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Defective Th Function Induced by a Dominant-Negative cAMP Response Element Binding Protein Mutation Is Reversed by Bcl-2

Feng Zhang,* Mercedes Rincon, † Richard A. Flavell, ‡ and Thomas M. Aune 2,∗§

cAMP response element binding protein (CREB) is a critical regulator of diverse stimulus-dependent transcriptional events. Following TCR stimulation, CREB is rapidly induced in CD4+ Th cell precursors, but not in effector Th cells. However, its role in mature T cell function is incompletely defined. Transgenic mice expressing a CREB dominant-negative (dn) mutation in the T cell lineage exhibited normal T cell development in the thymus, normal T cell homeostasis in the periphery, and normal T cell clonal expansion following Ag challenge. However, this mutation caused selective inhibition of Th cell function in vitro and in vivo, and increased susceptibility of Th cells to activation-induced cell death. Th cells expressing the CREB-dn mutation contained reduced levels of the inhibitor of programmed cell death, BCL-2; overexpression of BCL-2 in transgenic mice reversed both susceptibility to activation-induced cell death in CREB-dn T cells and the defect in effector cytokine production. Thus, CREB plays a critical role in Th cell function and development of Th cell-mediated adaptive immune responses, at least in part, by inhibiting stimulus-dependent cell death. The Journal of Immunology, 2000, 165: 1762–1770.

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D4+ T lymphocyte responses to TCR activation include proliferation, IL-2 production, differentiation into effector cells, effector cytokine production, unresponsiveness, and activation-induced cell death (1–6). This outcome depends upon a variety of factors. These include the type and quality of the TCR activation signal (7–9), the presence or absence of costimulatory signals (10, 11), the developmental stage of T cells (immature or peripheral T cells) (11), and the state of T lymphocyte differentiation (Th0, Th1, Th2) (12–14). Several models have been proposed to account for these different outcomes in the face of identical TCR stimuli (15). Thus, signals transduced by the TCR in cells of different responsive states may be identical, but differences in the transcriptional environment or the structure of chromatin may permit identical stimuli to induce differential gene expression and therefore different outcomes. Alternatively, differences in the quantity of signal transduced from the TCR may induce expression of entirely different sets of genes and lead to distinct outcomes; or the TCR in different T cell subsets may activate entirely different signal transduction pathways, and therefore induce expression of distinct gene sets. Each of these different models may function under different circumstances and is not mutually exclusive.

The process of Th cell differentiation from naive T cells to either IFN-γ-producing Th1 cells or IL-4-producing Th2 cells generates distinct T cell subsets which differ markedly in their response to TCR stimulation (12, 16–20). The cytokines IL-12 and IL-4 are important factors in stimulating differentiation into either Th1 or Th2 subsets, respectively. This differentiation process reflects, at least in part, an example of changes in the nuclear environment. Changes in both chromatin structure and in levels of nuclear transcription factors are associated with critical changes in gene transcription which mark T cell differentiation. Chromatin remodeling of both the IL-4 and IL-13 genes occurs during Th2 differentiation, and remodeling of the IFN-γ gene is associated with Th1 development (21). In addition, the transcription factors GATA-3 (22), JunB (23, 24), and c-maf (25) are expressed at high levels in Th2 cells but low levels in Th1 cells. It is unclear whether this is due to pre-existing differences in the nuclear environment or to differences in TCR-signaling pathways between these two cell populations.

The transcription factor, cAMP response element binding protein (CREB), a critical regulator of diverse stimulus-dependent transcriptional events (26–30), is highly induced in Th cell precursors (pTh cells), but not in Th1 or Th2 cells, by TCR stimulation (31). To test the hypothesis that CREB may be a critical regulator of Th cell differentiation, we examined the function of mature peripheral CD4+ T cells from transgenic mice expressing a dominant-negative (dn) form of CREB (32, 33). CD4+ T cells expressing CREB-dn developed normally in the thymus and exhibited normal homeostasis in the periphery. Mature peripheral CD4+ T cells from transgenic mice proliferated and produced IL-2 upon initial TCR stimulation. However, effector Th responses were markedly impaired, due in part to reduced levels of BCL-2 and increased susceptibility to cell death following TCR stimulation.

Materials and Methods

Transgenic mice

The CREB-dn mutation was generated by replacing Ser133 with Ala133 (32). This cDNA was subcloned downstream of the distal Lk promoter (34) and upstream of the human growth hormone intron and polyadenylation

Abbreviations used in this paper: CREB, cAMP response element binding protein, dn, dominant negative, pTh cell, Th cell precursor; cyt c, cytochrome c.

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sequences. Transgenic mice were prepared and screened as previously described (35). Reporter transgenic mice expressing the luciferase gene under the control of the distal transcriptional element from the IFN-γ promoter have been described elsewhere (36). TCR transgenic mice expressing the AND TCR specific for pigeon cytochrome c (Cyt c) peptide 81–104 in association with I-Ek (37) and the C57BL/6 B-2 transgenic mice (38) were obtained from The Jackson Laboratory (Bar Harbor, ME). CREB-dn mice and luciferase reporter transgenic mice had been backcrossed to B10.BR for four generations before intercrossing with Cyt c TCR mice, which were also B10.BR. Transgenic littermates were identified by Southern blot analysis or RT-PCR of tail DNA for the luciferase or CREB-dn cDNAs and by flow cytometry of peripheral blood using FITC-coupled anti-CD4 and PE-coupled anti-Vβ3.

Cell preparation and culture

Spleen cells, lymph node cells, or pooled spleen and lymph node cells were harvested from wild-type or transgenic animals. RBC were removed by hypotonic lysis. CD4⁺ T cells were purified by negative selection. IA⁺ cells and NK cells were removed by incubation with an anti-IE,IA mAb (m5/115, American Type Culture Collection, Manassas, VA) and an anti-NK mAb before anti-CD3 mAb restimulation

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Western blotting

Whole cell lysates or nuclear extracts were prepared by established procedures. For each experiment, equal amounts of protein were loaded in each lane, separated by SDS polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and incubated with specific primary Abs as outlined in the text. Membranes were washed and incubated with secondary HRP-conjugated anti-rabbit or anti-mouse Abs and developed with an enhanced chemiluminescent system (Amersham, Arlington Heights, IL) according to the vendor’s instructions.

Immunizations

CREB-dn transgene-positive mice and transgene-negative littermates were immunized with 0.1 ml of a suspension of 1 mg/ml OVA in CFA at 6 wk of age, i.p. Twelve weeks after primary immunization, mice were immunized with 0.1 ml of a suspension of OVA (1 mg/ml in IFA), i.p. Blood (~0.1 ml) was collected from nonimmunized and immunized mice by retro-orbital puncture, and serum samples were harvested by standard procedures. Levels of total and OVA-specific IgM, IgG1, IgG2a, IgG2b, and IgG3 in serum were determined by ELISA.

ELISAs

To measure cytokine levels in culture fluids, ELISAs were performed using Ab pairs recommended by PharMingen according to the manufacturers procedures. The lower limits of detection for each cytokine measured were in the range of 0.01–0.02 ng/ml. Total serum Ig levels were determined by coating plates with serum dilutions, followed by incubation with alkaline phosphatase-conjugated rat anti-murine IgM, IgG1, IgG2a, IgG2b, or IgG3 Abs, and by incubation with alkaline phosphatase substrate (Sigma, St. Louis, MO). Results are expressed as the reciprocal of the dilution required to yield a half-maximum OD₅₄₀. An indirect ELISA assay was used to determine titers of anti-OVA-specific Ab in serum samples. Briefly, ELISA plates were coated with 10 μg/ml OVA and blocked with PBS containing 10% FCS. Diluted serum samples were added and incubated for 1 h at 37°C, followed by incubation for 1 h at room temperature with alkaline phosphatase-conjugated goat anti-mouse IgM, IgG, IgG1, IgG2a, IgG2b, or IgG3 Abs, Color reactions were developed by alkaline phosphatase substrate (Sigma), and absorbance was determined at 405 nm. Results are expressed as the reciprocal of the serum dilution required to yield half-maximal OD.

Results

CREB protein levels are markedly increased in naive T cells following TCR stimulation but are not increased in effector T cells (Th0, Th1 or Th2) following identical TCR stimulation (31). Although phosphorylation of CREB at Ser133 is not required for CREB to bind to DNA CRE (26), it is required for CREB to bind to the coactivator CREB-binding protein and thus to activate transcription (39, 40). Therefore, we also wanted to determine whether

![FIGURE 1](http://www.jimmunol.org/) Levels of CREB and phosphorylated CREB in CD4⁺ pTh and Th cells following TCR stimulation. CD4⁺ T cells were stimulated with anti-CD3 mAb and APC for the indicated days. After 7 days, cultures were harvested and restimulated with anti-CD3 mAb. Nuclear extracts were prepared at the indicated times and analyzed for levels of total CREB and phosphorylated CREB by protein immunoblotting. Extracts from day 7 were prepared before anti-CD3 mAb restimulation
CREB was phosphorylated in CD4⁺ T cells following TCR stimulation. Protein immunoblotting experiments with a phosphorylated CREB-specific Ab demonstrated that levels of phosphorylated CREB were also markedly increased in naive T cells following TCR stimulation (Fig. 1). These data raised the possibility that CREB may be a critical regulator of transcriptional responses in either pTh cells or differentiating Th cells.

To investigate the role of CREB in T cell responses to TCR signaling, we prepared transgenic mice expressing a CREB-dn mutation (Ser133 to Ala (32)) in the T cell lineage, by placing this cDNA under the control of the distal lck promoter (34) (Fig. 2A). Protein immunoblotting experiments showed that purified resting splenic CD4⁺ T cells from transgenic mice contained higher levels of total CREB (wild type and mutant) than T cells from nontransgenic littermates (Fig. 2B). We performed RT-PCR using primers from the CREB gene and the human growth hormone gene to demonstrate that increased CREB protein levels in purified resting CD4⁺ T cells were actually due to expression of the mutant CREB transgene (Fig. 2C).

Transgenic mice contained normal percentages of CD4⁺ and CD8⁺ single-positive thymocytes, CD4⁺ and CD8⁺ double-positive thymocytes, and normal percentages of mature splenic CD4⁺ and CD8⁺ T cells (Fig. 2D). Transgenic mice also contained normal numbers of these different T cell subsets in thymus and spleen, normal sizes of lymph nodes, and normal levels of total serum IgM and IgG compared with littermate controls (data not shown).

To initiate an inquiry into possible defects in immune function induced by expression of the CREB-dn mutant, CD4⁺ T cells were purified from transgenic mice or transgene-negative littermates and were stimulated in vitro with anti-CD3 mAb. Two separate founder lines were employed to rule out the possibility of integration effects. T cells from transgenic mice or wild-type littermates yielded equivalent proliferative responses and produced equivalent amounts of IL-2 (Fig. 3, A and B). As expected, primary cultures containing T cells from either transgenic or wild-type mice contained very low levels of IFN-γ and undetectable levels of IL-4 (not shown). One CREB-dn transgenic line was also crossed to the TCR transgenic line which recognizes Cyt c peptide. CD4⁺ T cells from this cross were examined in a similar fashion. Expression of the CREB-dn mutation caused a partial decrease in proliferative responses to stimulation with Cyt c peptide and in IL-2 production when compared with TCR transgenic littermates, which lacked the CREB-dn transgene (Fig. 3, A and B).

To determine whether CREB is required for Th cell differentiation, primary CD4⁺ T cells were stimulated with anti-CD3 mAb and APC in the presence of medium, IL-12 (Th1 differentiation), or IL-4 (Th2 differentiation). After 5 days, T cells were harvested and equal numbers of cells were re-stimulated with anti-CD3 mAb or Ag. At the end of the 5-day primary culture period, similar numbers of viable CD4⁺ T cells were present in cultures from either wild-type or CREB-dn mice. Culture fluids were harvested 24 h after re-stimulation to analyze for levels of IFN-γ and IL-4 to determine whether the CREB-dn mutation had an impact on either Th cell differentiation or Th responses. Levels of IFN-γ were severely depressed in cultures from T cells expressing the CREB-dn mutation (Fig. 3C). This was the case even when primary cultures contained IL-12 to promote strong Th1 differentiation. Responses by Th cells were also inhibited at the transcriptional level as evidenced by the failure of T cells to activate the distal TCR response element from the IFN-γ promoter upon re-stimulation with anti-CD3 mAb (Fig. 3D). The CREB-dn mutation did not selectively inhibit Th1 responses. Production of IL-4 by Th2 cells re-stimulated with anti-CD3 mAb was also depressed in T cells expressing the CREB-dn mutation (Fig. 3E). The degree of inhibition of Th2...
responses (IL-4 production) was somewhat less than the degree of inhibition of Th1 responses (IFN-γ production) (compare Fig. 3C to Fig. 3E).

Double-transgenic mice expressing both the CREB-dn mutation and the single TCR were also investigated to examine the effects of the CREB-dn mutation on Th cell responses to Ag stimulation (Fig. 3, C–E). In general, these results were similar to results obtained using polyclonal T cells and the polyclonal TCR stimulus, anti-CD3 mAb. Both IFN-γ responses by Th1 cells and IL-4 responses by Th2 cells were severely depressed in the presence of the CREB-dn mutation.

Although the CREB-dn mutation resulted in inhibition of cytokine responses by both polyclonal CD4 T cells and monoclonal AND TCR CD4 T cells, the degree of inhibition was significantly greater in the AND TCR CD4 T cells. Whether this is due to differences in stimulation by anti-CD3 mAb vs antigenic peptide or is due to differences is susceptibility of the AND TCR monoclonal CD4⁺ T cell population vs the polyclonal CD4⁺ T cell population is not known.

Next we wanted to determine whether the CREB-dn mutation would alter T cell expansion and effector function following immunization with a protein Ag, OVA. For these experiments we used normal TCR wild-type mice rather than the AND TCR transgenic line. Recall proliferative responses to Ag stimulation, production of effector cytokines after in vitro Ag stimulation, and development of a humoral immune response were investigated. There was no detectable inhibition of the recall T cell proliferative response to challenge with OVA, in vitro (Fig. 4A). This suggests that similar levels of clonal expansion of OVA-specific T cells occurred in wild-type mice and in mice expressing the CREB-dn mutation. These data argue that OVA-specific T cells expressing a CREB-dn mutation undergo clonal expansion in response to Ag challenge and are able to proliferate in response to Ag stimulation in vitro. In contrast, immune T cells from OVA-immunized CREB-dn mutant mice failed to produce the effector cytokines IFN-γ and IL-10 in response to secondary challenge with OVA in vitro (Fig. 4B). Levels of IL-4 and IL-5 were below the limits of detection in cultures from both wild-type and CREB-dn mutant mice. Taken together, these data suggest that the OVA-immune T cells in the CREB-dn mutant line represent a population which is similar to a Th0 population and does not produce the effector cytokines IFN-γ and IL-10.

Development of humoral immune responses to T-dependent protein Ags depends upon both clonal expansion of small numbers of Ag-specific T cells and their differentiation into effector T cells. Serum was collected 1–2 wk after immunization to permit investigation of both IgM and IgG responses. Both OVA-specific IgM

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Expression of a CREB-dn mutation in the T cell lineage does not inhibit proliferation and IL-2 production following primary TCR stimulation but does inhibit expression of effector function. CD4⁺ T cells purified from two separate CREB-dn transgenic lines (nos. 4 and 33) or from one line (no. 33) intercrossed with the AND TCR transgenic line were stimulated with anti-CD3 mAb or Cyt c peptide and APC. Cultures were pulsed with [³H]TdR on day 2 and harvested on day 3 (A), or harvested on day 2 to measure levels of IL-2 in the cultures by ELISA (B). Cultures were also restimulated on day 5 with either anti-CD3 mAb or Cyt c peptide and APC and harvested 24 h later. Levels of IFN-γ (C) and IL-4 (E) were determined by ELISA; levels of IFN-γ promoter activity (D) were determined by measuring luciferase activity. In C and D, primary cultures were treated with or without IL-12 (5 ng/ml). In E, primary cultures were treated with or without IL-4 (10 ng/ml).
and IgG responses were markedly reduced in transgenic mice expressing the CREB-dn mutation. Expression of the CREB-dn mutation resulted in comparable inhibition of OVA-specific IgG1, IgG2a, and IgG2b responses (Fig. 5A). The 2-wk postimmunization serum titer is shown; comparable differences, but lower titers, were observed in the 1-wk postimmunization serum titer. The CREB-dn mutation still inhibited the humoral immune response to OVA following secondary immunization 2 mo after the primary immunization, although the degree of inhibition was somewhat less than observed following primary immunization (Fig. 5B).

In contrast to naive T cells, Th cells can rapidly undergo activation-induced cell death upon re-stimulation with anti-CD3 mAb or Ag (41–43). Increased cell death in Th cells expressing the CREB-dn mutation may contribute to reduced effector cytokine production following re-stimulation with anti-CD3 mAb or Ag. To test this possibility, the number of viable T cells was determined after secondary Ag or anti-CD3 mAb stimulation by trypan blue staining. There was a dramatic decrease in viable cell number within 16 h after secondary TCR-activation of Th cells expressing the CREB-dn mutation compared with Th cells from wild-type littermates (Fig. 6A). This decrease in viable cell number was independent of whether or not T cells were stimulated with IL-12 or IL-4 in primary cultures to promote selective Th1 or Th2 differentiation, respectively (data not shown). To investigate the rate of apoptosis, we utilized annexin V labeling of CD4+ T cells and flow cytometry. The rate and extent of annexin V was markedly increased in CD4+ T cells expressing the CREB-dn mutation. The observed loss in cell viability may contribute to the decrease in production of effector cytokines in these same cultures. This is in distinction to the primary cultures which exhibited equivalent viability in nontransgenic littermate T cells (3.2 ± 0.3 × 10^6/ml) and T cells expressing the CREB-dn mutation (3.4 ± 0.5 × 10^6/ml).

**Bcl-2** gene expression is induced by phosphorylated CREB during B cell activation, and BCL-2 protects B cells from activation-induced cell death (44). Therefore, we reasoned that Bcl-2 gene expression may also be dependent upon CREB in T cells and performed protein immunoblotting experiments to examine this possibility. Levels of BCL-2 were reduced in Th cells expressing the CREB-dn mutation compared with normal Th cells (Fig. 7A). Quantitation of the level of BCL-2 in the two populations showed that the CREB-dn mutation reduced BCL-2 levels by 5-fold. As a
control, levels of Fas were also compared in cell extracts from these two T cell populations and found to be similar. Reduced levels of BCL-2 may contribute to the decreased viability of Th cells expressing the CREB-dn mutation following re-stimulation with anti-CD3 mAb.

To examine this possibility, transgenic mice expressing the CREB-dn mutation were intercrossed with transgenic mice which overexpress Bcl-2 in the T cell lineage. Littermates expressing the CREB-dn transgene, the Bcl-2 transgene, or both transgenes were compared with littermates which did not express either of these transgenes. T cell cultures were established to measure the impact of the Bcl-2 transgene on the decrease in viability and in effector cytokine production induced by the CREB-dn mutation. The Bcl-2 transgene prevented the loss in cell viability induced by the CREB-dn mutation following restimulation of Th cells (Fig. 7B). Expression of the Bcl-2 transgene also largely prevented the induction of BCL-2.

Taken together, these results argue that T cells do not require CREB to undergo clonal expansion in response to Ag challenge in vivo or in vitro, but that T cells require CREB to differentiate into efficient effector Th cells capable of 1) producing effector cytokines, and 2) providing T cell help function to stimulate T-dependent Ig production by B cells. BCL-2 largely reverses the defect in T cell function induced by the CREB-dn mutation suggesting that a primary role of CREB may be for the efficient induction of BCL-2.

**Discussion**

CREB is a critical regulator of diverse stimulus-dependent transcriptional events (26–30). A TCR stimulus, such as anti-CD3 mAb, induces rapid phosphorylation of CREB at Ser133 in thymocytes (45) and certain transformed T cell lines (46), which permits direct coupling of CREB to the transcriptional machinery via the coactivator CREB-binding protein (39, 40). Expression of a dn form of CREB under the control of the CD2 promoter leads to general disruption of thymocyte function as measured by 1) decreased proliferative responses, IL-2 production, and induction of AP-1 transcription factors, and 2) increased rates of apoptosis in response to TCR stimulation (45).

In contrast to thymocytes, mature peripheral T cells contain extremely low levels of CREB. However, in mature peripheral CD4+ T cells, CREB protein is both up-regulated and also phosphorylated following TCR stimulation. Although this takes hours rather than minutes, it still raises the possibility that CREB may play an important role in the proliferation, differentiation, or function of mature T cells in the periphery. We reasoned that one means to test this hypothesis was to examine the function of T cells from transgenic mice which express a dn form of CREB (Ser133 to Ala133) under the control of the lck promoter which restricts expression of the transgene to the T cell lineage. In contrast to the above data, we did not see any effect of this CREB-dn mutation on thymocyte number, function, or apoptosis. The relatively low and late level of expression of the lck distal promoter may have contributed to the lack of a thymic phenotype. Thus, studies to investigate the role of CREB in peripheral CD4+ T cell function using this transgenic line should not be complicated by marked effects on thymocyte development.

Peripheral CD4+ T cells from CREB-dn mutant mice proliferate and produce IL-2 following initial stimulation by either Ag or by anti-CD3 mAb. Clonal expansion of CD4+ T cells following primary immunization also appear normal. However, CD4+ T cells from mice expressing the CREB-dn mutation are defective in their ability to produce effector cytokines in vitro and in vivo, and to participate in the humoral arm of the adaptive immune response. There are differences in the level of inhibition induced by the CREB-dn mutation in the different experimental systems. First, there is partial inhibition of proliferation and IL-2 production following primary stimulation. However, this degree of inhibition is very small compared with the degree of inhibition of effector cytokine production in the secondary cultures. Second, the CREB-dn mutation inhibits effector cytokine production in the AND TCR system to a greater degree than in the non-TCR transgenic system. This could reflect differences in the level of dependence upon CREB of CD4+ T cells stimulated by antigenic peptide vs anti-CD3 mAb. Alternatively, there could be some heterogeneity in the response of the CD4+ T cell population to inhibition of CREB function which may account for the differences in responses by monoclonal CD4+ T cells (AND TCR) and polyclonal CD4+ T cells.

Effector T cells produce effector cytokines and undergo activation-induced cell death in response to TCR signaling (4, 12–14, 47–52). A general view is that the majority of these effector T cells die after they deliver their effector function (53). However, effector T cells expressing the CREB-dn mutant exhibited both increased cell death in response to TCR signaling and a marked reduction in production of effector cytokines. This appears to be due, at least in part, to reduced levels of the anti-apoptotic protein, BCL-2, in effector T cells. Both defects, loss of cell viability and loss of

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**FIGURE 6.** Expression of the CREB-dn mutation increases loss of cell viability of Th cells following secondary TCR stimulation. A. Th cells, prepared as outlined in Materials and Methods, were harvested. Viable cells were obtained by gradient separation on Ficoll-Hypaque. Viable cells were cultured at 1 x 10⁶ cells/ml and stimulated with either anti-CD3 mAb or Cyt c peptide Ag and irradiated APC. Viable cell numbers were determined by trypan blue staining 16 h after re-stimulation. B. Th cells from anti-CD3 mAb-stimulated primary cultures were restimulated with anti-CD3. At the indicated times, cells were harvested and labeled with FITC-anti-CD3 mAb and PE-labeled annexin V. The percent of annexin V-positive cells was determined by flow cytometry.
effector cytokine production, were reversed by restoring BCL-2 levels by intercrossing CREB-dn transgenic mice with transgenic mice which overexpress BCL-2. This suggests that effector T cells expressing the CREB-dn mutation undergo activation-induced cell death before they produce effector cytokines.

Taken together, these data suggest that activation-induced cell death by the effector T cell population is linked to expression of effector function. One possibility is that separate death signals and signals to express effector cytokine genes are both induced by TCR activation. The death signals are delayed or reduced in amplitude by the presence of anti-apoptotic proteins, such as BCL-2. This could allow the TCR signals, which induce effector cytokine gene expression, to dominate early in the response, resulting in efficient adaptive immunity. Over time, the dominant death signals could eventually drive Th cells past their death checkpoint, resulting in apoptosis. In the face of reduced BCL-2 levels induced by the CREB-dn mutation or by other means, these cell death signals may not be delayed and may result in rapid cell death before delivery of effector functions. This could result in the induction of a weak adaptive immune response. A second possibility is that Th cells must pass through a death checkpoint following TCR stimulation before expressing effector function. High levels of CREB may direct expression of high levels of BCL-2 in differentiating Th cells, allowing them to avoid cell death and deliver effector functions. Whether similar checkpoints regulate the development of memory T cells is not known, but these data suggest that this experimental model will be suitable to test this possibility.

Gene knockout experiments have also demonstrated a key role for BCL-2 in normal homeostasis of both T and B cells (54, 55). Interestingly, both B and T cells develop normally in bcl-2-deficient mice. However, they are unable to maintain lymphocyte homeostasis due to the massive onset of apoptosis within a few weeks after birth. Up-regulation of BCL-2 in mature B cells following surface IgM cross-linking is associated with rescue from apoptosis (56). Widespread apoptosis is also observed in germinal centers and increased expression of BCL-2 is observed under conditions which prevent apoptosis of germinal center cells (57, 58). In mature B cells, Ig cross-linking induces the expression of BCL-2 by inducing CREB phosphorylation which activates the bcl-2 promoter through a series of CRE-binding sites (44). The fact that T cells which express a dn mutation of CREB have reduced levels of BCL-2 and exhibit increased susceptibility to cell death in response to TCR signaling is consistent with the B cell results.

Genetic manipulation of a number of transcription factors using a variety of strategies including gene knockouts, expression of dn mutations, or ectopic expression of lineage-specific transcription factors has been employed in an attempt to dissect the roles of individual transcription factors in developing acquired immune response. These studies group these transcription factors into several

FIGURE 7. Th cells from CREB-dn mice express reduced levels of BCL-2 and overexpression of BCL-2 rescues Th cells from CREB-dn-induced susceptibility to cell death and loss of effector function. A, Whole cell lysates were prepared from Th cells 5 days after primary stimulation with anti-CD3. Levels of BCL-2 were analyzed by protein immunoblotting. Levels of Fas were also analyzed to control for efficiency of the extraction procedure and protein quantitation and loading. Th1 and Th2 cells were prepared from littermates derived from a cross between CREB-dn-transgenic and BCL-2-transgenic mice as outlined in Materials and Methods. Th1 and Th2 cells (10^6/ml) were restimulated with anti-CD3 mAb. After 16 h, viable cell numbers (B) were determined and cell cultures fluids were harvested for analysis of IFN-γ (Th1) (C) and IL-4 (Th2) (D). At least three mice from each group were evaluated.
classes. Certain transcription factors, such as IFN regulatory factor 4, are required for the total function and homeostasis of mature T cells and B cells (59). Similarly, disruption of the NF-κB/c-rel signaling pathway in the T cell lineage through expression of a trans-dominant form of IκBα leads to increased apoptosis of thymocytes and mature T cells, as well as impaired T cell proliferation, IL-2 production, and effector cytokine production (60–62). In contrast to these studies, other transcription factors are specifically required for generation of either an effector Th1 or Th2 response but are not required for the initial activation and clonal expansion of naive T cells. For example, Stat4 (63, 64), IRF1 (65, 66), and a functional p38 pathway (67) are required to generate a Th1, but not required for generation of either a Th1 or Th2 response. In the presence of a CREB-dn mutation, normal numbers of T cells appear not to require CREB. It is well recognized that multiple exposures to foreign Ag result in the development of long-term memory in the brain. It is well established that development of long-term memory (brain) requires multiple training sessions (71, 72). Overexpression of CREB repressors, such as a CREB-dn mutation, or native CREB repressors (for example Aplysia CREB2 which is homologous to human CREB2 and murine ATF4), blocks acquisition of long-term memory in these models (73–76). Overexpression of CREB activators, such as native CREB, leads to acquisition of long-term memory in only one training session (77). This has led to the general hypothesis that the balance of CREB activators and CREB repressors comprise a molecular switch for the development of long-term memory. When CREB activators predominate, development of long-term memory ensues; if CREB repressors predominate, development of long-term memory is impaired. This general model has been confirmed in a variety of species including Aplysia, Drosophila, mice, and rats (71, 72, 76, 77). In the immune system, T cells appear not to require CREB for initial recognition and response to foreign Ag. However, their differentiation into effector cells and delivery of effector and long-term immune function does appear to require CREB. It is well recognized that multiple exposures to foreign Ag over spaced periods of time will yield the most effective adaptive immunity. It is possible that the same type of transcriptional model used to account for development of long-term memory in the brain may also apply to development of long-term adaptive immunity.

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