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*J Immunol* 2003; 170:2442-2448; doi: 10.4049/jimmunol.170.5.2442

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Distinct Thresholds for CD8 T Cell Activation Lead to Functional Heterogeneity: CD8 T Cell Priming Can Occur Independently of Cell Division

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To examine the bases for CD8 T cell functional heterogeneity, we analyzed responses to partial vs full agonist Ag. An extended period of interaction with APCs was required to set the threshold required for cell division in response to partial as compared with full agonist Ag. Acquisition of cytolytic function was restricted to the divided T cell population. In contrast, the threshold for commitment to produce IFN-γ and express some activation markers appeared lower and independent of cell division. Indeed, we characterized a T cell population stimulated in response to the partial agonist that was committed to produce IFN-γ, but failed to divide or secrete IL-2. Importantly, this activated nondivided population behaved as “primed” rather than “anergized,” indicating 1) that priming of CD8 T cells may be induced by suboptimal stimulation independent of cell division and 2) that encounter with Ag does not always induce a complete differentiation program in naive CD8 T cells, as previously reported. The Journal of Immunology, 2003, 170: 2442–2448.

The CD8 T lymphocytes are the main cellular effectors mediating elimination of virally infected or tumor Ag- and alloantigen-expressing cells. The response relies on the differentiation of a small number of specific naive CD8 T lymphocytes into potent effector CTLs having acquired a cytolytic machinery and the capacity to secrete cytokines, as well as on the clonal expansion of these effectors (1). Whether these phases of the immune response are triggered concomitantly or independently following engagement of the TCR complexes on naive CD8 T cells are important questions to answer to optimize vaccination protocols and antitumor immunotherapies. Recently, several studies (2–4) have suggested that a brief encounter with Ag (as little as 2 h of exposure to APCs) was sufficient for naive CD8 T cytotoxic precursors to initiate a complete differentiation program including proliferation, acquisition of effector functions, and the establishment of a pool of memory T cells.

However, several examples of dysfunctional (or partially functional) CD8 T cells have been documented in vivo both in humans and mice (5–9). It is not clear in these various examples whether the initial antigenic stimulation or secondary effects or the chronicity of antigenic stimulation are responsible for the observed deficiencies.

Altogether, it appears that the simple “autopilot” model proposed for naive CD8 T cell differentiation (10) does not account for the existence of partially functional CD8 T cells. Rather, the question of differential requirements for the acquisition of distinct effector functions and/or for long-term survival has still to be explored. In this respect, parameters such as 1) the strength of stimulation, 2) the duration of T cell/APC engagement, 3) the cell cycle status, and 4) the cytokine milieu may influence the acquisition of T cell functions. Concerning points 1) and 2), the nature of peptide-MHC complexes (agonist vs partial agonist) was shown to determine the activation program of CD8 T cells in vitro. Indeed, for both naive CD8 T cells (11) and CD8 T cell clones (12, 13), a partial agonist (also referred as an altered peptide ligand (14)) triggered only a subset of the effector functions induced by an agonist. These observations suggested that different activation thresholds were required to induce distinct CTL functions. Furthermore, as mentioned above, the initiation of an activation program may be dependent on cell proliferation (point 3), which is tightly linked to chromatin remodeling and gene expression. Indeed, primary induction of CTL could be dissociated in a differentiation step in the absence of DNA synthesis followed by blast transformation and proliferation (15). More recently, a correlation between CD8 T cell division and development of CTL activity has been suggested (16). A link between proliferation and cytokine production in CD4 T cells was also reported (17, 18) with distinct requirements for production of different cytokines. Whether the same observations can be applied to cytokines produced by CD8 T cells has still to be analyzed.

We took advantage of a model in which naive CD8 T cells expressing an H-2Kb-alloreactive TCR responded to a mutant alloantigen H-2Kbm8 by expressing a partial activation program (11), to define the requirements for the acquisition of different effector functions by CD8 T cells in relationship to avidity of TCR engagement and to cell division. Interestingly, we characterized an Ag-experienced nondivided population that behaved as a fully competent CD8 population in a secondary response.

Materials and Methods

Animals

Mice transgenic for the BM3.3 TCR (19) on the CBA/Ca background (tgTCR), C57BL/6 (B6) and C57BL/6.C-H-2bm8 (bm8) mice were bred in the Centre d’Immunologie de Marseille-Luminy animal facility.
Cell purification

CD8 cells were purified from lymph nodes of tgTCR mice by negative selection as previously described (11). In all experiments, CD8 T cells represented 90–98% of the enriched population. APCs were T-depleted irradiated splenocytes.

Flow cytometric analyses

Reagents used for immunofluorescence staining were: biotin-mAb T908, an anti-Fas mAb specific for the BM3.3 TCR (20) conjugated in the laboratory; PE-anti-CD44, PE-anti-Fas ligand (FasL),1 allophycocyanin-anti-IFN-γ, allophycocyanin-anti-IL-2, and PCP-Cy5.5-anti-CD8α (BD Pharmingen, San Diego, CA). For IFN-γ and IL-2 intracellular staining, cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA in the presence of 10 μg/ml brefeldin A. Cells were then fixed in 2% paraformaldehyde and permeabilized with 0.5% saponin. For FasL expression, cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA in the presence of a matrix metalloproteinase inhibitor, KB8301 (BD Pharmingen). After staining, 6 × 10⁴ viable cells in each sample were analyzed using a FACSCalibur cytometer (BD Biosciences).

CFSE staining and cell sorts

Determination of the number of T cell divisions was done by flow cytometry using the fluorescent dye CFSE that was shown to exhibit sequential halving of intracellular fluorescence intensity at each division step (21). Purified tgTCR CD8 cells were incubated for 10 min at 37°C with 5 μM CFSE (Molecular Probes, Eugene, OR). Cell sorts based on CFSE profiles were performed using a FACSSelection cell sorter (BD Biosciences).

Secondary stimulation

After cell sorting, cells were relabeled with 5 μM CFSE. Cells were then recultured in vitro with APCs.

Cytotoxic assays

Cytotoxic activity of tgTCR CD8 T cells was tested on RMA (H-2b) lymphoma cells during a 4-h incubation. To measure Fas-mediated lysis, effectors cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA. During this incubation, 10⁴ Fas-expressing targets (L1210-Fas (22)) or control cells (L1210) were added. All of the targets were labeled with ⁵¹Cr (New England Nuclear, Boston, MA).

Results

Heterogeneity within a “monoclonal” tgTCR CD8 population in the proliferative response to a partial agonist

In vitro cultures of CFSE-labeled tgTCR CD8 T cells with APCs expressing either the full (B6) or the partial (bm8) agonist were established and the division rate of tgTCR CD8 T cells was assessed by measuring CFSE intensities at different time points (Fig. 1a). As a control, syngeneic APCs (CBA) were also used, which did not induce any division in tgTCR CD8 T cells. The full agonist B6 sustained a massive proliferation of tgTCR CD8 T cells as most of these cells had divided four to five times by day 3. In contrast, the response to the partial agonist bm8 was heterogeneous as 1) part of the tgTCR CD8 T cell population went into division with slower kinetics (one to four divisions in 3 days) than observed in response to B6 and 2) more interestingly, a fraction of the tgTCR CD8 T cells did not divide at all. The fact that the percentage of the nondivided population dropped from day 3 to day 4 (Fig. 1a and Table I) suggested that some of these cells have a delayed entry in cell division. But in addition, there was a stable pool of 17% of the total population that did not divide even when the culture was maintained up to 5 days.

Lymphokine secretion by naive CD8 T cells is independent of T cell proliferation

We have previously shown that stimulation of the tgTCR CD8 T cells by the full agonist B6 was efficient to induce IFN-γ and IL-2 secretion, both being produced in excess in culture supernatants, whereas the partial agonist bm8 was only effective for IFN-γ production (11). In this study, we further showed that cytokine production occurred very early, 24 h after the initial stimulation, at a time when cell division has not begun yet. IL-2 was secreted during the first 24 h by nondivided cells in response to the full agonist B6 (Fig. 1b). A small amount of IL-2 was made by tgTCR CD8 T cells in response to the partial agonist bm8 (Fig. 1b) that was not detectable in culture supernatant (11). Similar to IL-2 secretion profiles, CD25 (IL-2Rα chain) was differentially up-regulated with a high vs a low expression upon B6 and bm8 stimulation, respectively (Fig. 2b). In the latter case, as proof that the limiting factor was the amount of IL-2, we were able to fully restore CD25 up-regulation by providing exogenous IL-2 in the culture in addition to bm8 APCs (Fig. 2b).

IFN-γ was produced in response to both full and partial agonist, first by nondivided tgTCR CD8 T cells and later by cells that had divided at day 2 (Figs. 1c and 2a) and day 3 (Fig. 2a). Clearly, part of the tgTCR CD8 T cells remained nondivided but differentiated into IFN-γ producers after 3 days of in vitro culture with bm8

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1 Abbreviations used in this paper: FasL, Fas ligand; CD40L, CD40 ligand; CD40L, CD40 ligand.

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FIGURE 1. Lymphokine secretion by naive CD8 T cells are independent of T cell proliferation. CFSE-labeled tgTCR CD8 T cells were cultured for the indicated period of time with either syngeneic (CBA) or B6 or bm8 APCs. a, CFSE histograms on gated tgTCR CD8 cells are shown for the BM3.3 TCR (20) conjugated in the laboratory; PE-anti-CD44, PE-anti-Fas ligand (FasL),1 allophycocyanin-anti-IFN-γ, allophycocyanin-anti-IL-2, and PCP-Cy5.5-anti-CD8α (BD Pharmingen, San Diego, CA). For IFN-γ and IL-2 intracellular staining, cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA in the presence of a matrix metalloproteinase inhibitor, KB8301 (BD Pharmingen). After staining, 6 × 10⁴ viable cells in each sample were analyzed using a FACSCalibur cytometer (BD Biosciences).

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FIGURE 2. Lymphokine secretion by naive CD8 T cells are independent of T cell proliferation. CFSE-labeled tgTCR CD8 T cells were cultured for the indicated period of time with either syngeneic (CBA) or B6 or bm8 APCs. a, CFSE histograms on gated tgTCR CD8 cells are shown for the BM3.3 TCR (20) conjugated in the laboratory; PE-anti-CD44, PE-anti-Fas ligand (FasL),1 allophycocyanin-anti-IFN-γ, allophycocyanin-anti-IL-2, and PCP-Cy5.5-anti-CD8α (BD Pharmingen, San Diego, CA). For IFN-γ and IL-2 intracellular staining, cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA in the presence of a matrix metalloproteinase inhibitor, KB8301 (BD Pharmingen). After staining, 6 × 10⁴ viable cells in each sample were analyzed using a FACSCalibur cytometer (BD Biosciences).

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FIGURE 3. Lymphokine secretion by naive CD8 T cells are independent of T cell proliferation. CFSE-labeled tgTCR CD8 T cells were cultured for the indicated period of time with either syngeneic (CBA) or B6 or bm8 APCs. a, CFSE histograms on gated tgTCR CD8 cells are shown for the BM3.3 TCR (20) conjugated in the laboratory; PE-anti-CD44, PE-anti-Fas ligand (FasL),1 allophycocyanin-anti-IFN-γ, allophycocyanin-anti-IL-2, and PCP-Cy5.5-anti-CD8α (BD Pharmingen, San Diego, CA). For IFN-γ and IL-2 intracellular staining, cells were restimulated for 4 h with 200 ng/ml ionomycin plus 10 ng/ml PMA in the presence of a matrix metalloproteinase inhibitor, KB8301 (BD Pharmingen). After staining, 6 × 10⁴ viable cells in each sample were analyzed using a FACSCalibur cytometer (BD Biosciences).
Table I. Evolution with time of the fraction of nondivided tgTCR CD8 cells upon stimulation by a partial agonist

<table>
<thead>
<tr>
<th>Day</th>
<th>B6</th>
<th>bm8</th>
<th>CBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2.75 ± 0.83 (n = 7)</td>
<td>27.92 ± 14.6 (n = 9)</td>
<td>97.4 ± 3.0 (n = 7)</td>
</tr>
<tr>
<td>4</td>
<td>0.44 ± 0.1 (n = 2)</td>
<td>19.74 ± 0.2 (n = 2)</td>
<td>95.1 ± 0.5 (n = 2)</td>
</tr>
<tr>
<td>5</td>
<td>0.26 ± 0.1 (n = 2)</td>
<td>16.53 ± 1.3 (n = 2)</td>
<td>94.5 ± 0.3 (n = 2)</td>
</tr>
</tbody>
</table>

Percentage of nondivided tgTCR CD8 cells assessed by CFSE labeling and FACS analysis.

We have previously observed that both B6 and bm8 APCs were able to induce the differentiation of naive tgTCR CD8 T cells into cytolytic effectors, but the efficiency of the latter stimulus was lower (Ref. 11 and Fig. 3a). This could be attributed to both a weaker perforin-mediated cytotoxicity and an absence of Fas-dependent cytolyysis (Fig. 3a) that was due to a defect of the partial agonist to induce the up-regulation of FasL (Fig. 3b). This defective FasL expression was also monitored at the mRNA level by quantitative RT-PCR (relative values (calculated as described in Ref. 17) are 21.1 ± 0.7 and 17.2 ± 0.9 for B6 activation at 48 and 72 h and 2.6 ± 0.4 and 2.6 ± 0.2 for bm8 stimulation). Moreover, the fact that addition of exogenous IL-2 could not correct the defect in FasL expression (result not shown) suggests that a weak TCR engagement by a partial agonist is a limiting factor for induction of FasL.

Upon sorting divided and nondivided tgTCR CD8 T cells, we observed that only the proliferating CD8 T cells were able to kill K b-expressing target cells (Fig. 4a, lower graph). A higher resolution cell sort showed (Fig. 4b) that tgTCR CD8 T cells had to complete at least two divisions to become potent CTLs.

Activation by a partial agonist required sustained TCR engagement

The existence of an Ag-experienced nondivided population in response to the partial agonist suggested that the threshold of TCR

![FIGURE 2](image)

FIGURE 2. Acquisition of activation marker by naive CD8 T cells is independent of T cell proliferation. CFSE-labeled tgTCR CD8 T cells were cultured with either syngeneic (CBA) or B6 or bm8 APCs. a, CD44 vs IFN-γ stainings are shown after applying a gate on CD8 T cells that are divided (upper row) or not (lower row). Percentage of CD44-positive cells are reported. b, Cultures were conducted for 3 days in the absence or presence of IL-2. CD25 profiles are shown, and means of relative fluorescence intensity are reported.

![FIGURE 3](image)

FIGURE 3. Failure of the K bm8 partial agonist to induce FasL expression. tgTCR CD8 T cells were cultured for 3 days with either syngeneic (CBA, △) or B6 (■, ●) or bm8 (□, ○) APCs. a, Cytolytic activity was assessed either on a K b target cell (RMA) or a MHC irrelevant target-expressing Fas (L1210-Fas, ■, □, thin lines) or not (L1210, ●, ○, dotted lines) as described in Materials and Methods. SD have been calculated on three independent experiments. b, FasL surface expression was measured and gray histograms represent the staining obtained after culture with syngeneic APCs.
The engagement required for induction of cell division was not reached for that population. We thus compared the duration of TCR engagement required in response to full or partial agonist to trigger cell division (Fig. 5a) or activation as measured by IFN-γ production (Fig. 5b). For this purpose, we added an anti-Kb mAb that recognizes equally Kb and Kb* molecules (23) at different times after the beginning of the stimulation of CFSE-labeled tgTCR CD8 T cells. The proliferation of the tgTCR CD8 T cells assessed after 3 days of culture was drastically blocked if the anti-Kb mAb was added either at the time of the stimulation (t = 0) or 4 or 18 h after this initial activation by bm8 APCs. The interruption of TCR engagement after 4 or 18 h also abrogated IFN-γ secretion. On the other hand, the anti-Kb mAb was less efficient at blocking the proliferation induced by the full agonist. Indeed, a complete inhibitory effect was only seen if the ratio of Kb APCs:tgTCR CD8 T was lowered to 1:1. At the commonly used 2:1 ratio, the proliferation was partially blocked if the anti-Kb mAb was added at the initiation of the culture and even less if the treatment was started 4 h later. At this time, only a partial effect on IFN-γ production could be observed. Altogether, we confirm that a very short interaction period with a full agonist is sufficient to commit naive CD8 T cells for acquisition of effector functions and cell division, as reported previously (2–4). In contrast, we show that for both induction of cell division and IFN-γ production, a sustained TCR re-engagement is required when stimulation of naive CD8 T cells is suboptimal.
Having identified a T cell population that was stimulated by the partial agonist, but failed to divide or secrete IL-2, properties similar to those of “anergized” T cell clones (24), we wondered whether this population would be able to respond in a secondary stimulation. In particular, it was of interest to analyze whether a second round of antigenic stimulation could overcome their defect in cell division. Therefore, after 3 days of a primary stimulation with bm8 APCs, divided (CFSElow) and nondivided (CFSEhigh) tgTCR CD8 T cells were sorted, relabeled with CFSE, and recultured for 48 h with either syngeneic or B6 or bm8 APCs. CFSE profiles analyzed after 2 days of this secondary stimulation (Fig. 6a) showed that the sorted divided population pursued a massive proliferation upon TCR re-engagement. In comparison, the nondivided sorted population also entered into division when its TCR was re-engaged either by full or partial agonist-expressing APCs, but at a nonsynchronized rhythm, and a small proportion of tgTCR CD8 T cells still failed to divide. For both T cell populations, proliferation required TCR re-engagement as shown by lack of cell division in the presence of syngeneic APCs. Hence, even the nondivided cells that were able to produce IFN-γ at day 3 (30%, see Fig. 4) failed to go on to divide unless recall Ag was provided.

We next addressed whether the proliferation observed after the secondary stimulation was linked to the recovery of an efficient CTL function. Indeed, both the sorted divided and nondivided populations were efficient killers of an H-2Kb-expressing target 48 h after TCR re-engagement in the secondary culture (Fig. 6b), conditions that failed to induce CTL in response to bm8 in primary stimulation (data not shown). In addition, the two sorted populations secreted very high levels of IFN-γ in a secondary activation by the full agonist B6 as compared with the weak response observed at that time (20 h) in a primary stimulation (Fig. 6c). Both sorted populations also mounted a response to the partial agonist bm8 that was significantly increased as compared with that of naive tgTCR CD8 T cells. Interestingly, the sorted nondivided CFSEhigh population showed a higher secondary response to bm8 than its CFSElow counterpart. This difference could not be explained by an alteration in tgTCR or CD8 expression (data not shown).

Altogether, the nondivided population of tgTCR CD8 T cells did not behave as anergic T cells or as cells induced in some state that would be refractory to cell division, but rather they behaved as primed T cells in terms of proliferation, cytotoxicity, and production of IFN-γ in a recall response.

**Discussion**

**Distinct activation thresholds define a novel hierarchy for acquisition of effector functions by CD8 T cells**

In this report, we have addressed the requirements for induction of functional programs in naive CD8 T cells with a main focus on the importance of the quality of the initial TCR engagement. We demonstrated that a low-avidity TCR ligand was able to drive IFN-γ production, to induce acquisition of activation markers such as CD44, and, most importantly, to fully prime these cells, in a manner independent of proliferation. Furthermore, we took advantage of a model where suboptimal TCR activation drove a fraction of the tgTCR CD8 cells into division, whereas another remained undivided, to explore the relationship between induction of CTL effector function and cell division. A correlation between the number of cell divisions and cytolytic activity assessed on a T cell population has been reported previously (16). In this study, we demonstrated on sorted cells that acquisition of cytolytic function was totally dependent on cell division. Finally, IL-2 production required a strong TCR engagement even though it appeared to be uncoupled from cell division.

Therefore, the activation threshold for the commitment to produce IFN-γ and to up-regulate certain surface molecules (CD44)
appeared lower than that required for entry into cell cycle and induction of CTL function. Indeed, a fraction of the cells that remain undivided has acquired the capacity to secrete IFN-γ.

Altogether, our data allow us to propose a novel hierarchy of effector functions induced in naive CD8 T cells that is different from the one suggested in a previous report (25). This discrepancy can be explained by the fact that lowering the concentration of the agonist peptide as was done in that study may not mimic TCR engagement by a partial agonist. Furthermore, we have analyzed the differentiation of naive CD8 T cells into CTL effectors, whereas Valitutti et al. (25) used established T cell clones and therefore addressed the sensitivity of the cytolytic effector phase. Other reports using CTL clones have shown that altered peptide ligands can activate Fas- but not perforin-dependent cytotoxicity (12, 13, 26). In the last of these three studies, the authors showed that the CTL clone constitutively expressed FasL and that weak stimulation allowed the translocation of preformed FasL at the cell membrane. In this study, using naive CD8 T cells that do not express FasL, we showed that the activation threshold required for priming of perforin-dependent cytotoxicity was lower than for FasL mRNA and protein expression. Therefore, the threshold for activation of a particular function may be different between naive and effector CD8 T cells. Consistent with the definition of a partial agonist (14, 27), the stimulation by peptide-K\textsubscript{LP} complexes led to a differential and heterogeneous response of the monoclonal tgTCR CD8 T cell population as compared with its homogeneous response to the full agonist. The heterogeneity included a partially activated dividing population and a more unusual partially activated nondividing subset.

**Nondivided activated CD8 T cells can be fully primed rather than energized**

In fact, a main focus of our functional study concerned this nondividing population of tgTCR CD8 cells stimulated by the partial agonist. In several models, lack of proliferation was correlated with induction of anergy (24). We were therefore interested by the ability of part of this nondividing cell subset (around 30%) to produce IFN-γ and up-regulate CD44. Although they acquired some functions, these nonproliferating tgTCR CD8 cells remained in G\textsubscript{0}-G\textsubscript{1} phase and did not increase in size (data not shown). They were also not committed to divide, since they had to re-engage their TCR to do so. Characterization of noncyling CD4 T cells that produce IFN-γ has also been reported (28, 29).

Furthermore, we showed that the undivided CD8 T cell population was fully primed and had acquired the ability to respond very quickly in a secondary stimulation (Fig. 6) with a complete functional program including IFN-γ secretion, CTL activity, and proliferation. Therefore, both the priming of CD8 T cells and cytokine secretion do not require cell division and blast transformation. These results demonstrate that under suboptimal stimulation, there is no link between the commitment to clonal expansion of naive CD8 T cells and differentiation into cytokine producers. They also suggest a novel mechanism for a partial CD8 T cell response where specific CTL precursors are present and activated in response to initial low-avidity stimulation, but fail to proliferate and to acquire cytolytic activity.

**Sustained TCR engagement by partial agonist compensates for weak signal**

Another characteristic of the stimulation by the partial agonist was the length of time required to reach the signal threshold for cell division (Fig. 5). Thus, although a very short period of TCR engagement by a full agonist was able to initiate a complete program of effector differentiation, in agreement with previous reports (2–4), TCR engagement by the partial agonist had to be sustained (>18 h) to drive the cells into division and effector differentiation. This brings the notion that a strong signal of short duration can be replaced by the accumulation over time of weak signals. This is probably a general concept in signaling that also applies to activation of naive CD4 T cells for which strength of stimulation, duration of priming, and number of cell divisions have been shown to contribute to cytokine gene expression (28). A delay in the detection of phospho-c-Jun reported for the response of CD8 T cells to a partial as compared with a full agonist OVA peptide also pointed to the notion that T cell responses are triggered by a cumulative signal (30). Similarly, we previously showed that AP-1 transactivation was particularly affected as compared with NF-κB activation in response to the partial agonist. Only with activated APCs could AP-1 complexes be detected in response to bm8, albeit with delayed kinetics (12 h for B6, 32 h for bm8 stimulation (11)). This is consistent with the reported mechanism of stepwise activation of AP-1 components of the Fos and Jun families, a property that allows for integration of the duration of signaling (31). Because the various signaling pathways that contribute to transcriptional activation may be sensitive to different levels of signal emanating from receptor engagement (11), a distinct pattern of gene expression may result from the triggering of the same receptor with ligands binding with different avidity. A gene profiling approach may reveal how divergent gene expression programs can be established in naive T cells (32). It may also provide hints as to whether coreceptor or cytosine receptor encoding genes are differentially up-regulated and may contribute to further divergence in T cell fate (G. Verdeil, D. Puthier, C. Nguyen, A. M. SchmittVerhulst, and N. Auphan-Anetzin, manuscript in preparation).

In summary, we showed here that distinct TCR ligands display strong vs low avidity required short vs long TCR engagement, respectively, to trigger T cell division, suggesting that duration may compensate for strength of signal. The commitment of a naive CD8 T cell into primed IFN-γ-producing cells by a low-avidity ligand was independent of cell division, indicating that different signaling thresholds drive IFN-γ production as compared with cell cycle entry and acquisition of cytolytic activity. Physiological situations of low stimulation of CD8 T lymphocytes may occur during the course of tumor development. Indeed, tumor cells are often poorly immunogenic, expressing nonmutated or mutated self-Ag, and may induce weakly or partially activated T cells. Our observation that partially activated tgTCR CD8 cells may become CTL effectors after a second challenge with either a low or a strong affinity ligand may open new approaches for antitumoral immunotherapies.

**Acknowledgments**

We thank C. Boyer, A. Guimezanes, L. Leserman, and B. Malissen for criticism on this manuscript. We also thank N. Brun for cell sortings.

**References**


