Identification of Kv1.1 Expression by Murine CD4<sup>+</sup>CD8<sup>+</sup> Thymocytes

A ROLE FOR VOLTAGE-DEPENDENT K<sup>+</sup> CHANNELS IN MURINE THYMOCYTE DEVELOPMENT*

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The patch-clamp recording technique and RNA-polymerase chain reaction were used to identify the voltage-dependent K<sup>+</sup> channels expressed by murine fetal and adult CD4<sup>+</sup>CD8<sup>+</sup> thymocytes. Two distinct currents, encoded by the genes Kv1.1 and Kv1.3 were identified based upon their biophysical and pharmacologic characteristics and confirmed with RNA-polymerase chain reaction. Peptide blockers of Kv1.1 and Kv1.3 gene products were also applied to a murine fetal thymic organ culture system to investigate the developmental role of these K<sup>+</sup> channels. Dendrotoxin (DTX) and charybotoxin (CTX), antagonists of Kv1.1 and Kv1.3 channels, respectively, decreased thymocyte yields in organ culture without affecting thymocyte viability. DTX-treated thymi contained 56 ± 8% (n = 8 experiments), and CTX-treated thymi contained 74 ± 4% (n = 7 experiments) as many thymocytes as untreated lobes. DTX and CTX also altered the developmental progression of thymocytes in fetal organ culture. These data provide the first evidence of Kv1.1 expression in a lymphoid cell and indicate that thymocyte voltage-dependent K<sup>+</sup> channels are critical to thymocyte preclonal expansion and/or maturation.

Membrane potassium channels subserve important physiologic functions of thymocytes and peripheral T lymphocytes. Although lymphocytes are not electrically excitable cells, they express potassium channels that are the primary determinants of the membrane potential (1, 2), and are critically important for production of the requisite T cell growth factor IL21 (3–5). Although lymphocytes are not electrically excitable cells, they express potassium channels that are the primary determinants of the membrane potential (1, 2), and are critically important for production of the requisite T cell growth factor IL21 (3–5).

Materials and Methods

Cell Isolation—Virus-free C57Bl/6 mice were obtained from Jackson Laboratories or the National Cancer Institute and were bred in the animal facility at the University of Pennsylvania. Thymic lobes were removed from fetal mice at desired gestational ages and were placed into RPMI medium containing 10% heat-inactivated fetal bovine serum, 50 mM 2-mercaptoethanol, 10 mM HEPES buffer, pH 7.4, 1 mM l-glutamine, and 100 U/ml penicillin and streptomycin. Thymocytes were isolated by teasing apart the lobes with sterile keratotomy knives.

Fetal Thymic Organ Culture—FTOC (8) was established in 24-well plates at 37 °C in a 5%CO<sub>2</sub>/95%O<sub>2</sub> environment. Surgical gel foam (3-cm<sup>2</sup>; Upjohn) was soaked in complete RPMI medium to which K<sup>+</sup>- blockers or additional reagents were added. Fetal lobes were placed onto sterile circular nitrocellulose filters (Millipore), which were supported by the gel foam. The fetal lobes (3/2 filter) were maintained on the air/medium interface and were bathed with medium daily. One half of the culture medium was changed on alternate days.

Thymocyte Phenotype Determination—2–5 × 10<sup>6</sup> cells were incubated with saturating concentrations of appropriate antibodies in 0.05 ml of staining medium (phosphate-buffered saline, 0.5% Bovine serum albumin) for 15 min at 4 °C. Staining was performed as a one-step procedure with phycoerythrin-conjugated L3T4 (anti-CD4, Pharmingen, San Diego, CA) and fluorescein-conjugated anti Ly-2 (anti-CD8<sub>β</sub>, Pharmingen). Flow cytometry was performed on a FACScan instrument (Becton Dickinson, San Jose, CA).

Electrophysiologic Recording—Murine fetal or adult CD4<sup>+</sup> CD8<sup>+</sup> thymocytes stained with fluorescein (CD8<sup>+</sup>) or phycoerythrin (CD4<sup>+</sup>)-tagged monoclonal antibodies (see above) were placed in a recording chamber (Brooks) mounted on an inverted fluorescent microscope (Diaphot, Nikon) and CD4<sup>+</sup> CD8<sup>+</sup> (non-staining) cells were patched. Patch pipettes (4–6 MΩ) were back-filled with solution containing 150 μg/ml nystatin.
RNA Preparation and Reverse Transcriptase PCR—Total cellular RNA was prepared according to the method of Chomczynski and Saachi (9). Contaminating genomic DNA was removed from RNA preparations by treating them with DNase I (Boehringer Mannheim) before cDNA synthesis. The polymerase chain reaction was used to amplify cDNA prepared from murine thymocyte RNA. Unique dinucleotide primer pairs were designed to 3′ regions of the K+ channel genes as follows: Kv1.1 (sense, cDNA clone MK3, bases 979–1007, ATCTTCAACTCTC-CGGCCACTCAAGGG; antisense, bases 1495–1476 of cDNA clone MK1, TTTGTCTTTTAAAACTCGT; probe, bases 1292–1321 of MK1, CCTAGGCTTCTGAAGTGACC (Ref. 10), Kv1.3 (sense, as above; antisense, bases 1558–1541 of cDNA clone KV3, ACTTCAAATGCTGAC-CACGG; probe, bases 1315–1336 of cDNA clone KV3, TTGGGTTAT-TGTTCTGT (Ref. 11)). PCR products were separated by electrophoresis in a 2% agarose gel (Life Technologies, Inc.) and capillary blotted onto GeneScreen Plus nylon membrane (DuPont NEN) with 0.5 M NaOH and 1.5 M NaCl. Oligonucleotide probes were 5′-end-labeled with [γ-32P]ATP using T4 polynucleotide kinase (Life Technologies, Inc.).

RESULTS

Pharmacological Characterization of Potassium Currents in CD4 CD8+ Thymocytes—To identify the voltage-dependent K+ channels expressed by CD4 CD8+ (double negative, DN) thymocytes, we employed K+ channel-selective peptide blockers with the nystatin variation of the whole cell patch-clamp technique. Immunocytochemically defined fetal (day 15 or 16) or adult murine thymocytes were voltage-clamped and K+ currents generated by step depolarizations. Experiments were performed on both fetal DN thymocytes, which are predominantly TCR-negative, and adult DN cells, which are both αβTCR-positive and -negative (16). Current amplitudes were stable in these cells for more than 45 min; the rate of current inactivation did accelerate somewhat over time, however, and was dependent on the series resistance (Rf) as previously reported (12). Fig. 1 shows a typical family of whole cell currents from an adult CD4 CD8+ thymocyte evoked by step depolarizations to potentials between –40 and +40 mV (20 mV steps). Potassium currents activated at approximately –40 mV and showed typical time-dependent activation and inactivation characteristics, similar to previous recordings from CD4 CD8+ cells (6, 7). Current inactivation was incomplete, however, and a small non-inactivating current component was a consistent feature of the currents in these cells. Total currents found in fetal and adult murine CD4 CD8+ thymocytes were comparable, but there was a substantially greater current density in the adult cells. The current density was 262 ± 252 μA/cm2 in adults (n = 29) and 95.4 ± 85.8 μA/cm2 in fetal cells (n = 30) for a voltage step from –80 to +50 mV.

Whole cell currents were pharmacologically characterized with several potassium channel-selective peptidyl toxins; outward currents were evoked by 250 or 500 ms depolarizing voltage steps from –80 to +50 mV before and after toxin addition. Previous reports have indicated that CD4 CD8+ thymocytes express Kv1.1 channels, which are completely blocked by CTX (6), which blocks Kv1.2, Kv1.3 and Kv1.6 gene products (11, 13, 36). We consistently observed a small, CTX-resistant (100 nm) current in fetal and adult cells, as shown in Fig. 2A. The CTX-insensitive current was 8.4 ± 2.7% (n = 6) of the original peak current in adult and 17.9 ± 3.8% (n = 6) in fetal cells. For adult CD4 CD8+ thymocytes, the peak CTX-resistant current was 60.4 ± 12.9 pA (n = 6), whereas for fetal cells the current was 36.3 ± 5.2 pA (n = 6). The CTX-resistant current was blocked by DTX (100 nm), an inhibitor of Kv1.1, Kv1.2, and Kv1.6 channels (11). The only gene known to encode a CTX-insensitive, DTX-sensitive, voltage-gated K+ current similar to that observed in CD4 CD8+ thymocytes is Kv1.1. The CTX-resistant current is also completely blocked by 0.8 mM tetraethylammonium (data not shown). Consistent with the presence of a charybdotoxin-insensitive current other than Kv1.3, the initial application of DTX to double negative thymocytes blocked a small component of the peak current (74 ± 9.8 pA, n = 7, Fig. 2B); the DTX-resistant current was then blocked by CTX (50 nm, Fig. 2B). Results from a third subtype-specific toxin were also consistent with the presence of a Kv1.1 channel current. KTX, which is selective for Kv1.3 at the concentration used (10 nm, Ref. 13), did not block all K+ current in CD4 CD8+ thymocytes. As with experiments using CTX and DTX, a small KTX-resistant current was observed (data not shown). Moreover, similar to experiments with CTX, KTX completely blocked outward current in the presence of DTX (Fig. 3). The KTX-resistant current was 18.5 ± 2.5% for adult and 16.7
Murine CD4<sup>+</sup>CD8<sup>+</sup> Thymocytes Express Kv1.1

**Fig. 2.** Pharmacological separation of potassium currents in CD4<sup>+</sup>CD8<sup>+</sup> thymocytes. The sensitivity of voltage-dependent potassium currents to charybdotoxin and dendrotoxin was determined. K<sup>+</sup> currents were elicited with 250 ms voltage-steps from a holding potential of −80 mV to +50 mV. A, each curve represents the average of two consecutive stimuli 30 s apart. Three superimposed responses represent stable currents obtained from the same cell (from largest to smallest current) in the absence of K<sup>+</sup> blocker, in the presence of 50 nM CTX, and in the presence of 50 CTX and 100 DTX. These data are representative of six separate experiments, which demonstrate CTX-sensitive, and CTX-resistant and DTX-sensitive components of the whole cell K<sup>+</sup> current. The experiment was conducted at 20–24°C under standard conditions (see "Materials and Methods"). The capacitance of this cell was 1.8 pF. The peak control current was 694 pA. The peak CTX-resistant K<sup>+</sup> current was 98 pA, and no substantial current remained after subsequent addition of DTX. Cell number 09220401. B, an adult CD4<sup>+</sup>CD8<sup>+</sup> thymocyte was subjected to voltage pulses from −80 mV holding potential to +50 mV. All parameters were as described for the thymocyte above. The capacitance of this thymocyte was 2.5 pF. The peak control current was 472 pA. After the addition of DTX the peak current was 472 pA. In this experiment a small residual K<sup>+</sup> current was observed after addition of 100 nM CTX. Cell number 08319404.

**Fig. 3.** DTx and KTX block the entire whole cell K<sup>+</sup> current of CD4<sup>+</sup>CD8<sup>+</sup> thymocytes. A day 15 fetal CD4<sup>+</sup>CD8<sup>+</sup> thymocyte was subjected to voltage pulses from −80 mV holding potential to +50 mV. All parameters were as described for the thymocyte in Fig. 2. The capacitance of this thymocyte was 2.8 pF. The peak control K<sup>+</sup> current was 367 pA. A combination of 100 nM DTX and 10 nM KTX completely blocked the whole cell K<sup>+</sup> current.

**Fig. 4.** The DTX sensitive component of the whole cell K<sup>+</sup> current does not exhibit use-dependent inactivation. An adult CD4<sup>+</sup>CD8<sup>+</sup> thymocyte was subjected to a single control voltage pulse (left panels) or a train of voltage-step pulses (200 ms, 50-ms interval) from −80 mV to +50 mV to induce use-dependent inactivation of the K<sup>+</sup> current (middle and right traces). Control currents were evoked in the absence (top left) and presence (bottom left) of 100 nM DTX. The peak K<sup>+</sup> current in the absence of blocker was 293 pA and in the presence of blocker was 260 pA. In the absence of DTX, a small use-independent K<sup>+</sup> current (peak = 64 pA) was observed (top right). 100 nM DTX blocked most of this current (bottom right). The capacitance of this thymocyte was 2.06 pF.

± 2.0% for fetal thymocytes. For adult CD4<sup>+</sup>CD8<sup>+</sup> thymocytes, the peak KTX-resistant current was 115.4 ± 14.1 pA (n = 5), whereas for fetal cells the current was 40.3 ± 3.8 pA (n = 9). Thus subtype-specific toxins demonstrate a DTX-sensitive and CTX/KTX-resistant current, consistent with the expression of Kv1.1 channels, as suggested by the expression of Kv1.1 mRNA (see below).

In addition to their unique pharmacologies, Kv1.1 and Kv1.3 are biophysically distinct in that Kv1.3 exhibits use-dependent inactivation, whereas Kv1.1 does not (13). As shown in Fig. 4, repeated depolarizations at short intervals (4 Hz) resulted in the progressive inactivation of one current component, which was DTX-insensitive. Conversely, a non-cumulatively inactivating current component existed, which was blocked by DTX. Similar to results with application of CTX, KTX, or DTX, the inactivating current (presumably Kv1.3) was the predominant current component.

Identification of mRNA for Kv1.1 and Kv1.3 in Day 15 Fetal Thymocytes—RNA-PCR was used to confirm the presence of mRNA in CD4<sup>+</sup>CD8<sup>+</sup> thymocytes for K<sup>+</sup> channels. Fetal thymocytes from day 15 gestation were used because they are almost exclusively CD4<sup>+</sup>CD8<sup>+</sup>. Using channel subtype-specific primers for Kv1.1 through Kv1.6, and for all members of the Kv2, Kv3, and Kv4 families, only PCR products of the appropriate size for Kv1.1 and Kv1.3 were obtained. Fig. 5 is an autoradiograph of a Southern blot of PCR products generated from Kv1.1 (lane 1) and Kv1.3 (lane 3) mRNA. The identity of the amplified cDNA was confirmed by hybridization with radiolabeled internal oligonucleotides specific for each of these genes (see "Materials and Methods"). Because these K<sup>+</sup> channel genes are intronless (10), we confirmed that these PCR products were not amplified from contaminating K<sup>+</sup> channel genomic DNA in the RNA samples. These data indicate that no PCR products were obtained for Kv1.3 and Kv1.1 when PCR was performed on untranscribed fetal thymocyte RNA (lanes 2 and 4, respectively). We were unable to detect mRNA for Kv1.2, Kv1.4, Kv1.5, Kv1.6, or the Kv2, Kv3 and Kv4 gene families in CD4<sup>+</sup>CD8<sup>+</sup> thymocytes. Hence, fetal CD4<sup>+</sup>CD8<sup>+</sup> thymocytes express Kv1.3 as previously shown, but also Kv1.1.
Thymocytes—Cellular expansion is a dominant feature of thy
tocyte development between days 15 and 17 of gestation, and
CD4 and CD8 both together caused an increase in the percentage of
tyve yields, we observed an effect on the CD4/CD8 phenotype of
ue due to inhibition of proliferation.
ence on the CD4/CD8 phenotype of murine thymo-
the 3′ translated and non-translated regions of Kv1.3 (first lane) and Kv1.1 (third lane). A portion of the day 15 derived total
 clears except for the omission of reverse transcriptase (−lanes) and was
alyzed products was made on the basis of their correct predicted sizes (Kv1.3, 550 base pairs; Kv1.1, 450 base pairs). Secondary confirmation was
отated with 32P-labeled unique internal DNA prim-
ors (see “Materials and Methods”).
 garnered CTX to have less effect than DTX on thymocyte yield.
the number of experiments. For individual experiments, the number of thymocytes
nents. Plots are displayed with CD4 fluores-
level of 0.01 (0.02) for differences between groups. Unpaired ANOVA followed by Dunnett’s test was used to confirm the presence of main effects.
ized may be requisite for subsequent development to
ons and non-translated regions of Kv1.3 (first lane) and Kv1.1 (third lane). A portion of the day 15 derived total
 RNA was isolated from day 15 fetal thymocytes (Fig. 6).
explained by hybridization with 32P-labeled unique internal DNA prim-
re (third lane) and was subjected to PCR with the same DNA primers for Kv1.3 (second lane) and Kv1.1 (fourth lane). Primary identification of the PCR-amplified
rhibited proliferation of thymocyte precursors and/or their pro-
K-range (180 base pairs) and Kv1.1 (450 base pairs) were used for PCR with the same DNA primers for Kv1.3 (350 base pairs) and Kv1.1 (450 base pairs). The bands were
or 200 nM DTX plus 200 nM CTX. Thymocytes isolated on day 2 of
ered variability within individual experiments, at least two identically treated
ed within rectangular gates. The percentage of thymocytes within each gate is indicated in the
mocytes are derived from immature proliferating CD4
hotoxin (Becton Dickinson). Plots are displayed with CD4 fluores-
and CD4/CD8 phenotype of murine thymocytes in situ, we characterized the effects of K+ channel blockers
shown that CTX and DTX inhibit proliferation of thymocyte precursors and/or their progression to the CD4+CD8− stage. That the combination of CTX and DTX induces a greater decrease in yield and the number of
phases was obtained from at least six separate experiments with each blocker.
reduction in cell yield induced by DTX or DTX and CTX is
cthymocytes in situ, after the effect of CTX or KTX was not different from control cultures (data not shown).
and CD8 fluorescence on the
duced to 59% ± 0.04% (n = 4) and 52% ± 0.14% (n = 4), respectively. For this experiment, the total number of thymocytes recovered per lobe was 384,300 for untreated thymi and 253,000,325,905, and 225,600 for thymi treated with
K+ channel blockers decrease cell yields from fetal
in situ

FIG. 5. rTPCR confirms that Kv1.3 and Kv1.1 are expressed by
day 15 fetal thymocytes. Total cellular RNA was isolated from day 15
fetal thymocytes, which comprise greater than 95% CD4+CD8+ thymo-
cytes. The RNA was treated with DNase I to remove contaminating

Potassium Channel Blockers Inhibit Proliferation of Fetal
Thymocytes—Cellular expansion is a dominant feature of thy-
moocyte development between days 15 and 17 of gestation, and
cell cycle activity may be requisite for subsequent development to
the CD4+CD8− phenotype (14). To determine whether Kv1.1 and
or Kv1.3 play a critical role during development of thymo-
cytes in situ, we characterized the effects of K+ channel block-
ers on thymocyte development in whole cultured day 15 fetal
thymic explants. In situ blockade of Kv1.3 or Kv1.1 played a critical role during development of thymo-
cytes in situ, and the inhibition of proliferation or toxicity. Apparent thymocyte via-
rability was unaffected by any of the blockers (>90% for all
ments. Moreover, neither CTX or DTX were directly toxic and the viability and CD4/CD8 phenotype of murine thymocytes
incubated in suspension culture for 48 h with CTX (200 nM) or DTX (200 nM) was not different from control cultures (data not shown).
ence, the reduction in cell yield is probably due to inhibition of proliferation.

In addition to the effects of CTX and DTX on thymocyte yields, we observed an effect on the CD4/CD8 phenotype of thymocytes in FTOC. Treatment of FTOC with CTX, DTX, or both together caused an increase in the percentage of CD4+CD8− and CD4+CD8− immature thymocytes (TREC-negative), both precursors of CD4+CD8+ thymocytes (15), and a decrease in the percentage of CD4+CD8+ thymocytes (Fig. 6).

However, as noted previously (Table I), we consistently ob-
erved CTX to have less effect than DTX on thymocyte yield.
The reduction in cell yield induced by DTX or CTX is
primarily a reflection of a reduction in the absolute number of
CD4+CD8− thymocytes (Table II). Even though the effect of
CTX alone on yield is less than DTX, its effect on phenotype is
imilar. Taken together, our observations that CTX and DTX
are not directly toxic to thymocytes, that the number of
CD4+CD8− thymocytes is decreased, and that CD4+CD8− thy-
mocytes are derived from immature proliferating CD4+CD8− and CD4+CD8− precursor pools suggest that these drugs in-
hibit proliferation of thymocyte precursors and/or their pro-
gression to the CD4+CD8− stage. That the combination of CTX and DTX induces a greater decrease in yield and the number of

![Fig. 5](image-url)  
**Fig. 5.** rTPCR confirms that Kv1.3 and Kv1.1 are expressed by day 15 fetal thymocytes. Total cellular RNA was isolated from day 15 fetal thymocytes, which comprise greater than 95% CD4+CD8+ thymocytes. The RNA was treated with DNase I to remove contaminating genomic DNA (see "Materials and Methods"). A portion of the RNA was used to generate cDNA (+lanes) and subjected to PCR with DNA primers specific to the 3′ translated and non-translated regions of Kv1.3 (first lane) and Kv1.1 (third lane). A portion of the day 15 derived total cellular RNA was treated identically as that subjected to DNA synthesis except for the omission of reverse transcriptase (−lanes) and was subjected to PCR with the same DNA primers for Kv1.3 (second lane) and Kv1.1 (fourth lane). Primary identification of the PCR-amplified products was made on the basis of their correct predicted sizes (Kv1.3, 550 base pairs; Kv1.1, 450 base pairs). Secondary confirmation was obtained by hybridization with 32P-labeled unique internal DNA primers (see "Materials and Methods").

| Table I Potassium Channel Blockers Decrease Cell Yields from Fetal Thymic Organ Cultures |
|----------------------------------|------------------|------------------|------------------|------------------|
| Dendrotoxin | Charybdotoxin | Kalotoxin | DTX + CTX | DTX + KTX |
| 0.56 ± 0.08 | 0.74 ± 0.04 | 0.53 ± 0.09 | 0.59 ± 0.04 | 0.52 ± 0.14 |

(n = 8) (n = 7) (n = 4) (n = 5) (n = 4)

**Fig. 6.** K+ channel blockers inhibit fetal thymocyte development. Day 15 fetal thymic lobes were cultured as described in methods in the absence of blocker, or in the presence of 200 nM DTX, 200 nM CTX, or 200 nM DTX plus 200 nM CTX. Thymocytes isolated on day 2 of culture were stained for surface CD4 and CD8 and analyzed on a flow cytometer (Becton Dickinson). Plots are displayed with CD4 fluorescence on the ordinate and CD8 fluorescence on the abscissa, and the individual thymocyte subpopulations are enclosed within rectangular gates. The percentage of thymocytes within each gate is indicated in the outside corner. This is one of three similar experiments showing that the percentage of CD4+CD8− and CD4+CD8+ thymocytes is increased by each of the blockers. However, each blocker also decreased thymocyte yields compared with untreated controls. For this experiment, the total number of thymocytes recovered per lobe was 384,300 for untreated thymi and 253,000,325,905, and 225,600 for thymi treated with DTX, CTX, and DTX + CTX, respectively. These data are representative of at least six separate experiments with each blocker.

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**K+ channel blockers decrease cell yields from fetal thymic organ cultures**

Thymic lobes were cultured in the absence or presence of indicated blockers (each at 200 nM). Each experimental yield (represented as 1 n) was obtained from at least two pooled identically treated lobes. Yields are expressed as the mean percentage (S.E.) from the indicated number of experiments. For individual experiments, the number of thymocytes recovered from K+ blocker-treated thymi was expressed as the percent recovered from untreated thymi from the same experiment. The values in the table are the means of the percentages so calculated, from the indicated number of experimental trials.

| Dendrotoxin | Charybdotoxin | Kalotoxin | DTX + CTX | DTX + KTX |
|-------------|---------------|-----------|-----------|-----------|
| 0.56 ± 0.08 | 0.74 ± 0.04   | 0.53 ± 0.09| 0.59 ± 0.04| 0.52 ± 0.14|

(n = 8) (n = 7) (n = 4) (n = 5) (n = 4)
The effect of K^+ channel blockers on the absolute number of thymocytes in each subpopulation

| Day 15 + 2 | CD4^+ CD8^- | CD4^+ CD8^+ | CD4^- CD8^- | CD4^- CD8^+ | Total |
|------------|-------------|-------------|-------------|-------------|-------|
| Control    | 16,100      | 9,672       | 133,976     | 1,612       | 161,180 |
| Dendrotoxin| 17,576      | 9,464       | 106,808     | 1,352       | 135,200 |
| Charybdotoxin| 26,625     | 10,650      | 136,675     | 1,775       | 175,725 |
| DTX + CTX  | 15,741      | 61,222      | 64,713      | 794         | 87,450  |

| Day 15 + 5 | CD4^+ CD8^- | CD4^+ CD8^+ | CD4^- CD8^- | CD4^- CD8^+ | Total |
|------------|-------------|-------------|-------------|-------------|-------|
| Control    | 19,215      | 11,529      | 334,341     | 18,215      | 384,300 |
| Dendrotoxin| 16,830      | 4,590       | 123,930     | 7,650       | 153,000 |
| Charybdotoxin| 19,557     | 9,779       | 280,317     | 16,298      | 325,951 |
| DTX + CTX  | 13,536      | 6,750       | 196,272     | 11,250      | 227,808 |

CD4^+ CD8^- thymocytes by day 2 than CTX or DTX alone suggests that they act synergistically.

DISCUSSION

We have identified a K^+ conductance not previously described in CD4^+ CD8^- thymocytes, which is encoded by the Kv1.1 gene. Patch-clamp studies demonstrated that the predominant channel in CD4^+ CD8^- thymocytes is Kv1.3, in agreement with previous findings in which the n current predominated (6, 7). However, our studies also indicate that Kv1.1 channels make up an appreciable current component. Thus, a DTX-sensitive current component was approximately 10% of the peak current. Unlike the Kv1.3 channel current, the Kv1.1 channel current activated slowly and displayed little time- or use-dependent inactivation. The extent to which the distinct kinetic and inactivation properties of these channels determine the membrane potential and calcium signaling in CD4^+ CD8^- thymocytes is not known.

We have also identified a developmental role for thymocyte potassium channels. Blockers of each of the identified voltage-dependent conductances inhibit proliferation of thymocytes within the thymus, suggesting that voltage-dependent potassium channels contribute to the signals that control early developmental events of thymus cellularity. The decrease in the absolute number of thymocytes by day 2 than CTX or DTX alone suggests that they act synergistically.

Several physiological roles have been defined for potassium channels in lymphoid cells. Voltage-dependent potassium channels are critical determinants of the transmembrane electrical potential for both thymocytes and peripheral blood T lymphocytes (17–20). For peripheral T lymphocytes, production of the T cell requisite growth factor (IL2) and the proliferative response to stimulation critically depend upon the transmembrane potential defined by these potassium channels (4, 5, 21). It is unlikely, however, that the inhibitory effect of potassium blockers on proliferation of CD4^+ CD8^- thymocytes is related to an effect upon IL2 production, since the production of IL2 is not critical for thymocyte development (22).

In conclusion, our data extend the observations of Lewis and Cahalan (6) and support the hypothesis that differential expression of potassium channel subtypes may play an important role in development (4). This could occur by several mechanisms. For example, modulation of the lymphocyte K^+ permeability exerts a substantial effect on membrane potential (1, 2), receptor-mediated transmembrane flux of calcium (5, 19, 27–32), and cytokine production (4, 5, 21, 33). Future efforts should be directed toward understanding why thymocytes have evolved such a complex K^+ channel phenotype and whether this phenotype is critical to the functional requirements of other thymocyte subpopulations during later developmental events such as T cell repertoire selection.

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