Low stringency screening of a Jurkat cDNA library with a rat brain K⁺ channel (RCK₁) probe has resulted in the isolation of HLK₃, a voltage-gated K⁺ channel. In *Xenopus* oocytes, the HLK₃ clone directs the expression of a rapidly activating transient outward K⁺ current similar to the type n K⁺ current recorded in Jurkat T cells. The HLK₃ gene is located on the short arm of human chromosome 1 (p13.3). Polymerase chain reaction was used to clone HIsK from Jurkat cDNA. The HIsK clone shares the same sequence with a previously described genomic clone (Murai, T., Kazikuka, A., Takumi, T., Ohkubo, H., and Nakanishi, S. (1989) *Biochem. Biophys. Res. Commun.* 161, 176-181). In *Xenopus* oocytes, it encodes a slowly activating, noninactivating K⁺ channel which cannot be recorded in Jurkat cells by conventional patch-clamp techniques.

Transcripts of both clones are present at a similar level before and after activation of purified human T lymphocytes and Jurkat cells, reflecting a constitutive expression of K⁺ channel messages. This finding is in good agreement with the electrophysiological results for type n K⁺ current density on the same cells. HIsK current is very sensitive to the scorpion toxin charybdotoxin (IC₅₀ = 0.8 nM). HIsK current is totally insensitive to this toxin but is blocked by the antiarrhythmic clofilium (IC₅₀ = 80 μM). While charybdotoxin has no effect on interleukin 2 mRNA induction, clofilium potently inhibits interleukin 2 mRNA expression upon mitogen-induced T cell activation. It is concluded that the HIsK channel is not an important component of the T cell mitogenic response. Other targets for K⁺ channel blockers, such as the HIsK protein, could be involved in the activation process.

Activation of T lymphocytes is a complex process requiring recognition of antigen by the T cell antigen-receptor complex (Ti-CD₃), as well as accessory signals generated by antigen presenting cells (1). Triggering T cell antigen receptors stimulates hydrolysis of phosphoinositides by phospholipase C within seconds to minutes (2). This in turn results in the production of diacylglycerol which activates protein kinase C (3), and of inositol triphosphate which releases Ca²⁺ from intracellular stores (4). These early events promote G₀ to G₁ transition, whereby the cells are made competent to proliferate by the expression of the genes encoding the T cell growth factor interleukin 2 (IL₂) and its receptor (1). The subsequent interaction of IL₂ with high affinity surface IL₂ receptors provides the signal that drives lymphocyte proliferation (1, 5). Along with these complex signal transduction pathways, changes in membrane ionic fluxes, including K⁺ fluxes, have been correlated with T lymphocyte activation by mitogens (6-11).

Voltage-dependent K⁺ channels resembling the delayed rectifier of nerve and muscle cells are found to be predominant in murine and human T lymphocytes. In mouse T cells at least three types of such channels are described and are called n, n', and l while in human T cells the type n is the only one present (10). Several lines of evidence suggest that voltage-gated K⁺ channels are involved in T cell activation and proliferation. Expression of type n K⁺ channels is increased following mitogen-induced activation of human and murine T cells (12, 13). K⁺ channel-dependent volume regulation correlates with K⁺ conductance (10, 14, 15). A role for K⁺ channels in target cell lysis by cytotoxic T lymphocytes and natural killer cells has been also suggested (16). In addition, K⁺ channel blockers inhibit cellular events associated with T cell activation, including secretion of IL₂ (17, 18) and mitogen-induced cell proliferation (8, 13, 17). Although the correlation between K⁺ channel blockade and inhibition of mitogenesis in very suggestive, the existence of a causal relationship between these events remains to be established.

Recent advances in molecular biology have allowed the cloning of several families of voltage-dependent K⁺ channels in mammals. From these studies the structure of two very different families emerged. The first class of mammalian K⁺ channel genes is homologous to *Drosophila* K⁺ channel genes called Shaker, Shab, and Shal (19-22). When expressed in Xenopus oocytes, these clones give rise to functional K⁺ channels with kinetic properties that vary from rapidly inactivating to slow or noninactivating currents (23). Interestingly, two recently characterized mouse and rat genomic clones MK₃ and RGK₃, respectively, were found to direct in

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The abbreviations used are: IL₂, interleukin 2; 4-AP, 4-aminopyridine; ChTX, charybdotoxin; PCR, polymerase chain reaction; PMA, phorbol 12-myristate 13-acetate; TMA, tetramethylammonium; kb, kilobase; SDS, sodium dodecyl sulfate; HPFS, N-2-hydroxyethylpipperazine-N'-2-ethanesulfonic acid; EGTA, [ethylenebis(oxyethyl)enitrilo]tetraacetic acid.

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††† Recipient of an Association pour la Recherche sur le Cancer fellowship.

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Xenopus oocytes. The expression of K⁺ currents whose biophysical and pharmacological properties are similar to those of voltage-gated type n K⁺ channels in T cells (24, 25). The second class of K⁺ channel structure only includes at the present time one single representative called IsK. This K⁺ channel protein is mainly found in epithelial cells (26), but it is also present in uterus and neonatal heart (27–29). When expressed in Xenopus oocyte, this K⁺ channel closely resembles the noninactivating delayed-rectifier K⁺ current recorded in cardiac cells (29, 30). Up till now, the IsK channel has only been expressed in Xenopus oocytes and the fact that it represents by itself a channel protein has been questioned. A recent report using site-directed mutagenesis has shown that mutation of the IsK gene can alter both ion selectivity and open channel blocks and has led the authors to conclude in favor of the pore nature of the protein (31).

In this report, we describe the cloning and expression of two voltage-gated K⁺ channels HLK3 and HIsK from human T lymphocytes. Steady-state mRNAs of both channels are following mitogen-induced T cell activation. The relative contribution of the two channels in the activation process is investigated by studying the effect of K⁺ channel blockers on HLK mRNA expression and T cell proliferation. In addition, the HLK gene mapping is performed.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—T cells were purified from peripheral blood cells from healthy volunteers. Human peripheral blood mononuclear cells were obtained by centrifugation over Ficoll-Hypaque gradients (Sigma). After 2 h of adherence to 150-mm plastic dishes (Integrid Falcon) at 37 °C in RPMI medium, nonadherent cells were collected and incubated for 30 min with L-leucine methyl ester (2.5 mM) (Sigma). Residual monocytes, B cells, and T cells isolated by this method were cultured by two cycles of treatment with nonototic rabbit complement (Beltrum, Federal Republic of Germany) in the presence of 5 μg/ml of anti-HLA class II monoclonal antibodies (G 157) as previously described by Hu et al. (32). The human leuemic T cell line Jurkat was cultured in RPMI 1640 supplemented with 5% fetal calf serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 2 mM l-glutamine. The human IM9 lymphocytes were cultured under the same conditions but the medium was supplemented with 10% fetal calf serum.

**RNA Isolation and Analysis**—Total cellular RNA was extracted (33) and poly(A)+ RNA isolated on oligo(dT)-cellulose. For Northern blot analysis, poly(A)+ RNA were resolved by electrophoresis through 5% acrylamide-8 M urea gels at 20°C in the presence of 1× SSC, 0.1% SDS, transferred to nitrocellulose filters, and hybridized with [32P]dCTP-labeled probes. Hybridization was performed at 5°C overnight in 50% formamide, 5× SSC, 50 μg/ml salmon sperm DNA, and 5× Denhardt's solution. Filters were washed stepwise to a final stringency of 0.1× SSC, 0.1% SDS at 65°C.

**Isolation of cDNA Clones**—An oligo(dT)-primed cDNA library, derived from poly(A)+ RNA isolated from peripheral blood lymphocytes, was constructed in the phage ZAP II (Stratagene). Recombinant phages were screened by plaque hybridization with the rat RCK DNA probe which was obtained by PCR using primer sequences corresponding to the previously described rat RCK channel (35). Plaque filters were hybridized in 50% formamide, 5× SSC, 4× 10-6 M solution, and washed according to the ZAP II protocol. DNA probes were radioactively labeled by nick-translation to a specific activity of 1.4 × 109 dpm μg⁻¹. The radiolabeled probe was hybridized to oocyte filters at the same stringency. Hybridization was monitored by autoradiography.

**DNA Sequence Determination**—A 37112 cDNA clone was used to select the genomic clone. One positive genomic clone was selected and subjected to nucleotide sequence analysis. Sequence was determined using T- RNA polymerase and the HIsK cDNA clone linearized by XhoI.

The HLK3 Gene Mapping—In situ hybridization was carried out on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 6-Bromo-deoxyuridine was used for the final 72 h incubation (0.2 μg/ml of medium). Filters were posthybridized with chromosomal banding of good quality. The HLK3 clone containing an insert of 1739 base pairs in Bluestripes was tritium labeled by nick-translation to a specific activity of 1.4 × 108 dpm μg⁻¹. The radiolabeled probe was hybridized to metaphase spreads at a final concentration of 25 μg/ml of hybridization solution at 37°C. After hybridization, the filters were washed stepwise to a final concentration of 0.1× SSC, 0.1% SDS, and 0.1% Triton X-100. Autoradiography was carried out for 3 days in the presence of 5% formamide, 0.5 μCi/well) 24 h prior lysis of the cells by sodium dodecyl sulfate and trypsin.

**Electrophysiological Measurements in Xenopus Oocytes**—Xenopus oocytes were purchased from CRBM (Montpellier, France). Pieces of the ovary were surgically removed and individual oocytes were dissected in a modified Barth's solution: 88 mM NaCl, 1 mM KCl, 2.4 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 0.41 mM MgSO₄, 10 mM HEPES (pH 7.4) with NaOH. To discard follicular cells, Stage V and VI oocytes were treated for 2 h with collagenase (1 mg/ml, Boehringer) in Barth's medium. After RNA injection (50 nl), oocytes were kept for 2–7 days in Barth's medium supplemented with 100 μM of penicillin and 100 μg/ml of streptomycin. In a 0.5 μl perfusion chamber, a single oocyte was impaled with two standard glass microelectrodes (0.5–2.0 mho) filled with 3 M KCl and maintained under voltage clamp using a Dagan 8500 amplifier. To discard follicular cells, Stage V and VI oocytes were treated for 2 h with collagenase (1 mg/ml, Boehringer) in Barth's medium. After RNA injection (50 nl), oocytes were kept for 2–7 days in Barth's medium supplemented with 100 μM of penicillin and 100 μg/ml of streptomycin.

**Proliferation Assays**—Purified human T lymphocytes were cultured for 3 days in RPMI 1640 supplemented with 10% fetal calf serum and 2 μM L-glutamine. Cultures were grown at 107 cells/0.2 ml in 96-well flat bottom tissue culture plates maintained at 37°C in humidified air containing 5% CO₂. Proliferation was initiated by adding 10 ng/ml of phorbol 12-myristate 13-acetate (PMA) and 0.1 μM A23187. K⁺ channel blockers were also added at the same time. DNA synthesis was determined from the incorporation of [3H]thymidine added to the cells (0.5 μCi/well) 24 h prior lysis of the cells by sodium dodecyl sulfate and trypsin.

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to record ionic currents. The external solution at pH 7.4 contained 140 mM tetramethylammonium (TMA) chloride, 1 mM CaCl₂, 2 mM MgCl₂, 5 mM KC1, 5 mM glucose, 10 mM HEPES/TMAOH. The pipette solution contained 140 mM K gluconate, 2 mM MgCl₂, 2 mM EGTA. This solution was buffered at pH 7.3 with 10 mM HEPES/KOH. Pipettes (2–5 megohm) were connected to the headstage of the patch-clamp amplifier (RK 300, Bio-Logic, France). The cell surrounding the medium was changed by a local perfusion system, a series of stainless tubes (100-μm inner diameter) connected to different test solutions and capped by a tapered glass capillary (200-μm tip inner diameter), followed at a constant rate of ∼0.2 ml/min in a 2.5-ml bath chamber. The fluid level was adjusted at a constant level by direct suction.

RESULTS

Isolation and Characterization of Two Human T Lymphocyte K⁺ Channel Clones—A human leukemic T cell line Jurkat cDNA library has been screened by hybridization under low stringency conditions with a 1.5-kb DNA fragment of the rat RCK, brain K⁺ channel (35). From 10⁶ recombinants, one positive clone was isolated and sequenced. The deduced open reading frame consisted of 337 amino acids, but was incomplete for the amino-terminal end of the derived protein. As we were not able to isolate another cDNA with which we could complete the open reading frame, we isolated the corresponding genomic clone from an EMBL3 human genomic library. A 2.2-kb Sall-KpnI restriction fragment, which hybridized with the amino-terminal end of the cDNA was selected for subcloning and sequencing. The genomic DNA sequence was co-linear with the cDNA sequence (out of 320 nucleotides). Accordingly, the sequence of the genomic clone fragment was aligned with the cDNA sequence in order to derive the complete open reading frame of the HLK3 clone (Fig. 1). The predicted product consists of a 523-amino acid polypeptide with a calculated molecular mass of 58,289. The nucleotide sequence surrounding the potential start codon agrees with the consensus translation initiation (40). HLK3 exhibits all the standard hallmarks of Shaker-related voltage-dependent K⁺ channels (19), including five hydrophobic segments (S₁, S₂, S₃, S₅, S₆) and one amphipathic segment (S₄) containing 7 positively charged amino acid residues at every third position (Figs. 1 and 2). The region connecting S₅ and S₆ segments which is intimately involved in the pore formation (41–43) is also well conserved. Based upon the topology previously proposed for voltage-gated K⁺ channels, the derived protein sequence contains one potential N-glycosylation site (Asn²²⁷) in the putative extracellular region connecting segments S₅ and S₆. A consensus site for cAMP-dependent phosphorylation is found in the carboxyl terminus portion of the channel sequence (Ser⁷⁰⁶). In the putative intracellular loop connecting segments S₅ and S₆, there is also a potential site for protein kinase C phosphorylation (Ser⁷⁴⁷). Fig. 2 shows that HLK₃ shares 70% amino acid sequence identity with the RCK, channel (35) which served as a probe for the cloning, and 97% identity with RCK₃ (23), also called RGK, by Douglass et al. (24). HLK₃ sequence is very similar to hPCN₃, a voltage-gated K⁺ channel, recently isolated from human insulinoma (44). HLK₃ and hPCN₃ differ by 9 out of 523 amino acids and display some different nucleotide sequences in 5' untranslated regions, suggesting that the two sequences may represent allelic variants of a single gene. HLK₃ cDNA probe binds to a single gene on genomic Southern blots under conditions of high stringency (not shown). Furthermore, restriction fragment analysis of the genomic clone demonstrates that the HLK₃ coding sequence is intronless.

Using a DNA fragment (1.74 kb) corresponding to a 1-kb coding sequence and 0.74-kb specific 3'-noncoding region, HLK₃ gene mapping was performed on human chromosomes by in situ hybridization (38). In the 100 metaphase cells examined after in situ hybridization, there were 411 silver grains associated with chromosomes and 91 of these (22.1%) were located on chromosome 1. The distribution of grains on this chromosome was not random since 81.3% of them (74/91) mapped to the [p11-p21] region of chromosome 1 short arm of the human chromosome. Using an in situ hybridization technique, the HLK₃ gene was mapped to the [p11-p21] region of chromosome 1.
putative channel is designated as HIsK in this study.

Functional Expression of HLKs and HIsK Channels in Xenopus Oocytes—The biophysical and pharmacological properties of K+ channels formed by HLK and HIsK proteins were characterized after injection of their respective cRNAs into Xenopus oocytes. HLK3 cRNA directed the expression of large voltage-activated K+ channels. Under voltage-clamp conditions, the K+ channel was activated at membrane potentials positive to ~50 mV. The calculated conductance was half-maximal at ~20 mV and the slope factor was 8.7 mV. The K+ channel was inactivated more than 80% at the end of a 3-s depolarizing pulse (Fig. 4, A and B). The inactivation kinetics could be fitted by a single exponential. The time constant of inactivation measured at +15 mV was 740 ± 22 ms (n = 25). This K+ current presented a use-dependent inactivation leading to a progressive decline of the peak current during repetitive stimulation at 0.2 Hz (data not shown). Raising the intracellular Ca2+ concentration by action of the Ca2+ ionophore A23187 (1 μM) resulted in a slight but consistent decrease of the K+ current amplitude (Fig. 4B). Table I and Fig. 4A show that the HLK3 current was blocked by various pharmacological agents including tetraethylammonium, 4-aminopyridine (4-AP), verapamil, the quaternary ammonium clofilium (45), and the scorpion toxin charybdotoxin (ChTX) (46). Clofilium and verapamil inhibition resulted in an acceleration of the inactivation phase as well as in a decrease of the peak current amplitude. HLK3 current was almost totally inhibited by 10 nM ChTX (Fig. 4A). The HLK3 channel shares all the biophysical and pharmacological properties of the type n K+ channel present in murine and human T lymphocytes.

Microinjection of HIsK cRNA led to the expression of a slowly activating K+ current which did not undergo inactivation. The calculated voltage for half-maximal activation was ~11 mV and the slope factor was 9 mV. Activation kinetics could be described by the sum of two exponentials. The two time constants of activation measured at +15 mV were 3 and 25 s. HIsK current was significantly increased in the presence of 1 μM A23187 (Fig. 4B).

FIG. 2. Amino acid sequence alignment of the cloned human lymphocyte K+ channels HLKs, RCK1, and RCK2. Boxed amino acids represent fully conserved motifs between the three channels. The putative transmembrane segments S1 to S6 are underlined. Putative N-glycosylation site (●), protein kinase C (○), and protein kinase A (★) phosphorylation sites are indicated.

Fig. 3. HLK3 chromosomal localization. Left, two partial human metaphases showing the specific site of hybridization to chromosome 1. Top, arrowheads indicate silver grains on Giemsa-stained chromosomes, after autoradiography. Bottom, chromosomes with silver grains were subsequently identified by R-banding. Right, idiogram of the human G-banded chromosome 1 illustrating the distribution of labeled sites for the HLK3 probe.

In order to know whether members of the second class of voltage-gated K+ channels, structurally related to IsK protein (26, 37), are present in human T lymphocytes, the PCR was used to clone the cDNA encoding such a protein from Jurkat T cell cDNA. After amplification and subcloning into a plasmid vector, four independent clones were sequenced. Their nucleotide sequences are identical to the recently characterized human genomic clone IsK (37) and encode a protein of 129 amino acids containing a single putative transmembrane domain surrounded by charged amino acid residues. This domain is surrounded by charged amino acid residues. This
purified T cells were pulsed with \(^{3}\text{H}\)thymidine 24 h prior to harvest.

 constants for the various blockers from fitting dose-response curves to Fig. 4. Proliferation was initiated by adding mitogens (10 ng/ml of curves. Unstimulated cells incorporated 2100 cpm/10^5 cells and stimulated cells incorporated 120,000 cpm/10^5 cells (n = 3). Except for 4-AP for which cell loss paralleled inhibition of \(^{3}\text{H}\)thymidine incorporation (50% cell loss at 1 mM 4-AP), the cell viability for the other blockers was >90% as measured by crystal blue exclusion.

### Table I

<table>
<thead>
<tr>
<th>Blocker</th>
<th>(K^+) current blockade</th>
<th>T cell proliferation</th>
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<tbody>
<tr>
<td>Charybdotoxin</td>
<td>(&gt;200 \mu\text{M})</td>
<td>(&gt;200 \mu\text{M})</td>
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<tr>
<td>Clofilium</td>
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<td>60 \mu\text{M}</td>
</tr>
<tr>
<td>TEA</td>
<td>20 mM</td>
<td>30 mM</td>
</tr>
<tr>
<td>4-AP</td>
<td>(&gt;3 \mu\text{M})</td>
<td>0.3 \mu\text{M}</td>
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<tr>
<td>Verapamil</td>
<td>50 \mu\text{M}</td>
<td>60 \mu\text{M}</td>
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### Fig. 5.

**Block of type K\(^+\) currents by ChTX and clofilium in Jurkat cells.** The currents were evoked by depolarizing pulses to 0 mV from a holding potential of -80 mV. A and B, nonactivated cells. A, control, after a 1-min application of 10 mM ChTX, and after washout of the toxin. B, control, after a 1-min application of 10 mM clofilium, and after washout of the drug. C and D, cells were activated for 48 h with 10 ng/ml of PMA and 0.1 \mu\text{M} A23187. C, control and after a 1-min application of 10 mM ChTX. D, control and after a 1-min application of 30 \mu\text{M} clofilium. Note the decreased intensities of the control currents in C and D compared to that of A and B.

### Fig. 6.

**Northern blot analysis of HLK3 and HIsK mRNAs in Human Lymphocytes**—Northern blot analyses were performed to determine the size and the level of expression of HLK3 and HIsK transcripts in the Jurkat T cell line, the IM9 human B cell line, and in purified human peripheral T lymphocytes. In addition, the steady-state of HLK3 and HIsK mRNA levels were analyzed upon T cell activation. Using a HLK3-specific DNA probe derived from the 3' non-coding DNA sequence, we examined the HLK3 hybridization pattern under high stringency conditions. Figs. 6 and 7 show that in Jurkat T cell lines, as well as in purified human T lymphocytes, the HLK3 cDNA probe hybridizes most strongly to a transcript of approximately 9.9 kb, and less intensely to transcripts of approximately 3 and 4.4 kb. This pattern of labeling was specific since no such hybridization signals were detected in samples from Jurkat T cell or purified human T lymphocyte poly(A)^+ RNA. Since the coding sequence consists of only 1569 base pairs, the detection of multiple hybridization signals may be due to alternative mRNA splicing in untranslated regions. Additionally, alternative transcription start sites or polyadenylation sites may be used. As shown in Fig. 6 no hybridization of the HLK3 probe was detectable in the IM9 human B cell poly(A)^+ RNA.

To detect HIsK mRNA, a full length HIsK cRNA antisense probe was generated and used at high stringency. The riboprobe hybridized specifically to a transcript of 1.1 kb in length.
both in Jurkat cells and in purified T lymphocytes. The same transcript size was also detected in IM9 human B cell poly(A)+ RNA. The HlsK riboprobe hybridized to an RNA species of larger size (~4.8 kb) which is most likely due to unspecific hybridization to the contaminating 28 S ribosomal RNA, as this signal was stronger in lymphocyte poly(A)+ RNA.

Since changes in K+ current density were found to precede mitogen-stimulated DNA synthesis in T cells (10, 12-14), it was important to examine whether these events could be accounted for by changes in K+ channel expression at the transcriptional level. For this purpose, Jurkat T cells, as well as purified peripheral T lymphocytes, were activated by the phorbol ester PMA and the Ca2+ ionophore A23187, a treatment which bypasses the requirement for T cell antigen receptor coupling to phospholipase C. Subsequently, the steady-state mRNA levels of HLK3 and HlsK channels were analyzed on Northern blots (Figs. 6 and 7). Ethidium bromide staining and rehybridization of the same blots with a β-actin probe confirmed that equal amounts of poly(A)+ RNA were loaded in each lane (data not shown). In Jurkat T cells, no quantitative changes in the major HLK3 mRNA species (9.9 kb) were found upon 12 h of mitogen-induced activation. However, a slight increase was observed with the minor transcripts (3 and 4.4 kb). Following 24 h of mitogen addition, there was a general decrease in HLK3 transcript abundance (~50% down-regulation). Roughly the same feature was found in purified human T lymphocytes. Forty-four hours after mitogen addition, a time at which IL2 mRNA was highly expressed, no quantitative changes in the major HLK3 mRNA species (9.5 kb) were observed, while an up-regulation of the minor mRNA species (3 and 4.4 kb) was noted (Fig. 7). After 68 h, a general down-regulation of HLK3 transcript levels was found in purified T lymphocytes.

For HlsK protein expression, no significant changes in the 1.1-kb HlsK mRNA levels were observed at any time of mitogen-induced activation of either Jurkat cells or purified T lymphocytes, when compared to unstimulated control cells (Figs. 6 and 7).

Modulation of IL2 mRNA Levels and [3H]Thymidine Incorporation by K+ Channel Blockers—Because expression of the IL2 gene plays a central role in the T lymphocyte proliferative response (1, 3), it is important to understand how IL2 mRNA levels are regulated upon T cell activation. Recently, charybdotoxin, a blocker of the lymphocyte voltage-gated type K+ channel, was found to inhibit IL2 production in human peripheral blood lymphocytes (18). Knowing the existence of HLK3 channels and HlsK mRNA in human T cells, it was of interest to examine whether charybdotoxin, 4-AP, and clofilium would modulate IL2 mRNA levels following T cell activation. Fig. 8 shows that upon activation of Jurkat T cells with PMA and A23187 for 8 h there is a potent induction of IL2 mRNA. When cells were simultaneously treated with charybdotoxin (56 nM), no significant change in the induction of IL2 mRNA was observed. Similarly, 4-AP at a dose (2 mM) which depressed HLK3 current, and tetrodotoxin, a blocker of voltage-dependent Na+ channels, had no effect on mitogen-induced IL2 mRNA (not shown). Conversely, the quaternary ammonium clofilium dose dependently inhibited mitogen-induced IL2 mRNA (IC50 ≈ 10 μM). All these different drugs were also tested for their potency to inhibit [3H]thymidine incorporation into activated peripheral blood T lymphocytes (Table I). Again, charybdotoxin was without effect while clofilium blocked the incorporation with an IC50 of 10 μM. At concentrations lower than 0.2 mM, 4-AP had no effect and then, at higher concentrations, abruptly inhibited [3H]thymidine incorporation. However, in this type of experiment which necessitates cultivation of cells for 3 days in the presence of the drugs, 4-AP was found to be very toxic. The percentage of inhibition was in close correlation with the proportion of dead cells identified by Trypan blue exclusion.

**DISCUSSION**

The aim of the present study was to identify voltage-gated K+ channels expressed in human T lymphocytes by means of molecular cDNA cloning, to study the biophysical properties of the clones functionally expressed in *Xenopus* oocytes, and to examine their levels of transcriptional expression following T cell activation. Low stringency hybridization screening of a human Jurkat T cell line cDNA library with a rat RCK1 brain K+ channel probe (38) has resulted in the isolation and characterization of HLK3. This is the first cloning achieved using conventional screening of a T lymphocyte cDNA library. The predicted amino acid sequence of the HLK3 clone indicates that it exhibits all the well-conserved structural features of Shaker-related voltage-dependent K+ channels. Comparison of HLK3 amino acid sequences with that of the rat brain K+ channel cDNA clone RCK1 (29), or the rat genomic clone RGK1 (24) indicates that HLK3 represents the human counterpart of the same type of K+ channel.

The chromosomal location of the HLK3 gene on the human chromosome 1 contrasts with the previous finding of Grissmer et al. (25) on chromosome 13 using MK3, the mouse counterpart of HLK3 as a probe. The reasons for this discrepancy are unclear. In spite of our careful examination for the presence.
of staining on chromosome 13 with the HLK3 probe, we were unable to reveal such localization in our experiments. It is possible that the difference of chromosomal localization techniques used accounts for the different results.

When injected into Xenopus oocytes, cRNA transcribed in vitro from the HLK3 clone directs the expression of outward K⁺ currents which inactivate during sustained depolarization. The inactivation rate of HLK3 K⁺ current (τ = 740 ms) is between typical "A" currents and delayed rectifiers (49, 50). Inactivation accumulates during repetitive depolarizing pulses because recovery from inactivation during the interpulse is incomplete. These biophysical properties are very similar to those of K⁺ currents that we recorded in Jurkat T cells and are characteristic of type n K⁺ channels described in both mice and human T lymphocytes (8, 10, 13, 51). The effects of Ca²⁺ and the pharmacological properties displayed by the K⁺ current derived from HLK3 cRNA confirm that the HLK3 gene encodes the type n K⁺ channel of human T lymphocytes.

Raising intracellular Ca²⁺ by adding the Ca²⁺ ionophore A23187 resulted in a decrease in HLK3 current amplitude. The same effects were previously described in purified human T cells and were suggested to be due either to a decreased probability of channel opening or to a reduced number of channels capable of being activated (52).

HLK3 expressed in oocytes and type n K⁺ channels in T lymphocytes also have similar pharmacological profiles. K⁺ currents were 50% inhibited at similar concentrations of charybdotoxin, tetraethylammonium, 4-AP, and verapamil in oocytes and Jurkat cells. Interestingly, the quaternary ammonium clofilium, which exhibits potent class III antiarrhythmic activity in animals and humans (45), depressed with a similar potency HLK3 channels expressed in Xenopus oocytes and type n K⁺ channels recorded in Jurkat T cells. Inhibition of outward currents was characterized by a reduction of peak current amplitude and an acceleration of current inactivation. This latter effect most probably reflects a block of K⁺ channels in an open conformation.

Our results have revealed the expression of the HIsK message in human T lymphocytes. The HIsK predicted structure differs completely from that of other known ionic channels and consists of 129 amino acids with a single putative transmembrane domain. Its presence in human B and T lymphocytes is demonstrated not only by PCR experiments but also by Northern blot analysis. However, we failed to record HIsK currents in Jurkat cells. The reason for this failure is not really known. Several possible explanations can be formulated such as: (i) the channel is in fact present but requires activation conditions which have not been found; (ii) the IsK protein needs another or several other protein components to form the active channel; and (iii) the IsK protein is mainly localized in Jurkat cells in the internal membrane system from which it cannot be recorded with the technique used. Our present work is devoted to expressing the IsK protein in other heterologous systems than Xenopus oocytes in order to bring a clearer answer to these questions.

In view of previous observations reporting an increased K⁺ conductance upon mitogen-induced T cell activation (7, 12), the presence of two very different types of voltage-gated K⁺ channels in human T lymphocytes raises the question of knowing the importance and the relative contribution of these K⁺ channels to the activation process. To try to answer these questions we analyzed the amount of transcript levels for both channels at different stages of activation. Since Jurkat cells are leukemic cells which have lost control of their proliferation, the analysis was also performed on purified peripheral blood human T lymphocytes. For both channel types, our data suggest that there is no transcriptional induction upon T cell activation. It rather seems that even at the resting state of human T cells, there is a constitutive expression of both HLK3 and HIsK messages. The lack of increase of HLK3 mRNA is not very surprising. In contrast to the situation encountered in mouse T cells, very subtle if any variation of current density were observed in human T cells upon mitogen activation (for review, see Ref. 10). Under our culture conditions, the electrophysiological study on Jurkat T lymphocytes identified a decrease, instead of an increase, of outward currents. The increase in charybdotoxin-binding site density reported by Deutsch et al. (48) is rather small and could be due to the procedure of activation which used phytohemagglutinin P instead of PMA plus A23187.

Several studies on a variety of mouse and human T lymphocytes have previously suggested that K⁺ channel blockers inhibit mitogen-stimulated proliferation (13, 14, 17, 53). We also observed these effects in human T cells. In view of the lack of inhibition of proliferation by charybdotoxin in the presence of serum (Ref. 18, and this study) and of the failure of this toxin to prevent IL2 mRNA induction, it seems unlikely that blockade of type n K⁺ channels can be the only K⁺ channel involved in the inhibitory effects of the other K⁺ channel blockers. Then a blockade of the HIsK channel could be reasonably invoked to explain these inhibitory effects. The pharmacological profiles of the slowly activating (HIsK) channels on one hand and of the inhibition of T cell proliferation on the other hand (Table I) would suggest such an hypothesis. The previous observation showing that no more than 50% of the proliferative response can be inhibited by charybdotoxin (18) is in line with the existence of at least one charybdotoxin-insensitive channel which may also contribute to the mitogenic response. The existence of other rapidly activating K⁺ channels in lymphocytes involved or not involved in the activation process is still an open question. More work is clearly necessary to elucidate this point.

The mechanism whereby the K⁺ channel blocker clofilium modulates IL2 gene expression and the subsequent proliferative response is not clear. It is known that IL2 gene expression is dependent on the combinatorial effects of different transcription factors, some of them being controlled by Ca²⁺-dependent processes (54). Since Ca²⁺ influx via second messenger-operated (voltage-independent) Ca²⁺ channels (4) depends directly on the driving force for Ca²⁺ across the cell membrane, hyperpolarization due to an enhanced K⁺ conductance would increase Ca²⁺ influx and thus possibly contribute to mitogenesis (55). The fact that the HIsK K⁺ current in Xenopus oocyte is activated by a Ca²⁺-dependent mechanism favors a role for this putative channel in the activation process. Stimulation of the HIsK K⁺ conductance during the activation process would tend to maintain T lymphocytes at a hyperpolarized resting potential. Conversely, clofilium blockade of the HIsK channel would result in a depolarization and decrease in the driving force for Ca²⁺ entry and then to a reduced increase in intracellular Ca²⁺ upon mitogen addition which would then result in a decreased IL2 mRNA induction.

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