Indian J Physiol Pharmacol 2018; 62(1) : 97–104

Original Article

Current Density of Voltage-gated Proton Currents Decreases During Differentiation of Human Peripheral Blood Monocytes to Macrophages in Culture

S. Snekalatha[1]* and Praghalathan Kanthakumar[2]

[1]Department of Physiology,
ESIC Medical College and PGIMSR,
Chennai, Tamil Nadu

[2]Department of Physiology,
Christian Medical College,
Vellore, Tamil Nadu

Abstract

Voltage gated proton channels play an important role during the respiratory burst in phagocytic cells. Proton channels have been earlier described in all leucocytes, including THP-1 monocytes. In this study, proton currents in peripheral blood monocytes (PBMs) and the changes that occur in these currents during differentiation into macrophages in culture were studied. The proton currents in PBMs were similar to proton currents described in other mammalian phagocytic cells in terms of their threshold potential, zinc sensitivity, activation and inactivation profiles. The proton currents in PBMs were larger than the currents previously reported in THP-1 monocytes. There was a remarkable increase in cell size during differentiation of monocytes under appropriate culture conditions to monocyte derived macrophages. This light microscopic finding was supported by an increase in cell capacitance. The current density decreased significantly as the monocytes differentiated into monocyte derived macrophages. The results of the study indicate that differences exist between PBMs and THP-1 monocytes with respect to proton current amplitude and kinetics. Hence THP-1 cells may not be an accurate model to study electrophysiological characteristics of monocytes. Further, monocytic differentiation lead to a decrease in proton current density which could be related to functional changes that occur in these cells during differentiation.

Introduction

Voltage gated proton channels have been demonstrated in neutrophils, lymphocytes, eosinophils, basophils and alveolar epithelial cells (1-6). Phagocytes generate superoxide radicals within their phagosomes to kill invading pathogens. This process is termed the “respiratory burst”. This involves a coordinated action of an enzyme complex called NADPH oxidase, voltage-gated proton channels and several other membrane transporters including proton pumps (7). The redox reaction mediated by NADPH oxidase transfers negatively

***Corresponding author :**
S. Snekalatha, Department of Physiology, ESIC Medical College and PGIMSR, Chennai – 600078, Telephone: 044-24748959
E-mail: sneka_r@yahoo.co.in

(Received on August 1, 2017)

charged electrons out of the cytoplasm. This leads to accumulation of positively charged H^+ ions in the cytoplasm, causing membrane depolarization and intracellular acidification. Therefore, H^+ extrusion through proton channels helps to prevent depolarization of the cell during the respiratory burst and is essential to maintain the activity of the enzyme NADPH oxidase (8, 9). Proton channels are also expressed in phagosomes of granulocytes (10) which is consistent with its proposed role during the respiratory burst. Proton channels also help to prevent intracellular acidification in neutrophils during respiratory burst (11). Voltage gated proton channels present in the brain microglia contribute to neuronal death after ischemia (12).

Proton currents in peripheral blood monocytes have been studied in perforated patch configuration after stimulation with phorbol myristate acetate (PMA) and exposure to glucose. An increase in proton conductance, faster channel activation and slow deactivation were observed on activation with PMA, while glucose did not have any effect on the proton currents (13). Proton currents have been described in THP – 1 monocyte (a monocyte leukemic cell line) and after their differentiation to macrophages with PMA (14). The total current as well as current density was found to decrease after PMA induced differentiation.

THP – 1 monocytes, derived from a human monocytic leukemia cell line, are considered to be a more differentiated cell line than PBMs (15, 16). Monocyte derived macrophages are also closer in phenotype and function to tissue macrophages when compared to macrophages obtained by PMA induced differentiation (16). This study investigates the changes in proton currents during culture-induced differentiation of PBMs to macrophages.

Materials and Methods

The work was conducted at the Department of Physiology, Christian Medical College, Vellore after obtaining approval from the institutional review board.

Isolation of monocytes from peripheral blood

Peripheral blood mononuclear cells were isolated by density gradient centrifugation. 10 ml of heparinised peripheral venous blood collected from healthy human volunteers was diluted with an equal amount of RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA). The diluted blood was layered over 10 ml of Ficoll-paque (Sigma-Aldrich, St. Louis, MO, USA) and centrifuged at 400 g at 20ºC for 30 min. The mononuclear cells were harvested from the interface between Ficoll and the supernatant plasma and washed twice with phosphate buffered saline (PBS) containing 2 mM EDTA. The cells were re-suspended in PBS with EDTA. Monocytes were isolated from peripheral blood mononuclear cells by magnetic-activated cell sorting (MACS) technique using anti CD 14 coated magnetic beads (Miltenyi Biotec, Aurburn, CA, USA).

Culture of monocytes

The monocytes were cultured in 35 mm tissue culture dishes at a concentration of 2×10^5 cells/ml in RPMI supplemented with 10% fetal bovine serum (FBS)(Gibco, Auckland, New Zealand), 100 U/mlPenicillin/Streptomycin, 2.5 µg/ml of Amphotericin-B at 37ºC in a humidified 5% CO_2 incubator for 3-6 days. Cells were harvested on day 3 or day 6 of culture. Harvesting was done by gentle flushing of the culture dishes with PBS containing EDTA, and the cells were washed and re-suspended in PBS. Patch clamp experiments were performed on the day of isolation, day 3 and day 6 of culture.

Patch clamp experiments

The data were acquired using the Axopatch 200B patch-clamp amplifier and digitized with the Axon Instruments Digidata 1322A A-D converter. Micropipettes were fabricated using borosilicate glass capillary tubes (Kimax Borosilicate Capillaries, Fischer Scientific, USA). Tip resistance ranged between 3-5 MΩ when immersed in bath solution.

Voltage gated proton currents were recorded in monocytes and monocyte derived macrophages in whole-cell configuration. The bath solution contained

60 mM CsCl, 100 mM HEPES, 2 mM $CaCl_2$, 1 mM EGTA and 10 mM glucose. The osmolarity of the bath solution was made up to 300 mOsm/L and pH titrated to 7.5 with 1M tetramethyl ammonium hydroxide. The pipette solution had 100 mM MES, 35 mM CsCl, 3 mM $MgCl_2$, 1 mM EGTA and 10mM glucose. The pH of pipette solution was titrated to 6.0 with concentrated methane sulphonic acid. Voltage-gated proton currents were recorded using depolarizing step voltages. All experiments were performed at room temperature (25°C).

Series resistance compensation (60-70%) was applied before each recording. Data were sampled at 10 kHz. Offline filtering of data was done when required. Igor Pro version 5.0.4.8 (Wave Metrics, Inc.) was used for data representation and offline analysis.

Statistical analysis

All data are expressed as mean±SD. Capacitance, absolute current and current densities on different days of culture were compared using Mann Whitney – U test. SPSS version 17 was used for statistical analysis. $p<0.05$ was considered to be significant. The activation time constant (τ_{act}) and the tail current time constant (τ_{tail}) were obtained by fitting raw current tracings with a single exponential curve. Clampfit version 9.2 (Axon Instruments) was used for curve fitting. As depolarizing potentials positive to +20 mV resulted in drooping of outward currents during the later phases of the voltage pulse, curve fitting was done only on the initial segment.

The Boltzmann function used to fit the conductance-voltage (g-V) relationship used the following formula,

$gH = gH.max/[1 + \exp((V_{1/2} - V)/V_{slope})]$, where gH is the conductance of hydrogen channels, gH.max is the maximum conductance, $V_{1/2}$ is the membrane voltage at which conductance is half the maximum value; and V_{slope} is the slope factor related to the steepness of the curve.

Results

Slowly activating outward currents were recorded when depolarizing pulses were applied to monocytes (Figure 1a). They were inhibited by addition of 0.5 mM $ZnCl_2$ to the bath solution (Figure 1b).

The outward currents activated at –30 mV (Figure 2a and 2b) and reversed at –70 mV (Figure 3). The conductance-voltage (g-V) relationship as shown in Figure 2b depicts an increase in conductance with membrane depolarization. Data were best fitted with a standard Boltzmann fit, commonly used to describe g-V curves.

The mean peak proton current density in peripheral blood monocytes recorded at +60 mV was found to be 113.56±37.6 pA/pF (n=21).

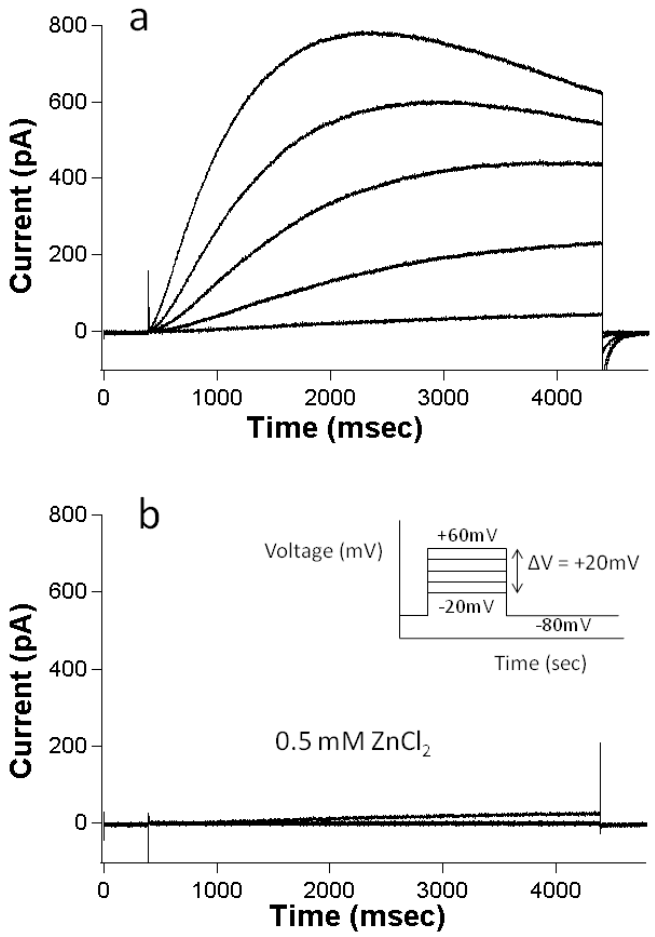

Fig. 1 : Zinc-sensitive outward currents in a human monocyte a) A family of slow-activating outward currents recorded from a human monocyte using step voltage pulses. V_{Hold} = –80mV; test potentials range from –20 mV to +60 mV at 20 mV increments. b) Recording made from the same cell after addition of 0.5 mM ZnCl2 shows inhibition of outward currents. The voltage protocol used is shown in the inset. Filtered at 1 kHz.

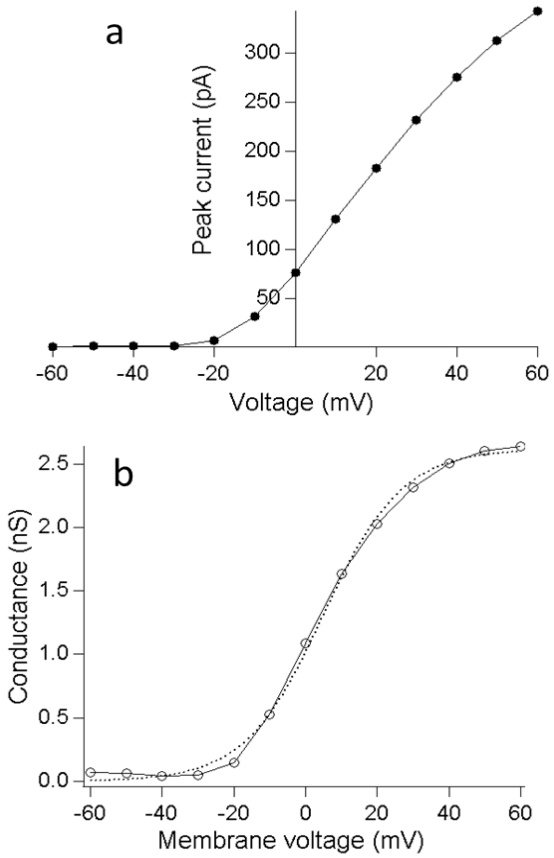

Fig. 2 : I-V and g-V relationships of proton currents recorded in human monocyte a) The current-voltage relationship of a representative proton current recording. The activation threshold of proton currents is observed to be around –30 mV. The bath pH (pHo) was 7.5 and the internal pH (pHi) was 6.0. b) The conductance-voltage relationship of the proton channels plotted using the data shown above. The solid line with markers represents the conductance as calculated from the data. The conductance increases in a sigmoid fashion with membrane depolarization. The dotted line represents asigmoid fit of the g-V curveusing Boltzmann function. Boltzmann fit parameters are: $V_{1/2}$ = 4.9±0.86 mV; V_{Slope} = 11±0.8 mV and $g_{H.max}$ = 2.6±0.06 nS.

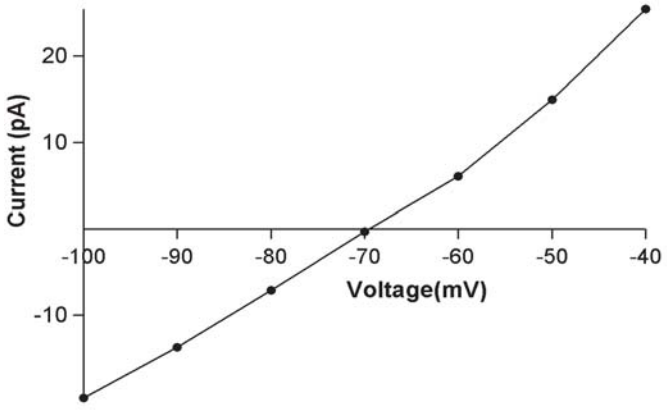

Fig. 3: Tail current-voltage relationship of proton currents. A representative tail current-voltage relationship of proton currents obtained from a human monocyte on the day of isolation, showing reversal around –70 mV. The membrane was held at –80 mV and depolarized to 0 mV. Test pulses ranging from –100 mV to –40 mV at 10 mV increments were applied.

Changes in culture

Light microscopy showed an increase in cell size as the cells differentiated in culture. Increase in cell size is accompanied by an increase in the surface area of the cell membrane. Cell membrane acts as a good capacitor due to its extreme thinness and low electrical conductivity. Therefore, an increase in the surface area of a cell would increase its electrical capacitance. This line of reasoning is supported by the increase in the cell capacitance observed on day 3 (12.3±1.4 pF) and day 6 (21.2±2.6 pF) of culture compared to the day of isolation (4.4±1.1 pF) ($p<0.001$). The increase in cell capacitance on day 6 compared to day 3 was also significant ($p=0.002$) (Fig 4).

There was an increase in the peak current amplitude recorded at +60 mV on day 3 (849±365.8 pA) and day 6 (772±180.9 pA) of culture compared to day 0(510.8±241.8 pA) (Figure 5a). However, there was a drop in the peak current amplitude on day 6 compared to day 3 which was not statistically significant.

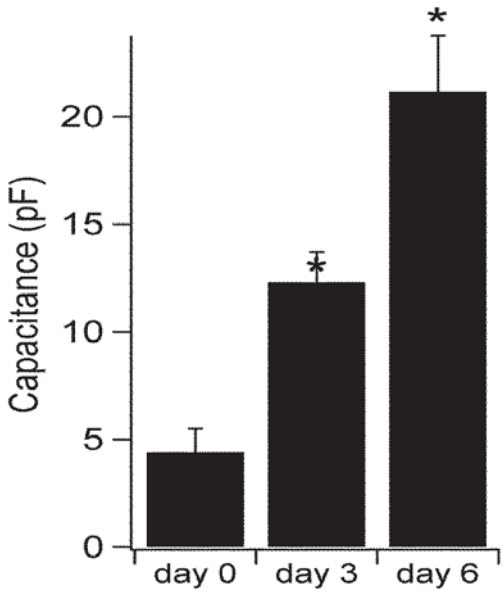

Fig. 4 : Cell capacitance during differentiation of monocytes in culture into monocyte derived macrophages. Capacitance of monocytes recorded on the day of isolation, third and sixth days during culture. The capacitance values are given as mean±SD. The cells had grown significantly larger during the culture as seen by their increase in cell capacitance. (n = 11 on day 0; n = 8 on day 3 and n = 6 on day 6). *p<0.001 with Mann-Whitney U test.

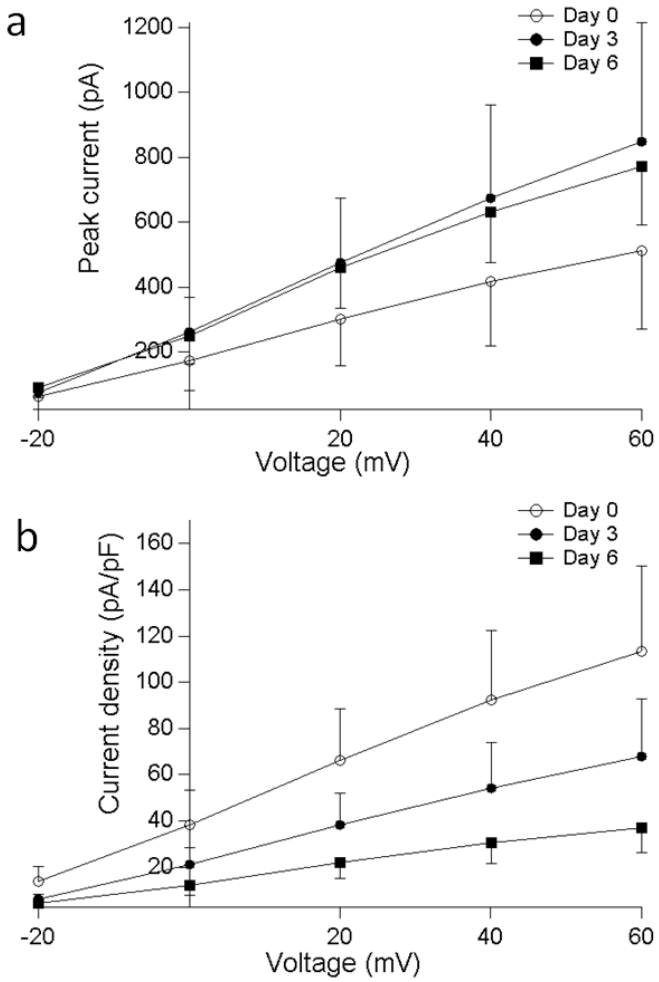

Fig. 5 : I-V relationship of proton currents in monocytes and monocyte derived macrophages a) The relationship between absolute current magnitude of proton currents and membrane voltage at day 0, 3 and 6 of culture. The currents recorded on day 3 and 6 were significantly larger than those recorded on the day of isolation. b) The relationship between proton current density and membrane voltage on three different days of culture (day of isolation, 3 and 6 days during culture). The current density showed significant reduction on day 3 and day 6 of culture as compared to the day of isolation ($p<0.05$ with Mann-Whitney U test). Proton currents were recorded with pHo = 7.5 and pHi = 6.0. (n = 11 on day 0; n = 8 on day 3 and n = 6 on day 6).

The peak current density at +60 mV was reduced to 60% and 32% on day 3 and day 6 of culture respectively, when compared to the current density on day of isolation ($p<0.05$) (Figure 5b).

Gating kinetics

The activation time constants for each voltage were obtained by fitting the raw current tracings with a single exponential curve. The activation time constants (τ_{act}) decreased progressively as the membrane voltage was stepped up to more depolarizing potentials, which indicates faster activation with depolarization (Figure 6a). The deactivation time constant (τ_{tail}) increased with depolarizing pulses showing slowing of proton channel deactivation at depolarizing voltages (Figure 6b). The activation kinetics was not significantly different before and after culture.

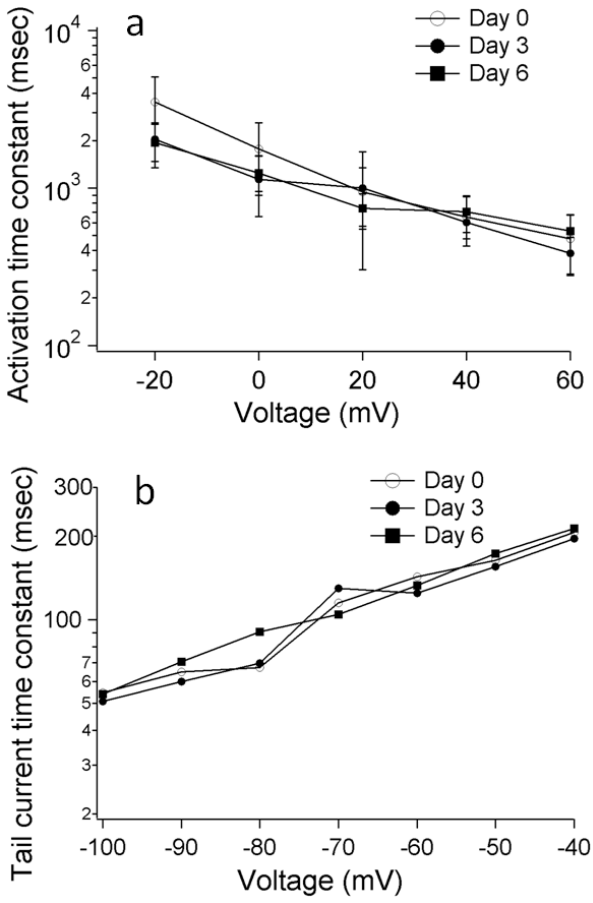

Fig. 6 : Activation and deactivation time constants of proton currents during differentiation of monocytes in culture to monocyte derived macrophages. The time constants were obtained by fitting raw current tracings with a single exponential curve. The y-axes are shown in logarithmic scale. a) The relationship between activation time constant of proton currents and membrane voltage on day 0, 3 and 6 during the culture. Data shown as mean±SD. Activation was observed to be faster with progressive membrane depolarization as shown by a decrease in activation time constant. No significant difference in channel activation was observed during culture. (n = 11 on day 0; n = 8 on day 3 and n = 6 on day 6). b) The tail current time constants plotted against membrane voltage. The figure shows 3 representative data from day 0, day 3 and day 6 of culture. Deactivation time constant increased with progressive membrane depolarization.

Discussion

Voltage gated proton currents have been previously described in THP-1 monocytes, which is considered to be a more differentiated cell line than peripheral blood monocytes (15,16). Differentiation of monocytes to macrophages is accompanied by changes in the phagocytic capacity and respiratory burst activity, which are closely related to the function of proton channels. This study aims to describe proton currents in freshly isolated human peripheral blood monocytes, and the changes that occur during their differentiation to macrophages in culture.

Proton currents in THP -1 monocytes and peripheral blood monocytes (13,14) has been described earlier and were found to be similar to proton currents described in other phagocytic cells in terms of their voltage dependence, kinetics and zinc sensitivity. In the present study, voltage-gated proton currents were studied in peripheral blood monocytes in whole-cell configuration. The threshold potential for the recorded outward currents was observed to be around –30 mV when the proton gradient between the pipette and bath solutions was 1.5 pH units with the proton gradient directed outward. This is comparable to the data previously published on proton channel activation in alveolar epithelial cells (18). The outward currents recorded were blocked by 0.5 mM Zn^{2+}, a known blocker of voltage-gated proton channels. At membrane potentials positive to +20mV, the outward currents exhibited a discernible droop towards the later phase of the voltage pulse. As the proton channels do not exhibit inactivation, the cause for the droop is probably intracellular proton depletion (19).

Proton channel conductance increased in a sigmoid fashion as the membrane voltage was progressively depolarized as seen in Figure 2b. Proton channel activation was found to occur faster with progressive membrane depolarization (Fig. 6a). Deactivation was hastened with progressive membrane hyperpolarization (Fig. 6b). Analysis of the tail current-voltage relationship showed that the reversal potential (E_{rev}) of the recorded outward currents was approximately –70 mV. The calculated H^+ reversal potential (E_H) was –90 mV (Fig. 3). This discrepancy between the observed and calculated reversal potentials could be due to the depletion of hydrogen ions on the intracellular side as a result of continuous proton efflux. This is expected to occur at the depolarizing membrane potential used to measure E_{rev}. The resultant alkalinization of the intracellular environment would shift the proton reversal potential to a less negative value as observed in the tail current-voltage relationship.

The peak proton current density at +60 mV was recorded in 21 freshly isolated peripheral blood monocytes. The peak current density was about 5 to 6 times higher in the freshly isolated human monocytes when compared to that reported in THP-1 monocytes in a previous study (14) (Fig. 5b). Though the THP-1 monocytes have many characteristics similar to peripheral blood monocytes, they are considered to be more differentiated cells than peripheral blood monocytes (15, 16). This could be the cause for the difference in density of voltage-gated proton currents between the two cell types. The activation time constant was 2 fold smaller in the peripheral blood monocytes when compared to that of THP-1 cells, which implies faster activation in PBMs.

Peripheral blood monocytes are known to differentiate into macrophages, when cultured in medium containing serum (20). Differentiation of monocytes to macrophages is associated with changes in phenotype and function (20). Changes in expression of ion channels have also been reported during differentiation. Expression of potassium channels has been found to change during differentiation of THP -1 cells into macrophages under the influence of PMA (21). Another study reports changes in density of proton currents during PMA-induced differentiation of monocytes to macrophages (14).

In the present study, monocytes were cultured for 6 days under appropriate culture conditions in the presence of FBS. Analysis of the cells under light microscopy revealed progressive increase in cell size, suggesting differentiation of monocytes into macrophages. However, this was not confirmed by functional or phenotypic assays. A significant and progressive increase in cell capacitance was noticed

during culture which corroborates the light microscopic finding of increase in cell size. The mean current amplitude increased on day 3 when compared to the day of isolation. However a small reduction in current magnitude was observed beyond day 3 in spite of a 1.7-fold increase in cell size from day 3 to day 6. The current density decreased on day 3 and day 6 of culture compared to the day of isolation at all recorded membrane voltages (Figure 5b). This suggests that density of voltage gated proton channels decreased on the monocyte membrane during their differentiation into macrophages. The results of the present study are consistent with the results of a previous study in which PMA-induced differentiation of THP-1 monocytes was accompanied by a reduction in the density of voltage gated proton currents to half (14).

The results of the present study indicate that proton current density decreases when peripheral blood monocytes differentiate into macrophages in culture. These results are comparable to the earlier reports on THP-1 monocytes. However, these findings are contrary to that found during granulocytic differentiation studied in PLB-985 cells, which was accompanied by a parallel increase in expression of Hv1 proton channels and Nox2 (an enzyme of the NADPH oxidase complex) (22). Phagocytic cells exhibit a correlation between the activities of the proton channel and the enzyme NADPH oxidase (23) that generates superoxide radicals during respiratory burst. PMA-induced activation of phagocytes activates NADPH oxidase and increases proton channel activity by enhancing their gating characteristics. Cells that express high levels of NADPH oxidase show increased gating enhancement of proton channels during PMA activation.

Differences have been reported between monocytes and macrophages in their capacity to generate superoxide radicals and efficiency to kill intracellular pathogens. The observed decrease in proton current density of monocytes upon differentiation can be explained by the comparatively lesser ability of macrophages to produce superoxide radicals (24). It suggests a probable decrease in the expression of NADPH oxidase during differentiation. However, this demands further experimentation and functional assays.

Conclusion

Differentiation of peripheral blood monocytes to macrophages in culture is accompanied by a decrease in proton current density, which could be related to functional changes accompanying differentiation. Differences exist between PBMs and THP-1 monocytes with respect to proton current amplitude and kinetics. Hence THP-1 monocytes may not be a perfect model to study electrophysiological characteristics of monocytes.

Acknowledgements

The authors are thankful to Christian Medical College Fluid Research Grant for funding this project.

Conflict of interest

None

References

1. Decoursey TE. Voltage-Gated Proton Channels and Other Proton Transfer Pathways. *Physiol Rev* 2003 Apr 1; 83(2): 475–579.
2. DeCoursey TE, Cherny VV. Potential, pH, and arachidonate gate hydrogen ion currents in human neutrophils. *Biophys J* 1993 Oct; 65(4): 1590–1598.
3. Schilling T, Gratopp A, DeCoursey TE, Eder C .Voltage-activated proton currents in human lymphocytes. *J Physiol* 2002 Nov 15; 545(Pt 1): 93–105.
4. Bánfi B, Schrenzel J, Nüsse O, Lew DP, Ligeti E, Krause KH, et al. A novel H(+) conductance in eosinophils: unique characteristics and absence in chronic granulomatous disease. *J Exp Med* 1999 Jul 19; 190(2): 183–194.
5. Musset B, Morgan D, Cherny VV, MacGlashan DW Jr, Thomas LL, Ríos E, DeCoursey TE. A pH-stabilizing role of voltage-gated proton channels in IgE-mediated activation of human basophils. *Proc Natl Acad Sci* USA 2008 Aug 5; 105(31): 11020–11025.
6. DeCoursey TE. Hydrogen ion currents in rat alveolar epithelial cells. *Biophys J* 1991 Nov; 60(5): 1243–1253.
7. DeCoursey TE. Voltage-gated proton channels find their dream job managing the respiratory burst in phagocytes. Physiology (Bethesda). 2010 Feb; 25(1): 27–40.

8. Femling JK, Cherny VV, Morgan D, Rada B, Davis AP, Czirják G Enyedi P, England SK, Moreland JG, Ligeti E, Nauseef WM, DeCoursey TE. The antibacterial activity of human neutrophils and eosinophils requires proton channels but not BK channels. *J Gen Physio* 2006 Jun; 127(6): 659–672.
9. DeCoursey TE, Morgan D, Cherny VV. The voltage dependence of NADPH oxidase reveals why phagocytes need proton channels. *Nature* 2003 Apr 3; 422(6931): 531–534.
10. Okochi Y, Sasaki M, Iwasaki H, Okamura Y. Voltage-gated proton channel is expressed on phagosomes. *Biochem Biophys Res Commun* 2009 May 1; 382(2): 274–279.
11. Morgan D, Capasso M, Musset B, Cherny VV, Ríos E, Dyer MJS, et al. Voltage-gated proton channels maintain pH in human neutrophils during phagocytosis. *Proc Natl Acad Sci* USA. 2009 Oct 20; 106(42): 18022–18027.
12. Wu L-J, Wu G, Akhavan Sharif MR, Baker A, Jia Y, Fahey FH, Luo HR, Feener EP, Clapham DE. The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. *Nat Neurosci* 2012 Mar 4; 15(4): 565–573.
13. Musset B, Cherny VV, DeCoursey TE. Strong glucose dependence of electron current in human monocytes. *Am J Physiol Cell Physiol* 2012 Jan 1; 302(1): C286–C295.
14. DeCoursey TE, Cherny VV. II. Voltage-activated Proton Currents in Human THP-1 Monocytes. *J Membr Biol* 1996 Jul; 152(2): 131–140.
15. Auwerx J. The human leukemia cell line, THP-1: a multifacetted model for the study of monocyte-macrophage differentiation. *Experientia* 1991 Jan 15; 47(1): 22–31.
16. Daigneault M, Preston JA, Marriott HM, Whyte MKB, Dockrell DH. The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages. PLoS One 2010 Jan 13; 5(1): e8668.
17. Thomas F. Tedder and Paul.J.Jansen . Immunologic Studies in Humans. Supplement 23. *Curr Protoc Immunol* 1997. 7.32.1.
18. Cherny VV, Markin VS, DeCoursey TE. The voltage-activated hydrogen ion conductance in rat alveolar epithelial cells is determined by the pH gradient. *J Gen Physiol* 1995 Jun; 105(6): 861–896.
19. DeCoursey TE. Voltage-gated proton channels: what's next? *J Physiol* 2008 Nov 15; 586(Pt 22): 5305–5324.
20. Zuckerman SH, Ackerman SK, Douglas SD. Long-term human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocyte-macrophage cell cultures. *Immunology* 1979 Oct; 38(2): 401–411.
21. DeCoursey TE, Kim SY, Silver MR. III. Ion Channel Expression in PMA-differentiated Human THP-1 Macrophages. *J Membr Biol* 1996 Jul; 152(2): 141–157.
22. Petheo GL, Orient A, Baráth M, Kovács I, Réthi B, Lányi A, Rajki A, Rajnavölgyi E, Geiszt M. Molecular and functional characterization of Hv1 proton channel in human granulocytes. PloS One 2010 Nov 23;5(11): e14081.
23. Musset B, Cherny VV, Morgan D, DeCoursey TE. The intimate and mysterious relationship between proton channels and NADPH oxidase. *FEBS Lett* 2009 Jan 5; 583(1): 7–12
24. Vulcano M, Dusi S, Lissandrini D, Badolato R, Mazzi P, Riboldi E, Borroni E, Calleri A, Donini M, Plebani A, Notarangelo L, Musso T, Sozzani S. Toll receptor-mediated regulation of NADPH oxidase in human dendritic cells. *J Immunol* 2004 Nov 1; 173(9): 5749–5756.