Increase in voltage gated potassium currents of human lymphocytes on culture

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Voltage gated potassium channels present in T lymphocytes play an important role during lymphocyte activation. Though an increase in potassium currents has been reported in activated lymphocytes, changes in potassium currents in culture without activation by antigen or mitogen has not been reported. The peak potassium current densities on day 1 and day 5 of culture have been compared in this study. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation. Lymphocytes were separated from PBMCs by negative selection using anti-CD14 coated magnetic beads and cultured under appropriate conditions without antigenic or mitogenic stimulation. Lymphocytes were patched on day 1 or day 5 of culture. Voltage gated potassium currents were recorded by whole cell patch clamp technique using a depolarizing protocol. The mean of peak current densities recorded at +60 mV on day 1 of culture was 228.12± 89.39 pA/pF (n=7) and on day 5 of culture was 468.96 ± 192.07 pA/pF (n=7). The difference between the current densities on day 1 and day 5 was found to be significant. Change in electrophysiological characteristics can lead to functional changes in the lymphocytes and this should be considered when culturing lymphocytes in vitro for research and clinical use.

Keywords: Culture, Lymphocyte, Patch clamp, Potassium currents

Voltage gated potassium channels have been described in human T and B lymphocytes and they have been found to be essential for T lymphocyte activation. Potassium exit through voltage gated potassium channels and calcium activated potassium channels during lymphocyte activation prevent depolarization of the lymphocyte membrane. Depolarization of the lymphocyte membrane decreases calcium entry through calcium release activated channels (CRAC). An increase in intracellular calcium is central for lymphocyte activation and therefore the voltage gated potassium channels play an important role in activation of lymphocytes. Voltage gated potassium channels in lymphocytes are also involved in the maintenance of resting membrane potential and regulation of cell volume.

Markers of voltage gated potassium channels in vitro have been found to decrease lymphocyte activation, proliferation and cytokine production. Further, blockade of voltage gated potassium channel Kv1.3 in vivo has been found to inhibit delayed type hypersensitivity reaction and inhibit development of T cell subsets in thymus. Changes in expression of voltage gated potassium channels in lymphocytes on antigenic stimulation in culture have also been reported. Voltage gated potassium currents have been found to increase on lymphocyte activation and expression of voltage gated potassium channels have been found to change when the T lymphocyte differentiates into central or effector memory T cells on antigenic stimulation. The present communication reports changes in potassium current densities in culture without antigenic or mitogenic stimulation by comparing current densities on day 1 and 5 of culture.

Materials and Methods

Isolation of peripheral blood mononuclear cells—Peripheral venous blood (10 mL) collected from healthy human volunteers was heparinised and diluted with an equal volume of RPMI-1640 (Sigma-Aldrich, St.Louis, MO, USA). It was then layered over 10 mL of Ficoll-Histopaque (Sigma-Aldrich, St.Louis, MO, USA) in a centrifuge tube and centrifuged at 1500 rpm for 30 min. The layer of mononuclear cells (which contains lymphocytes and monocytes) at the interface between Ficoll and plasma was harvested and washed twice with phosphate buffered saline (PBS). The cells were suspended in 180 μL of PBS containing 0.5% fetal bovine serum (FBS) and 2 mM EDTA.

Isolation of lymphocytes—Lymphocytes were separated from the mononuclear cells by negative
selection using a magnetic assisted cell sorter (MACS). Cell suspension (180 μL) was incubated with 20 μL anti-CD14 coated magnetic beads (Miltenyi Biotec, Auburn, CA, USA). The cell suspension was then passed over a magnetic column. The bound cells (CD14+ cells which are monocytes) remain in the column whereas the unbound cells, which are the lymphocytes flow out of the column. The effluent cells (lymphocytes) were collected and cultured.

**Culture of lymphocytes**—Lymphocytes (1×10⁶ cells/mL) were cultured in RPMI–1640 media supplemented with 10% FBS, 100 U/mL penicillin/streptomycin, 2.5 μg/mL amphotericin B and incubated in humidified 5% CO₂ incubator at 37 °C. The cells were harvested after 24 h or on day 5 of culture, washed with PBS and patch clamp experiments were performed.

**Patch clamp experiments**—Voltage gated potassium currents were recorded in the whole cell configuration. Micropipettes (Kimax Borosilicate Capillaries, Fischer Scientific, USA) were pulled using a Narishige PP-83 pipette puller. The pipette resistances were between 3-5 MΩ. The pipettes were polished using a GlasswoRx 500 fine point microforge. The cells were loaded on petridishes and the bath volume was made up to 2 mL with the bath solution. The bath solution had 140 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose and 10 mM HEPES. The pipette solution contained 140 mM KCl, 1 mM CaCl₂, 10 mM EGTA and 10 mM HEPES. The osmolarities of the pipette and bath solutions were between 290 to 300 mosm/L. The cells were patch clamped and studied in whole cell configuration. Depolarizing pulses from -60 mV to +80 mV were applied for 200 ms in 20 mV increments from a holding potential of -80mV.

The tail current reversal potential and sensitivity of outward currents to external tetraethylammonium (TEA, a potassium channel blocker) were determined to establish that potassium was the ionic carrier. Tail currents were recorded by applying a depolarizing pulse of +40 mV for 200 ms and then test pulses from -120 mV to -70 mV in 10 mV increments. Sensitivity of the currents to TEA was determined by adding TEA (10 mM) to the bath. Series resistance compensation of 60 to 70% was done for all recordings. The analysis was done using Clampfit 9.2 and IgorPro version 5.04B.

**Statistical analysis**—The results are expressed as mean±SD. The peak current densities recorded at +60 mV and cell capacitances on day 1 and day 5 of culture were compared using Mann Whitney U test. The P value <0.05 was considered to be significant.

**Results**

Outward currents were recorded when depolarizing pulses were applied to the cell (Fig. 1). Tail current...
analysis showed that the currents reversed close to the calculated equilibrium potential \( (E_K) \) for potassium (-90mV) for the given solutions (Fig. 2). Further, the currents were 50% inhibited with 10 mM TEA in the bath solution (Fig. 3). Hence these outward currents were considered to be potassium currents. The difference between the current densities on day 1 and day 5 was found to be significant \( (P<0.05; \text{Fig. 4a}) \). The cell capacitance on day 5 was not significantly different from that of day 1 (Fig. 4b).

**Discussion**

The aim of this study was to determine changes in voltage gated potassium currents that may occur during culture of lymphocytes without antigenic or mitogenic stimulation by patch clamp technique using whole cell configuration. A significant increase in the potassium current density was found in lymphocytes on day 5 of culture when compared to day 1. Previous studies report an increase in voltage gated potassium currents on lymphocyte activation on mitogenic stimulation, while no change in these currents was observed in cells kept in culture for 20 to 28 h without mitogen\(^9\). In the present study a two fold increase in potassium current density in lymphocytes was observed on day 5 of culture without antigen or mitogen when compared to day 1. The change in current density, which reflects the channel density on the cell membrane, was not due to a change in the cell size since there was no change in the cell capacitance between day 1 and day 5. Also, the absence of significant change in cell capacitance precludes the possibility that the cells had undergone activation during culture because activation is accompanied by an increase in cell size\(^9,11\).

Differentiation of naïve lymphocytes to effector memory T cells on repeated antigenic stimulation during \textit{in vitro} culture has been reported to be accompanied by an increased expression of voltage gated potassium channels Kv1.3\(^10\). Previous studies have also reported phenotypical and morphological changes in other cell types like dendritic cells and Langerhan’s cells during short term culture\(^12,13\).

The increase in potassium current density observed in this study is most likely to have occurred due to increased expression of voltage gated potassium channels. This could have resulted due to...
differentiation of lymphocytes under culture conditions. Further studies are required to assess whether the electrophysiological changes observed in lymphocytes during culture are accompanied by phenotypical and functional changes.

Lymphocytes are now being cultured in vitro for research and clinical purposes including cell based therapies for viral infections and cancer. The changes in electrophysiological characteristics in culture can have functional consequences since voltage gated potassium channels play a central role in lymphocyte activation. Hence the possibility that functional changes could occur in the lymphocytes while maintaining them in culture should be considered when using lymphocytes for research and clinical purposes.

Conclusion
There is significant increase in the voltage gated potassium currents in human lymphocytes on day 5 of culture, when compared to day 1. Change in electrophysiological characteristics can lead to functional changes in the lymphocytes and it should be considered when culturing lymphocytes in vitro for research and clinical use.

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References
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