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CD4-Dependent Signaling Is Required for a Late Checkpoint during Th2 Development Associated with Resistance to Activation-Induced Cell Death

Zohreh Tatari-Calderone,* Jennifer L. Brogdon,† Kevin W. Tinsley,* Anahita Ramezani,* and David Leitenberg2*‡

Previous studies have found that class II-restricted T cells from CD4-deficient mice reconstituted with a tail-less CD4 transgene have a specific defect in the development of Th2 effector cells; however, the reason for this defect was not clear. Following stimulation with a high potency peptide and exogenous IL-4, CD4-dependent signaling is required for optimal generation of a Th2 effector population. However, initial IL-4 and GATA-3 transcription is appropriately induced, suggesting that the initial stages of Th2 development are intact and independent of CD4 after priming with a strong agonist peptide. In addition to the defect in Th2 development, CD4 mutant T cells are also relatively resistant to activation-induced cell death (AICD). Furthermore, inhibition of AICD in wild-type T cells causes a defect in Th2 development similar to that seen in the CD4 mutant T cells. These data support the hypothesis that CD4-dependent signaling pathways regulate a distinct checkpoint in the expansion and commitment phase of Th2 development, which is related to dysregulation of AICD.

The development of Th lymphocyte effector function is a multistep process that occurs over several days following initial recognition of peptide ligand presented by APCs. Over the last several years it has become clear that Th subset development is regulated by a complex interplay between TCR, costimulatory and cytokine receptor signaling events, which in turn regulate the expression and activity of a variety of transcription factors that control the distinct patterns of gene expression necessary for Th2 effector cell function (1–3). Despite extensive study, many issues regarding the relative roles, timing, and potential cross-talk between TCR, costimulatory and cytokine receptor signals involved in controlling Th development remain unclear.

CD4 plays an important role in regulating T cell activation, by serving as a coreceptor for the T cell Ag receptor during initial Ag recognition and promoting the activation and recruitment of the src family kinase lck to the CD3/TCR complex. In addition, CD4 has been shown to play a less well-understood role in regulating the function and development of effector and memory T lymphocytes (4–6). Several years ago, we and other groups independently found that CD4-dependent signaling was required for the optimal development of IL-4-secreting Th2 effector cells in vitro and in vivo but not for the development of Th1 effector cells (7–9). The specific failure in Th2 development was observed in class II MHC-restricted, CD4-deficient T cells (or CD4-deficient T cells reconstituted with a tail-less CD4 transgene), as well as in situations where wild-type CD4+ T cells were primed with peptide presented by mutant class II MHC molecules unable to interact with CD4. Surprisingly, the requirement for CD4 expression was not overcome by the addition of large amounts of exogenous IL-4 and/or following stimulation with anti-CD3 or strong agonist peptides which do not require CD4 coreceptor function (8, 9). These data suggest that the role of CD4 in promoting Th2 development is a relatively late event in the differentiation pathway.

In support of this hypothesis, we have found that CD4 mutant and wild-type T cells produce similar levels of IL-4 and GATA-3 messenger RNA early after priming with a strong agonist peptide and exogenous IL-4, despite exhibiting a defect in Th2 cytokine production upon restimulation. These data further indicate that initial Th2 priming events are intact in the absence of CD4 signaling and that CD4-dependent signaling pathways define a previously unrecognized late checkpoint in Th2 development, which is independent of a coreceptor requirement for initial TCR activation. Coincident with the defect in Th2 development there is also a significant defect in Fas signaling and activation-induced cell death (AICD) in CD4 mutant T cells. Our data suggest that the defect in AICD is linked to Th2 development because inhibition of AICD after T cell priming in wild-type T cells inhibits Th2 development and IL-4 production similar to that seen in CD4 mutant T cells. We propose a model in which the differential susceptibility to AICD among Th development is a critical selection step, which enriches for committed Th2 effector cells from a diverse pool of Th2 precursor cells.

Materials and Methods

Mice

AND TCR transgenic mice in which CD4+ T cells express a TCR specific for carboxyl terminus of moth cytochrome c (pMCC) peptide in the context of I-Eα or I-Eβ have been previously described (10), and are maintained in our breeding colony as heterozygotes on a B10.BR background. The CD4Δ2Δ mice were originally provided by D. Littman (New York

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University, New York, NY) (11) and were backcrossed seven to nine times onto a B10.BR background with the AND TCR transgenic mice as previously described (9).

Purification of APC and CD4+ T cells

T cell-depleted APC were prepared by Ab-mediated complement lysis from total splenocytes as described (39). The APC were treated with 50 μg/ml mitomycin C (Sigma-Aldrich) before use. CD4+ CD8+ T cells from lymph nodes and spleens of transgenic mice were isolated by immunomagnetic negative selection, using Abs against CD8, CD32/CD16, B220, and MHC class II, followed by incubation with anti-mouse and anti-rat Ig-coated magnetic beads (Polysciences). Purity of the recovered Vα11+/CD4+ T cells as determined by staining with anti-CD4 and anti-Vα11 mAb is usually 85–95%.

T cell stimulation and detection of IL-4 and IFN-γ production

Induction of naive T cell differentiation was performed as previously described with slight modifications. Briefly, purified T cells (0.5 × 10⁶/ml) from AND TCR transgenic wild-type or Δcyt mice and mitomycin C-treated, T cell-depleted splenocytes (1 × 10⁶ cell/ml) were incubated with 5 μg/ml pMCC (agonist peptide of moth cytochrome c (81–103), pMCC = VFAGLKKANERADLIAYLKQATK) under neutral conditions in the presence of rmIL-2 (25 U/ml) or under Th2 skewing conditions (with IL-2, rmIL-4 (10–20 ng/ml) (Harlan Bioproducts), or Th1 skewing conditions (with IL-2, rmIL-2 (10 ng/ml), anti-IL-4 (11B11)). After 4 days of priming, T cells were harvested, and dead cells were removed by using gradient centrifugation. Viable cells were then incubated for a rest period of 2 days with fresh APCs only. For the secondary culture, rest T cells (0.5 × 10⁶/ml) were restimulated with pMCC (5 μg/ml) and fresh APC (1 × 10⁷/ml) for an additional period of 2 days. Cytokine concentrations in the supernatants were determined by ELISA kit (Pierce-Endogen). Supernatants were diluted serially in duplicate, and the concentration of cytokine determined in relation to a reference standard supplied by the manufacturer. In some experiments, we added Jo-2 anti-mouse Fas mAb or anti-Fas ligand (Fasl) mAb (BD Pharmingen) at varying times after initial stimulation.

Intracellular cytokine analysis was performed following 12–16 h of stimulation with agonist peptide and the addition of monensin (Golgistop; BD Biosciences) for the final 4–6 h of culture. Cells were harvested and surface labeled with anti-CD4 before fixation and permeabilization (Fix and Perm; Caltag Laboratories), and labeling with Abs to IL-4 and IFN-γ (BD Pharmingen).

RNase protection assay

Two or 3 days after stimulation of CD4+ T cells with APC and peptide, the cells were harvested and dead cells removed by gradient centrifugation. In some experiments, CD4+ T cells were further isolated by immunomagnetic negative selection as described above. The purity of CD4+ T cells was then determined by FACS analysis and was >98% by CD4 and Vα11 labeling. Total RNA was isolated using TRIzol (Invitrogen Life Technologies).

FIGURE 1. Specific defect in Th2 development in the absence of CD4 signaling. Purified CD4 T cells from wild-type (WT) or Δcyt mice expressing AND TCR transgene were primed with 5 μg/ml agonist peptide (pMCC) and 25 U/ml IL-2 under Th2, Th1, or neutral skewing conditions for 4 days as described in Materials and Methods. Cells were then washed and rested for an additional 2 days before challenging with agonist peptide and APC. A, After 48 h, culture supernatants were collected and analyzed for IL-4, IFN-γ, IL-5, and IL-10 by ELISA. This experiment is representative of more than three independent experiments. B, Flow cytometric intracellular cytokine analysis of cells primed under Th2 or Th1 conditions after overnight stimulation with agonist peptide and APC. These data are representative of three independent experiments.

FIGURE 2. Induction of early IL-4 and GATA-3 transcription is not dependent upon CD4 signaling. Purified WT or Δcyt CD4 T cells were stimulated with pMCC and the indicated IL-4 concentrations. A, After 48 h, the CD4 T cells were harvested, and total RNA was isolated. Cytokine RNA levels were analyzed by RNase protection assay (RPA); 10 μg of total RNA were used in each reaction. Equal loading of RNA was assessed using the housekeeping genes L32. B, Purified CD4 WT or Δcyt T cells were stimulated as above, and total RNA was extracted at day 3 or day 4 and assessed for GATA-3 expression by Northern blot; 10 μg of RNA were loaded per lane, and equal sample loading was confirmed by UV light shadowing of 28S and 18S rRNA. These data are representative of two independent experiments.
FIGURE 3. Tyrosine phosphorylation of STAT-6 and JAK-3 following IL-4 signaling is independent of CD4 expression. Purified CD4 \( \delta \)cyt or WT controls were stimulated with agonist peptide in the presence or absence of IL-4. Three days after stimulation, the viable T cells were isolated and rested for 3 h and then 5 \( \times \) 10^5 cells group were restimulated with the indicated dose of IL-4 for 10 min and immunoprecipitated with antisera to STAT-6 or JAK-3. Immunoprecipitated proteins were separated by 8% SDS-PAGE and assessed for tyrosine phosphorylation by Western blot. The same membranes were reprobed for total STAT-6 or JAK-3. These data are representative of three independent experiments.

Northern blot analysis
After 2 days in the primary culture, cells were harvested and dead cells were moved by gradient centrifugation. Total cellular RNA was isolated using TRIzol reagent (Invitrogen Life Technologies), and 10 \( \mu \)g of total RNA from each sample were fractionated on 1.2% agarose/formaldehyde CT. UV shadowing of the membrane to visualize 28S and 18S rRNA was conducted according to the manufacturer’s instructions. In some experiments, 150 ng/ml aprotinin, and 1 \( \mu \)g/ml cross-linker (Alexis Biochemicals Axxora) were added at the time of stimulation. Analysis was based on a FACSCalibur (BD Biosciences) and analyzed with CellQuest software.

Caspase 8 activity analysis
To evaluate caspase 8 activation after restimulation with agonist peptide, CD4 wild-type and \( \delta \)cyt blasts were generated and restimulated with agonist peptide as described above in the AICD assay. After 16 h of restimulation, the cells were washed and stained for caspase 8 activity using carboxyfluorescein FLICA, apoptosis detection kit II (BD Biosciences) and CD4-allophycocyanin (BD Biosciences) according to the manufacturer’s instructions. In some experiments, 150 ng/ml rhFasL and 1 \( \mu \)g/ml cross-linker (Alexis Biochemicals Axxora) were added at the time of restimulation. Analysis was based on a FACSCalibur (BD Biosciences) and analyzed with CellQuest software.

CD4 8 regulation of Th subset differentiation
To investigate the role of CD4 in regulating peripheral T cell differentiation, we have used T cells from CD4-deficient mice, reconstituted with a mutant CD4 transgene, which contains a deletion of the cytoplasmic tail of CD4 (CD4 \( \delta \)cyt) and inter-crossed with AND TCR transgenic mice. T cells from AND TCR transgenic mice recognize an agonist peptide from moth cytochrome c (pMCC) in the context of I-E^k MHC molecules and are activated in the absence of CD4 coreceptor function (9, 12). Importantly, thymic development of functional “CD4 lineage” AND TCR transgenic T cells also occurs relatively efficiently in the absence of CD4 signaling when selected on a homozygous I-E^k background (9, 13).

To evaluate the developmental potential of these cells, purified CD4^+ CD8^- T cells from the spleen and lymph nodes were primed in vitro with agonist peptide (pMCC) under neutral or Th2 skewing conditions. After 4 days of stimulation, and a 2 day “rest” period, the cells were then harvested and normalized for cell number and restimulated with pMCC for an additional 48 h. In cell cultures stimulated under Th2 skewing conditions, there is a significant decrease in IL-4, IL-5, and IL-10 production in CD4 \( \delta \)cyt T cells compared with the wild-type control cells, whereas IFN-\( \gamma \) production is suppressed similarly in both groups (Fig. 1). In contrast, when the cells were primed under neutral or Th1 skewing
conditions, both wild-type CD4 and CD4 δcyt cells produce a similar amount of IFN-γ (Fig. 1). Th2 cytokines are not significantly induced in both wild-type and CD4 mutant T cells following stimulation with agonist peptide under neutral conditions. Consistent with previous results, this data suggests that CD4-dependent signaling is not required for the development of Th1 effector cells, yet is critical for the optimal development of Th2 effector cells (9). Although the production of Th2 cytokines is decreased in the CD4 mutant T cells, it is important to note that Th2 development is not completely abrogated. As seen in the ELISA data and following intracellular single cell analysis of cytokine production when the CD4 mutant T cells are primed under Th2 conditions, IFN-γ production is suppressed and there is continued production of Th2-associated cytokines, although at a much lower level than in wild-type CD4 T cells (Fig. 1, A and B). Thus, it appears that in the absence of CD4-dependent signaling, cells are not converted into Th1 or Th0 cells but simply fail to develop as efficiently into a population of Th2 effector cells capable of high rate cytokine synthesis.

Defect in Th2 development is independent of initial TCR signal transduction events

The defect in Th2 differentiation after priming with a strong agonist peptide does not appear related to a defect in initial TCR-mediated signals, because the agonist peptide used in these studies is able to stimulate early biochemical signaling events in the absence of CD4 expression (9). Consistent with this finding, we also found no defect in cytokine gene transcription early after priming. As shown by RNase protection assays, wild-type and CD4 δcyt cells produced equivalent levels of IL-4 mRNA 48 h after initial priming with agonist peptide plus exogenous IL-4 (Fig. 2 A).

As GATA-3 is both a critical regulator as well as a hallmark of Th2 development, we also examined GATA-3 expression in the CD4 mutant T cells. Similar to the RNase protection assay data, induction of GATA-3 transcription following agonist peptide and IL-4 stimulation is also unchanged in wild-type and CD4 δcyt cells (Fig. 2 B). Thus, these results suggest that the initial signaling events induced by TCR recognition of peptide by CD4 δcyt T cells in conjunction with exogenous IL-4 are sufficient for the development of a Th2 precursor population. It also suggests that the defect in Th2 differentiation, seen in the absence of CD4 signaling, occurs during the maturation and/or clonal expansion phase of Th2 development. In total, this data lead to a model in which there are at least two checkpoints that regulate Th2 development. One is during the initial development of a Th2 precursor population, which is CD4-signaling independent if T cells are activated using a high potency stimulus. The other is at a later checkpoint that promotes the selection of a mature Th2 effector cell population and is CD4 dependent.
Consistent with our previous observations that CD4 mutant T cells are more resistant to AICD than wild-type cells resulting in prolonged survival and enhanced cell division. Indeed, when wild-type or CD4 δcyt T cell blasts were restimulated with agonist peptide, the wild-type cells underwent apoptotic cell death to a much greater extent than the CD4 mutant T cells (Fig. 5A). Importantly, although the overall level of cell death is lower in cells primed for 3 days under Th2 skewing conditions there remains a significant decrease in death in the absence of CD4 signaling compared with wild-type CD4 T cells (Fig. 5B). This defect in AICD is also seen when the CD4 mutant blasts are stimulated with recombinant soluble FasL followed by cross-linking (Fig. 5C). These data indicate that there is a defect in AICD in the CD4 mutant T cells, which is at least due in part to a defect in the Fas signaling pathway.

One possible explanation for the decreased AICD in the CD4 mutant T cells is failure to express components of the death receptor signaling pathways (e.g., FasL), and/or overexpress negative regulators of AICD (e.g., FLIP). In semiquantitative RT-PCR assays, and/or in RNase protection assays we have not found any evidence for decreased expression of Fas, FasL, Fas-associated death domain protein, caspase 8, TRAIL, TNFR, or increased expression of FLIP (Fig. 6). Consistent with this data, we have found that FasL expression as well as expression of other death receptor signaling components is more sustained over time in CD4 mutant cells compared with wild-type T cells. Typically, expression of FasL is transient on T cells because membrane expression allows cis-interactions with Fas resulting in cell death of the FasL expressing cells. When FasL membrane expression was examined in wild-type and CD4 mutant T cells, we found that expression peaked at day 1 after restimulation of wild-type T cells but was not detectable at day 2. In contrast, the CD4 mutant T cells exhibited sustained detectable levels of FasL expression through day 2 (Fig. 7). Flow cytometric analysis of Fas expression was also equivalent in CD4 wild-type and CD4 mutant T cells (data not shown). In total, this data suggests that in the absence of CD4-dependent signaling, there is a defect in the signaling events mediating AICD independent of Fas and FasL expression.

The defect in Fas signaling in the CD4 mutant T cells appears to occur before activation of caspase 8 in the cell death pathway, because caspase 8 activation was also significantly decreased in the CD4 mutant cells (Fig. 8). Activation of caspase 8 occurs early on in the death receptor signal transduction cascade upon assembly of the death-inducing signaling complex (DISC). This process requires appropriate oligomerization of death effector domains within Fas-associated death domain protein following association with Fas. Thus failure to activate caspase 8, in conjunction with appropriate Fas and FasL expression, suggests that the defect in AICD in the CD4 mutant T cells may be related to dysregulation of the assembly or composition of the DISC.

**CD4 mutant T cell defect in AICD is linked to the defect in Th2 development**

In experiments designed to link the defect in AICD described above to the defect in Th2 development described earlier, we have inhibited AICD using anti-Fas or anti-FasL Abs in wild-type CD4

![FIGURE 7](http://www.jimmunol.org/) Sustained increase in FasL expression in CD4 mutant T cells. Purified wild-type and δcyt CD4 T cells were stimulated as in Fig. 5 and then restimulated with agonist peptide. Flow cytometric analysis of FasL expression in wild-type (dark line) and δcyt (pale line) CD4 T cells was done 1 and 2 days after restimulation. Data shown are FasL expression using a “gate” on CD4+ T cells. The shadowed histogram represents labeling with an isotype control Ab.

**FIGURE 8.** Purified wild-type or δcyt CD4 T cells were primed under neutral conditions as described in Fig. 5 and then left unstimulated (−) or restimulated with agonist peptide (+) for 14 h. Caspase 8 activation was detected using a fluorescent reagent specific for active caspase 8 (FAM-LETD-FMK) obtained from Immunochrometry Technologies. This reagent is cell permeable and binds to active caspase 8. In the absence of caspase 8 activity unbound reagent is washed away. The percentage of cells containing fluorochrome-bound active caspase 8 was determined by flow cytometric analysis after gating on CD4+ T cells.
cells and assessed the subsequent impact on Th2 development. Initial experiments were done to confirm that anti-Fas and anti-FasL Abs would block AICD in wild-type CD4 T cells to the low levels seen in the 8cyt T cells following Ag restimulation (Fig. 9, A and C). Subsequent experiments went on to assess the effect of blocking AICD with Fas or FasL Ab during Th2 priming. In these experiments, wild-type CD4\(^+\) T cells were first stimulated under Th2 cytokine skewing conditions as described in Fig. 1. One or 2 days after the initial stimulation, anti-Fas (Jo-2) or anti-FasL (MFL3) was added. When cells were analyzed for IL-4 production after being treated with Ab during priming only and then restimulated with peptide, there was a defect in IL-4 production similar to that seen in the CD4 8cyt T cells after challenge (Fig. 9, B and D). There was little or no effect on Th1 development as shown by IFN-\(\gamma\) production in cells primed under neutral or Th1 skewing conditions following treatment with anti-Fas or anti-FasL (Fig. 9, B and D). No significant IFN-\(\gamma\) production was detected in cells primed under Th2 conditions upon treatment with anti-Fas or anti-FasL as shown by ELISA or intracellular cytokine analysis (data not shown).

In total, these data suggest that inhibition of AICD after initial priming of naive CD4 T cells specifically inhibits the development of Th2 effector cells. Furthermore, this data suggests that the defects we have described in the regulation of AICD in the absence of CD4 signaling are related to the defect in Th2 development.

**Discussion**

The role of CD4 as a coreceptor that promotes initial T cell Ag receptor-mediated activation events is well established. However, the role of CD4 in regulating previously activated T cells and ongoing immune responses is less well understood. In the present study, we have found that CD4 signaling is required for a distinct checkpoint during Th2 effector cell development, which is independent of initial T cell stimulation. Although production of Th2-associated cytokines is significantly diminished in T cells primed under Th2 conditions in the absence of CD4 signaling, initial Th2 development appeared intact as defined by the appropriate induction of IL-4 and GATA-3 transcription. These findings are quite distinct from other studies in itk, SAP, and fyn-deficient mice in which Th2 development was compromised in cells primed under

**FIGURE 9.** Inhibition of AICD causes decreased Th2 development. A and C, WT and 8cyt CD4 T cells were assayed for AICD as described in Fig. 5 in the presence or absence of anti-Fas (2 \(\mu g/ml\) Jo-2) or anti-FasL (5 \(\mu g/ml\)) added during restimulation with agonist peptide. B, Purified CD4 T cells from AND TCR transgenic mice were stimulated under neutral or Th2 skewing conditions as in Fig. 1. Two days after initial stimulation, 2 \(\mu g/ml\) anti-Fas or isotype control Ab were added to the cultures. The cells were maintained in culture for an additional 2 days, then harvested, washed, and rested for 2 days before restimulation with agonist peptide and APC. After 48 h, culture supernatants were collected and analyzed for the detection of IL-4 or IFN-\(\gamma\) production by ELISA. IFN-\(\gamma\) production was below detectable limits in cells primed under Th2 skewing conditions under all conditions. D, Cells were stimulated under Th2 or Th1 skewing conditions as described in Fig. 1, and 1 day after priming 5 \(\mu g/ml\) anti-FasL or isotype control Ab were added to the cultures. Following 4 days of stimulation, the cells were harvested, washed, and restimulated for 2 additional days with agonist peptide and APC only. Supernatants were collected and analyzed for IL-4 and IFN-\(\gamma\) by ELISA. These data are representative of at least two independent experiments.
neutral conditions, but was recovered upon the addition of exogenous IL-4 in conjunction with a high potency TCR stimulus (14–16). In these cases, the defect in Th2 development appears related to initial T cell signal transduction events that are required for IL-4 production. In contrast, CD4 mutant T cells appear to be initially primed to undergo Th2 differentiation after stimulation with a strong agonist peptide and exogenous IL-4, but do not become a fully developed Th2 effector population when compared with wild-type CD4 T cells.

In addition to the defect in Th2 development, T cells unable to signal through CD4 are relatively insensitive to AICD and Fas-mediated death receptor signaling. The defect in Th2 development and AICD appear linked as shown in experiments in which inhibition of Fas-mediated cell death in wild-type T cells also results in a defect in Th2 development. These data suggest that the appropriate regulation of AICD during Th development is an important factor in controlling the selection of different effector cell subsets. It has been recognized for some time that mature Th2 effector populations are relatively resistant to AICD (17, 18). We propose that the development of this resistant phenotype is an important factor in enriching for committed Th2 effector cells in a mixed population. In this model, cells that are more committed to the Th2 developmental pathway will be correspondingly more resistant to AICD and will have a survival advantage over less committed Th cells. In Th precursor cells that do not signal efficiently through CD4, there is an inappropriate resistance to AICD removing the survival advantage of the more committed Th2 cells. This creates a more mixed population of effector cells after several days of cell expansion (and cell death), with a relative decrease in the proportion of high-rate IL-4 producing Th2 effector cells.

This model is supported by another report describing the paradoxical effect of IFN-γ in promoting Th2 development (19). Bocek et al. found that cells in an IFN-γ-deficient environment were less efficient in developing Th2 effector cells. Similar to our model, the authors proposed that IFN-γ promotes susceptibility to AICD in uncommitted Th precursors, which allows more committed Th2 cells that are relatively resistant to AICD to have a selective advantage. The regulatory role of Fas and AICD in selecting for Th effector cell subsets is also consistent with data in young MRL/lpr (Fas deficient) mice which exhibit a propensity toward Th1 development and IFN-γ production compared with MRL control mice (20).

The defect in AICD seen in the absence of CD4 signaling is similar to that observed in some previous reports; however, the molecular basis for this defect has not been clear (21, 22). In contrast to the results of Hamad et al. (22), we do not find any evidence of a decrease in FasL expression in the CD4 mutant T cells. Instead, our data suggests that CD4 signals are important to render Fas competent to initiate death receptor signaling and initiate caspase activation. It has been known for some time that expression of Fas and FasL is not sufficient for the induction of cell death in T lymphocytes, but that additional signals from the CD3/TCR complex provide a critical competency signal (23, 24). The precise nature of this signal is not yet known; however, it closely correlates with the oligomerization of Fas molecules and Fas residence in lipid raft membrane microdomains (25). Our current data suggest that CD4 signaling provides a necessary part of this competency signal.

Although CD4 signaling may affect other components of death receptor signaling, our hypothesis that CD4 promotes oligomerization of the Fas signaling macromolecular complex, is consistent with our previous data indicating that CD4 signaling is required for the association of the TCR complex with lipid rafts after T cell activation (26). CD4 may play a similar role in promoting Fas association with lipid rafts and/or in promoting Fas oligomerization. The mechanism(s) by which CD4 (and presumably CD4-associated lck) may promote oligomerization and effective Fas signaling are also not yet clear. One possibility is that CD4 signaling provides a positive signal required for DISC oligomerization. For example in the absence of CD4 there may be a defect in protein kinase C activation, which has been suggested to be an important signaling event in developing competence for Fas-mediated cell death following CD4 and/or CD3/TCR cross-linking (24, 27). Alternatively, CD4 signaling may be required to inhibit signaling pathways that constitutively down-regulate Fas signaling pathways. One possibility in this scenario would be inappropriate activation of the ras/raf/ERK pathway due to a requirement of CD4-associated lck for the activation of dok family members and ras-GAP activation, which normally would inhibit ERK activation. Inappropriate activation of ERK has been associated with resistance to Fas-mediated cell death (28); thus CD4-dependent signaling may be normally required to down-regulate ERK activation, which would in turn enhance sensitivity to Fas-mediated cell death.

Regardless of the mechanism by which CD4 promotes AICD, our data suggest that this is a critical step in the development and selection of Th effector populations. These data may have important implications for controlling Th development in vivo. Following initial T cell priming, developing T cells will typically be in a complex microenvironment with a variety of competing signals and cytokines capable of influencing Th1 and Th2 effector cell development. Our finding that dysregulation of AICD in CD4 mutant T cells affects Th2 development, suggests that the differential development of susceptibility and resistance to AICD seen in Th1 and Th2 cells can be a factor in selecting for biased Th1 and Th2 effector populations. Therapeutic strategies that alter susceptibility to AICD during the Th development may be an effective means of biasing outcomes of T cell activation.

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Disclosures
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