Requirement for Efficient Interactions Between CD4 and MHC Class II Molecules for Survival of Resting CD4$^+$ T Lymphocytes In Vivo and for Activation-Induced Cell Death

Rosario Maroto, Xiaoli Shen and Rolf König

*J Immunol* 1999; 162:5973-5980; 
http://www.jimmunol.org/content/162/10/5973

**References**
This article cites 45 articles, 23 of which you can access for free at: 
http://www.jimmunol.org/content/162/10/5973.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at: 
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at: 
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at: 
http://jimmunol.org/alerts
Requirement for Efficient Interactions Between CD4 and MHC Class II Molecules for Survival of Resting CD4+ T Lymphocytes In Vivo and for Activation-Induced Cell Death

Rosario Maroto, Xiaoli Shen, and Rolf König

Regulation of homeostasis in the immune system includes mechanisms that promote survival of resting T lymphocytes, and others that control activation-induced cell death (AICD). In this study, we report on the use of a transgenic mouse model to test the role of CD4-MHC class II interactions for the susceptibility of CD4+ T lymphocytes to AICD, and for the survival of resting CD4+ T cells in peripheral lymphoid organs. The only I-A\textsubscript{\textmu} gene expressed in these mice is an A\textsubscript{\textmu}k transgene with a mutation that prevents MHC class II molecules from interacting with CD4. We showed increased apoptosis in CD4+ T lymphocytes derived from wild-type, but not from mutant A\textsubscript{\textmu}k transgenic mice following stimulation with staphylococcal enterotoxin A. Therefore, AICD may be impaired in CD4+ T cells derived from mutant A\textsubscript{\textmu}k transgenic mice. Importantly, we observed much higher apoptosis in resting CD4+ T cells from mutant A\textsubscript{\textmu}k transgenic mice than from wild-type mice. Furthermore, resting CD4+ T cells from mutant A\textsubscript{\textmu}k transgenic mice expressed higher levels of cell surface CD95 (Fas, APO-1). Ab-mediated cross-linking of CD95 further increased apoptosis in CD4+ T cells from mutant A\textsubscript{\textmu}k transgenic mice, but not from wild-type mice, suggesting apoptosis involved CD95 signaling. When cocultured with APC-expressing wild-type MHC class II molecules, apoptosis in resting CD4+ T lymphocytes from mutant A\textsubscript{\textmu}k transgenic mice was reduced. Our results show for the first time that interactions between CD4 and MHC class II molecules are required for the survival of resting CD4+ T cells in peripheral lymphoid organs. The Journal of Immunology, 1999, 162: 5973–5980.
AICD. However, unstimulated CD4+ T cells from E137A/V142A mutant A$k b transgenic mice undergo apoptosis at a much higher rate than do CD4+ T lymphocytes from wild-type A$k b transgenic mice. Our results show for the first time that interactions between CD4 and MHC class II molecules are required for survival of resting CD4+ T cells in peripheral lymphoid organs.

Materials and Methods

Materials

All chemicals, of the highest purity available, were purchased from Sigma (St. Louis, MO), unless otherwise stated. Staphylococcal enterotoxin A (SEA) was from Toxin Technologies (Madison, WI). Tissue culture media and supplements were from Life Technologies (Gaithersburg, MD).

Media and cell lines

DMEM containing 5% heat-inactivated FCS, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM HEPES, and 50 μM 2-ME were used for all cell culture experiments with mouse lymphocytes. This medium is henceforth called D5. The anti-mouse CD4 hybridoma GK1.5, the anti-mouse CD8 hybridoma 2.43, the anti-mouse MHC class II hybridoma 39E, and the IL-2-dependent indicator cell line CTLL-2 were obtained from the American Type Culture Collection (Manassas, VA) and cultured according to their recommendations.

Generation of transgenic mice

The generation of transgenic mice expressing wild-type or mutant A$k b has been described (18). Briefly, the double mutation Ala for Glu125 and Ala for Val133 was introduced into A$k b cDNA using PCR (12). Wild-type and mutant A$k b cDNAs were cloned into the pDOI plasmid within the rabbit β-globin gene under control of the Eα promoter (25). Fragments containing the Eα promoter, the A$k b wild-type or mutant cDNA, and a polyadenylation site were excised with BglII. Fragments free of vector sequences were used for microinjection into fertilized eggs from (C57BL/6 × 129V)F1 heterozygous females. Founder lines that transmitted the transgene were established. Transgenic mice were bred to A$k b mice. The only MHC class II molecules expressed on the cell surface in these lines are A$k b, b heterodimers. Lines that displayed normal tissue distribution of expression were selected. Henceforth, the wild-type A$k b transgenic line will be referred to as W A$k b, and the mutant line as M A$k b. Transgenic mice were maintained in a conventional animal care facility (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International), according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Isolation and culture of cells from lymph nodes and spleens

Cell suspensions from lymph nodes (LNs) and spleens were prepared as described (18, 26). Cells used for functional analyses were always isolated by negative selection. Briefly, purified populations of CD4+ T lymphocytes and CD4-depleted splenocytes were prepared by incubating with specific Abs (2.43, anti-CD8 mAb; 39E, anti-MHC class II mAb; GK1.5, anti-CD4 mAb), followed by incubation with anti-IgG Ab-coated magnetic beads (Dynabeads M-450; Dynal, Oslo, Norway), and magnetic depletion (26). Cell purity was routinely tested by fluorescence-activated flow cytometry using a FACScan (Becton Dickinson, Mountain View, CA).

In vitro culture of lymphocytes, axillary, brachial, cervical, inguinal, popliteal, and mesenteric LNs were dissected from unprimed mice. To measure apoptosis, we incubated LN cell preparations in 24-well plates at 2 × 10^6 cells/well in 0.8 ml of D5 at 37°C and 7% CO2. Alternatively, cell preparations were depleted of MHC class II and CD8+ cells with mAb 39E and 2.43, respectively (26). CD4-enriched preparations were incubated at 1–2 × 10^6 cells/well with 1–2 × 10^6 T cell-depleted splenocytes from either W A$k b or M A$k b mice. Cytokine production and cellular proliferation were measured as described previously (18, 26). Briefly, LN T cells (LNTCs) were incubated in flat-bottom 96-well plates at 1 × 10^5 cells/well in 0.2 ml of D5. To measure cellular proliferation, 1 μCi of [3H]thymidine was added 16 h before harvesting cells on a Packard Filtermate 196 cell harvester (Packard, Downers Grove, IL). Incorporation radioactivity was measured in a Packard Direct Matrix Beta Counter with a counting efficiency of 5%. Concentrations of secreted IL-2 were measured by determining the ability of a 1/4 dilution of culture supernatants to support growth of the IL-2-dependent cell line CTLL-2.

Flow-cytometric analysis and apoptosis assay

Apoptosis was measured by two- and three-color flow cytometry on a FACScan (Becton Dickinson). Cell preparations (1–2 × 10^6 cells) were stained with R-PEabeled anti-CD4 mAb (Caltag, South San Francisco, CA) and FITC-labeled Annexin V (Boehringer Mannheim, Indianapolis, IN), according to the manufacturer’s recommendations. Viable lymphocytes were electronically gated on forward and side scatter parameters characteristic for lymphocytes. In addition, viable cells were gated on their ability to exclude the dye 7-amino actinomycin D (27, 28). Expression of CD95 and the IL-2Rα-chain (IL-2Ra, CD25) was measured by staining with fluorescein-labeled anti-CD95 mAb Jo2 (PharMingen, San Diego, CA) and fluorescein-labeled anti-CD25 mAb 7D4 (PharMingen), respectively. Acquired data were analyzed with the CellQuest program (Becton Dickinson).

Results

Lack of AICD in CD4+ T lymphocytes from E137A/V142A MHC class II mutant mice

Activation of naive CD4+ T lymphocytes by Ag or bacterial superantigens can cause peripheral deletion of mature T lymphocytes (29, 30). We have reported previously that interference with CD4-MHC class II interactions using synthetic peptides that correspond to the CD4 binding site on the MHC class II β2-domain blocks AICD (20). Therefore, we hypothesized that the E137A/V142A mutation in A$k b may prevent cell death in CD4+ T lymphocytes induced by activation with SEA. To test this idea, we measured in vitro effects of SEA on CD4+ LNTCs from W A$k b and M A$k b mice. Apoptotic cells are rapidly cleared from circulation in vivo (31). However, an early and ubiquitous event in cells undergoing apoptosis is exposure of phosphatidylserine at the cell surface (32). In nonapoptotic cells, phosphatidylserine is located on the cytoplasmic side of the plasma membrane. Cell surface-exposed phosphatidylserine can be detected by a phospholipid-binding protein, Annexin V (32, 33). Thus, fluorescence-activated flow cytometry can be used to measure apoptosis. Viable apoptotic CD4+ cells can be detected by Annexin V staining and their ability to exclude the dye 7-amino actinomycin D (28). Using this technique, we could measure the apoptotic index in freshly isolated CD4+ LNTCs and after in vitro culture.

Activation-induced apoptosis was detectable in lymphocytes from W A$k b mice 72 h after stimulation with SEA, showing an increase in a dose- and time-dependent manner in CD4+ LNTCs from W A$k b, but not from M A$k b mice (Fig. 1). We could not detect any differences in the apoptotic index of CD4+ LNTCs from M A$k b mice whether the cells were incubated with SEA up to 5 μg/ml or without SEA. However, it was readily apparent that in unstimulated CD4+ LNTCs from M A$k b mice, apoptosis was enhanced as compared with CD4+ LNTCs from W A$k b mice (Fig. 1).

The E137A/V142A mutation in MHC class II does not prevent activation of CD4+ T lymphocytes

One possible explanation for the lack of AICD in CD4+ T lymphocytes from M A$k b mice following SEA stimulation was that the mutation in MHC class II prevented activation of CD4+ T cells. Although CD4+ LNTCs from M A$k b mice could proliferate in response to stimulation with bacterial superantigen, they required 10- to 100-fold higher concentrations of SEA for responses equivalent to those observed in CD4+ LNTCs from W A$k b mice (Fig. 2A). However, when incubated longer, LNTCs from M A$k b mice reached proliferation rates comparable with LNTCs from W A$k b mice, even in response to low SEA concentrations (Fig. 2B). Therefore, LNTCs from M A$k b mice could be activated by SEA, and thus lack of AICD in CD4+ LNTCs from M A$k b mice was not due to an inability to respond to SEA.
CD4⁺ T lymphocytes from E137A/V142A MHC class II mutant mice are deficient in IL-2 production

Maximal levels of IL-2 in cultures of SEA-stimulated LN T cells from W⁺ and M Aβk mice differed by almost 5-fold. Cultures of LN T cells from M Aβk mice never achieved IL-2 concentrations similar to those found in W⁺ Aβk cultures (Fig. 3). Even at the highest SEA concentrations used, IL-2 levels were very low in cultures of LN T cells from M Aβk mice. Furthermore, we could not detect cytoplasmic IL-2 by intracellular staining in CD4⁺ LN T cells from M Aβk mice after 4 or 24 h of SEA stimulation, whereas a large proportion of CD4⁺ LN T cells from wild-type mice contained intracellular IL-2 after 4 h of SEA stimulation (not shown). These data confirmed previous observations made in the M Aβk mice using SEA and the protein Ag, keyhole limpet hemocyanin (18).

Because IL-2 has been reported to promote AICD (34), it was possible that a dearth in IL-2 secretion prevented AICD in SEA-stimulated LN T cells from M Aβk mice. To test this possibility, we incubated LN T cells from W⁺ and M Aβk mice with SEA (5 µg/ml) in the presence or absence of exogenous IL-2 (20 U/ml) for 72 h. Exogenous IL-2 enhanced apoptosis in SEA-stimulated CD4⁺ LN T cells from both W⁺ and M Aβk mice (Fig. 3D), suggesting that insufficient IL-2 production was responsible for the lack of AICD in LN T cells from M Aβk mice.

Enhanced apoptosis in resting CD4⁺ T lymphocytes from E137A/V142A MHC class II mutant mice

During the experiments conducted to determine whether the E137A/V142A mutation in MHC class II would affect AICD in CD4⁺ LN T cells, we observed a higher percentage of apoptotic cells in resting CD4⁺ LN T cells from M Aβk mice than in CD4⁺ LN T cells from W⁺ Aβk mice (Fig. 1). It was suggested previously that survival of resting CD4⁺ T lymphocytes in vivo may depend on interactions with MHC class II-expressing cells (35). Because our mouse model introduced only a mutation in the CD4 binding site of Aβk without affecting Ag-presenting functions of the MHC class II molecule, it was ideally suited to determine whether CD4⁺ T lymphocytes required CD4-MHC class II interactions for survival.

Therefore, we measured the proportion of apoptotic CD4⁺ T cells in freshly isolated LN from W⁺ and M Aβk mice. The number of apoptotic CD4⁺ LN T cells was 2- to 3-fold higher in lymphocyte populations freshly isolated from M Aβk mice than in lymphocytes from W⁺ Aβk mice (Fig. 4A, 0 time point). This suggested that in vivo, apoptosis-inducing signals were delivered at a higher rate in M Aβk than in W⁺ Aβk mice. Furthermore, when LN cell preparations were cultured in vitro without Ag stimulation, this difference in the apoptotic index between CD4⁺ LN T cells from M Aβk and W⁺ Aβk mice remained (Fig. 4). Because apoptotic cells eventually shrink and lose their cell membrane integrity, we used electronic gating, based on cell size and ability to exclude 7-amino actinomycin D, to eliminate dead cells. Therefore, our
results suggested that rates of apoptosis differed between CD4+ LNTCs from the two mouse strains.

Coculture with APC that express wild-type MHC class II reduces the apoptotic index in CD4+ T lymphocytes from E137A/V142A MHC class II mutant mice, but does not restore the ability to undergo AICD.

To determine whether the increased apoptotic index in resting CD4+ LNTCs from M Aβk mice was due to an intrinsic defect in the LNTCs (e.g., inability to receive signals required for survival) or rather to the lack of signals necessary for survival provided by the environment, we depleted LN cells from M Aβk mice of CD8+ and MHC class II+ cells. The resulting cell fraction was 95% CD4+. CD4+ LNTCs were cocultured for 72 h with CD4+ T cell-depleted splenocytes from W1Aβk and M Aβk mice. The apoptotic index of CD4+ LNTCs from M Aβk mice was significantly lower after coculture in the presence of W1Aβk-expressing cells (Fig. 5A).

No difference in the percentage of apoptotic CD4+ LNTCs from

FIGURE 3. Lack of IL-2 secretion by lymphocytes from M Aβk mice in response to SEA. Lymphocytes were incubated at 1 × 10^6 cells/well in 200 µl of D5 media with various concentrations of SEA in flat-bottom 96-well plates. Culture supernatants were collected after 48 (A), 72 (B), and 96 (C) h of incubation. IL-2 concentrations were determined by the ability of a 1/4 dilution of the supernatants to support the growth of the IL-2-dependent cell line CTLL-2. Data are expressed as the mean ± SD of triplicates, and are representative of three independent experiments. D, Endogenous IL-2 enhances AICD in SEA-stimulated CD4+ LNTCs from W1Aβk and M Aβk transgenic mice. Lymphocytes were incubated with 5 µg/ml of SEA in the presence or absence of exogenous IL-2 (20 U/ml) for 72 h. Apoptotic cells in the CD4+ T cell population were measured as described in the legend to Fig. 1. When cultured in the presence of IL-2, the mean FITC-fluorescence intensity of viable SEA-stimulated CD4+ T lymphocytes increased 1.4-fold and 2.1-fold for LNTCs derived from W1Aβk and M Aβk mice, respectively. Data are from two independent experiments.

FIGURE 4. Enhanced apoptosis in CD4+ T lymphocytes from M Aβk mice. A, The percentage of apoptotic CD4+ LNTCs in freshly isolated LN cells (0 time) and after various lengths of in vitro culture (48–96 h) was measured by two-color flow cytometry using R-PE-labeled anti-CD4 mAb and FITC-labeled Annexin V. Viable lymphocytes were electronically gated, based on their forward and side light scatter characteristics. Data represent the mean ± SD of three to six independent experiments. Similar data were obtained by three-color flow cytometry and additional gating of viable cells on their ability to exclude 7-amino actinomycin D. The percentage of apoptotic CD4+ LNTCs in lymphocytes from W1Aβk and M Aβk mice was compared using Student’s unpaired t test. **, p < 0.01; ***, p < 0.001. B, Freshly isolated LN cells from M Aβk mice were stained with anti-CD4 mAb-R-PE, Annexin V-FITC, and 7-amino actinomycin D. Cells were analyzed by three-color flow cytometry, and viable lymphocytes were gated on their forward and side light scatter characteristics. The graph shows Annexin V versus 7-amino actinomycin D staining of CD4+ cells. The numbers refer to the percentages of CD4+ LNTCs in the respective quadrants.
W+ Aβk mice was observed whether the cells were incubated as whole LN cell preparations or as purified CD4+ LNTCs supplemented with W+ Aβk-expressing splenocytes. This result demonstrated that interactions between CD4 and MHC class II molecules were required for survival of CD4+ T lymphocytes, and suggested that in resting CD4+ T cells, signals via CD4 may block apoptosis.

To determine whether the inability to undergo AICD in response to SEA stimulation was also due to the lack of a signal mediated by the interaction of MHC class II molecules with CD4, we cocultured purified CD4+ LNTCs from M Aβk mice with CD4+ T cell-depleted splenic APC from W+ Aβk mice. CD4+ LNTCs from M Aβk mice were not sensitive to AICD when stimulated by SEA in the presence of APC from W+ Aβk mice (Fig. 5B). This result was not unexpected because the cells are defective in their ability to respond to SEA with AICD. On the other hand, CD4+ LNTCs from W+ Aβk mice were sensitive to AICD when stimulated by SEA from M Aβk mice. In this experimental situation, inability of the mutant MHC class II molecules to engage CD4 presumably did not allow full activation of CD4+ LNTCs.

Exogenous IL-2 cannot protect CD4+ T lymphocytes from apoptosis in E137A/V142A MHC class II mutant mice

The apparent defect in IL-2 production by CD4+ T lymphocytes from M Aβk mice (Fig. 3) (18) may have contributed to their enhanced apoptosis in vivo. For example, although IL-2 promotes AICD (34, 36, 37), it can also rescue T lymphocytes from apoptosis, depending on their activation status (38–40). Therefore, we injected 8000 IU of rIL-2 twice daily for 5 days into W+ Aβk and M Aβk mice. Treatment with IL-2 led to a slight increase in the percentage of apoptotic CD4+ lymphocytes in W+ Aβk mice, whereas in M Aβk mice the percentage of apoptotic CD4+ lymphocytes was reduced after IL-2 treatment in vivo. However, these changes were small, and IL-2 injections into M Aβk mice could not diminish apoptosis in CD4+ lymphocytes to the level observed in uninjected W+ Aβk mice (data not shown).

We established that the injected dose of IL-2 was biologically effective by measuring CD25 (IL-2Rα) expression in IL-2-injected and uninjected mice (Fig. 6). In both W+ Aβk or M Aβk mice, IL-2 treatment up-regulated CD25. This observation is in agreement with previous reports describing rescue of CD25 expression by exogenous IL-2 in lpr/lpr mice (41). However, in our M Aβk mice, regulation of CD25 expression was not solely dependent on IL-2 secretion, because constitutive expression of CD25 was higher in M Aβk than in W+ Aβk mice (Fig. 6A). Together, these results suggest that inability to produce IL-2 may be a contributing factor to the enhanced apoptosis of CD4+ T cells in M Aβk mice. Nevertheless, exogenous IL-2 could not substitute for the lack of CD4-MHC class II interactions.

CD4+ T lymphocytes from E137A/V142A MHC class II mutant mice express higher levels of CD95 than lymphocytes from wild-type MHC class II transgenic mice

To further elucidate the mechanism responsible for enhanced apoptosis of CD4+ T lymphocytes in M Aβk mice, we measured cell surface expression of CD95 (Fas, APO-1) on CD4+ lymphocytes...
FIGURE 6. Flow-cytometric analysis of CD25 (IL-2Rα) and CD95 (Fas, APO-1) expression in CD4+ T lymphocytes from W+ Aβk (blue line) and M Aβk mice (red line). A. CD25 expression on freshly isolated CD4+ LNTCs from W+ Aβk and M Aβk mice. Mean fluorescence intensity was 58 and 125 channels for CD4+ LNTCs from W+ Aβk and M Aβk mice, respectively. Region M1 identifies cells that express high levels of CD25 (W+ Aβk, 25% CD25high; M Aβk, 56% CD25high). B. CD25 expression on CD4+ LNTCs from W+ Aβk and M Aβk mice that were injected with IL-2. Mice were injected i.p. with 8000 U of IL-2 per day for 5 days. Mean fluorescence intensity was 90 and 150 channels for CD4+ LNTCs from W+ Aβk and M Aβk mice, respectively. Region M1 identifies cells that express high levels of CD25 (W+ Aβk, 42% CD25high; M Aβk, 58% CD25high). C. CD95 expression on freshly isolated CD4+ LNTCs from W+ Aβk and M Aβk mice. Mean fluorescence intensity was 53 and 153 channels for CD4+ LNTCs from W+ Aβk and M Aβk mice, respectively. D. CD95 expression on CD4+ LNTCs from W+ Aβk and M Aβk mice after stimulation with SEA (5 μg/ml for 72 h). Mean fluorescence intensity was 105 and 167 channels for CD4+ LNTCs from W+ Aβk and M Aβk mice, respectively. LN cells were stained with anti-CD4 R-PE and either anti-CD25 FITC or anti-CD95 FITC. Viable lymphocytes were electronically gated using forward and side scatter parameters characteristic for LNTCs. Only cells staining with anti-CD4 R-PE are shown.

from W+ and M Aβk mice. Signals through CD95 initiated by CD95 ligand mediate apoptosis in susceptible cells (42, 43). Expression of CD95 was higher on CD4+ lymphocytes from M Aβk mice than on CD4+ cells from W+ Aβk mice (Fig. 6C). These differences remained detectable in CD4+ lymphocytes that survived in vitro culture for 72 h (not shown). In CD4+ cells from W+ Aβk mice, incubation with SEA (5 μg/ml for 72 h) strongly up-regulated CD95 expression (Fig. 6D). This SEA-induced up-regulation of CD95 was less pronounced in CD4+ cells from M Aβk mice. These results showed increased cell surface expression of CD95 in unstimulated CD4+ T lymphocytes from M Aβk mice, and reduced up-regulation of CD95 expression following SEA stimulation.

Because the higher cell surface expression of CD95 in CD4+ LNTCs from M Aβk mice did not necessarily imply that apoptosis was Fas mediated, we tested the susceptibility of CD4+ LNTCs to Fas signaling. We incubated LN cells from M Aβk and normal mice in culture plates that were either coated with the anti-Fas mAb Jo2 or left untreated. To determine susceptibility to Fas-mediated apoptosis, we measured the apoptotic index in CD4+ LNTCs after 24 h in culture. Treatment with Jo2 enhanced apoptosis in CD4+ LNTCs from M Aβk but not from normal mice (Fig. 7). We also attempted to inhibit Fas-mediated apoptosis by blocking Fas ligand in LN cell cultures with the anti-Fas ligand mAb MFL3. Culturing LN cells from M Aβk mice with MFL3 for 24–72 h reduced the apoptotic index in CD4+ LNTCs to 87 ± 3.8% of the value measured in LNTCs incubated in the absence of MFL3 (mean ± SD of three experiments). We conclude that Fas-mediated signals contribute to the observed increased apoptosis in CD4+ LNTCs from M Aβk mice.

Discussion

The E137A/V142A mutation in the Aβk chain affects only interactions between CD4 and MHC class II molecules, but not interactions between the TCR and MHC class II (12, 17, 18). Nevertheless, in M Aβk mice, which only express an E137A/V142A mutant Aβk transgene, both thymic selection of CD4+ T cells (18) and survival of peripheral CD4+ T lymphocytes were reduced. Thus, although TCR-mediated signals initiated by complexes between MHC class II and self peptides were sufficient for positive selection of some CD4+ T cells in the thymus, these signals alone could not salvage peripheral CD4+ T cells. It is unlikely that CD4+ T cells selected on M Aβk thymic epithelium had a decreased ability to signal through the TCR, because no quantitative differences in CD3 expression could be detected between M Aβk, W+ Aβk, or normal B10.A mice (data not shown). Therefore, our data suggest that CD4+ T cells require engagement of CD4 by MHC class II molecules for their long-term survival in peripheral lymphoid organs, whereas thymic selection can occur in the absence of such interactions. Thus, the CD4 coreceptor is not restricted to enhancing TCR-mediated signals, but also regulates long-term peripheral survival of T cells. Future studies must now address molecular signaling pathways that mediate this regulatory role of CD4.
Thus, MHC class II-TCR interactions are not sufficient for the survival of resting CD4\(^+\) T cells, removal of the potential for these interactions should eventually lead to increased apoptosis in CD4\(^+\) LNTCs from normal mice. However, the reported \(t_{1/2}\) of about 26 days for CD4\(^+\) T lymphocytes in MHC class II-deficient mice (45) suggested that ex vivo cultures would not be adequate to address this question. Indeed, when we cocultured resting CD4\(^+\) LNTCs from W\(^+\) A\(_b\)^b mice with T cell-depleted APC from M A\(_b\)^k mice for up to 96 h, we did not observe an increase in apoptosis (Fig. 5B).

A possible mechanism responsible for the enhanced apoptosis in CD4\(^+\) LNTCs from M A\(_b\)^k mice may be via the CD95-induced pathway (42, 43). CD4\(^+\) LNTCs from M A\(_b\)^k mice expressed higher cell surface levels of CD95 than did CD4\(^+\) LNTCs from W\(^+\) A\(_b\)^k mice (Fig. 6). Furthermore, mAb-mediated cross-linking of cell surface CD95 enhanced apoptosis in resting CD4\(^+\) LNTCs from M A\(_b\)^k, but not from W\(^+\) A\(_b\)^k mice, demonstrating susceptibility to CD95-mediated apoptosis (Fig. 7).

We also found that CD4\(^+\) T lymphocytes from M A\(_b\)^k mice were defective with regard to the regulation of antigenic responses. This defect was manifested by deficient IL-2 secretion, a lack of AICD, and reduced up-regulation of CD95 following stimulation with SEA. However, exogenous IL-2 added in vitro could partly correct the defect to undergo AICD. Thus, the inability of CD4\(^+\) T cells from M A\(_b\)^k mice to undergo AICD may be the consequence of a defective regulation of expression of cell surface receptors (e.g., CD95, CD25). The failure of exogenous IL-2 to fully restore competence to regulate CD95 and to respond with AICD to SEA stimulation may reflect a requirement for CD4 signaling. Alternatively, positive selection in the absence of CD4-MHC class II interactions may select for CD4\(^+\) T cells that possess signaling capacities on the extreme borders of the normal distribution. Again, this question can only be answered by molecular analyses of signaling pathways.

The inability to undergo AICD following SEA stimulation in vitro probably reflects a similar inability to respond to antigenic stimulation with AICD in vivo. We have shown previously that in M A\(_b\)^k mice, primary and secondary immunizations with keyhole limpet hemocyanin expand Ag-responsive CD4\(^+\) T cells that secrete enhanced amounts of IFN-\(\gamma\) following in vitro restimulation. In W\(^+\) A\(_b\)^k mice, a primary keyhole limpet hemocyanin immunization primes CD4\(^+\) T cells to respond to in vitro restimulation with IFN-\(\gamma\) secretion to the same extent as in M A\(_b\)^k mice, but a secondary immunization causes a drastic reduction in the ability to secrete IFN-\(\gamma\) in response to in vitro restimulation (18). This difference in IFN-\(\gamma\) secretion after a secondary stimulation with Ag probably reflects in vivo AICD in W\(^+\) A\(_b\)^k mice, but not in M A\(_b\)^k mice.

In conclusion, we report that CD4-MHC class II interactions are required for maintenance of CD4\(^+\) T cells in peripheral lymphoid organs and for AICD. These observations suggest that signals through CD4 independent or in combination with TCR-mediated signals facilitate long-term survival of peripheral CD4\(^+\) T cells. Furthermore, CD4\(^+\) T lymphocytes selected in the absence of CD4-MHC class II interactions appear defective in their ability to regulate antigenic responses, as exemplified by their divergent regulation of CD25 and CD95, and their lack of IL-2 secretion following stimulation with SEA.

**Acknowledgments**

We thank Drs. Nancy Van Houten and Lynn Soong for discussions and critical comments on the manuscript, Wenhong Zhou and Timothy L. Denning for discussions, and Mardelle Susman for editorial assistance.
References


