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The Poststimulation Program of CD4 Versus CD8 T Cells (Death Versus Activation-Induced Nonresponsiveness)\textsuperscript{1}

Ee Loon Tham\textsuperscript{*†} and Matthew F. Mescher\textsuperscript{2‡}

Both CD8 and CD4 T cells undergo autocrine IL-2-induced proliferation and clonal expansion following stimulation with Ag and costimulation. The CD8 T cell response is transient because the cells rapidly become activation-induced nonresponsive (AINR) and exhibit split anergy. In these cells, the capacity for IL-2 production is lost, but TCR-mediated IFN-γ production and cytotoxicity are maintained. At this point, the CTL become dependent on IL-2 provided by CD4 Th cells for continued expansion. If IL-2 is available to support expansion for a brief period, AINR is reversed and the cells regain the ability to produce IL-2. In this study, we show that CD4 T cells do not become AINR, but instead are rendered susceptible to Fas-mediated activation-induced cell death following stimulation through TCR and CD28. Using z-VAD-fmk or anti-Fas ligand mAb to inhibit cell death, we demonstrate that previously activated CD4 T cells retain the ability to up-regulate c-Jun N-terminal kinase activity and IL-2 mRNA levels upon TCR engagement and no longer require costimulation. This rewiring of signaling pathways is similar to that seen following reversal of AINR in CD8 T cells. Thus, CD8 and CD4 T cells appear to use distinct mechanisms, AINR and activation-induced cell death, respectively, to limit excessive clonal expansion following a productive response, while permitting important effector functions to be expressed. \textit{The Journal of Immunology,} 2002, 169: 1822–1828.

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D4 T cells can provide help for CTL responses by two principal mechanisms, one involving conditioning of APCs through CD40-CD40 ligand interaction (1–3), and the second being provision of growth or survival factors, such as IL-2 (4). Conditioning of APCs by CD4 T cells enhances the ability of the APCs to prime naïve CD8 T cells and thus initiate CTL responses. In many cases, however, this form of help is not required and a helper-independent CTL response can be initiated. These helper-independent responses are limited, however, and often insufficient to clear Ag. CD4 T cells can provide help at this stage, in the form of IL-2, to expand and sustain the CTL responses.

Recent studies have suggested the basis for the requirement for CD4 T cell help to expand and sustain CTL responses. The initial helper-independent, autocrine IL-2-induced CD8 T cell response to full stimulation with Ag and costimulation is transient, because within 3–4 days the cells become activation-induced nonresponsive (AINR),\textsuperscript{3} unable to up-regulate IL-2 mRNA and protein upon further stimulation. Unless exogenous IL-2 or CD4 T cell help is available to these AINR cells, their clonal expansion ceases and survival declines (5, 6). Biochemical analyses have revealed that the defect in IL-2 production in AINR CD8 T cells is at least partly attributable to a block in activation of the mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK), and p38 (6). TCR-mediated signaling can still occur, however, because AINR CD8 T cells can produce IFN-γ and carry out cytotoxic effector function upon recognition of Ag-bearing target cells (5, 7). The AINR state is reversed when the cells are allowed to undergo a brief period of expansion (1–2 days) in response to exogenous IL-2. Once reversed, the cells again can make a sustained and effective response to Ag (4). Upon reversal, the cells regain the ability to up-regulate MAPKs and produce IL-2 to drive a sustained response to Ag in the absence of any further help. Furthermore, some rewiring occurs upon reversal so that unlike naïve cells, these reversed cells do not require co-stimulation to activate the MAPKs and IL-2 production; TCR engagement is sufficient.

The induction and subsequent reversal of AINR in CD8 T cells are observed in a variety of in vivo (5, 7, 8) and in vitro conditions (4–6), suggesting that it is unlikely to result from tolerization by a specific viral or tumor Ag. In addition, the development of AINR in CD8 T cells is not the result of CTLA-4 engagement (5, 8) or down-regulation of TCR, CD28, or LFA-1 (5). Thus, AINR appears to be an integral part of the normal CD8 T cell development program, and acts as a regulatory checkpoint at which the autonomous CD8 response is converted to one requiring IL-2 from CD4 T cell for continued expansion.

Whether CD4 T cells experience a refractory period following initial stimulation, similar to AINR in CD8 T cells, has not been clear. Functional nonresponsiveness or clonal anergy is observed in CD4 T cell clones that are stimulated through the TCR in the absence of costimulation. These cells are characterized by an inability to produce IL-2 and proliferate following restimulation through the TCR, even if costimulation is present (9, 10). Furthermore, studies using in vivo models have shown that a transient clonal expansion can precede the induction of Ag-specific nonresponsiveness in CD4 T cells (11–16). Although these studies suggest that a nonresponsive state similar to AINR might occur in CD4 T cells, they do not clearly distinguish whether the refractoriness to restimulation is a consequence of inappropriate initial stimulation or a regulatory mechanism that is inherent to the cells,
as appears to be the case for CD8 T cells. Various mechanisms of tolerance induction have been described for CD4 T cells, including activation-induced cell death (AICD), functional nonresponsiveness, or active suppression by regulatory cells or cytokines (17, 18). These mechanisms are not necessarily mutually exclusive.

In this study, we demonstrate that CD4 T cells, unlike their CD8 counterparts, do not become AINR following optimal stimulation through the TCR and costimulatory receptors. They do, however, undergo a rewiring similar to that seen in reversed AINR CD8 T cells. Thus, they retain the ability to rapidly up-regulate IL-2 production upon restimulation, but this no longer requires a costimulatory signal. In addition, the activated CD4 T cells become highly sensitive to Fas-mediated AICD upon restimulation, again differing from activated CD8 T cells in this respect. These results suggest that activated CD4 T cells retain the ability to produce IL-2 to provide help to AINR CD8 T cells upon re-encountering Ag, but may then be eliminated due to induction of AICD.

Materials and Methods

Cells and reagents

C57BL/6 mice (National Cancer Institute, Frederick, MD) were maintained in the specific pathogen-free facility at the University of Minnesota and were used at 6–12 wk. Single cell preparations were made by homogenizing lymph nodes with a tissue grinder and lysing RBC in a buffer containing 11 mM KHCO3 and 152 mM NH4Cl. The resulting suspension was adherence cell depleted at 37°C for 1 h. CD4 or CD8 T cell enrichment was conducted on negative selection columns (Cedarlane Laboratories, Hornby, Ontario, Canada), according to the protocols provided by the suppliers. Purity of the cells was assessed by flow cytometry and was typically 95–98% and 90–95% for CD4 and CD8 T cells, respectively.

Anti-CD3e (2C11) and anti-Fas ligand (FasL; MFL3) mAbs were purchased from BD Pharmingen (San Diego, CA). Murine B7-1-Fc and B7-2-Fc recombinant proteins were obtained from R&D Systems (Minneapolis, MN). ICAM-1 proteins were purified, as previously described (19, 20). Human rIL-2 was used at 2.5 U/ml. z-VDAC-fmk (Calbiochem, La Jolla, CA), anti-FasL, and anti-IL-2R (PC61.3) mAbs were used at 100 μM and 10 and 75 μg/ml, respectively. Anti-B7-1 (16-10A1) and anti-B7-2 (GL1) blocking Abs were used at 50 μg/ml.

Cell proliferation

Cell-size microspheres used for stimulating responder cells were prepared as described (6). The final concentrations of 2C11, B7-1 (or B7-2), and ICAM-1 were 0.75, 0.5, and 0.25 μg/ml/105 microspheres, respectively. A total of 5 × 105 responder cells and 1 × 105 microspheres (per well) were cultured in triplicate in 96-well flat-bottom culture plates in a total volume of 0.2 ml medium. Cells were pulsed with 1 μCi [3H]thymidine/well for the last 8 h of the indicated time and were subsequently harvested onto glass fiber filters (Wallac, Turku, Finland). [3H]Thymidine incorporation was measured using a Betaplate liquid scintillation counter (Wallac). Results are expressed as the mean (±SD) of triplicate wells.

IL-2 mRNA measurement

CD4 and CD8 T cells used in the experiments were either freshly isolated or stimulated with 2C11/B7-1/ICAM-1 microspheres for the indicated number of days. For IL-2 mRNA measurement, cells and microspheres having immobilized 2C11 mAb or 2C11 mAb and B7 ligands were centrifuged in 96-well V-bottom plates at 1500 rpm for 10 min (1.5 × 105 cells and 3 × 105 microspheres per well). The pellets were incubated at 37°C for 7 h in the presence of 100 μM z-VDAC-fmk. Total RNA was prepared from the cells using the RNAqueous kit (Ambion, Austin, TX), according to the manufacturer’s protocol. RNA was then reversed transcribed, and PCR was conducted using the IL-2 and actin primers and conditions, as previously described (6). The number of cycles was confirmed to be within the linear amplification range for the assay.

Results

Kinetics of CD8 and CD4 T cell activation

The proliferative responses of CD8 and CD4 T cells to antigenic stimulation were examined over the course of 8 days. Purified CD8 or CD4 T cells were stimulated with cell-size microspheres bearing BSA as a control, anti-CD3 mAb (2C11), or anti-CD3 mAb and costimulatory molecules (2C11/B7-1/ICAM-1). Stimulation with 2C11 alone resulted in little or no proliferation in CD8 (Fig. 1A) and CD4 T cells (Fig. 1B), while coimmobilization of B7-1 and ICAM-1 results in optimal proliferation of both subsets of T cells (Fig. 1, A and B). The proliferative response of 2C11/B7-1/ICAM-1-stimulated CD8 T cells peaked at day 3 and rapidly declined thereafter (Fig. 1A). In contrast, the CD4 T cell response peaked on day 4–5, and was more vigorous and prolonged due to more efficient IL-2 production and clonal expansion (Fig. 1B) (19, 20).

Secondary responses of CD8 and CD4 T cells to anti-CD3 mAb or anti-CD3 mAb and IL-2

Previously, we have shown that CD8 T cells make a transient response to Ag and costimulation. Within 3–4 days, clonal expansion and viability decline as the cells become AINR, unable to produce IL-2 and proliferate in response to Ag and costimulation (5). AINR CD8 T cells, however, are able to respond to exogenous IL-2 (5). It is uncertain whether CD4 T cells undergo similar state of nonresponsiveness following a productive response to Ag and costimulation. To examine responsiveness of CD8 and CD4 T cells following the initial response, cells stimulated with 2C11/B7-1/ICAM-1 microspheres for 3 days were harvested, washed, and restimulated under conditions described for primary stimulation (Fig. 1). Proliferation was assessed by measuring [3H]Thymidine incorporation during the last 8 h of the indicated day. Results are expressed as the mean (±SD) of triplicate determinations.

FIGURE 1. Time course of CD8 and CD4 T cell proliferation in response to stimulation with anti-CD3 mAb and costimulation. Freshly isolated CD8 (A) or CD4 T cells (B) were stimulated with microspheres having immobilized BSA, anti-CD3 mAb (2C11) or anti-CD3 mAb, B7-1, and ICAM-1 (2C11/B1). Proliferation was assessed by measuring [3H]Thymidine incorporation during the last 8 h of the indicated day. Results are expressed as the mean (±SD) of triplicate determinations.

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incorporation on day 2 following restimulation. As expected, recently activated (AINR) CD8 T cells did not respond to stimulation with BSA, 2C11, or 2C11/B7-1/ICAM-1, but continued to proliferate in response to IL-2 or 2C11 and IL-2 (Fig. 2A). Similarly, activated CD4 T cells did not respond to stimulation with BSA, 2C11, or 2C11/B7-1/ICAM-1 (Fig. 2B). However, unlike activated (AINR) CD8 T cells, activated CD4 cells proliferated only in response to IL-2, but not to the combination of 2C11 and IL-2 (Fig. 2B). Thus, TCR engagement suppressed or inhibited the proliferation of activated CD4, but not activated CD8 T cells to exogenous IL-2.

**FIGURE 2.** Ag-experienced CD8 and CD4 T cells differ in their responses to restimulation with anti-CD3 mAb and IL-2. CD8 or CD4 T cells stimulated with 2C11/B7-1/ICAM-1 microspheres for 3 days were harvested, washed, and restimulated with BSA, anti-CD3 mAb, B7-1 and ICAM-1 (2C11/B7-1), human IL-2 (IL-2) or anti-CD3 mAb, and IL-2 (2C11 + IL-2). Human IL-2 was used at 2.5 U/ml. Proliferation was assessed by measuring [3H]thymidine incorporation during the last 8 h of the second day following restimulation. Results are expressed as the mean (±SD) of triplicate determinations. The results are representative of three independent experiments.

**FIGURE 3.** Blocking of Fas-mediated cell death restores proliferation of activated CD4 T cells restimulated through the TCR. CD4 T cells become susceptible to Fas-mediated cell death following initial stimulation. CD4 T cells stimulated with 2C11/B7-1/ICAM-1 microspheres for 3 days were harvested, washed, and restimulated with microspheres having various immobilized ligands, as indicated, with or without z-FMK-VM and anti-FasL mAb. Z-FMK-VM and anti-FasL mAb were used at 100 µM and 10 µg/ml, respectively. Proliferation was assessed by measuring [3H]thymidine incorporation during the last 8 h of the second day following restimulation. Results are expressed as the mean (±SD) of triplicate determinations. The results are representative of five independent experiments.

**FIGURE 4.** Kinetic analyses have shown that there are comparable levels of Fas and FasL expression in CD4 and CD8 T cells that are activated for 3 days (28, 29). Activated CD8 T cells, however, in contrast to activated CD4 T cells, are relatively insensitive to Fas-mediated cell death. Instead, the AICD in CD8 T cells may involve engagement of TNF receptors (22). Our earlier studies showed that the addition of anti-TNF-β or anti-FasL mAbs does not prevent nor reverse the development of AINR in CD8 T cells (5). Taken together, these results suggested that activated CD4 T cells are rendered susceptible to Fas-mediated cell death following activation, whereas activated (AINR) CD8 T cells are more prone to passive cell death due to cytokine withdrawal.

**CD4 T cells do not become AINR**

Freshly isolated (day 0) CD8 T cells up-regulate IL-2 mRNA within 7 h of being stimulated with 2C11/B7-1/ICAM-1 (Fig. 4A). In contrast, cells that have become AINR (day 3) show no increase in IL-2 mRNA levels upon re-engagement of TCR and costimulatory receptors. To determine whether a similar defect occurs in...
CD4 T cells, cells either freshly isolated (day 0) or activated for 2, 3, or 5 days were examined for their ability to up-regulate IL-2 mRNA following restimulation with 2C11/B7-1/ICAM-1 microspheres at 37°C for 7 h.

Freshly isolated CD4 T cells (day 0) express little or no IL-2 mRNA and, as expected, a significant amount of IL-2 mRNA was detected following a 7-h stimulation with 2C11/B7-1/ICAM-1 microspheres (Fig. 4B). In contrast to activated CD8 T cells (day 3), CD4 T cells that were stimulated for 2, 3, or 5 days retained the ability to up-regulate IL-2 mRNA upon restimulation, thus demonstrating that AINR does not occur in CD4 T cells (Fig. 4B).

These observations also support the conclusion that Fas-mediated cell death and IL-2 production are two independent TCR-mediated events in activated CD4 T cells, and that IL-2 production can occur when Fas-mediated cell death is prevented.

Altered costimulation requirements for IL-2 mRNA up-regulation in activated CD4 T cells

The AINR state in CD8 T cells is reversed when cellular proliferation is sustained by exogenous IL-2 for a period as brief as 1–2 days. Following reversal of AINR, CD8 T cells can again make IL-2 and proliferate in response to Ag and costimulation. Furthermore, the reversed cells are no longer dependent on costimulation for up-regulation of mitogen-activated protein kinase (extracellular signal-regulated kinase, JNK, and p38) activities and IL-2 mRNA (4). We therefore examined whether activated CD4 T cells, like reversed CD8 T cells, up-regulate IL-2 mRNA upon TCR engagement alone. Freshly isolated lymph node CD4 T cells are typically 85–92% naive (CD45Rb$^{high}$), and the remaining display the memory phenotype (CD45Rb$^{low}$). Stimulation of increased IL-2 mRNA expression by freshly isolated CD4 T cells required both 2C11 anti-TCR mAb and B7-1 or B7-2 ligand on microspheres, and was blocked by the respective anti-B7 mAbs (Fig. 5A). In contrast, CD4 T cells that had been activated 2 days previously up-regulated IL-2 mRNA expression in response to just 2C11 anti-TCR mAb (Fig. 5B). Furthermore, stimulation by microspheres having just anti-TCR mAb was not blocked by anti-B7-1 and B7-2 mAbs, thus ruling out the possibility that the activated CD4 T cells might be expressing B7 ligands and providing trans-costimulation through T:T interactions (30). Thus, activated CD4 T cells are able to up-regulate IL-2 mRNA in response to signal 1 alone, most likely as a result of rewiring of TCR and costimulatory receptor pathways.

Rewiring of TCR and costimulatory signaling pathways

As for naive CD8 T cells, $c$-jun terminal protein kinase (JNK) activity is minimally up-regulated in naive CD4 T cells in response to TCR engagement alone; optimal JNK activation requires TCR engagement and costimulation (6, 33, 34) (Fig. 6). CD8 T cells in the AINR state experience a block in JNK activation, but following reversal of AINR the cells up-regulate JNK activity in response to TCR engagement (4). Rewiring of TCR and costimulatory receptor signaling pathways has occurred, in that costimulation is no longer required for JNK and IL-2 mRNA up-regulation. To determine whether a similar rewiring of TCR and costimulatory pathways has also occurred in activated CD4 T cells, we examined naive cells or cells activated for 2, 3, or 5 days, for their ability to up-regulate JNK activity in response to TCR engagement (4). Rewiring of TCR and costimulatory receptor signaling pathways has occurred, in that costimulation is no longer required for JNK and IL-2 mRNA up-regulation. To determine whether a similar rewiring of TCR and costimulatory pathways has also occurred in activated CD4 T cells, we examined naive cells or cells activated for 2, 3, or 5 days, for their ability to up-regulate JNK activity in response to 2C11 or 2C11/B7-1 (Fig. 6). Cells were stimulated with microspheres bearing the indicated ligands at 37°C for 10 min before being lysed. JNK activity in the cell lysates was determined using recombinant $c$-jun protein as a substrate. In contrast to naive CD4 cells, cells that were previously activated for 2 days were able to fully activate JNK in response to stimulation with 2C11, as well as to 2C11/B7-1. Similar results were observed with cells that were activated for 3 or 5 days. These results suggest that activated CD4 T cells, despite not becoming AINR following
stimulation with Ag and costimulation, undergo a similar rewiring of TCR and costimulatory signaling pathways as occurs upon reversal of the AINR state in CD8 T cells. Furthermore, the rewiring of signaling pathways in CD4 T cells occurred as early as 2 days after the initial stimulation.

Discussion

Murine CD8 T cells become AINR after being optimally stimulated with Ag and costimulation (5, 7, 8). The induction of AINR occurs regardless of the nature of the initial stimulus, and it appears to act as an intrinsic regulatory mechanism for limiting excessive proliferation of potentially harmful CD8 effector cells. Despite their inability to produce IL-2 upon restimulation AINR, CD8 cells are able to respond to exogenous IL-2 (5). The AINR state is reversed when continued proliferation of AINR cells is supported by exogenous IL-2 for 1–2 days (4). In vivo studies examining tumor-specific CD8 T cell responses showed that the reversal of the AINR state in CD8 T cells occurs via a CD4 T cell- and IL-2-dependent mechanism, and that once reversal has occurred the cells can make a prolonged response without further help from CD4 T cell help (8).

CD4 T cells activated in the same way as CD8 T cells also become refractory to restimulation through both TCR and costimulatory receptors. However, in contrast to CD8 T cells, the recently activated CD4 T cells respond to IL-2, but not to the combination of IL-2 and anti-CD3 mAb (Fig. 2B). The lack of responsiveness to IL-2 and anti-CD3 mAb in these CD4 T cells correlates with decreased cell viability (data not shown). These results are not surprising because numerous reports have shown that CD4 T cells become highly susceptible to TCR-mediated AICD following activation (24, 35, 36). Thus, it was unclear whether the refractoriness of activated CD4 T cells to restimulation is the result of AICD alone or if the cells that are susceptible to AICD also become AINR.

The primary pathways responsible for AICD in CD4 and CD8 T cells involved Fas and TNF, respectively (22). Our previous results show that the decline in viability of CD8 T cells following the induction of AINR is predominantly due to the lack of IL-2 production (passive cell death) and not AICD, because the addition of a general caspase inhibitor, z-VAD-fmk, anti-FasL, or anti-TNF–β mAbs to restimulation cultures does not prevent the nonresponsiveness. Furthermore, AINR cells that are undergoing passive cell death could be readily rescued by exogenous IL-2 (5). In contrast, the addition of z-VAD-fmk or anti-FasL mAb to restimulation cultures restores the ability of activated CD4 T cells to respond to IL-2 and anti-CD3 mAb, as well as to anti-CD3 mAb or anti-CD3 mAb/B7-1 (Fig. 3 and data not shown). The conditions that lead to Fas-mediated cell death have been delineated in great detail (37). Fas receptor is ubiquitously expressed in a variety of cells, but FasL expression is limited to effector cells such as activated T cells and NK cells (38). Therefore, Fas-mediated cell death in activated T cells most likely occurs in a fratricidal or autonomous manner (39). In resting T cells, FasL expression is weakly up-regulated upon TCR engagement, but activated T cells only become highly sensitive to Fas-mediated cell death after cycling in high levels of IL-2 for 2–3 days (24, 35). Thus, the massive Fas-mediated fratricide or suicide that is observed following in vitro stimulation of CD4 T cells is likely due to highly localized IL-2 and repetitive anti-CD3 mAb stimulation (23, 40). There is evidence suggesting that in vivo, the elimination of Ag-specific CD4 T cells after the peak of clonal expansion could be either Fas dependent or Fas independent, depending on the concentration and persistence of the Ag. If Ag is present at low concentrations or is rapidly cleared, the loss of Ag-specific T cells is caused by growth factor withdrawal. In contrast, if the Ag is chronically present, Fas-mediated cell death occurs (37, 40–42).

In CD4 and CD8 T cells, CTLA-4 is up-regulated 2–3 days following T cell activation (45). The interaction of CTLA-4 and its ligands, B7-1 and B7-2, has been implicated in the induction of peripheral T cell tolerance (46–48). Our previous study shows that CTLA-4 is not involved in the induction of AINR in CD8 T cells because the addition of anti-CTLA-4 mAb does not prevent nor reverse the nonresponsiveness (5). It is also unlikely that CTLA-4 is responsible for the lack of responsiveness in activated CD4 T cells; the addition of z-VAD-fmk or anti-FasL mAb to the restimulation culture fully restores proliferation (Fig. 3) and IL-2 mRNA up-regulation (Fig. 4) in the activated CD4 T cells. A number of receptors with costimulatory activity have recently been identified on activated T cells that may act subsequently to primary stimulation and may extend responses (49, 50). It remains to be determined whether signaling through any of these receptors might alter the nonresponsiveness of AINR CD8 T cells, or the susceptibility to AICD of CD4 T cells.

Based on our previous and current work, a model can be suggested to explain the role of CD4 T cells in providing help to
expand and sustain CTL responses in the face of persisting Ag. For effective CTL priming, both CD4 Th cell and precursor CTL must recognize Ags on the same APC (51, 52). Precursor CTLs that are activated by cross-presentation undergo clonal expansion in the lymph node for ~3 days. At the peak of clonal expansion, the CTLs begin to migrate out of the lymph node into the spleen, blood, and sites of Ag deposition (53). By this time, the activated CD8 T cells have become AINR and exhibit split anergy, able to carry out effector functions, but no longer able to respond autonomously to Ag stimulation. Naïve CD4 T cells similarly undergo clonal expansion upon recognition of Ag presented by activated APCs in the lymph node, and at the peak of clonal expansion they also leave the lymph node and migrate to peripheral tissues (54). In contrast to effector CTLs, activated CD4 T cells do not become AINR after activation; rather, they retain the ability to produce IL-2 upon restimulation. Furthermore, these cells no longer require costimulation for IL-2 production. Thus, activated CD4 T cells migrating to peripheral tissues are armed to produce IL-2 efficiently upon Ag re-encounter. Although dendritic cells are required to optimally prime naïve CD4 T cells in the lymph node, Ag-bearing macrophages or B cell at sites in peripheral tissues may effectively stimulate activated CD4 T cells to produce IL-2, even when they are not conditioned to provide optimal costimulation. At the site of Ag deposition, continued proliferation of AINR effector CD8 T cells is most likely supported by IL-2 produced by activated CD4 T cells. Activated CD4 T cells only need to produce IL-2 briefly, before undergoing Fas-mediated cell death, because AINR in the CD8 T cells is rapidly reversed. Following the reversal of AINR, the effector CD8 T cells can resume their responses to persisting Ag.

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