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Immunology

Differential expression of four members of the POU family of proteins in activated and phorbol 12-myristate 13-acetate-treated Jurkat T cells

(interleukin 2)

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ABSTRACT **The POU family of proteins binds specifically to octamer DNA motifs present in the promoters of several genes and regulates their expression. We identified the presence of four members of the POU family of proteins, Oct-1, Oct-2, Oct-T1, and Oct-T2, in the human T-cell line Jurkat. To obtain insight into the physiological role played by these proteins in T cells, we studied the time course of expression of these genes in resting, activated, and phorbol 12-myristate 13-acetate (PMA)-treated cells. The expression of the gene encoding Oct-1 (now assigned the name *OTF1* for octamer-binding transcription factor 1) remained unchanged and the levels of Oct-T2 mRNA decreased with increasing time of incubation to undetectable amounts in all three states of T-cell growth. The levels of Oct-2 mRNA and protein were increased in activated cells, were increased to a lesser extent in the PMA-treated cells, and were undetectable in resting cells. The levels of the Oct-T1 transcripts increased dramatically in PMA-treated cells but not in resting or activated cells. Sequence analysis of the Oct-T1 cDNA showed an open reading frame coding for a POU domain-containing protein of 42.7 kDa. Transient transfection of the gene encoding Oct-T1 decreased the activity of the interleukin 2 gene promoter in activated Jurkat cells. Further, there is evidence for an additional octamer-binding protein, Oct-T3, in Jurkat T cells.**

Activation of T lymphocytes requires two distinct stimuli. The first stimulus is provided through the interaction of the T-cell receptor complex with a specific antigen presented on the surface of an antigen-presenting cell (APC). The second stimulus is provided by the interaction of monomorphic receptors on T cells, such as CD28, with their ligands on the APCs (1).

Upon activation of T lymphocytes, the gene encoding interleukin 2 (IL-2) is one of the early genes to be induced. The IL-2 gene promoter, extending from nucleotide 52 to nucleotide 326 upstream of the transcription initiation site, contains recognition sites for several DNA-binding factors and functions as a T-cell-specific enhancer (2, 3). Two octamer-binding sites centered around positions −74 (ATGTAAAACA; proximal) and −251 (ATGCAATTAA; distal) are present in the IL-2 promoter. Deletion of the proximal or the distal binding site results in a 95% and 59% reduction, respectively, in IL-2 promoter activity (4), suggesting a major role for octamer-binding transcription factors in the expression of the IL-2 gene.

The POU family of transcription factors bind to octamer DNA elements. They have a conserved sequence motif consisting of an amino-terminal POU-specific domain and a carboxyl-terminal homeo domain linked through a stretch of variable amino acids (5). Two members of this family—Oct-1, a ubiquitous transcription factor (6), and Oct-2, a factor predominantly expressed in B-cells (7)—have been shown to bind *in vitro* to the octamer sites of the IL-2 promoter (8). Oct-1 has been the only detectable octamer-binding protein in Jurkat T cells (1, 8, 9).

We report the expression of four different members of the POU family of proteins—Oct-1, Oct-2, Oct-T1,* and Oct-T2*—in the human T-cell line Jurkat (10). We studied their expression in resting T cells (untreated) and in cells treated with either phorbol 12-myristate 13-acetate (PMA) alone, which leads to a state of nonresponsiveness (11), or with PMA and phytohemagglutinin (PHA), which leads to activation of cells (12). We confirmed that expression of the ubiquitous factor Oct-1 remains constant during the activation process of Jurkat cells. Contrary to earlier observations (7, 8), we found that the expression of the gene encoding Oct-2 (now designated *OTF2* for octamer-binding transcription factor 2) is induced in activated Jurkat cells. The gene encoding Oct-T1 is expressed at much higher levels in PMA-treated cells than in fully activated or resting Jurkat cells. The Oct-T1 cDNA has an open reading frame encoding a protein of 42.7 kDa, a member of the POU family of proteins. In cotransfection experiments, the Oct-T1 protein represses the IL-2 promoter activity in activated Jurkat cells. Expression of the fourth POU domain-containing factor, Oct-T2, is induced at low levels upon serum starvation of Jurkat cells and is repressed by serum replenishment. Furthermore, we present evidence for the presence of a distinct octamer-binding factor, Oct-T3, in T cells.

MATERIALS AND METHODS

Materials. Anti-Oct-1 antibodies were kindly provided by Winship Herr (Cold Spring Harbor Laboratory); anti-Oct-2 antibodies, by Robert G. Roeder (Rockefeller University); Jurkat cells, by Paula Kavathas (Yale University); pRSVCAT, by Nancy Ruddle (Yale University); and IL-2CAT constructs, where CAT is the gene for chloramphenicol acetyltransferase, by Gerald Crabtree (Stanford University). Polyclonal anti-Oct-T1 antibodies were raised against the peptide Gly-Ala-Gln-Arg-Glu-Lys-Met-Asn-Lys-Pro-Glu-Leu-Phe-Asn-Gly by Research Genetics (Huntsville, AL).

Stimulation of Jurkat Cells. Jurkat cells were grown in RPMI 1640 medium containing 10% (vol/vol) fetal bovine serum. Prior to stimulation, the cells were serum-starved in RPMI 1640 medium for 24 hr and replenished with 10% fetal bovine serum at a density of 0.2×10^6 cells per ml. After 3

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; IL-2, interleukin 2; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility-shift assay; SV40, simian virus 40.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L20433 for Oct-T1 and L20434 for Oct-T2).

hr, either 50 ng of PMA alone or in combination with 2 μg of PHA were added per ml. Cells were harvested at different time intervals, washed with phosphate-buffered saline, and processed for the preparation of RNA or the nuclear protein extracts.

Ribonuclease Protection Assay. To generate ^{32}P-labeled antisense RNA probes, DNA fragments (obtained by PCR) corresponding to different genes were cloned in the plasmid pBluescript (Stratagene), linearized, and transcribed with either phage T3 or T7 RNA polymerase. A typical transcription reaction contained 10 μM of nonradioactive CTP and 50 μCi (1 μCi = 37 kBq) of [α-^{32}P]CTP (3000 Ci/mmol). Low-specific-activity RNA probes for γ-actin and β_2-microglobulin were made in the presence of 500 μM nonradioactive CTP. The ribonuclease protection assay was done as described by Zinn *et al.* (13).

DNA Transfections and CAT Assays. Transient DNA transfections and the CAT enzyme assays were done as described by Banerji *et al.* (9).

Electrophoretic Mobility-Shift Assay (EMSA). EMSA was done as described (14) with a few modifications. A typical binding reaction contained 1 μg of nuclear protein extract, 10 fmol of the ^{32}P-labeled oligonucleotide probe, 2 μg of poly(dI-dC), 1.5 μg of bovine serum albumin (BSA), 10% (vol/vol) glycerol, 60 mM KCl, and 50 mM Hepes buffer (pH 7.0). Incubation was done at 30°C for 20 min. The DNA–protein complexes were separated on a 4% polyacrylamide gel in 0.25× TBE buffer (1× TBE = 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). Nuclear protein extracts were prepared by the method of Schreiber *et al.* (15). Double-stranded oligonucleotides 5′-CATGCAATTAAC-3′ and 5′-TATGTAAAACAT-3′ corresponding to the distal and the proximal octamer-binding sites of the IL-2 promoter were used. A double-stranded DNA (39-mer) having the immunoglobulin octamer site ATGCAAAT was a gift from Robert Roeder (Rockefeller University).

cDNA Library Construction. Poly(A)$^+$ RNA prepared from Jurkat cells treated with PMA for 36 hr was primed with oligo(dT), and the cDNA synthesis was carried out as described (16). Oligonucleotide linkers 5′-GCTTGAATTC-CAAGC-3′ were ligated to the cDNA molecules, digested with *Eco*RI and *Hin*dIII and ligated to the *Eco*RI/*Hin*dIII-cut, dephosphorylated λpSH4K vector (17).

PCR. PCR amplification was done by using as template the cDNA prepared from the poly(A)$^+$ RNA from the Jurkat cells that had been treated for 36 hr with PMA. Degenerate oligonucleotides representing all possible codons for the conserved amino acids [Phe-Lys-(Val or Gln)-Arg-Arg-Ile-Lys-Leu-Gly and Trp-Phe-Cys-Asn-(Arg or Gln)-Arg-Gln-(Arg or Lys)-(Glu or Gln)] from either end of the POU domain were used as the primers (18). *Eco*RI sites were introduced at the 5′ ends of each oligonucleotide.

DNA Sequencing. Deletion clones of the Oct-T1 cDNA were sequenced by the dideoxy chain-termination method (19).

RESULTS

Identification of Four POU Domain-Encoding Transcripts in Jurkat Cells. To identify members of the POU family of transcription factors expressed in Jurkat cells, degenerate primers corresponding to the conserved amino acids from either end of the POU domain were used in a PCR reaction. Amplification of cDNA made from the poly(A)$^+$ RNA of Jurkat cells resulted in DNA fragments of about 420 bp. Cloning of the *Eco*RI-digested PCR products and sequence analysis of 60 clones identified the POU domains of the products of the ubiquitous Oct-1 gene, the B-cell-enriched Oct-2 gene, and two other genes, Oct-T1 and Oct-T2. The nucleotide sequence of the PCR product corresponding to the Oct-T1 gene is 87% homologous to that of the Oct-T2 gene, and the products of both genes belong to class IV of the POU family of proteins (18).

Characterization of Oct-T1 cDNA Clones. To obtain Oct-T1 cDNA clones, we used the Oct-T1 DNA fragment to screen an oligo(dT)-primed cDNA library made from the poly(A)$^+$ RNA of Jurkat cells that had been treated for 36 hr with PMA. A 3.8-kilobase (kb) DNA sequence was assembled from three different clones. Oct-T1 cDNA has an open reading frame starting from nucleotide 1 and ending at nucleotide 1497. The G+C content of the coding region is 72% and that of the 3′ untranslated region is 47%. The A+T-rich sequence ATTATTTATTTAATTAT present at nucleotide residues 3302–3318 has a dimer of the pentanucleotide ATTTA that is implicated in the posttranscriptional down-regulation of mRNA levels (20).

The first in-frame ATG codon at position 235 of the Oct-T1 cDNA sequence is part of an optimal translation initiation sequence, GCCACCATGA (21). We tentatively fixed this ATG to be the initiation codon of the Oct-T1 protein. Based on this assignment, the deduced Oct-T1 protein is 420 amino acids long with an estimated size of 42.7 kDa. The POU domain is present at the extreme carboxyl terminus of the protein, and the amino-terminal region is extremely rich in glycine and alanine residues. In agreement with this assignment of the open reading frame, antibodies raised against an Oct-T1 peptide (deduced from the cDNA sequence) detected an inducible 40-kDa protein from the nuclear extracts of Jurkat cells treated for 43 hr and 72 hr with PMA (unpublished data).

Northern analysis of poly(A)$^+$ RNA from activated Jurkat cells showed the size of the Oct-T1 transcript to be 4.4 kilobases (Fig. 1). Based on this size, a total of 600 nucleotides of sequence is missing from the ends of the Oct-T1 cDNA. When one considers that the poly(A) tail is 150–200 nucleotides long in most eukaryotic mRNAs and the polyadenylylation signal, AATAAA, is not present within 30 nucleotides of the 3′ end of the Oct-T1 cDNA sequence obtained, it is possible that most of the missing sequence is from the 3′ end. Determination of the exact position of the 5′ end of Oct-T1 mRNA by the primer-extension assay was hampered by the high G+C content of the 5′ end. A primer complementary to nucleotides 250–267 resulted in a weak

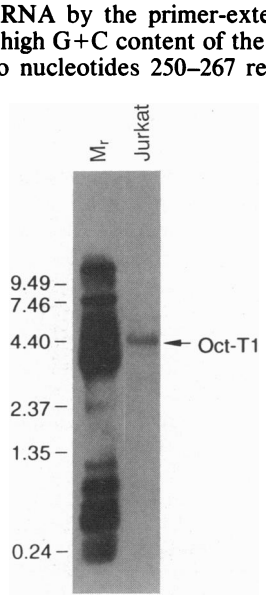

FIG. 1. Oct-T1 mRNA in Jurkat cells. Five micrograms of poly(A)$^+$ RNA from Jurkat cells treated with PMA (50 ng/ml) for 36 hr was analyzed by Northern blotting (22). Cells were grown in RPMI 1640 medium containing 10% fetal bovine serum to a density of 0.2 × 10^6 cells per ml before the addition of PMA. RNA was prepared as described earlier (23). The blot was hybridized with ^{32}P-labeled Oct-T1 cDNA and exposed to x-ray film for autoradiography. Sizes of the RNA molecular weight markers (BRL) are indicated in kilobases on the left.

extension product about 265 bases long, as expected from the sequence of Oct-T1 cDNA (unpublished data).

IL-2 Gene Induction in Synchronized Jurkat Cells. Total RNA isolated at different times from either the untreated or stimulated Jurkat cells was analyzed by the RNase protection assay (13). A 180-nucleotide-long RNase-protected fragment corresponding to the IL-2 mRNA was first detected after 1 hr, reached a maximum level after 4 hr, and then declined to undetectable levels after 62 hr of PMA/PHA treatment (Fig. 2*B*). IL-2 mRNA was not detected in either the untreated Jurkat cells or cells treated only with PMA, consistent with earlier reports (1).

Oct-1 Gene Expression Remains Constant. Oct-1 transcripts were present at a constant level in untreated Jurkat cells or when treated with either PMA or with PMA/PHA (Fig. 2 *A–C*).

Oct-2 Gene Expression Is Induced in PMA/PHA- and PMA-Treated Cells. Expression of the Oct-2 gene varied significantly under different treatment conditions. In Jurkat cells maintained in serum-depleted medium for 24 hr, Oct-2 transcripts were present at a very low level. Upon serum replenishment, the level of Oct-2 transcripts gradually declined to undetectable levels over 51 hr (Fig. 2*A*). In the PMA/PHA-treated Jurkat cells, Oct-2 transcripts increased 8-fold after 6 hr and then declined to the baseline level after 62 hr (Fig. 2*B*). When Jurkat cells were treated with PMA alone, a smaller increase in the level of Oct-2 transcripts was seen compared with the PMA/PHA-treated cells. The transcripts increased after 2 hr, reached a peak of 4-fold induction after 4 hr, and then declined over 48 hr (Fig. 2*C*).

Oct-T1 Gene Expression Is Induced by PMA Treatment. In PMA-treated cells, the level of Oct-T1 mRNA began to increase after 8 hr, peaked at a 40-fold increase after 36 hr, and remained at high levels for at least 62 hr (Fig. 2*C*). In PMA/PHA-treated cells, the Oct-T1 mRNA level did not change significantly up to 48 hr and then increased 2-fold at 62 hr (Fig. 2*B*). In resting cells, the level of Oct-T1 mRNA decreased marginally over a period of 51 hr (Fig. 2*A*).

Oct-T2 Transcripts Are Present at Low Levels. Oct-T2 mRNA was present at low levels in untreated and in PMA/PHA-treated cells and declined to undetectable levels after 36 hr (Fig. 2 *A* and *B*). However, it remained at low but detectable levels for at least up to 62 hr of PMA treatment (Fig. 2*C*).

Oct-T1 Transcripts Decline in Activated Peripheral Blood T Cells. Total RNA isolated from activated human peripheral blood T cells was analyzed for the expression of Oct-1, Oct-T1, and IL-2 genes (Fig. 3). Expression of the Oct-1 gene did not change in resting or activated T cells. Oct-T1 transcripts were present in resting cells but not in cells activated with diacylglycerol and ionomycin for 4 hr, a time at which the level of the IL-2 transcripts had increased 100-fold.

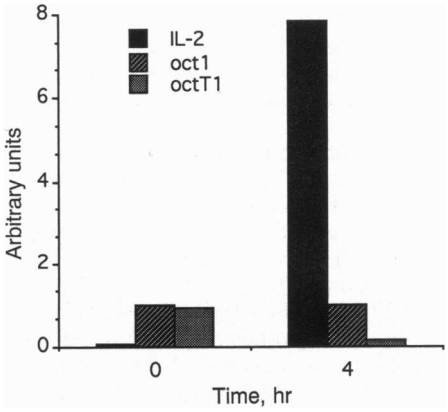

FIG. 3. An inverse correlation exists between the mRNA levels of IL-2 and Oct-T1 in activated human peripheral T cells. Peripheral blood T cells were prepared by the method described earlier (24). Total RNA was prepared from resting T cells and from those treated with diacyl glycerol (10 μg/ml) and ionomycin (1 μM) for 4 hr. RNA was analyzed by the RNase protection assay as described under Fig. 2.

Expression of Octamer Motif-Binding Proteins in Jurkat Cells. We next analyzed the presence of octamer motif-binding proteins in Jurkat cells. Nuclear protein extracts prepared from resting, PMA/PHA-, and PMA-treated cells were analyzed by EMSA using a radiolabeled oligonucleotide (ATGCAAAT) (Fig. 4). The Oct-1 protein level remained unchanged in resting, PMA/PHA-, or PMA-treated Jurkat cells. A band corresponding to Oct-2 protein gradually increased in PMA/PHA-treated Jurkat cells (lanes JTP6, JTP8, and JTP50), which supershifted when polyclonal anti-Oct-2 antibody was added to the reaction mixture (lane A2/JTP50). After 50 hr of treatment with PMA/PHA, the level of Oct-2 protein in Jurkat cells reached 50% of the level that was seen in the lymphoblastoid B-cell line JY.

In addition to the bands corresponding to Oct-1 and Oct-2 proteins, another band (Oct-T3) was observed from the nuclear extracts of Jurkat cells but not from HeLa or JY cells (Fig. 4). The level of Oct-T3 gradually decreased in Jurkat cells treated with either PMA or PMA/PHA (lanes JT42, JTP8, and JTP50). A qualitatively similar pattern of binding

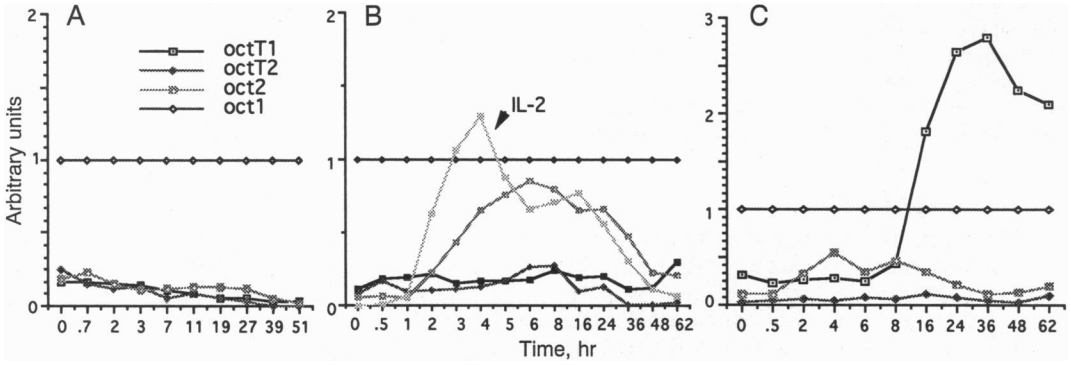

FIG. 2. Expression of IL-2, Oct-1, Oct-2, Oct-T1, and Oct-T2 genes in Jurkat cells. Graphs corresponding to untreated (*A*), PMA/PHA-treated (*B*), and PMA-treated (*C*) cells are shown. The RNase-protected fragments corresponding to IL-2, Oct-1, Oct-2, Oct-T1, Oct-T2, γ-actin, and β_2-microglobulin mRNAs were separated on either 5% or 7.5% polyacrylamide/7M urea gels and quantitated by using a phosphor imager (Molecular Dynamics). A low-specific-activity antisense probe of γ-actin was hybridized with all of the RNA samples isolated from untreated and activated cells; a β_2-microglobulin antisense probe was hybridized with the RNA samples isolated from PMA-treated cells to normalize the losses that occur during the handling of different samples. We divided the normalized values obtained at each time point by the normalized values of Oct-1, which remained constant throughout. The ratios obtained for each gene when divided by the Oct-1 values were plotted against the time points indicated on the graph.

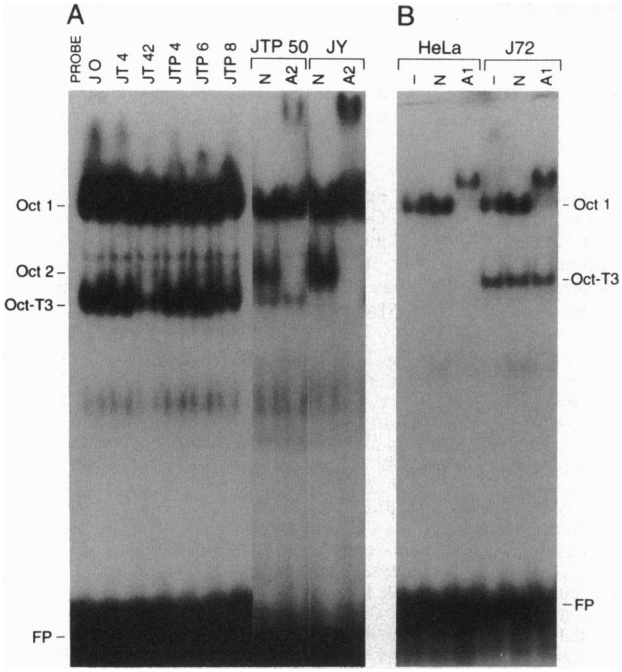

FIG. 4. Expression of octamer-binding proteins in Jurkat cells. EMSA of nuclear extracts prepared from Jurkat (J), JY (B lymphoblastoid cells), and HeLa cells using the radiolabeled double-stranded oligonucleotide (ATGCAAAT) probe was performed. Extracts were incubated with normal rabbit serum or Dulbecco's modified Eagle's medium (lanes N) or rabbit anti-Oct-2 antibodies (lanes A2) or monoclonal anti-Oct-1 antibodies (lanes A1). Nuclear extracts from untreated Jurkat cells (lane J0), PMA-treated Jurkat cells (lanes JT4 and 42) and PMA/PHA-treated cells (lanes JTP 4, 6, 8, and 50). The numbers indicate the time of treatment in hr. The positions of the Oct-1, Oct-2, and Oct-T3 bands and of free probe (FP) are shown. The autoradiograms in *A* and *B* were exposed for different time periods.

proteins was observed with the proximal (ATGTAAAACA) octamer-binding motif present in the IL-2 promoter.

Oct-T1 Expression Decreases the IL-2 Gene Transcription in Activated Jurkat Cells. We assessed the ability of Oct-T1 protein to modulate the expression of the reporter CAT gene driven by the IL-2 promoter in activated Jurkat cells. On cotransfecting the Oct-T1 cDNA under the control of the simian virus 40 (SV40) promoter reduced the activity of the IL-2 promoter (from position −319 to +42) by 90% in activated Jurkat cells (Fig. 5). The activity of the Rous sarcoma virus promoter remained unchanged in the presence of Oct-T1 protein.

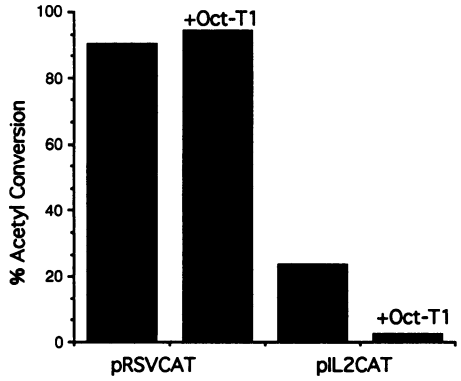

FIG. 5. Repression of the IL-2 promoter activity by transfection of an Oct-T1-expressing plasmid. Jurkat cells were transfected with CAT vectors driven by either the Rous sarcoma virus promoter (pRSVCAT) or the IL-2 promoter (pIL2CAT) in the presence or absence of the Oct-T1 expression vector driven by the SV40 promoter. Twenty-four hours after transfection, the cells were stimulated with PMA (25 ng/ml) and ionomycin (1 μM) for 18 hr. Extracts were made and assayed for CAT activity for 16 hr. Results from the chromatography were quantitated by using a phosphor imager (Molecular Dynamics). Results are expressed as the percentage of acetylated material. Data are representative of three separate experiments.

DISCUSSION

Characterization of POU Proteins in Jurkat Cells. We identified four members of the POU family of transcription factors by PCR amplification of cDNA from Jurkat cells. Two of these have been well studied: Oct-1, a ubiquitously occurring transcription factor (6), and Oct-2, a factor predominantly found in B cells (7). The products of two other POU domain-encoding clones were named Oct-T1 and Oct-T2 because of their isolation from Jurkat T cells. The amino acid sequence of the POU domains of Oct-T1 and Oct-T2 were identical to that of Brn-3 (18) and Brn-3B (25), respectively. Both Brn-3 and Brn-3B were identified as PCR-amplified products from murine nervous tissues, and no information about the amino acid sequence outside their POU domains is available. The POU domains of Oct-T1 and Oct-T2 differed at seven amino acid positions, one of which (valine to isoleucine) is in the conserved POU–homeo region. The POU family of factors are known to interact with one another through their POU–homeo domain (26, 27), and a change from valine to isoleucine may cause Oct-T1 and Oct-T2 proteins to have different protein–protein interacting properties. The Oct-T1 amino acid sequence is 87% homologous to the human RDC-1 protein from nervous tissue (28), but the first 45 amino acids show no homology. Oct-T1 and RDC-1 differ at three amino acid residues in the highly conserved POU–homeo domain, and may be the products of differential splicing of closely related genes.

Possible Role of POU Proteins and Oct-T3 in IL-2 Gene Regulation. To obtain insight into the role played by the four POU domain-containing transcription factors Oct-1, Oct-2, Oct-T1, and Oct-T2 and an octamer-binding protein, Oct-T3, in the physiology of T cells, we studied the pattern of their expression in Jurkat T cells under different conditions of stimulation and growth. The addition of PHA and PMA to the growth medium of Jurkat cells mimics the complex process of T-cell activation via the antigen receptor complex and the protein kinase C pathway (1). Exposure of T cells to PMA alone in the absence of any antigenic stimulus leads to an initial increase in protein kinase C activity and then to its down-regulation (29). We observed that the level of expression of Oct-1 transcripts did not change either in untreated Jurkat cells or when they were treated with either PMA/PHA or PMA alone. Though the levels of Oct-1 remained constant, it has been suggested that Oct-1 plays a significant role in the activation of the IL-2 promoter by binding to a 40-kDa inducible protein, a member of the Jun family of proteins (30, 31). Alternatively, Oct-T3 protein is also a candidate activator of IL-2 gene expression because it binds to the proximal octamer-binding site of the IL-2 promoter and its binding activity decreases when the level of IL-2 transcripts begins to fall in activated Jurkat cells. Ullman *et al.* (30) observed a band of similar mobility from Jurkat cells that did not react with antibodies raised against the amino-terminal peptides (amino acid residues 1–32 and 69–93) of the Oct-1 protein. When we used anti-Oct-1 antibodies specific for the linker region between the POU and homeo domains of Oct-1 or used anti-Oct-2 antibodies specific for the amino-terminal region of Oct-2 protein, the Oct-T3 band did not shift in EMSA. Further characterization of the Oct-T3 protein is required to establish its identity and role in the IL-2 gene regulation.

Oct-2 and Increased Responsiveness in T Cells. The levels of Oct-2 mRNAs varied significantly under the treatment conditions we used. The decrease in Oct-2 transcripts with serum replenishment could account for earlier findings in which neither Oct-2 transcript nor Oct-2 protein was detected in Jurkat cells (7, 8), although Oct-2 has been found in some T-cell lines such as murine EL4 (7). The detection of Oct-2 in Jurkat cells suggests that its regulated expression in T cells may be a general phenomenon. Exposure of the Jurkat cells to PMA/PHA resulted in an increase in the level of Oct-2 transcripts that did not precede activation of the IL-2 gene; hence, its role in the initial phase of IL-2 expression in naive T cells is not clear. Similar kinetics of Oct-2 induction were seen after the onset of IL-2 gene expression in mouse AE7 cells (32). The level of Oct-2 mRNA is maximal after 6 hr of PMA/PHA treatment, whereas the maximum DNA binding activity is seen after 50 hr of treatment, suggesting that the Oct-2 protein is stable and can accumulate in activated T cells or the Oct-2 protein is modified to bind the octamer DNA motif more efficiently. Kamps *et al.* (8) found that Oct-2 protein binds to the IL-2 promoter *in vitro* and potentiates the expression of the IL-2 promoter in Jurkat cells treated with PMA/PHA. Thus, the late accumulation of the Oct-2 DNA-binding activity in activated Jurkat cells could play a role in increasing the responsiveness of T cells to subsequent exposure of antigen.

Oct-T1, a Mediator of IL-2 Gene Repression? Treatment of T cells with PMA alone (11) or the occupancy of the antigen receptor alone (33) leads to a state of tolerance or anergy in that these cells do not produce IL-2 after subsequent exposure to a stimulus that would have been effective in naive cells. However, the cells respond to the addition of IL-2 protein by activation and proliferation (11, 34), suggesting that repression of the IL-2 gene could be one of the reasons for the induction of T-cell anergy or tolerance. Restimulated anergized cells express the transcription factors associated with the expression of IL-2 gene but still do not express IL-2. This suggests, among other possibilities, the existence of a single-stimulus-induced repressor of the IL-2 gene (34, 35). Expression of Oct-T1 dramatically increases in Jurkat cells treated with PMA alone and may play a role in the repression of IL-2 gene expression. In support of this hypothesis, transfection into activated Jurkat cells of the Oct-T1 cDNA under the control of the SV40 promoter severely inhibited IL-2 expression. Also, during the stimulation of human peripheral blood T cells, we observed an inverse correlation between the levels of Oct-T1 and IL-2 transcripts. In contrast, the expression of Nil2A (36), a zinc finger protein, characterized as a repressor of the IL-2 gene did not change significantly in Jurkat cells treated with PMA (unpublished data).

The bacterially expressed Oct-T1 protein binds with greater affinity to the distal than the proximal octamer binding site present in the IL-2 promoter (unpublished data). However, we did not find any PMA-inducible protein band in our gel retardation assays despite the detection of an inducible 40-kDa protein band when using Oct-T1-specific antibodies on an immunoblot. This could be because of the low affinity of the Oct-T1 protein towards the octamer sites present in the IL-2 promoter. Thus, in the absence of direct binding, one possible explanation for the inhibition of the IL-2 promoter activity in cotransfection experiments is that Oct-T1 is indirectly repressing the IL-2 gene by interacting with an activator at the protein level.

More generally, the relationship between the induction of the Oct-T1 gene and the induction of tolerance or anergy in T cells needs further investigation. Also, the differential expression of at least four different POU proteins that may have the ability to interact with each other (26) and the additional octamer binding activity could play a potentially important role in the sequential expression of other responsive genes.

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