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# cAMP inducibility of transcriptional repressor ICER in developing and mature human T lymphocytes

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**ABSTRACT** **Stimulation of the cAMP-dependent signaling pathway exerts an inhibitory effect on the proliferation and effector functions of T cells. The ability of T cells to form high intracellular levels of cAMP is acquired during development in the human thymus and is retained by the majority of mature peripheral T lymphocytes. Here we show that elevated cAMP levels in T cells correlate with the expression of the potent transcriptional repressor ICER (inducible cAMP early repressor) previously described in the hypothalamic–pituitary–gonadal axis. Further, in transcriptional assays *in vivo*, ICER inhibits calcineurin-mediated expression of the interleukin 2 promoter as well as Tax-mediated transactivation of the human T-lymphotropic virus type I (HTLV-I) promoter. Thus, the induction of ICER in T cells may play an important role in the cAMP-induced quiescence and the persistent latency of HTLV-I.**

The inhibitory effects of the second messenger cAMP on T-cell proliferation and effector functions are well recognized (1). However, little is known about the mechanisms by which the inhibitory effects occur. Recently, a novel cAMP-inducible transcriptional repressor protein has been described (2, 3). This transrepressor, ICER, is expressed in neuroendocrine tissues such as the pineal, pituitary (2, 3), and thyroid (4) glands under rhythmic, circadian control. ICER binds to cAMP response elements (CREs) that diverge considerably from the canonical CRE motif, TGACGTCA, and thereby inhibits CRE-binding transactivator proteins. These CREs include the cAMP autoregulatory response elements located in the promoter of the ICER gene. The expression of the ICER gene is stimulated by cAMP-activated transcription factors and is then repressed by negative autoregulatory feedback, thereby establishing a circadian control of ICER gene expression.

Because of the known circadian fluctuations in the proliferation and activities of lymphocytes (5, 6), we sought to determine whether high levels of intracellular cAMP in developing T lymphocytes could induce the expression of ICER and so implicate a potential role for ICER in the observed inhibitory effects of cAMP on mature T cells (1). Here we report that prostaglandin $E_2$ ($PGE_2$) and forskolin, known agonists for cAMP signaling, rapidly induce ICER mRNA in human medullary thymocytes and mature peripheral blood T lymphocytes, but not in immature cortical thymocytes or peripheral B lymphocytes. Further, we show that in transfection expression experiments *in vivo*, ICER inhibits the transactivation of the interleukin-2 (IL-2) promoter as well as the promoter for the human T-cell-specific lymphotropic virus type I (HTLV-I). Inasmuch as IL-2 is critical for the proliferation of T cells, these findings suggest that the induction of the ICER may be a mechanism to explain the antiproliferative effects of cAMP signaling on T cells. The capability for ICER to bind to a wide variety of divergent CREs raises the possibility that ICER may regulate the expression of several or many genes in response to cAMP signaling during T-cell development and/or proliferation.

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## MATERIALS AND METHODS

**Preparation of Human Thymocytes and Peripheral Blood T and B Lymphocytes.** Human thymus glands were obtained from children (ages 3 months to 4 years) undergoing corrective cardiac surgery. Thymocytes were fractionated over discontinuous Percoll gradients (Pharmacia) (7). Cells with densities of $1.060 < o < 1.070$ and $P > 1.070$ were collected and classified into large and small cells, respectively. Cell size profiles were determined on a FACScan flow cytometer (Becton Dickinson) using a linear scale. Cell surface staining and flow cytometry was performed (8), with fluorescein isothiocyanate conjugated monoclonal antibody anti-CD3 (UCHT1, Immunotech, Westbrook, ME), anti-CD4-PE (MT310, Dako), or anti-CD8-FITC (SK1, Becton Dickinson). Buffy-coat leukocytes were obtained from healthy adult blood donors, and peripheral blood mononuclear cells were isolated by using Ficoll–Hypaque (Pharmacia). Monocytes were depleted by adherence to plastic, 2 × 45 min in RPMI 1640 medium (GIBCO/BRL) at 37°C. Recovered cells were depleted of B cells by using magnetic cell sorting (MACS, Miltenyi Biotec, Sunnyvale, CA). Staining with anti-CD19-microbeads and separations on MACS columns were performed according to manufacturer's instructions, except that the buffer used was RPMI 1640 medium with 1.0% bovine serum albumin. The T-cell-enriched populations typically contained less than 4% $CD20^+$B cells, less than 3% $CD14^+$ monocytes, and 15–23% $CD16^+$ natural killer cells, as revealed by flow cytometry (data not shown).

**cAMP Assay.** To measure cAMP formation, cells were treated with the indicated concentrations of agonists and incubated in RPMI 1640 medium with 1.0% bovine serum albumin. Measurements of intracellular cAMP concentrations were performed according to established procedures (9). Error bars represent standard deviations of average values calculated from three or more independent experiments.

**Reverse Transcription (RT)–PCR Cloning and Sequencing.** Human Jurkat T cells induced by 0.1 mM forskolin were harvested 3 h post-induction. Total RNA was isolated and subjected to RT–PCR. The sequences of the 5′ and 3′ primers were: 5′-ATG GCT GTA ACT GGA GAT GAA ACT-3′ and 5′-CTA ATC TGT TTT GGG AGA GCA AAT GTC-3′, respectively. The 5′ and 3′ primers were selected to begin with the start codon (ATG) and stop codon (antisense CTA),

Abbreviations: ICER, inducible cAMP early repressor; IL-2, interleukin 2; HTLV-I, human T-lymphotropic virus type I; $PGE_2$, prostaglandin $E_2$; PBL, peripheral blood lymphocyte; bZIP, basic leucine zipper; PKA, protein kinase A; CRE, cAMP response element; PMA, phorbol 12-myristate 13-acetate ("12-*O*-tetradecanoylphorbol 13-acetate").

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respectively. RT–PCR cloning and sequencing were performed by using standard procedures (10).

**RNase Protection Analysis.** RNA extraction was performed as described (10). RNA probe 1 is generated from pCREM-hRIBO3 by *Bam*HI digestion and *in vitro* transcription. RNA probe 2 was prepared from pJL5 by *Xho* I digestion and corresponds to full-length cDNA of human ICER II. In the data described in Fig. 3*B*, an RNA probe complementary to human α-globin was included with the ICER RNA probe to serve as an internal control. RNA probes, uniformly labeled with [α-$^{32}$P]CTP, were prepared by using reagents from an RNA probe kit (Ambion). These probes were used for RNase protection studies, according to the protocol provided by Ambion (RPAII Ribonuclease Protection Assay kit). X-ray films were scanned and evaluated by densitometry with IMAGE-QUANT (Molecular Dynamics). Results have been reproduced in several independent experiments.

**Immunocytochemistry.** Jurkat T cells were treated with forskolin (0.1 mM), attached to slides after 24 h by using a micro-cytocentrifuge (StatSpin, Norwood, MA), and fixed for 5′ in 4% paraformaldehyde in saline. Immunocytochemistry was performed by using Vectastain horseradish peroxidase ABC kit (Vector Laboratories). The ICER-specific antiserum, used for immunocytochemical staining, was raised in a guinea pig (Hazelton Research Products, Denver, PA) against an ICER-specific peptide (Ala-Val-Thr-Gly-Asp-Glu-Thr-Gly-Gly-Cys) (see Fig. 2*A*), conjugated to keyhole limpet hemocyanin.

**Transient Overexpression in Jurkat T Cells.** Transfection assays were performed by the DEAE-dextran technique. Typically, $10^7$ Jurkat cells were transfected with 2 μg of the reporter and the same amount of Tax or protein kinase A (PKA) expression vectors. Chloramphenicol acetyltransferase assays and quantification methods are described elsewhere (11). The percent conversion of [$^{14}$C]chloramphenicol to its acetylated forms (Ac) was quantified by using a Betagen Betascope (Mountain View, CA). The results shown here are representative of a series of several independent experiments.

## RESULTS AND DISCUSSION

**Maturation of PKA Pathway During T-Cell Development in Human Thymus.** Thymus glands were obtained from children undergoing corrective heart surgery, and thymocytes were separated by centrifugation through Percoll gradients (7). Fractionated thymocytes consisted of either small, immature cortical cells ($CD3^{-/low}CD4^+CD8^+$) (Fig. 1 *A–C*), or large cells, containing mature medullary $CD3^{high}CD4^+CD8^-$ or $CD4^-8^+$ thymocytes (Fig. 1 *A, B,* and *D*) (12). To examine their differences, these fractionated cells were treated with the adenylyl cyclase agonists forskolin or $PGE_2$ within 12 h of obtaining the thymus gland. Both agonists caused a striking elevation of cAMP levels in medullary thymocytes, but relatively low elevations in small cortical thymocytes (Fig. 1 *E* and *F*). $PGE_2$ or forskolin also markedly stimulated cAMP levels in mature PBL T cells, whereas little or no response was observed in B cells (Fig. 1 *G* and *H*). These large differences in the responses of separated thymocytes led us to examine whether the elevations of cAMP that are measured in medullary thymocytes and mature PBL T cells could be sufficient to induce the transcriptional repressor ICER.

**Human ICER Sequence Is Highly Conserved.** To test whether elevated levels of intracellular cAMP in medullary thymocytes induce ICER, all four isoforms of human ICER were cloned by RT-PCR from forskolin-treated human Jurkat T cells and were sequenced (Fig. 2). Alignment of the human and mouse sequences of ICER II shows a high degree of conservation at both the nucleotide and amino acid levels, particularly in the bZIP domains (Fig. 2*B*).

**Induction of ICER Correlates with cAMP Response in Medullary Thymocytes.** The ICER cDNAs allowed for the construction of RNase protection probes specific for the detection of ICER mRNAs expressed in thymocytes. Treatment of medullary thymocytes with forskolin for 3 h yielded a

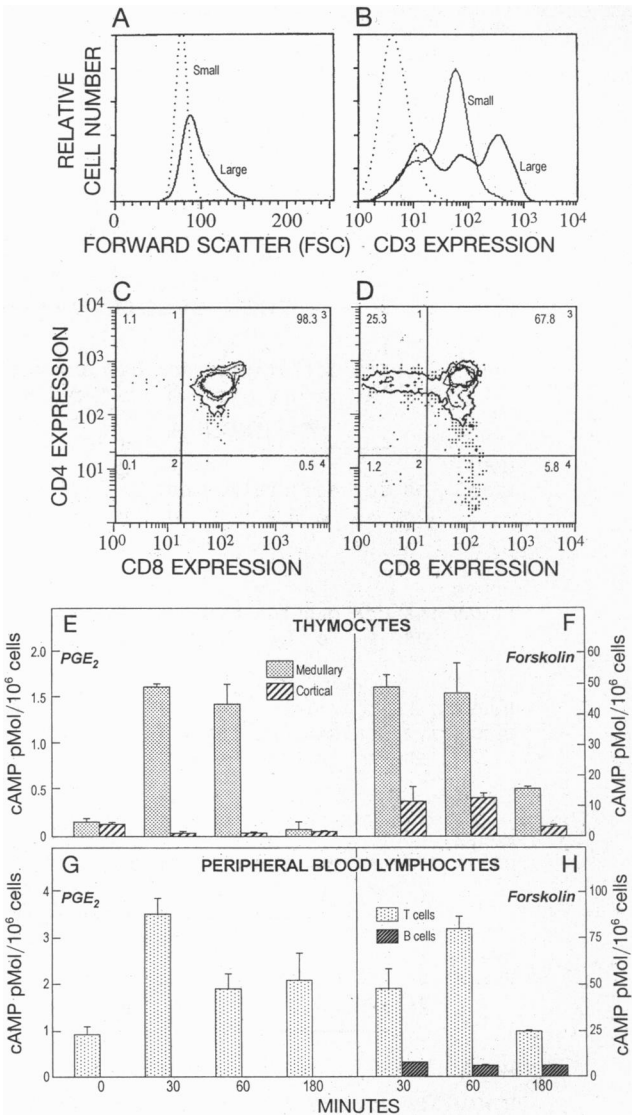


FIG. 1. Stimulation of cAMP levels in thymocytes and peripheral blood lymphocytes (PBLs) by either $PGE_2$ or forskolin. (*A*) Forward light scatter (FSC) profiles of thymocyte populations obtained after separation over density gradients (7). The dotted line represents small, low-density thymocytes; the filled line represents large, high-density thymocytes. Large thymocytes represent approximately 15% of total thymocytes. (*B*) CD3 expression of small and large thymocytes measured by flow cytometry. Small thymocytes expressed no detectable, or relatively low, levels of CD3, while the large thymocyte population contained cells with different levels of CD3 expression, including a portion of $CD3^{high}$ cells. The dotted line represents a negative control staining with an isotype matched antibody. (*C*) Small thymocytes were stained with anti-CD4-PE and anti-CD8-FITC. Shown are two-color histograms, indicating the percentage of positive cells in each quadrant. Small thymocyte populations contained more than 98% cortical $CD4^+CD8^+$ thymocytes. (*D*) The flow cytometry of large thymocytes reveals an enrichment of medullary $CD4^+8^-$ and $CD4^-8^+$ thymocytes. Small and large thymocytes are referred to as cortical and medullary thymocytes, respectively. (*E*) Comparison of relative cAMP levels in the medullary versus cortical thymocytes ($10^6$ cells) without treatment (0′), or after $PGE_2$ treatment (500 ng/ml), at 30-, 60-, and 180-min time intervals. (*F*) Relative cAMP levels measured in medullary or cortical thymocytes after forskolin treatment (0.1 mM). (*G*) Comparison of relative cAMP levels in human PBL T cells after $PGE_2$ treatment (500 ng/ml). (*H*) Relative cAMP levels measured in PBL T cells versus B cells after forskolin treatment (0.1 mM).

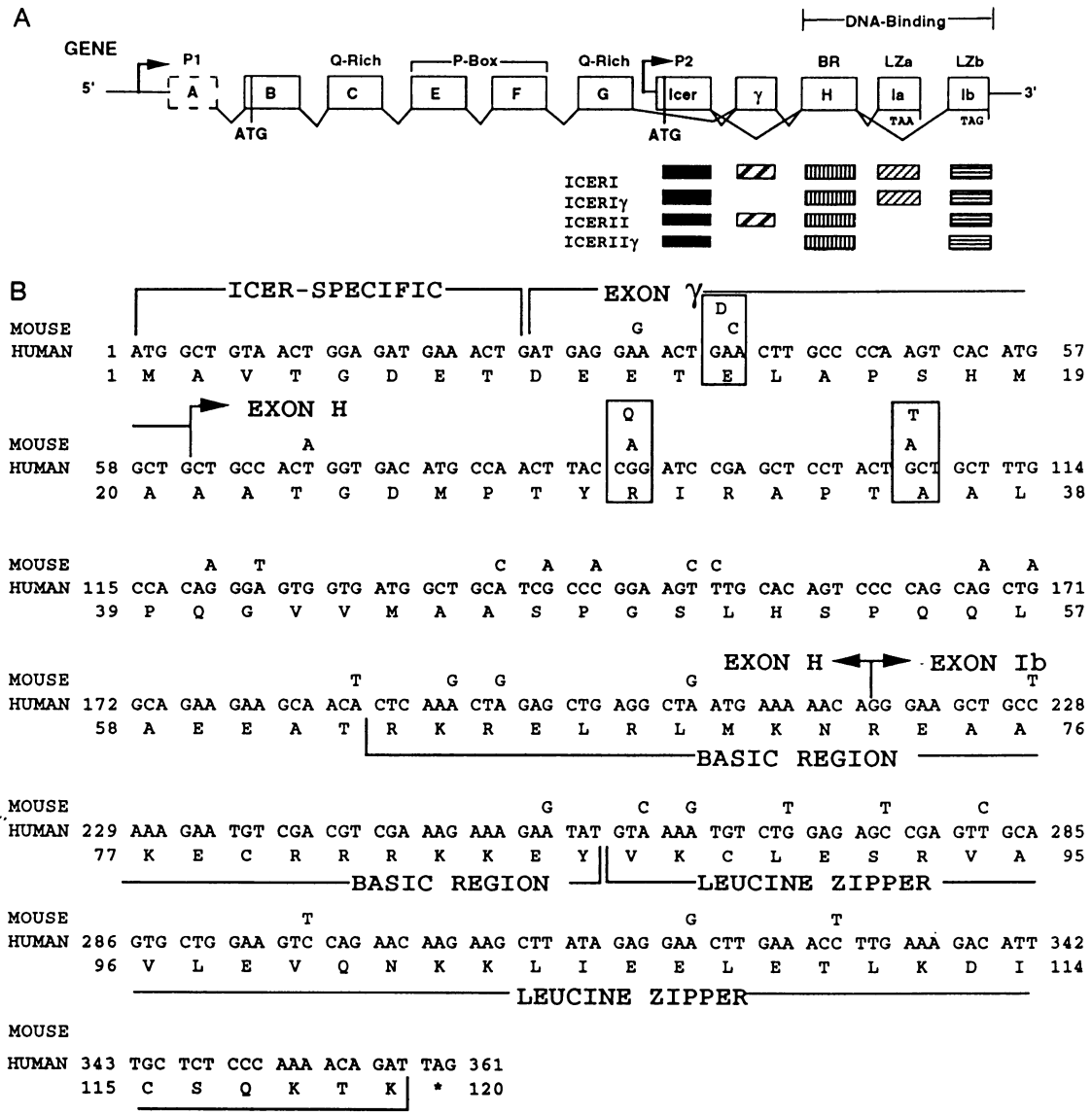


FIG. 2. ICER cDNA sequences obtained from forskolin-induced Jurkat T-cell RNA by RT–PCR. (*A*) Schematic representation of the CREM gene. The alternative P1 and cAMP-responsive P2 promoters are depicted by arrows. The BRLZa corresponds to the DNA-binding domain basic leucine zipper bZIP I (CREB-like), and BRLZb corresponds to bZIP II (CREM-like). The phosphorylation domain (P-box) and Q-rich domains are indicated. RNA splicing generates the four isoforms of ICER, with alternatively spliced exons encoding the DNA-binding bZIP domains (ICER I or ICER II containing exons Ia or Ib, respectively) and the amino proximal γ exon (ICER I and ICER II containing γ exon, and ICER Iγ and ICER IIγ lacking the γ exon). All four isoforms of ICER are schematically represented below. (*B*) Alignment of human and mouse homologues of ICER II cDNAs. Upper numbers in the margins refer to adjacent nucleotides; lower numbers in the margin are amino acids. The +1 corresponds to A of the ATG initiation codon. Single-point nucleotide substitutions outside of the bZIP II domain yielded three amino acid changes between human and mouse homologues in positions $Glu^{13}$-Asp, $Arg^{30}$-Gln, and $Ala^{36}$-Thr, framed in squares. The cDNA sequences of the three other human ICER isoforms that were cloned and sequenced from human Jurkat T cells (ICER IIγ, ICER I, and ICER Iγ) are not shown.

strong ICER IIγ and weaker ICER II-specific mRNA signals with the human ICER II RNA probe (probe 2) (Fig. 3 *A* and *B*). Notably, no significant forskolin-mediated induction of ICER mRNA was detected in either cortical thymocytes or unfractionated (total) human thymocytes (Fig. 3*B*). These findings correlate with the differences in cAMP levels observed after treating medullary and cortical thymocytes with forskolin (Fig. 1*F*) and confirm that ICER induction can be detected only in a subset of mature medullary thymocytes.

Of importance is that ICER is transcribed and translated exclusively from an internal promoter (P2) whose activity is stimulated by cAMP-responsive autoregulatory enhancers (3) (Fig. 2*A*). To confirm that ICER is the only CREM isoform induced in thymocytes and that the constitutive P1 promoter is not used, a human CREMγ probe (probe 1) that contains exons from the 5′ portion of the CREM gene upstream of the ICER-specific P2 promoter was used in the RNase protection assays (Fig. 3 *A* and *C*). The length of protected ICER IIγ fragment by the CREMγ probe corresponded exactly to that observed with the ICER II RNA probe, indicating the absence of 5′ sequences (P1 promoter induced) upstream of the P2 promoter in forskolin-induced CREM transcripts (Fig. 3*C*). These results demonstrate that ICER IIγ is the prevalent CREM isoform in the forskolin-treated Jurkat leukemic T-cell line (Fig. 3 *B* and *C*).

**Autoregulatory Inhibition of ICER in T Cells.** Since ICER expressed in medullary thymocytes, as well as in Jurkat cells, specifically uses the cAMP-responsive P2 promoter in the human CREM gene, we questioned whether an autoregulatory loop, similar to that described in the pineal and pituitary glands

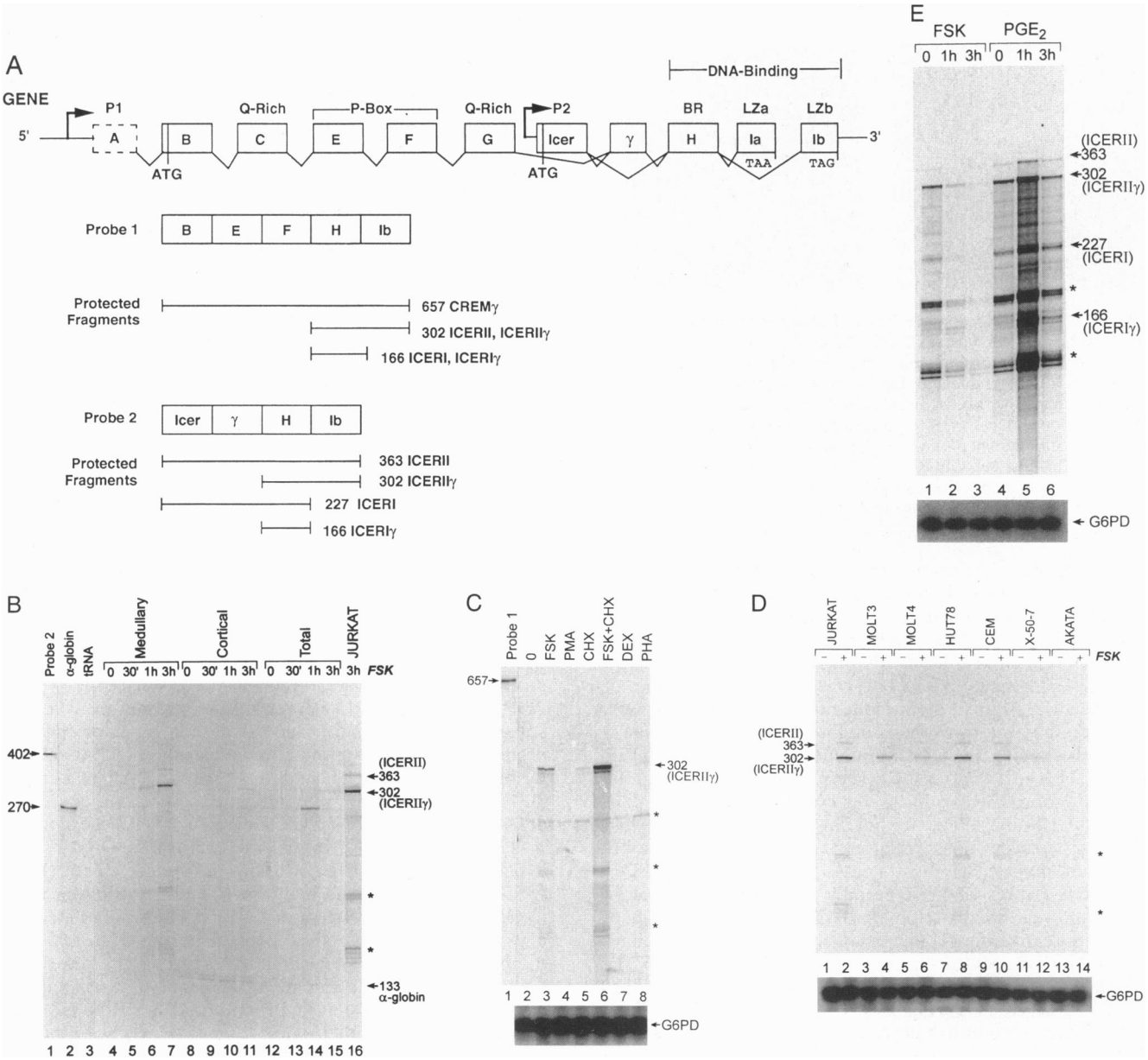


FIG. 3. Differential inducibility of ICER transcripts during T-cell development. (*A*) Schematic representation of probes used in ICER RNA analysis by RNase protection assays. The DNA-binding domains and the phosphorylated region (P-box) are indicated. The extent of partially protected probes obtained during the RNase protection analysis is indicated by horizontal bars. Probe 1 corresponds to human CREMγ (13), initiated at the P1 promoter. Probe 2 corresponds to human ICER II, initiated at the P2 promoter. The sizes in nucleotides of the protected RNA are indicated. (*B*) RNase protection analysis of total RNA from medullary, cortical, or total thymocytes treated with forskolin for the times indicated (lanes 4–15), and total RNA from Jurkat cells after 3 h of forskolin treatment (lane 16). The sizes of the bands, corresponding to specific ICER-protected fragments, are shown (nt). Asterisks denote nonspecific background bands. The bands of 363 nt and 302 nt correspond to ICER II and ICER IIγ, respectively; both contain the bZIP II domain. Probe 2 (402 nt) (lane 1) and internal probe of α-globin (270 nt) (lane 2) did not yield any detectable signal with tRNA (lane 3). The band of 133 nt corresponds to the protected fragment of the α-globin probe. The enhancement of the 270-nt α-globin band in the total thymocyte RNA 1 h after treatment with forskolin is unexplained. (*C*) ICER expression is induced specifically by the cAMP signal transduction pathway. ICER expression following 6 h of treatment was scored with probe 1 (lane 1) in untreated Jurkat cells (0; lane 2) or Jurkat cells treated with 0.1 mM forskolin (FSK; lane 3), 10 μg/ml PMA (lane 4), 10 μg/ml cycloheximide (CHX; lane 5), or cycloheximide in combination with forskolin (FSK+CHX; lane 6), $10^{-7}$ M dexamethasone (DEX; lane 7), and 10 μg/ml phytohemagglutinin (PHA) (lane 8). ICER expression is induced only upon stimulation of the cAMP pathway by forskolin (lane 3), or after co-treatment with cycloheximide, which resulted in superinduction (lane 6). No products corresponding to utilization of the P1 promoter were detected. (*D*) Immediate, early ICER expression is specifically induced in T-cell lines, but not in B-cell lines. RNase protection analysis of ICER expression (probe 2) in five different T-cell lines and two different B-cell lines, either untreated or after 3 h of forskolin treatment, shows induction of ICER IIγ (302 nt) and ICER II (363 nt) in Jurkat cells (lanes 1 and 2), MOLT3 (lanes 3 and 4), MOLT4 (lanes 5 and 6), HUT78 (lanes 7 and 8), and CEM (lanes 9 and 10). ICER induction was only faintly detectable under these conditions in the B-cell lines X-50–7 (11) (lanes 11 and 12), and AKATA (12) (lanes 13 and 14). T-cell lines were obtained from the American Type Culture Collection. (*E*) ICER expression in PBL T cells is downregulated by forskolin (0.1 mM), but upregulated by $PGE_2$ (500 ng/ml). After 1 h, forskolin (lane 2) or $PGE_2$ (lane 5) caused significant diminution or enhancement of ICER expression in PBL T cells, respectively. A significant shift from ICER II (302 and 363 nt) to ICER I (227 and 166 nt) isoforms was also observed after $PGE_2$ treatment. ICER expression was scored by RNase protection analysis using probe 2. Equal loading and quality of RNA were monitored either by using human α-globin as an internal probe (protected fragment 133 nt) (14) or by G6PD RT-PCR controls (15). These results are representative of a series of many experiments.

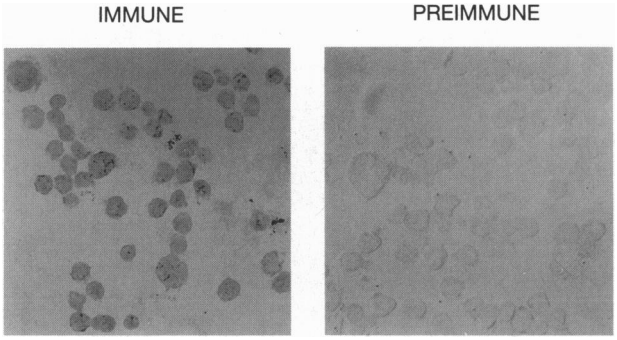


FIG. 4. ICER expression in T cells. Immunocytochemical analysis of forskolin-treated Jurkat T cells after 24 h of treatment with forskolin reveals intense staining in nuclei with ICER-specific antiserum, but no detectable staining with pre-immune serum. Jurkat T cells were treated with forskolin (0.1 mM) and attached to slides after 24 h. Immunocytochemistry was performed with ICER specific antiserum raised in guinea pigs against a synthetic ICER-specific peptide (Ala-Val-Thr-Gly-Asp-Glu-Thr-Gly-Gly-Cys) (see Fig. 2*A*) using horseradish peroxidase. Preabsorption of ICER-specific antisera with the ICER peptide (100 μg/ml) abolished the immunostaining (data not shown).

of rodents (2, 3), results from ICER binding to its own promoter in human T cells. The specificity of the cAMP signaling pathway in the induction of the P2 promoter in T cells was therefore examined. Activation of signal transduction pathways other than the cAMP pathway by treatment with phorbol 12-myristate 13-acetate (PMA), dexamethasone, or phytohemagglutinin, did not induce ICER mRNAs (Fig. 3*C*). However, the protein synthesis inhibitor cycloheximide markedly potentiated the forskolin-induced elevation of ICER mRNA (FSK+CHX) (Fig. 3*C*). These findings suggest that the inhibition of ICER synthesis by cycloheximide in T cells renders ICER unavailable for binding to the P2 promoter, and are consistent with autoregulatory inhibition, as described in the rodent model (3).

**ICER Is Expressed Preferentially in T Cells.** Induction of immediate, early expression of ICER mRNA after 3 h of forskolin treatment was detected in all five human T-cell lines tested (Jurkat, MOLT-3, MOLT-4, HUT78, and CEM), whereas ICER was only faintly detectable in the B-cell lines (X-50-7 and AKATA) (Fig. 3*D*). This suggests an immediate, early T-cell preferential expression of ICER. Moreover, a marked increase of ICER mRNA levels was observed in purified human PBL T cells in response to $PGE_2$ (Fig. 3*E*, lane 5). Notably, a shift in the prevalence of isoforms was observed: ICER II and ICER IIγ shifted to ICER Iγ and, to a lesser extent, to ICER I. In these mature PBL T cells, a forskolin-mediated inhibition of ICER mRNA levels is consistent with the mechanism of autoregulatory inhibition of the P2 promoter (Fig. 3*E*, lanes 2 and 3). The relatively high levels of ICER mRNA observed in untreated, purified PBL T cells may be either a consequence of the activation of cAMP signaling pathways during the preparation of T lymphocytes, or may reflect a normal physiologic property of resting PBL T cells. This conjecture correlates with the elevated basal levels of cAMP observed before the addition of $PGE_2$ or forskolin (note the difference in zero time points in Fig. 1 *E* and *G*). A further activation of the cAMP pathway by forskolin is predicted to repress ICER mRNA levels via the negative autoregulatory actions of the ICER repressor of the P2 promoter. The inducibility of the ICER promoter under physiological conditions *in vitro* suggests an important role of ICER in T-cell functions *in vivo*. The specific expression of ICER protein, directed by the cAMP-responsive P2 promoter in T cells, is further supported by *in situ* immunocytochemical analysis of Jurkat cells (Fig. 4) with an antiserum raised against a synthetic peptide that corresponds to the ICER-specific exon (Fig. 2) and is present in ICER, but not other CREM proteins. Detection of ICER after 24 h of forskolin treatment indicates a relatively high stability of the protein expressed in the T cells.

**ICER Inhibits Calcineurin-Mediated IL-2 Expression.** $PGE_2$ is one of the numerous physiologic inhibitors of T-cell proliferation that elevate intracellular levels of cAMP (16, 17). $PGE_2$ is also known to inhibit IL-2 expression in T cells by counteracting calcineurin-dependent activation of the IL-2 promoter, mediated by the cooperative interactions of transcription factors NFAT and the Fos/Jun (AP-1) complex (18–20). To determine whether ICER expression could supplant the effect of $PGE_2$ in the downregulation of the activated IL-2 promoter, ICER II, and, as a control, a DNA-binding deficient mutated ICER II without the leucine zipper (ICER II $LZ^-$), were expressed in Jurkat T cells in transient transfection assays. Expression of ICER II downregulated the human IL-2 promoter activated by the combined treatment of the cells with ionomycin and PMA (PMA+I), a treatment known to activate the calcineurin-dependent signal pathway (18), whereas expression of the mutated ICER II $LZ^-$ had little effect on activation (Fig. 5*A*). Thus, ICER can be induced by, and substituted for, $PGE_2$ in the transcriptional downregu-

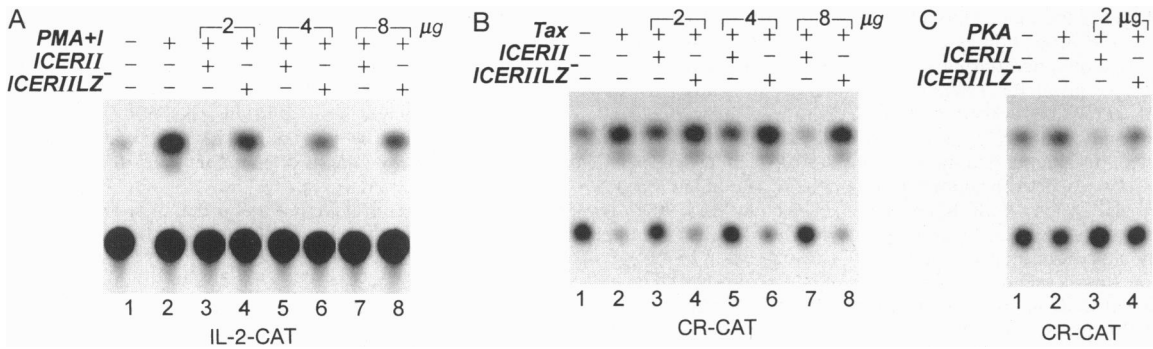


FIG. 5. ICER represses calcineurin-dependent activity of IL-2 promoter and Tax or PKA-mediated HTLV-I transactivation. (*A*) Jurkat cells were transfected with either the human IL-2-CAT reporter (20) or with the HTLV-I LTR-CAT (CR-CAT) reporter (*B* and *C*) (21). To activate the calcineurin-dependent signal pathway, Jurkat cells were treated with PMA (10 ng/ml) and ionomycin (1 μg/ml) 16 h posttransfection (PMA+I); to induce the HTLV-I promoter, Jurkat cells were cotransfected with an expression vector of Tax (21) (*B*), or with an expression vector of the catalytic subunit of PKA (22) (*C*). Co-transfection with 2, 4, or 8 μg of the ICER II expression vector (pKWJL5) represses promoter activation (*A* and *B*, lanes 3, 5, and 7, respectively). No comparable repression can be seen with the DNA-binding deficient ICER mutant, ICER II $LZ^-$ expression vector in parallel transfections using the same amounts of DNA (*A* and *B*, lanes 4, 6, and 8). (*C*) PKA activated CR-CAT reporter (lane 2) is almost completely repressed by 2 μg of ICER II (lane 3). To confirm the specificity of ICER inhibition, pGl2(3×GAL4)-CAT reporter (23) was transactivated by the GAL4VP16 (24) in combination with ICER II and ICER II $LZ^-$ expression vectors. No significant inhibition in transient transfections with any of the above-mentioned plasmids was observed (data not shown).

lation of the calcineurin-dependent, NFAT/AP-1-mediated transactivation of the IL-2 promoter, when induced by PMA and ionomycin. Although transcription factors other than ICER may contribute to the inhibition of IL-2 expression by cAMP generating agonists, the data presented here support the idea that ICER is a negative regulator of IL-2 gene transcription. The similarities between the bZIP domains of ICER and Fos/Jun suggests the possibility that ICER may interfere with Fos/Jun in the NFAT complex, and so inhibit activation of the IL-2 promoter.

**ICER Downregulates Tax-Mediated HTLV-I Expression.** The Tax protein encoded by HTLV-I has been shown to enhance the binding of the bZIP transcription factors (25), such as the strongly cAMP-responsive transactivator CREB (26), to CRE-like sequences in the 21-bp repeats contained in the promoter of HTLV-I. These observations suggest a role for ICER in HTLV-I-infected T cells. Therefore, we examined whether the expression of ICER would inhibit HTLV-I promoter activity. Indeed, in transcriptional assays, *in vivo* expression of ICER II effectively downregulates both Tax- and PKA-mediated transactivation of the HTLV-I promoter (Fig. 5 *B* and *C*). The results of our studies suggest that the induction of ICER by cAMP signaling is a potentially important mechanism in the down regulation of certain genes in response to cAMP. cAMP is known to inhibit pathways upstream of transcription, e.g., cAMP-dependent phosphorylation inactivates Raf-1 (27). We suggest, however, that ICER could be a dominant transcriptional repressor in T cells and thus, under certain circumstances, override effects on transcription mediated by other upstream pathways. The findings that the expression of ICER in T cells downregulates the IL-2 and HTLV-I promoters provides a potential link between the quiescent state of T cells and the persistent latency of HTLV-I.

**Note Added in Proof.** It has been reported recently (28) that the targeted expression of a dominant negative isoform of the CRE binding protein to thymocytes of transgenic mice results in a profound proliferative defect characterized by a markedly decreased IL-2 production.

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