked, with responses to all four epitopes being readily detected. Striking differences were found in the OR 1/2 for the various epitope-specific cell populations. In BALB/c mice, half the $CD8^+$ T cells specific for the NP118 epitope had initiated IFN-γ production as soon as ~50 min after peptide exposure (see red drop-arrow). In contrast, the remaining two populations of epitope-specific cells responded much more slowly to peptide contact, and their OR 1/2 averaged between 140 and 175 min. Analyses of responses in C57BL/6 mice also revealed marked differences in OR 1/2 among the four epitope-specific populations; the two dominant populations had OR 1/2 of ~180 min, whereas the responses of the two subdominant populations were delayed by ~1 h. To determine whether the relative relationships among epitope-specific populations would be maintained in mice that could respond to all these epitopes, we crossed BALB/c mice with C57BL/6 mice to generate CB6 mice (H-2^{bd}). Remarkably, the overall dominance hierarchy was well maintained in these mice (Fig. 1, *upper right panel*). The strong response to NP118 correlates with reduced responses to the four epitopes presented by H-2^b alleles; indeed, the response to the weakest epitope (NP205) was almost undetectable in CB6 mice. The lower H-2^b-restricted responses are consistent with the idea that a strong $CD8^+$ T cell responses to a dominant epitope is associated with reduced $CD8^+$ T cell responses to subdominant epitopes, as we have previously demonstrated (19). The relationships between the on-rates of the epitope-specific populations in CB6 mice were similar to those seen in the parental mouse strains; the NP118-specific cells had by far the shortest OR 1/2, and all the other populations showed markedly delayed responses.

All $CD8^+$ memory T cell populations respond rapidly to Ag contact

Between days 8–30 postinfection, virus-specific $CD8^+$ T cell numbers rapidly decline, resulting in a relatively stable population of memory cells that remains detectable throughout the life of the animal (reviewed in Ref. 20). To investigate whether there might be differences between the on-rate kinetics of primary $CD8^+$ T cells and memory $CD8^+$ T cells of the same epitope specificity, we conducted analyses similar to those described above, using splenocytes from long term immune mice of all three strains. Mice were infected with LCMV, and at least 5 wk later the IFN-γ on-rate kinetics of their memory T cells were analyzed. The overall dominance hierarchies in immune mice (Fig. 2, *upper row*) are similar to those seen at the peak of the immune response, although, as expected, the numbers of cells were much reduced in the long term immune animals. Most strikingly, however, all the epitope-specific $CD8^+$ memory T cells responded very quickly to Ag contact; the OR 1/2 for all populations was between 40 and 90 min (Fig. 2, *lower row*). Six of the seven epitope-specific populations showed at least a 2-fold decrease in OR 1/2, and in some cases (e.g., the population specific for gp276) the response was accelerated by >4-fold. The only population that did not show an increased response rate was that specific for NP118; these T cells were by far the fastest responding population at the peak of infection (Fig. 1), suggesting that T cells with an OR 1/2 of ~1 h may be unable to further accelerate their response to Ag contact.

Acceleration of Ag-triggered IFN-γ synthesis is complete by ~21 days postinfection

When exposed to Ag, $CD8^+$ memory T cells initiate IFN-γ production more rapidly than do primary $CD8^+$ T cells. To determine when in the course of infection this maturation occurs, C57BL/6

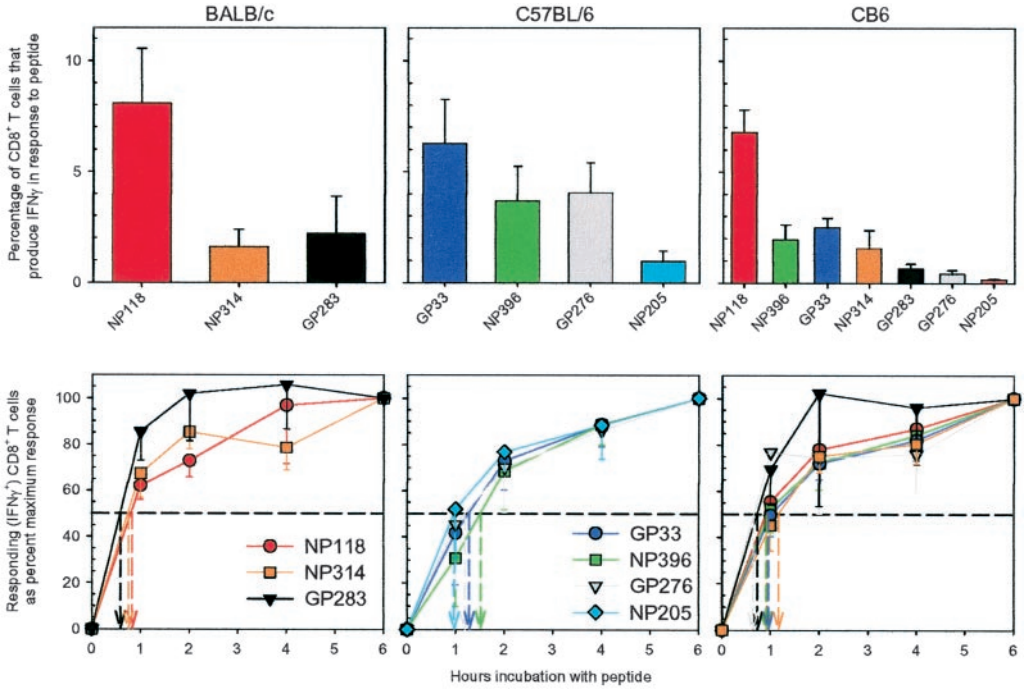

FIGURE 2. Response rates of various epitope-specific $CD8^+$ T cell populations at >5 wk postinfection. Mice of the indicated strains were infected with LCMV, and > 5 wk later, their epitope-specific $CD8^+$ T cell responses were measured by ICCS as described. The data represent results from four to eight mice; the arithmetic means are shown, and the error bars represent 1 SD. *Upper panels*, Epitope-specific responses as percentages of total splenic $CD8^+$ T cells. *Lower panels*, IFN-γ production after 1, 2, 4, or 6 h of peptide stimulation, shown as a proportion of the number of cells responding at 6 h. For each epitope-specific population, a drop-arrow indicates the OR 1/2.

mice were infected with LCMV, and their $CD8^+$ T cell responses were analyzed on days 8, 12, 21, and >30 postinfection. Representative results for the NP396 and gp276 epitopes are shown in Fig. 3; findings for other epitopes were similar in all mouse strains (not shown). The OR 1/2 decreased markedly between days 8 and 12, and by 21 days postinfection the cell populations had become maximally responsive to Ag contact (OR 1/2, ~1 h), with no subsequent improvement being noted in cells from long term immune animals. Therefore, the fast-onset phenotype of memory cells appears stable. However, we and others have previously shown that some aspects of memory cell effector function rapidly revert to an effector phenotype if the mice are once again exposed to virus (secondary infection); for example, their cytokine expression profiles revert to those observed in primary cells (8, 11), and their direct ex vivo cytolytic activities are rapidly up-regulated (21–23). To determine whether secondary exposure to virus in vivo would influence the response speed of memory cells, long term immune mice were infected with LCMV, and 4 days later, the OR 1/2 of the cell populations were determined. As shown in Fig. 3 (■), no significant increase in response speed was detected, and this was confirmed by subsequent analyses conducted 30 days after secondary infection (data not shown). Therefore, once a memory T cell has acquired a rapid cytokine induction phenotype, this characteristic is maintained during secondary challenge, even though other aspects of their biological function revert to those seen during the primary response.

The IFN-γ on-rate of the primary $CD8^+$ T cell population is more heterogeneous than the on-rate of the $CD8^+$ memory cell population

The slower onset of IFN-γ synthesis in primary cell populations compared with memory cell populations could be explained in at least two ways. First, the primary cell population may be relatively homogenous, with all the cells being slow in making IFN-γ; in this case, the faster responses by memory cells would imply that individual primary cells must differentiate, acquiring the ability to respond more quickly as they enter the memory phase. Second, the primary population may be heterogeneous, containing both fast and slow cells; if this is the case, the accelerated responses seen in memory cells might be explained not by the differentiation of individual cells, but possibly by the selective expansion/survival of the fast-responding cells in the primary population. To examine this issue we evaluated the heterogeneity of the primary and memory cell populations by studying the onset of IFN-γ production at various times after Ag exposure. Cells were incubated with peptide Ag for 1, 2, 3, 4, or 6 h and were analyzed by ICCS. Representative data are shown for cells exposed to peptide gp276 (Fig. 4). Many responding cells are readily detected in both primary and memory populations after only 1 hour of Ag exposure, indicating that both primary and memory populations contain fast-responding cells. However, the memory cell population appears much more homogeneous. Most memory cells rapidly initiate IFN-γ production and thereafter continue to accumulate the cytokine (in the presence of brefeldin A), resulting in an ascending cloud of cells; at later time points very few new IFN-γ-positive cells are recruited to the memory population, consistent with there being very few slow-onset cells in the memory pool. To evaluate the onset of IFN-γ^+ synthesis in a more quantitative manner, the IFN-γ^+ cells were divided into five strata (designated A–E) based on their IFN-γ content. At each time point after Ag contact, the proportion of responding cells within each stratum was calculated. The resulting bar-charts illustrate the homogeneity of the memory population. For example, by 6 h postexposure most memory cells are in strata A and B, and very few cells have recently initiated IFN-γ synthesis (stratum E). In contrast, the primary T cell population is much more heterogeneous. There are many fast-responding cells, shown by the rapid appearance of cells in strata A and B; however, slow-responding cells continue to enter the IFN-γ^+ population even long after the addition of peptide Ag. The heterogeneity of the primary response is clearly demonstrated by the similarity in cell numbers in all five strata, even at the later time points. These data lead us to speculate that the faster responsiveness of memory cell populations may result from the enhanced survival of those primary cells that are programmed to have the fastest onset of IFN-γ synthesis. Analogous analyses of dominant T cells (e.g., NP118-specific cells) showed that the population was relatively homogeneous during both the acute and memory phases, consistent with our observation that these cells are almost uniformly fast onset (data not shown).

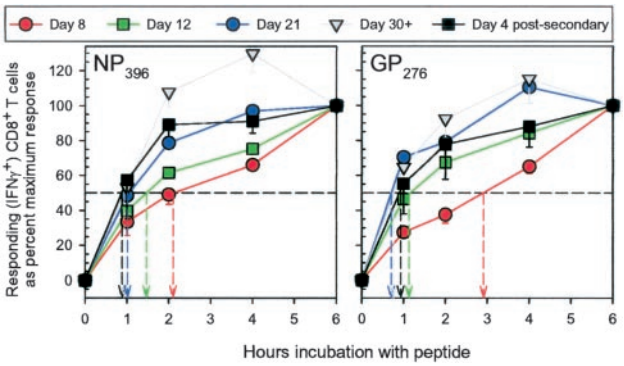

FIGURE 3. IFN-γ production accelerates over the course of primary infection, but not after secondary exposure to virus. C57BL/6 mice were infected with LCMV, and the maturation of effector function was followed by determining the OR 1/2 at various times thereafter. OR 1/2 values for two epitope-specific cell populations at 8, 12, 21, and >30 days after primary virus infection and at 4 days after secondary virus infection are shown by drop-arrows. The data represent results from four to eight mice; the arithmetic means are shown, and the error bars represent 1 SD.

Direct correlation between the speed with which IFN-γ production is initiated by epitope-specific $CD8^+$ T cell populations and their immunodominance

The data in Fig. 1 suggest that there is a relationship between the abundance (immunodominance) of an epitope-specific population and the rapidity with which its cells initiate IFN-γ production; cells that produce IFN-γ very quickly after Ag contact (e.g., NP118-specific cells in BALB/c and CB6 mice) are more abundant than cells that initiate IFN-γ production more slowly. To determine whether there is a statistically significant correlation between OR 1/2 and immunodominance during the acute phase of the immune response, the mean OR 1/2 was plotted against the mean cell abundance for each of the epitope-specific populations of day 8 cells (Fig. 5, *upper panel*). The correlation is clear and highly significant ($p = 0.0002$). A similar plot was prepared for each individual mouse, and in each case the dominant cell population was faster than the subdominant populations, and T cells of a similar hierarchical rank demonstrated similar on-rate kinetics (not shown). The OR 1/2 of subdominant cells could not be accurately determined before 8 days postinfection for two reasons. First, at earlier times postinfection the subdominant cells are rare; responding cells are undetectable at early time points in the direct ex vivo assay, preventing the acquisition of a reliable OR 1/2. Second, this problem was compounded by the fact that at earlier times postinfection, there is some remaining viral Ag in the preparation, which increases the background of spontaneous IFN-γ production.

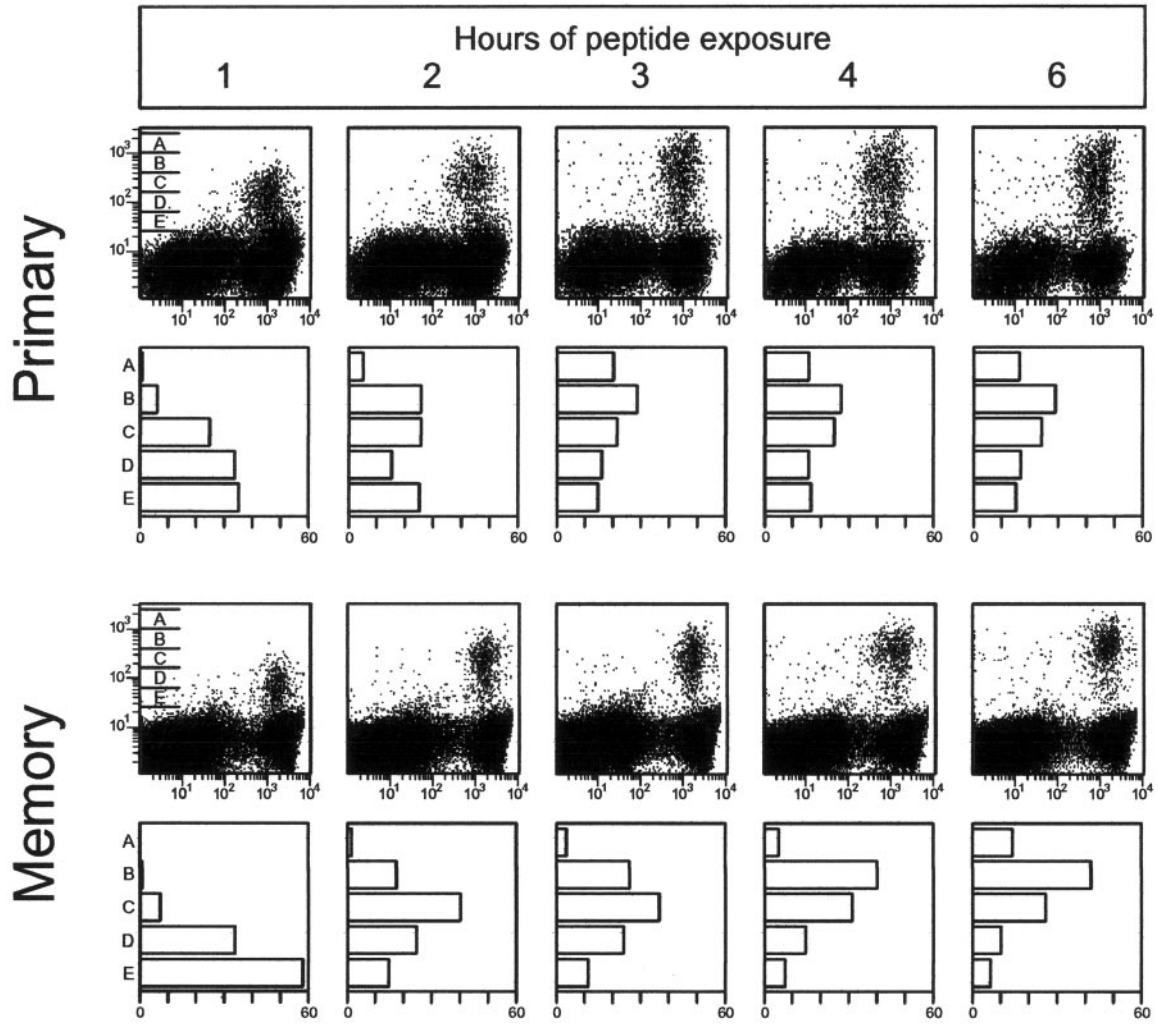

FIGURE 4. The IFN-γ on-rate of the primary CD8+ T cell population is more heterogeneous than the on-rate of the CD8+ memory T cell population. Primary cells (8 days postinfection) or memory cells (30 days postinfection) were incubated with the gp276 peptide for the indicated times, and the cells were analyzed by ICCS. Representative dot plots show the responding CD8+ T cells (*x*-axis, CD8 expression; *y*-axis, IFN-γ expression). The level of IFN-γ fluorescence was divided into five strata (*A–E*, shown in the *leftmost* dot plots), and for each dot plot, a bar chart illustrates the proportion of cells in each stratum, expressed as the percentage of all responding (IFN-γ+) gp276-specific cells.

When the mean values for memory cells were plotted (Fig. 5, *lower panel*), no significant difference in their kinetics of IFN-γ production were found ($p = 0.20$), thereby highlighting our observation that the OR 1/2 of most epitope-specific CD8+ T cell populations have been similarly maximized by the time the cells pass into the memory phase (Figs. 2–4).

Discussion

Studies of virus infections and of vaccinations suggest that CD8+ T cells confer maximal biological benefit upon the host if they can identify and act upon infected cells very soon after infection, before the virus has had the chance to produce infectious progeny. For instance, many of the most effective CD8+ T cell responses are specific for epitopes from viral proteins that are synthesized immediately postinfection (24–27); in some cases, CD8+ T cells may even recognize viral structural proteins that are introduced into the MHC class I pathway immediately after virus entry, without any requirement for de novo synthesis within the cell (28). Furthermore, engineering a viral CD8+ T cell epitope to cause its expression earlier in the viral life cycle increases the antiviral effects of the cognate CD8+ T cells (29). Moreover, CD8+ memory T cells induced by antiviral vaccines appear to exert their antiviral effects very soon after challenge (30). Taken together, these findings suggest that the speed with which CD8+ T cells can respond to individual virus-infected cells is an important criterion of their biological efficacy.

How might CD8+ T lymphocytes maximize the speed with which they can exert their effects on an infected cell? We have previously shown that the ability of CD8+ T cells to respond to low doses of Ag (which we termed their functional avidity) undergoes a dramatic improvement between days 4 and 8 postinfection, over which period the amount of Ag required to trigger CD8+ T cell responses drops ~80-fold (15). Thus, one facet of CD8+ T cell maturation is their enhanced ability to recognize cells at a very early stage of infection, when Ag presentation has just begun. How might CD8+ T cells further increase their value to the host? We show here that primary CD8+ T cell populations of different epitope specificities differ in the speeds with which they initiate IFN-γ production after Ag contact, but for many populations, the OR 1/2 is ~3–4 h. These slow-onset populations accelerate their IFN-γ expression over a period of ~21 days postinfection until they approach an OR 1/2 of ~1 h. Although this improvement may at first appear modest, it is likely that it would confer a substantial biological advantage. For most viruses, a single round of

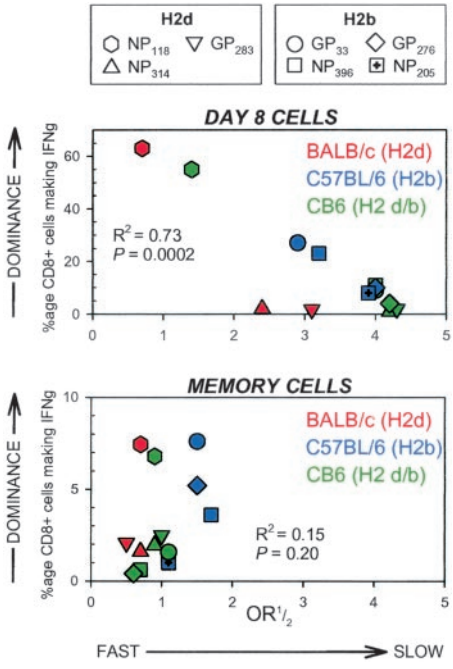

FIGURE 5. During acute infection, the OR 1/2 of an epitope-specific $CD8^+$ T cell population is correlated with its abundance. *Upper panel*, the kinetics of IFN-γ production (OR 1/2) are plotted against the abundance of the corresponding peptide-specific T cells (immunodominance) for each epitope-specific population at 8 days postinfection. *Lower panel*, An analogous plot for each epitope-specific population of memory cells (>5 wk postinfection). Each symbol represents the average score obtained from more than eight mice. The r^2 and p values for the day 8 and memory populations were determined by linear regression analysis.

propagation (from infection, through replication, to release of infectious progeny) takes place over a short time period (usually ~6–24 h); consequently, even a small increase in the rapidity with which a triggered $CD8^+$ T cell can express an antiviral function might have a substantial effect on virus production by the infected cell. We infer from two observations that there may be a physiological limit beyond which there can be no additional reduction in the OR 1/2. First, we see no significant improvement in the speed of response during the maturation of cells specific for NP118, which already have attained a fast OR 1/2 during the acute phase of infection (Figs. 1 and 2). Second, we see no significant additional improvement in OR 1/2 when memory cells are re-exposed to virus Ag in vivo (Fig. 3, ■). In summary, during maturation of the antiviral response, $CD8^+$ T cells increase the speed with which they can exert their effector functions upon infected cells, using two related, but distinct, mechanisms: 1) they can be triggered by lower levels of Ag, thereby responding to cells soon after they become infected (15); and 2) after triggering takes place, their effector functions are expressed more rapidly (this study).

In this study we report three observations concerning IFN-γ synthesis by virus-specific $CD8^+$ T cells. First, $CD8^+$ T cells are not all created equal; the on-rates differ among the various primary $CD8^+$ epitope-specific populations. Second, the slower populations mature over a ~21-day period by accelerating their initiation of IFN-γ production. Third, there is a correlation between the IFN-γ OR 1/2 of primary cells and their immunodominance. What molecular mechanisms might underpin these biological events? The reasons for the differences in OR 1/2 early in infection are unknown. Every population of epitope-specific $CD8^+$ T cells comprises a collection of clonotypes, each of which has a TCR with a different affinity for the MHC/peptide complex. This raises the possibility that clonotypic cells with high affinity TCR might initiate IFN-γ synthesis more rapidly. However, we consider this explanation to be unlikely, because structural (TCR-based) avidity maturation does not occur to any considerable degree in the LCMV model (15), and only minor changes in the structural avidity of peptide-specific T cells have been reported in other models (12, 13). We considered the possibility that the changes reported here might result from the selective expansion of T cells with the highest functional avidity. However, this is unlikely, because functional avidity maturation is completed by ~8 days postinfection (15), whereas the improvement in the OR 1/2 of subdominant cells continues to increase well beyond this time point. Furthermore, several studies have shown that the TCR of primary cells and memory cells are phenotypically similar (31, 32), and that changes in TCR representation do not occur after the primary infection (33, 34). These observations also suggest that the maturation of $CD8^+$ T cell responses in the ~21 days after infection is unlikely to rely on a preference for cells bearing high affinity TCR. The maturation events could be explained in at least two ways, which are not mutually exclusive: 1) by differentiation, in which individual cells acquire the heritable capacity to more rapidly initiate IFN-γ synthesis; and 2) by selection, in which the improvement in OR 1/2 results not from the acceleration of IFN-γ production by individual cells, but, rather, from the selective expansion and survival of fast-onset cells in the epitope-specific population, with attrition of slow-onset cells. Our previous analyses of functional avidity (i.e., a cell's ability to be triggered by a low dose of Ag) shows it to be complete by ~8 days postinfection, and it appears to be reliant on differentiation (hard-wiring of the signal transduction machinery) (Ref. 15 and data not shown), although we have not excluded the possibility that selection, too, plays some part in avidity maturation. In this manuscript we demonstrate that the OR 1/2 matures over time, and our data show that (for most epitope-specific populations) the primary pool is heterogeneous and moves toward homogeneity as the cells pass into the memory phase (Fig. 4). These data are consistent with differentiation, because the slow-onset cells present on day 8 may accelerate their IFN-γ on-rate over the succeeding ~2 wk, thereby contributing to the homogeneity of the memory cell population. However, the data also are consistent with the second mechanism, wherein the fast-onset cells, present in all the epitope-specific primary populations, are at a selective advantage; they and their progeny may preferentially expand, survive, and enter the memory cell population. Regardless of which mechanism underlies the maturation process, our finding that the constitution of the $CD8^+$ T cell pool continues to evolve until ~21 days postinfection may explain the progressive changes in gene expression that have been observed in populations of $CD8^+$ T cells after virus Ag has been cleared (35).

The third observation reported herein, that there is a correlation between OR 1/2 of primary cells and their position in the immunodominance hierarchy (Fig. 5, *upper panel*), can be considered from the same mechanistic perspective. A role for IFN-γ in regulating immunodominance was first proposed by Harty et al. (36), who showed that the relative abundance of epitope-specific populations was altered in mice lacking this cytokine, and these findings were extended by our laboratory's evaluation of $CD8^+$ T cell responses in DNA-immunized mice (19). Thus, although many factors contribute to immunodominance (reviewed in Ref. 16), it is clear that IFN-γ plays a significant role in regulating $CD8^+$ T cell abundance during virus infection (19). The extent of this IFN-γ-mediated effect and its underlying mechanism are under active investigation, but our current data lead us to propose that the rapidity with which a cell produces IFN-γ is a major criterion that

determines the cell's survival and future expansion. Thus, we propose a unifying hypothesis that links the immunodominance hierarchy of CD8$^+$ T cells, their subsequent maturation, and their entry into the memory phase. We suggest that rapid IFN-γ production confers a selective advantage upon CD8$^+$ T cells, thereby explaining 1) why the fastest-onset cells are the most abundant during the primary response; 2) how epitope-specific T cell populations improve their OR 1/2 until they have reached a limit; and 3) the ability of DNA vaccine-induced memory cells, all of which have a fast IFN-γ on-rate (37), to preferentially expand upon secondary exposure to Ag (thus contributing to "T cell original antigenic sin") (19). Consistent with the idea that IFN-γ may play a central role in the regulation of CD8$^+$ T cell responses, a very recent in vivo analysis has shown that naive T cells begin to produce IFN-γ within hours of encountering dendritic cells in lymph nodes (38). This hypothesis also may explain why, as described above, virus-specific CD8$^+$ T cells tend to be targeted toward virus proteins that are expressed early in infection. We suggest that the overwhelming dominance of CD8$^+$ T cell responses to, for example, herpesvirus immediate-early proteins, occurs because CD8$^+$ T cells of that specificity are the first to receive antigenic stimulation and therefore are the first to elaborate IFN-γ. There is an obvious biological benefit to the host, because the CD8$^+$ T cells that can produce IFN-γ most rapidly in response to early viral Ags will be selectively expanded; in this way, the host ensures that the dominant primary CD8$^+$ T cell response to virus infection will comprise fast-onset cells that are specific for Ags expressed early in the viral life cycle.

In conclusion, we propose that the rapid onset of IFN-γ production by CD8$^+$ T cells has two distinct functions: it will enhance the T cells' antiviral effect, and it will play a role in regulating their abundance. During primary infection, the virus-specific T cells that can most rapidly respond to Ag contact will be preferentially expanded, and fast-onset cells preferentially enter the memory T cell pool. Indeed, we find this aspect of the hypothesis particularly attractive; evolution has ensured that the dominant primary CD8$^+$ T cells and the surviving CD8$^+$ memory T cells are those that most rapidly elaborate an important antiviral effector molecule and thereby will be most capable of controlling virus infection.

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