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Patch-Clamp Studies in Human Macrophages: Single-Channel and Whole-Cell Characterization of Two K^+ Conductances

Elaine K. Gallin and Leslie C. McKinney
Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814-5145

Summary. Human peripheral blood monocytes cultured for varying periods of time were studied using whole-cell and single-channel patch-clamp recording techniques. Whole-cell recordings revealed both an outward K current activating at potentials >20 mV and an inwardly rectifying K current present at potentials negative to −60 mV. Tail currents elicited by voltage steps that activated outward current reversed near E_K, indicating that the outward current was due to a K conductance. The *I-V* curve for the macroscopic outward current was similar to the mean single-channel *I-V* curve for the large conductance (240 pS in symmetrical K) calcium-activated K channel present in these cells. TEA and charybdotoxin blocked the whole-cell outward current and the single-channel current. Excised and cell-attached single-channel data showed that calcium-activated K channels were absent in freshly isolated monocytes but were present in >85% of patches from macrophages cultured for >7 days. Only 35% of the human macrophages cultured for >7 days exhibited whole-cell inward currents. The inward current was blocked by external barium and increased when $[K]_o$ increased. Inward-rectifying single-channel currents with a conductance of 28 pS were present in cells exhibiting inward whole-cell currents. These single-channel currents are similar to those described in detail in J774.1 cells (L.C. McKinney & E.K. Gallin, *J. Membrane Biol.* **103**:41–53, 1988).

Key Words: potassium · patch clamp · K conductance · macrophage · ion channel

Introduction

Several voltage-dependent K conductances have been described in macrophages (Gallin & McKinney, in press). These include 1) an inwardly rectifying K conductance described in long-term cultures (5 to 30 days) of mouse peritoneal and spleen macrophages (Gallin, 1981; Gallin & Livengood, 1981), and adherent J774.1 cells (a mouse-derived macrophage-like cell line) (Gallin & Sheehy, 1985), 2) an outwardly rectifying K conductance in J774 cells 1 to 4 hr following adherence (Gallin & Sheehy, 1985) and in mouse peritoneal cells cultured for 1 to 5 days (Ypey & Clapham, 1984), and 3) a calcium-activated K conductance described at the single-channel level in long-term cultured human macrophages (Gallin, 1984; McCann, Keller & Guyre, 1987). These data imply that the K conductances expressed by the macrophage are different depending on the source of the macrophage, the culture conditions and the age of the cells. These differences are not surprising in view of the large variations in function that have been described in macrophages from different sources (Van Furth, 1984).

This study characterizes, on both the single-channel and the whole-cell levels, two voltage-dependent K conductances present in human monocytes that have been grown in tissue culture for varying periods of time. An outward current is described which has a similar voltage dependence and pharmacology to the calcium-activated K single-channel currents previously described (Gallin, 1984). Single-channel experiments indicate that this conductance is not present in freshly isolated peripheral blood monocytes but is expressed after several days of culture. In addition, we demonstrate for the first time that about one-third of human macrophages cultured for >7 days exhibit an inwardly rectifying K conductance similar to that described in mouse macrophages (Gallin, 1981; Gallin & Sheehy, 1985).

Materials and Methods

Cells

Human peripheral blood monocytes were isolated by density centrifugation on Ficoll-Hypaque® and Percoll® gradients (Metcalf et al., 1986). Cells were cultured either directly on glass cover slips or in Teflon® jars for several days and then plated. All cells were grown in RPMI 1640 supplemented with 5% glutamine, 5% fetal bovine serum, and 100 U/ml of penicillin-streptomycin. After varying times in culture the glass cover slip con-

taining macrophages was placed in a recording chamber containing 1 ml of recording solution (*see below*). Recordings were done at room temperature (21 to 25°C).

Patch-Clamp Techniques

Patch electrodes were prepared as previously described (McKinney & Gallin, 1988). Seal resistances ranged from 10 to 50 GΩ. Either an EP7 (List Instruments, West Germany) or an Axopatch (Axon Inst., Calif.) patch-clamp amplifier was used in these studies. Voltage pulses were generated by a computer and ramp stimuli by a Kron-Hite (Avon, Mass.) model 5200A waveform generator. During ramp stimuli voltages were changed from a pipette potential of 0 to 100 mV, then to −100 mV and back to 0 mV. Single-channel data were filtered at 500 Hz (−3 dB) with a 4-pole bessel filter and recorded on an FM tape recorder (bandwidth 0 to 5 kHz). Data were digitized off line at 1000 Hz using an Indec systems unit (Sunnyvale, Calif.) and a PDP 11/23 computer. Amplitude histograms showing open and closed current levels were constructed from single-channel current records 33 or 66 sec in duration. Analytical methods are described in the preceding paper (McKinney & Gallin, 1988).

For whole-cell recordings data were digitized on line at 500 Hz. In most recordings, capacitance and series resistance were compensated electronically, although a small residual capacitative transient sometimes remained. In the studies where capacitance and series resistance were not compensated currents were <1.5 nA. Since series resistance (measured directly from the amplifier after capacitance compensation) ranged from 3 to 8 MΩ the maximum possible error for the studies in which no compensation was used was 12 mV. In some cases, residual capacitative and leak currents were eliminated from current tracings by subtracting appropriately scaled current responses of the opposite polarity. When the current peaked rapidly peak current amplitude was measured after the settling of the capacitative transient.

The zero-current potential measured immediately (~30 sec) after obtaining the whole-cell configuration was taken to be a reasonable estimate of the resting membrane potential (resting V_m). Resting V_m for monocytes cultured for 11 to 15 days was −51 ± 3 mV (n = 27). Cells were held within several mV of the zero-current potential and stepped to varying potentials every 4 to 8 sec unless otherwise noted. Membrane resistance measured from steps to −40 or −60 mV was 1.8 ± 0.2 GΩ (n = 25). No consistent shift in the voltage at which the inward or outward current activated was noted during the first 10 min of recording. In some cases an increase in the magnitude of the outward currents was evident during the first 5 min of recording, presumably due to the high (3×10^{-6} M) $[Ca]_i$ in the pipette solution. Recordings were usually stable for 10 to 25 min.

Membrane capacitance was calculated by integrating the capacitative transients recorded in the absence of filtering. Human monocytes increase in size in culture so that by 11 to 14 days in culture cells were ~22 μ in diameter, compared to 10 to 14 μ in diameter when first isolated. Capacitance measurements ranged from 40 to 100 pF and averaged 79 pF. The value for the specific membrane capacitance of the macrophage membrane calculated from the surface area of a 22 μ sphere and the cell capacitance was 5.2 μF/cm², 5 times greater than the expected 1 μF/cm² value for the capacitance of most biological membranes. Thus the value of membrane surface area is probably an underestimate. This may be due to the prominent membrane infoldings evident in scanning electron micrographs of macrophages.

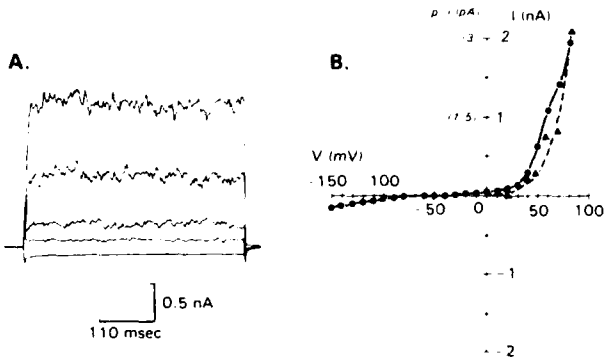

Fig. 1. (*A*) Whole-cell currents from a cell grown in tissue culture for 10 days. Patch electrode contained 3×10^{-6} M [Ca], in KCl Hanks'. Input resistance of cell measured for step to −40 mV was 1.6×10^{9} Ohms. Capacitance = 83 pF. V_h = −33 mV. Voltage steps to 20, 40, 60, 80, and −140 mV. (*B*) Peak *I-V* relation from cell in 1(*A*). Dashed line represents mean single-channel current [steady state open probability (from Fig. 3*B*) × single-channel current) obtained from fourteen excised inside-out patches with NaCl Hanks' in electrode and KCl Hanks' (3×10^{-6} M Ca) in bath

Solutions

NaCl Hanks' contained (in mM) 150 NaCl, 4.5 KCl, 1.6 $CaCl_2$, 1.13 $MgCl_2$, and 10 HEPES, pH 7.3. KCl Hanks' contained 150 KCl, 10 NaCl, 1.13 $MgCl_2$, 10 HEPES, 1.1 EGTA and varying concentrations of calcium, pH 7.2. For final ionized calcium levels of 3×10^{-6} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M, the calcium concentrations were 1.07, 1.0, 0.55 and 0.1 mM, respectively. In studies where higher $[Ca]_i$ levels were required both EGTA and calcium were omitted, yielding $[Ca]_i$ levels (assessed with a calcium-sensitive electrode (WPI, Conn.)) of ~10^{-5} M. In a few studies, equimolar Kisethionate was substituted for KCl.

Tetraethylammonium chloride (TEA) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Partially purified charybdotoxin, obtained from Dr. C. Miller, was diluted in the appropriate Hanks' solution to a concentration of approximately 50 nM.

Results

Calcium-Activated Potassium Conductance

Whole-Cell Outward Currents

Stable (10 to 20 min) whole-cell currents could not be obtained routinely from freshly isolated or 1- to 2-day cultured human monocytes. The incidence of successful whole-cell recordings increased with time in culture. Therefore the whole-cell currents described in this paper were done on monocytes grown in culture for at least 5 days. Figure 1(*A*) shows the whole-cell currents of a human macro-

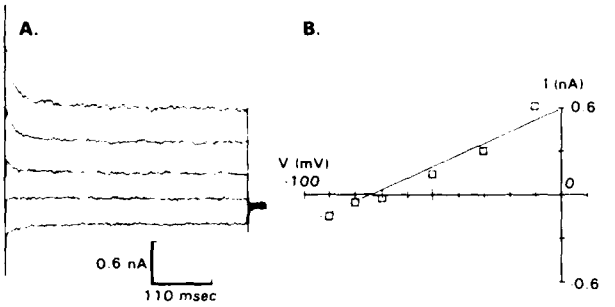

Fig. 2. (*A*) Tail currents. V_h = −75 mV. Cell was stepped to 50 mV for 200 msec and then to −10, −30, −50, −70 and −90 mV. (*B*) *I-V* relationship of tail currents was measured as difference in current 10 msec after initiation and 10 msec before the end of second step

phage cultured for 10 days. Voltage steps of >20 mV elicited outward currents characterized by a noisy baseline and no time-dependent inactivation. Hyperpolarizing voltage steps (−80 to −150 mV) produced small inward currents that were only slightly greater than the leak currents measured at −50 mV. The *I-V* relationship of this cell (solid line in Fig. 1*B*) shows prominent outward rectification.

In order to determine the reversal potential of the outward current, tail currents were measured in three different cells exhibiting large (~5 nA) outward currents. Cells were first stepped to potentials that activated the outward current (either 50 or 70 mV) and then stepped to a series of potentials ranging from −10 to −90 mV (Fig. 2*A*). Figure 2(*B*) plots tail current amplitude versus potential of the second step; the resulting *I-V* curve reverses at a potential of −75 mV. Similar data were obtained from two other cells. Under these recording conditions, calcium and sodium have positive reversal potentials, and the chloride reversal potential is 0 mV. The only ion with a negative reversal potential is potassium (E_K = −85 mV), providing strong evidence that the outward current must be carried predominantly by K.

Single-Channel Outward Currents

Previous studies in this laboratory have demonstrated that human macrophages grown in tissue culture express large conductance K channels that are activated by both intracellular calcium and voltage (Gallin, 1984). In 150 mM symmetrical KCl the *I-V* relationship and channel conductance is 240 pS. Under conditions of asymmetric K (150 mM $[K]_i$ and 150 $[Na]_o$) the *I-V* curve is nonlinear, single-channel conductance is reduced and the reversal potential shifts towards E_K (Gallin, 1984). This usually is the most prevalent channel present at depolarized potentials in patches of membrane from cultured (5 to 30 days) macrophages.

In order to compare the voltage dependence of this channel with that of the macroscopic outward current shown in Fig. 1(*A*), the steady-state channel open probability was determined in excised inside-out patches under ionic conditions which were similar to the whole-cell recordings (the patch electrode and bath contained NaCl Hanks' and KCl Hanks' [3 × 10^{-6} M calcium], respectively). Amplitude histograms were obtained from current records of 14 different patches (4 patches contained 1 channel, 7 patches contained 2 channels, 2 patches contained 3 channels and 1 patch contained 5 channels). Two representative histograms from a single patch are shown in Fig. 3(*A*). It was assumed that the channels behaved independently. Therefore, the open-state probability (p) was calculated from the equation:

$$p = \sum_{n=0}^{N} n \cdot Pn/N \qquad (1)$$

where Pn (the area under peak n) is the probability that n channels are open simultaneously, and N is the number of channels in the patch. The open-state probability plot is shown in Fig. 3(*B*). Before using these data to calculate the mean single-channel amplitude ($p \cdot i$), p was estimated by fitting the data to a sigmoidal equation:

$$p(V) = A + B/(1 + \exp(-(V - C)/D)) \qquad (2)$$

where A is the minimum open-state probability, $A + B$ is the maximum open-state probability, C is the midpoint of the curve, and D is the slope. These values were 0.001, 1.0, 98 and 12, respectively.

There was considerable variability between patches. For example, open-state probability at 60 mV ranged from 0.03 to 0.24. There was no correlation between the open-state probability at a given potential and the age of the macrophage in the 14 patches studied. Despite the variability, it is apparent that depolarization beyond 30 mV was needed to significantly activate the channels even though $[Ca]_i$ (3×10^{-6} M) was considerably higher than the $[Ca]_i$ values reported in resting phagocytes (Stickle, Daniele & Holian, 1984; Young, Ko & Cohn, 1984). In several studies in which $[Ca]_i$ was elevated to ~10^{-5} M, large increases in open-state probability were noted. At potentials of 20 and 40 mV, p increased to 0.21 and 0.75, respectively.

The voltage dependence of the single-channel current is compared with whole-cell data in Fig.

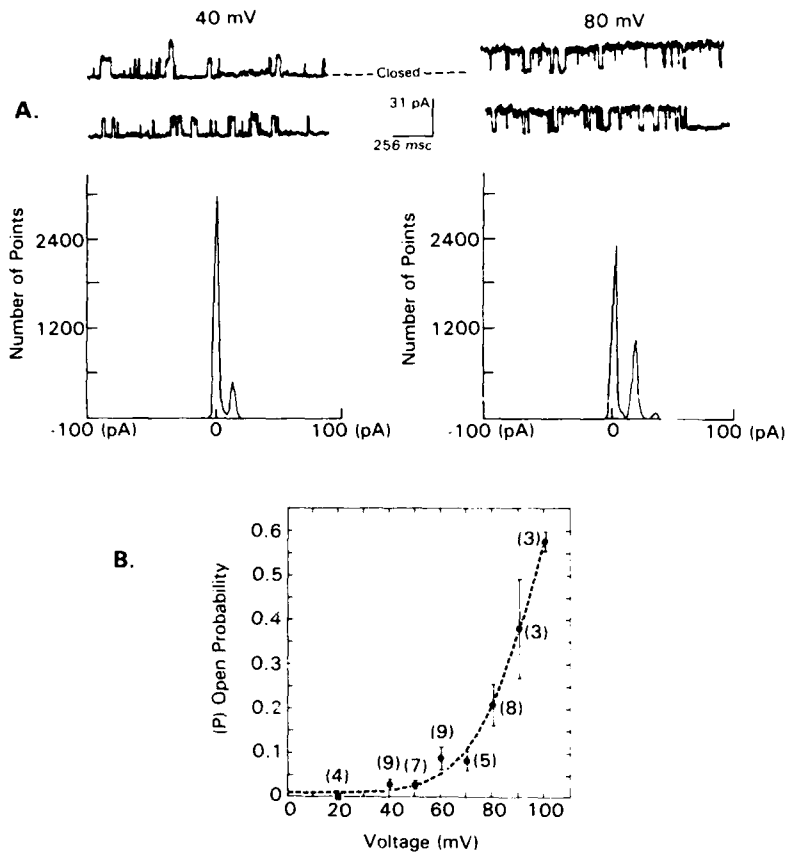

Fig. 3. (*A*) Single-channel activity from an excised inside-out patch from a cell cultured for 12 days. Electrode and bath contained NaCl Hanks' and KCl Hanks' 3×10^{-6} M $[Ca]_i$, respectively. Amplitude histograms determined for 60 sec of data at each potential are depicted under representative current tracings. Single-channel current records were leak subtracted. (*B*) Steady-state open probability versus voltage recorded under same conditions as in (*A*). Data points are mean ± SEM. Number of patches used to determine each point shown in parentheses. Curve fit to a sigmoidal relationship had a multiple R square of 0.99

1(*B*). The dashed line is a plot of the mean single-channel current amplitude ($p \cdot i$) calculated from the open-state probability (Fig. 3*B*) and the single-channel current amplitude. It is evident that the single-channel and the whole-cell currents have similar voltage dependencies. The number of channels in the cell depicted in Fig. 1 can be estimated from the relationship $I(V) = N \cdot p(V) \cdot i(V)$ where I is the whole-cell current amplitude at potential V, and N is the number of channels. The cell had approximately 660 channels.

Effect of TEA

The effect of TEA, which is a well-known blocker of large conductance calcium-activated K channels in other tissues (Iwatsuki & Petersen, 1985; Guggino, et al., 1987), was studied on the single channel and the macroscopic outward currents. Whole-cell outward currents were measured before and after bath addition of TEACl (15 to 20 mM) in 11 different cells. Data from one of these cells are shown in Fig. 4(*A*). The outward current for a step to 80 mV in the presence of TEACl was reduced by >90% whereas leak or inward currents were only slightly affected. Similar results were obtained in the other 10 cells studied.

Figure 4(*B*) depicts data from an experiment in which currents from calcium-activated K channels were recorded from a cell-attached patch before and after the electrode was perfused with TEACl (10 mM). Channel activity was abolished after exposure to TEACl. Because perfusion of the electrode often led to disruption of the patch, related studies were done in which channel activity was assessed in cell-attached patches with and without TEACl in the electrode. Eight patches obtained without TEACl in the electrode contained calcium-activated K channel activity (with 2 to 4 channels per patch). No large outward current fluctuations were present in the five patches obtained with electrodes containing 5 mM TEACl. Exposure of the inner surface of the membrane of excised inside-out patches to TEACl (5 mM) did not block channel activity, although there was a slight (10 to 20%) decrease in the channel conductance, similar to that reported in other preparations (Iwatsuki & Pererson, 1985).

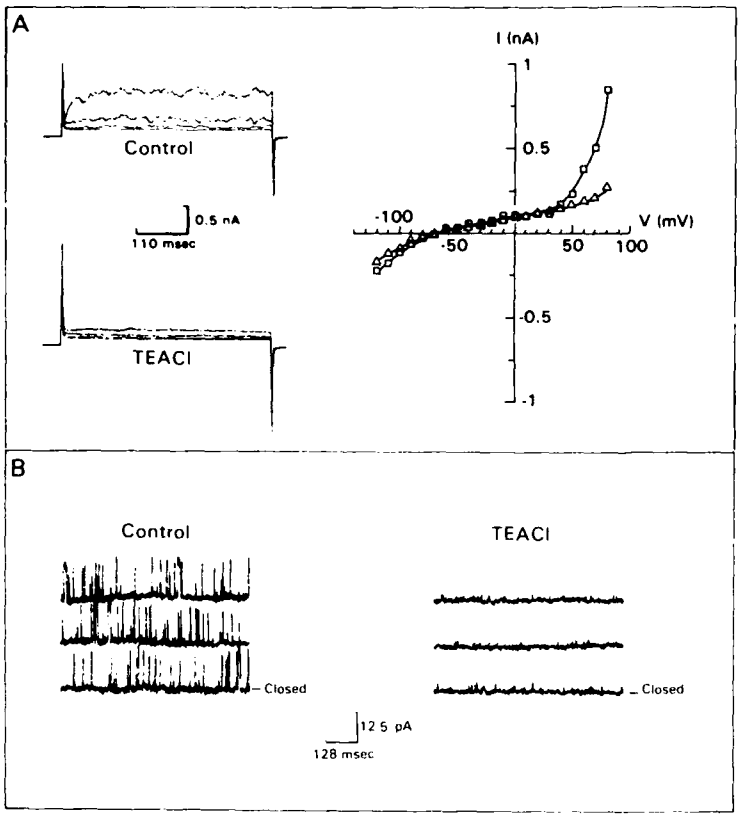

Fig. 4. (*A*) Effect of TEA on whole-cell currents. Macrophage was cultured for 7 days. V_h = −60 mV. Voltage steps were to 20, 40, 60, and 80 mV before and after TEACl (15 mM). *I-V* relationship before (□) and after (△) addition of TEACl. (*B*) Effect of TEACl on calcium-activated K channels. Cell-attached patch from a human macrophage cultured for 21 days before and after electrode was perfused with 10 mM TEACl. Bath and electrode contained KCl Hanks' solution. V_h = 80 mV

Effect of Charybdotoxin

Charybdotoxin, isolated from scorpion venom, is a high-affinity (K_d ~ 10 nM) blocker of the large conductance calcium-activated K channel (Miller et al., 1985, Guggino et al., 1987). The effects of charybdotoxin on whole-cell currents from a macrophage are shown in Fig. 5(*A*). Outward current was present for voltage steps to 70 and 90 mV. After the addition of charybdotoxin to the bath (~25 nM), the outward current was almost completely eliminated. This cell also exhibited inward currents for hyperpolarizing step to −100 mV or more negative. As shown in the *I-V* curves in Fig. 5(*A*), the inward current was not significantly affected by the addition of charybdotoxin. Similar results were obtained in two other experiments.

In other studies we tested the effect of charybdotoxin on single-channel currents. Excised inside-out patches were bathed in symmetrical KCl with the inside of the membrane exposed to 3×10^{-6} M $[Ca]_i$. Patches were obtained from human macrophages grown in tissue culture for at least one week. Three to four channels were present in each of seven patches in which the electrode contained only KCl Hanks' (Fig. 5*B*). In five other patches, done under the same ionic conditions, charybdotoxin (~25 nM) was present in the electrode. In three of these patches channel activity was absent at all potentials, while in two patches, channel openings were evident only at potentials >90 mV (Fig. 5*B*). The maximum channel amplitude at the holding potential of 100 mV corresponded to the channel size expected for the calcium-activated K channel under these conditions. However, no measurement of conductance was made in the presence of charybdotoxin since channels often did not open fully and were present only at extremely depolarized potentials. Exposure of the inside surface of the patch membrane to charybdotoxin did not affect channel activity.

Expression of the Calcium-Activated K Channel

Human peripheral blood monocytes grown in tissue culture differentiate into macrophages during the first week in culture (Bainton & Golde, 1978). In order to determine whether calcium-activated K channel expression changes during differentiation

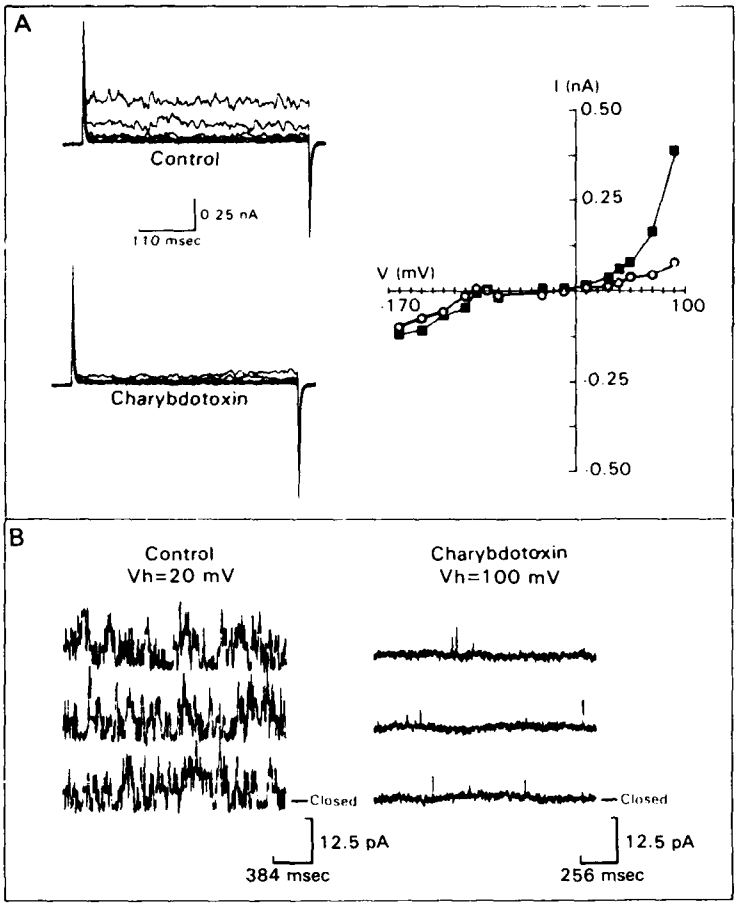

Fig. 5. (*A*) Effect of charybdotoxin on whole-cell currents. Outward current from a macrophage cultured for 4 weeks before and after addition of ~25 nM charybdotoxin. V_h = −40 mV. Voltage steps to 30, 40, 50, 70, and 90 mV. *I-V* relationship before (■) and after (○) addition of charybdotoxin. (*B*) Effect of charybdotoxin on calcium-activated K channels. Data from two excised inside-out patches in symmetrical KCl Hanks'. Inside surface of membranes was exposed to solution containing 3 × 10 $^{-6}$ M [Ca]. Charybdotoxin (25 nM) was present in electrode for tracings on the right

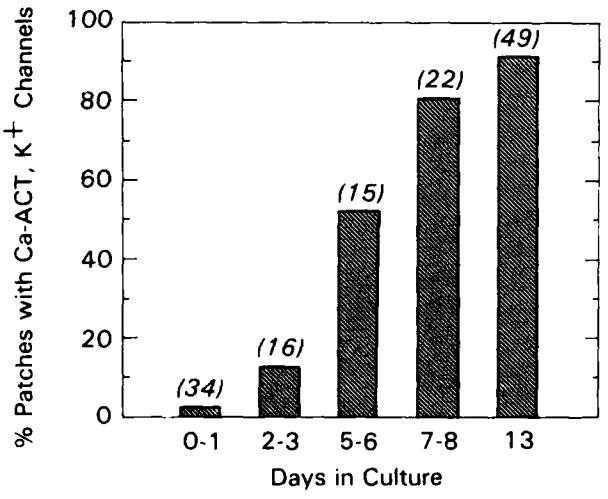

Fig. 6. Relationship between time in culture and presence of Ca-activated K channels. Data are from excised inside-out patches in symmetrical KCl with 3 × 10 $^{-6}$ M [Ca] in bath. Number of patches in each group are indicated in parentheses

cell-attached and excised inside-out patches were obtained from human monocytes cultured for differing periods of time. Only 1 out of 32 cell-attached patches from macrophages cultured for 24 hr or less contained calcium-activated K channels in response to depolarizing voltage steps. In contrast, 92% of cell-attached patches from monocytes cultured for >13 days exhibited these channels. In cell-attached patch experiments, the resting potential and $[Ca]_i$ (two parameters that influence channel activity) were uncontrolled, and may vary in cells cultured for different times. Therefore, experiments also were done on excised inside-out patches in symmetrical KCl with 3 × 10^{-6} M $[Ca]_i$. Under these recording conditions only one in 34 patches from cells cultured for <24 hr contained a calcium-activated K channel while 89% of the patches from cells cultured for >13 days had calcium-activated K channels. The data summarized in Fig. 6 indicate that the percentage of patches with calcium-activated K

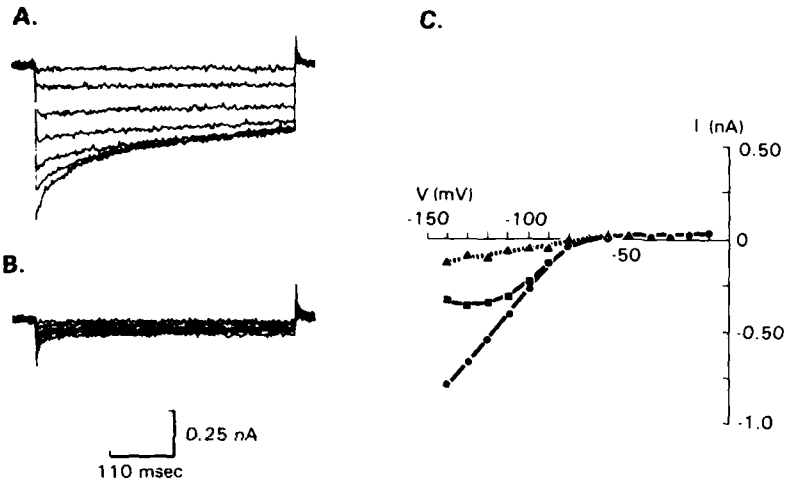

Fig. 7. (*A*) Currents recorded from a human macrophage cultured for 18 days. $V_h = -70$ mV. Input resistance for voltage step to −30 mV was 4×10^9 Ohms. Steps to −100, −110, −120, −130, and −140 mV before (*A*) and after (*B*) addition of 2 mM BaCl. (*C*) (●) Peak and (■) steady-state *I-V* curves. Dashed line shows peak current after BaCl

channel activity increased over the first week in culture.

To determine if the expression of calcium-activated K channels in human macrophages is affected by adherent versus suspended growth conditions monocytes were grown either in Teflon® dishes (nonadherent conditions) or in adherent conditions for 2 weeks. Teflon-grown cells were added to the recording chamber, and patch clamped within minutes of adherence and the recordings were compared to those obtained from adherent cells. Calcium-activated K channels were present in both groups of cells, indicating that adherent growth conditions were not required for channel expression.

Inwardly Rectifying Current

Whole-Cell Inward Currents

Approximately one-third of the cells studied under whole-cell recording conditions exhibited prominent ($G > 0.6$ nS at −110 mV) inward currents in responses to hyperpolarizing voltage steps. The current tracings and *I-V* curves from one of these cells are shown in Fig. 7. No outward currents were evident for voltage steps to 30 mV. Hyperpolarizing voltage steps produced inward currents. Time-dependent inactivation, typical of inward-rectifying K currents (Gallin & Sheehy, 1985; Leech & Stanfield, 1981), was evident for voltage steps to −100 mv or more negative. The ratio of the inward current measured at the peak of the current and the end of the voltage step declined from 0.81 to 0.37 between −100 and −140 mV, respectively (Fig. 7).

Previously we stated that the inward-rectifying conductance is important in setting the resting V_m of the macrophage (Gallin & Livengood, 1981; Gallin & Sheehy, 1985). The V_m of cells exhibiting prominent ($G > 0.6$ nS) inward rectification was compared to cells recorded on the same day that showed little or no inward rectification. Seventeen cells exhibiting inward-rectifying conductances (ranging from 0.7 to 3.8 nS) had an average resting V_m of -56 ± 4 mV while 19 cells in which this conductance was absent or quite small had an average resting V_m of -42 ± 2.5 mV.

Effect of Barium

The inward-rectifying K current in mouse peritoneal macrophages (Gallin & Livengood, 1981), the macrophage-like cell line J774 (Gallin & Sheehy, 1985) and other cells (Standen & Stanfield, 1978) is blocked by external barium. Figure 7 shows the *I-V* relationships for inward current in a human macrophage before and after addition of barium to the bath. The inward current was completely blocked whereas leak current was unaffected.

Effect of $[K]_o$

A characteristic of the inward-rectifying conductance is that the inward current increases with the square root of $[K]_o$. Figure 8 depicts inward currents from a cell in which $[K]_o$ was reduced from 148 to 31 mM. In 148 mM K/10 mM Na, currents were large and inactivation was largely absent, even for steps to −180 mV (Fig. 8*A*). In contrast, in 31 mM K/134 mM Na the inward currents were smaller, and inactivation was prominent for steps more negative than −120 mV. The peak *I-V* curve obtained

in each solution is plotted in Fig. 8(*C*). The whole-cell conductance was 17 nS in 31 mM $[K]_o$ and 26 nS in 148 mM $[K]_o$. The data agree with previous findings in the macrophage-like cell line, J774.1, that the inward-rectifying K conductance is proportional to the square root of $[K]_o$ (Gallin & Sheehy, 1985).

Single-Channel Inward Currents

Inward single-channel current fluctuations were observed in 37% of the cell-attached patches from human macrophages cultured for 7 to 20 days (Fig. 9).

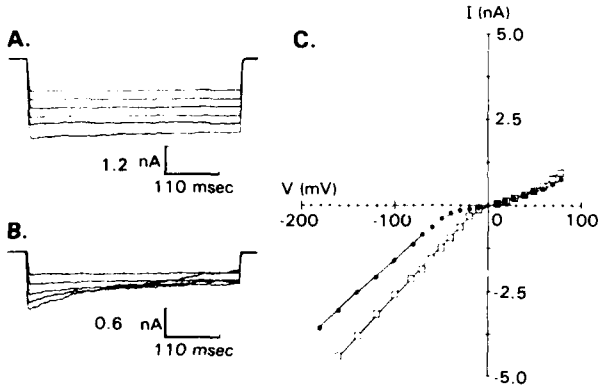

Fig. 8. Effect of $[K]_o$ on inward currents. Macrophage cultured for 8 days. V_h = 0 mV. Currents are leak subtracted. Steps to −80, −100, −120, −140, −160, and −180. (*A*) Currents were recorded in medium containing 148 mM KCl/10 mM NaCl. (*B*) Currents were recorded in media containing 31 mM KCl/134 mM NaCl. (*C*) Peak *I-V* curves for cell shown in (*A*) (□) 148 mM K, and in (*B*) (●) 31 mM K

Currents were observed at zero or negative pipette potentials and did not reverse. The single-channel *I-V* curve for the patch is shown in Fig. 9(*A*). The zero-current potential of this cell was not determined so the voltages represent the electrode holding potential only. Assuming the cell has a resting membrane potential of −51 mV (the average V_m) the channel reversal potential (E_{rev}) is 0 mV. Under these recording conditions, the equilibrium potential for $[K]_o$ will be close to zero, assuming $[K]_i$ is 158 mM. [The value of $[K]_i$ has not been measured in cultured human macrophages, therefore we used the value for $[K]_i$ in J774.1 cells (Sung et al., 1985).] The average channel conductance was 28 ± 1 pS (n = 12), similar to that reported for inwardly rectifying K channels in J774.1 cells (McKinney & Gallin, 1988).

Measuring the reversal potential of small channels, or channels that rectify can be difficult (*see* preceding paper by McKinney and Gallin). In addition to estimating the reversal potential by extrapolation of the *I-V* curve we also used voltage ramps (0.5 mV/sec) to determine the reversal potential. One of these studies is shown in Fig. 9(*B*). The resting potential of this cell was determined by breaking through the patch after the voltage ramp was completed. Therefore the voltage axis in Fig. 9(*B*) represents the true membrane potential across the patch. For clarity, the closed-current level was superimposed on the voltage axis and the open-current level was depicted by the dashed line. Inward currents that did not reverse polarity as the patch membrane was depolarized are evident in the ramp. The channel reversal potential determined from the intersec-

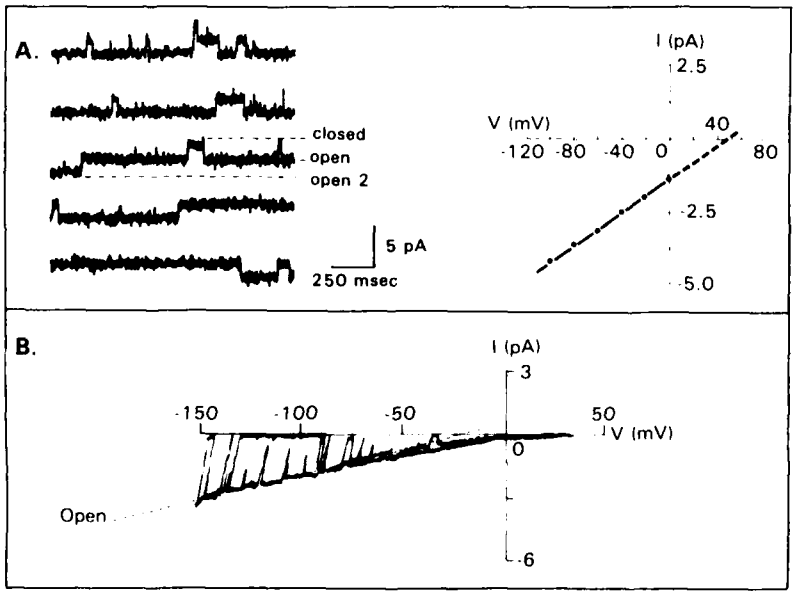

Fig. 9. Inward currents in cell-attached patches from human macrophages cultured for 20 and 22 days. Bath contained NaCl Hanks'; electrode contained KCl Hanks'. (*A*) Single-channel currents at holding potential, −70 mV. *I-V* relationship of single-channel currents shown on right. G = 28 pS. Potential represents electrode potential and does not include resting V_m. (*C*) *I-V* relationship from a different patch obtained with voltage ramp (0.36 mV/sec). Potential represents total potential across patch membrane and includes resting V_m which was −45 mV. Current trace was shifted so that the closed-current level was superimposed on the voltage axis. Dotted line was drawn through the open-current level between −150 to −100 mV. G determined from dotted line = 26 pS

tion of the closed- and open-current levels is close to 0 mV. In contrast, the dashed line superimposed on the open-current level in the region of −150 to −100 mV intersects the voltage axis at −20 mV rather than 0 mV. This is because there is a slight rectification of the *I-V* curve near the reversal potential.

To test for chloride permeability of the channel, several experiments were done with Kisethionate Hanks' in the patch electrode. Inward channels were present that had conductances of 28 pS and extrapolated reversal potentials of −10 and −8 mV. Assuming that isethionate is an impermeant anion, the results rule out chloride as a current carrier, since E_{Cl} under these recording conditions would be 30 mV [assuming $[Cl]_i \simeq 40$ mM (Melmed, Karaman & Berlin, 1981)].

Two representative current tracings at −80 and −100 mV, together with amplitude histograms, are shown in Fig. 10 for a cell-attached patch containing two channels. The actual potential across the patch membrane was determined from the electrode holding potential and the zero-current holding potential of the cell obtained after the channel recordings were completed. Channels are closed more at −100 mV than at −80 mV. These data along with additional data from the same patch at different holding potentials were used to determine the steady-state open probability (using Eq. 1) plotted in Fig. 10(*B*). As the potential was made more negative the open-state probability decreased, so that at −160 mV, it was only 10%.

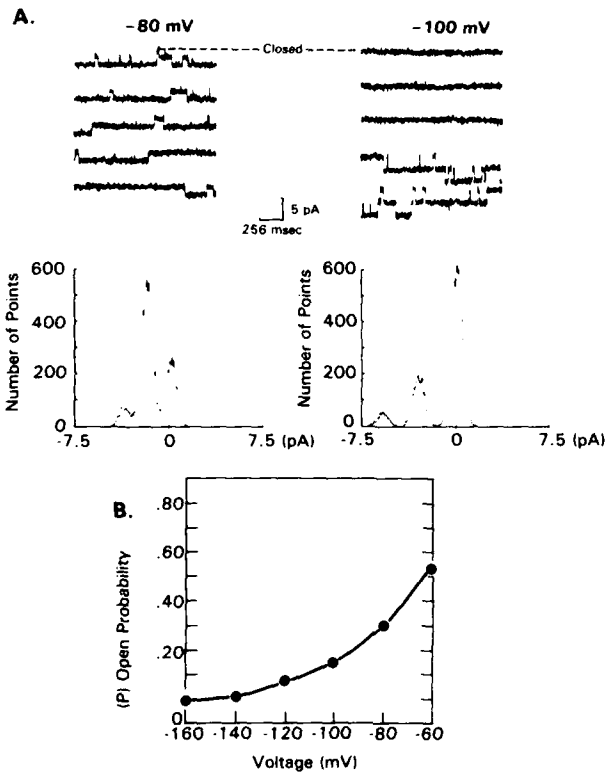

Fig. 10. (*A*) Inward currents in a cell-attached patch from a macrophage (20 days in culture) at membrane potentials of −80 and −100 mV. Ionic conditions were the same as in Fig. 9. Amplitude histograms (drawn as a line connecting individual points) for each potential obtained from 60 sec of channel data are shown under current tracings. (*B*) Steady-state open probability versus voltage for channels shown in (*A*)

Other Conductances

Two different amplitude single-channel outward currents ($G > 30$ and > 15 pS) sometimes were noted at depolarized potentials with KCl in the patch electrode. In several instances, patches were obtained that contained both of these small conductance channels as well as the calcium-activated K channel. If other outward currents exist in macrophage we would expect to see these under whole-cell recording conditions. However, only 2 out of >60 whole cell recordings revealed additional outward currents. In those two cases, inactivating outward currents that activated at voltages positive to −50 mV were seen. These currents were similar to those previously described in mouse peritoneal macrophages (Ypey & Clapham, 1984) and J774 cells (Sheehy & Gallin, 1985).

At negative or zero electrode potentials with KCl in the patch electrode and NaCl Hanks' in the bath, a second type of inward rectifying channel was recorded. The channel had a slightly larger single-channel conductance (ranging from 32 to 44 pS), than the 28 pS channel, did not reverse polarity and exhibited bursting activity. It was distinct from the inward 28 pS channel, occurring both in patches with and without the 28 pS channel. Preliminary experiments indicate that these channels can be activated by bath addition of the calcium ionophore, ionomycin (Gallin, 1988).

Discussion

Outward Calcium-Activated K Current

Previous patch-clamp studies of human macrophages have demonstrated the presence of large conductance calcium-activated K channels in human macrophages (Gallin, 1984). In this paper, we extend those observations to demonstrate whole-cell outward currents in macrophages that correspond to the activation of these channels. This view is supported by several findings. First, the reversal

potential of the tail currents indicated that the outward current was a K current. Second, as shown in Fig. 1(*B*), with similar $[Ca]_i$, the whole-cell *I-V* and the mean single-channel current ($p \cdot i$) *I-V* relationships have a similar voltage dependence, activating at voltages > 30 mV. In a few whole-cell studies, the $[Ca]_i$ was varied in an attempt to demonstrate the calcium sensitivity of the outward currents. Unfortunately there was so much variability in the amplitude of the outward currents from cell to cell that it was not possible to draw a correlation with $[Ca]_i$.

The $[Ca]_i$ sensitivity of calcium-activated K channels varies considerably in different cell types. For example, in rat skeletal muscle, the open-state probability at 20 mV for 3×10^{-6} M $[Ca]_i$ is 0.2 (Barrett, Magleby & Pallotta, 1982), while in pancreatic acinar cells, p is ~1.0 at 20 mV (Maruyama et al., 1983). In macrophages, although there was variability from patch to patch, this channel was quite insensitive to $[Ca]_i$, having an open-state probability of 0.004 at 20 mV in 3×10^{-6} M $[Ca]_i$. Increasing $[Ca]_i$ to $\sim 10^{-5}$ M increased p to 0.21 at 20 mV. Assuming that the channel in situ has the same $[Ca]_i$ and voltage sensitivity as it does in excised patches, it is likely to play a minor role in ionic regulation, functioning primarily when $[Ca]_i$ changes from 10^{-6} to 10^{-5} M. Alternatively, it is possible that the calcium sensitivity in situ can be modulated, or that these channels are important in intracellular compartments where calcium levels may be quite high (Klempner, 1985). However, the observation that calcium-activated K channels are not present in 0- to 1-day-old peripheral blood monocytes indicates that these channels are not required (on the cell surface) for the monocyte to carry out phagocytosis, chemotaxis, and secretory processes which occur in these cells.

Outward current was reduced by bath addition of either TEA or charybdotoxin at concentrations that blocked single-channel activity also supporting the view that calcium-activated K channels underly the outward current. In cells treated with charybdotoxin, a small residual outward current usually remained at very depolarized potentials. Single calcium-activated K channel activity also was present at very positive voltages in the presence of charybdotoxin (Fig. 5). These two observations are consistent with studies in bilayers indicating that at high $[K]_i$ there is a voltage-dependent knockoff of charybdotoxin from calcium-activated K channels (MacKinnon & Miller, 1987). It is less clear why a small residual outward current sometimes remained following the addition of TEA (10 mM) since similar concentrations of TEA completely blocked the single channels. One possibility is that there is a small TEA-insensitive outward current in these cells.

Inward-Rectifying K Current

The inwardly rectifying whole-cell currents present in human macrophages are similar to those described in the macrophage-like cell line, J774 (Gallin & Sheehy, 1985; McKinney & Gallin, 1988) and mouse spleen and peritoneal macrophages (Gallin & Livengood, 1981). It is likely that the 28 pS channel described in this paper is responsible for the macroscopic inward current for several reasons. First, in cases where single-channel and whole-cell recordings were done on the same cell, the presence of 28 pS inwardly rectifying channels was always associated with inwardly rectifying whole-cell current. Second, the 28 pS single-channel currents described here are identical in conductance, and in the voltage dependence of activation and inactivation to the inwardly rectifying channels we characterized in detail in the preceding paper in J774 cells (McKinney & Gallin, 1988).

Human macrophages that express this conductance generally have a more negative resting V_m (−56 versus −42 mV), although not as negative as J774 cells (Gallin & Sheehy, 1985; McKinney & Gallin, 1988). This agrees with previous findings that the inward-rectifying K conductance was important in setting the resting V_m of mouse macrophages and the macrophage cell-line, J774 (Gallin & Livengood, 1981; Gallin & Sheehy, 1985). The rectifying *I-V* relationship of these cells enables them to have a resting V_m near E_K but remain sensitive to small depolarizing currents. Our value of −52 mV for the average resting V_m of cultured human peripheral blood monocytes agrees with that of Nelson et al. (1985) who reported an average resting V_m of −55 mV in human alveolar macrophages cultured for 1 day or longer, and −14 mV in freshly isolated cells.

Channel Expression

Human monocytes grown in tissue culture differentiate into macrophages over a period of 1 week. During this time changes occur in morphology (Zuckerman, Ackerman & Douglas, 1979) and function, although they are essentially a nondividing population (J. Sechler, *personal communication*). Functional changes include a decrease in peroxidase activity (Nichols, Bainton & Farqhuar, 1971), changes in surface antigens (Zwadlo et al., 1985), an increase in phagocytic ability (Wuest et al., 1981) and an initial increase followed by a decrease in H_2O_2 secreting ability (Nakagawara, Nathan & Cohn, 1981). It is thought that this differentiation process parallels the differentiation that normally

occurs when monocytes emigrate from the bloodstream into tissues. The data presented here provide evidence that an ionic conductance on the monocyte/macrophage surface also changes during this maturation period. That is, freshly isolated monocytes do not exhibit large conductance calcium-activated K channels whereas monocytes cultured for 5 days or longer do. The inward-rectifying channel was noted in only one-third of the patches from long-term cultured macrophages. Patches from cells cultured for 1–3 days frequently contained inwardly rectifying channels, but preliminary evidence indicates these are primarily the 32 to 44 pS channel; therefore, the expression of the 28 pS channel was not systematically investigated.

Other Currents

Under our whole-cell recording conditions the two K conductances described in this paper were the major voltage-dependent conductances present in human macrophages cultured for 5 to 25 days. Inactivating outward currents similar to those described in mouse peritoneal macrophages (Ypey & Clapham, 1984) and J774 cells (Gallin & Sheehy, 1985) were noted in only two cells. The single-channel small conductance outward currents present in some patches may be related to the inactivating outward currents seen in two of the cells. In a preliminary report, Nelson et al. (1986) reported a 4-aminopyridine-sensitive outward current activating at potentials above −30 mV in human macrophages cultured 1 to 10 days. However, the percentage of cells exhibiting this current was not discussed.

Our cell-attached patch single-channel recordings also revealed a second inwardly rectifying channel (when KCl is in the electrode and NaCl in the bath). We have not fully characterized this channel but it has a similar conductance (~36 pS) to a K channel described in human macrophages by McCann et al. (1987). However, in contrast to that study where channel reversal was noted, this channel did not reverse polarity in our studies. Preliminary studies indicate that this channel is activated by addition of ionomycin to the bath (Gallin, 1988). Whether this channel contributes to the whole-cell inward rectification described in this paper remains to be determined in future studies.

In conclusion, this paper characterizes two voltage-dependent K conductances at both the whole-cell and single-channel level in cultured human macrophages. It also demonstrates that the expression of at least one of these conductances, the 240 pS calcium-activated K conductance, changes during the time that the peripheral blood monocyte is maturing into the macrophage.

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