Phagocytosis by Human Macrophages Is Accompanied by Changes In Ionic Channel Currents

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Abstract. The present study has shown that changes in ionic channel currents accompany the phagocytosis of particles by mononuclear phagocytes. The patch-clamp technique in the cell-attached configuration was applied to human monocyte-derived macrophages to measure the activity of single transmembrane ionic channels in intact cells. During such measurements, IgG-opsonized and non-opsonized latex particles were offered for phagocytosis under continuous video-microscopical observation. Single particles were presented to the phagocytes at a membrane location some distance from that of the patch electrode. After a lag period following particle attachment, enhanced inward and outward time-variant single channel currents coinciding with particle engulfment were observed. On the basis of current-voltage characteristics and membrane potential measurements, the outward-directed channels were identified as K^+ channels. Phagocytosis was also accompanied by slow transient changes in background membrane currents, probably due to changes in the membrane potential of the phagocytosing cell. Phagocytosis of IgG-coated latex particles differed from phagocytosis of uncoated or albumin-coated particles by a shorter lag time between particle attachment and the onset of enhanced ionic channel activity.

THE ability of mononuclear phagocytes (monocytes and macrophages) and granulocytes to phagocytose and kill invading pathogens forms an important part of host defense. Phagocytosis is either mediated by opsonins that interact with specific membrane-bound receptors, such as Fc and complement receptors, or relies on the surface properties of particles (26). In Fc-receptor-mediated phagocytosis, engulfment of particles is accomplished by a continuous interaction between IgG molecules present on the particle surface and Fc-receptors in the macrophage membrane (7, 25). Membrane potential changes in macrophages have been shown to be associated with such functions as chemotaxis (5) and phagocytosis (2, 15, 19, 28). Use of the patch-clamp technique (8) has led to the identification of various classes of ionic channels in different types of mononuclear phagocyte (4, 6, 13, 20, 23, 24, 30). However, a role played by ionic channels in phagocytosis has not been established. The aim of the present study was to use the patch-clamp technique (8) to find out whether phagocytosis of latex particles by human monocyte-derived macrophages is associated with ionic channel activity. The cell-attached patch mode (8) was chosen because this configuration leaves the cells intact and undamaged, and does not disturb the phagocytic process. Fc-receptor-mediated and non-specific-receptor-mediated phagocytosis were compared in studies using particles with different coatings. Preliminary results of parts of this work have appeared elsewhere (27).

Materials and Methods

Cells

Peripheral blood monocytes from healthy human donors were isolated as described elsewhere (16) and cultured on glass coverslips in petri dishes (12). The culture medium consisted of medium 199 (Microbiological Associates, Bethesda, MD) supplemented with 20% heat-inactivated newborn calf serum (Gibco, Grand Island, NY), 2,000 U penicillin G/ml (Mycofarm, Delft, The Netherlands), and 50 µg streptomyocin/ml (Mycofarm). During one to three weeks of culture the monocytes become macrophage-like cells, here called macrophages, which were used for the experiments.

Opsonization of Latex Beads

Approximately 15×10^6 monodispersed polystyrene latex beads (Polysciences, Warrington, PA, USA) in 0.01 M borate-buffered saline (BBS)[1] (pH 8.0) were incubated with 2 mg/ml purified human serum albumin (HSA) or 2 mg/ml purified human IgG for 16 h at 20°C. After centrifugation for 30 min at 1,500 g and 4°C, the supernatant was analyzed to detect non-bound residual IgG. The amount of IgG bound to the beads was calculated to be about 4×10^4 IgG molecules per bead. To block residual binding sites on the IgG-coated latex particles, the beads were re-incubated with an excess of 10 mg/ml HSA under continuous rotation for 20 h at 20°C, washed six times with BBS, and finally resuspended at a concentration of ~4 × 10^9 beads per ml. These beads were called HSA/IgG-coated particles. For the experiments, uncoated, HSA-coated, or HSA/IgG-coated particles were used. Prior to the electrophysiological experiments a suspension containing

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1. *Abbreviations used in this paper:* BBS, borate-buffered saline; HSA, human serum albumin; rms, root mean square.

~1 × 10³ polystyrene latex beads was added to the bathing solution of cells.

Electrophysiology

Glass coverslips with attached macrophages were secured to a teflon dish which was placed on the stage of an inverted microscope. This arrangement, which allows the use of 100× objective magnification with maintenance of free access of electrodes to the cells (12), permitted high magnification video time-lapse recordings of cells phagocytosing latex particles during patch-clamp measurements. Latex particles were maneuvered under microscopical control by means of a micropipette with a forged hook. Video observations were made with an RCA black and white video camera, type TC2014/CX and time-lapse recordings with a Panasonic video time-lapse cassette recorder type NV-8050.

Patch pipettes were drawn from thin-walled borosilicate glass and filled with the same solution as used to bathe the cells. This solution was composed of 150 mM NaCl, 3 mM KCl, 4 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes-NaOH (pH 7.2). Currents were measured with an I-V converter built according to Hamill et al. (8) and recorded on a Racal Store 4DS Recorder (Southhampton, U.K.). Currents were filtered (low-pass cutoff frequency 800 Hz) with a Model 3550 Krohne-Hite filter (Avon, MA). Root mean square (rms) measurements were made with a true rms electronic voltmeter (Type 2425; Bruel and Kjaer, Copenhagen, Denmark) after currents were passed through a high pass filter (cut-off frequency 0.5 Hz).

Patch-clamp measurements were made in the cell-attached patch configuration (8). The potential of the patch pipette was held at 0 mV to clamp the patch potential to the membrane potential. In accordance with convention, outward channel currents were plotted upwards (positive direction) and inward currents downward (negative direction). Seals between the patch pipette and the membrane patch were of the order of gigaohms (i.e., giga seals), as judged by the combined resistance of seal and patch (measured by application of 10-mV pulses to the pipette), which ranged between 5 and 100 Gohms. The resting membrane potential of cells was determined by measurement of the peak value of the fast potential transient occurring in the first few milliseconds after microelectrode entry into cells (11). Such measurements provide a reliable measure of the resting membrane potential and were made as described elsewhere (11). All experiments were carried out at room temperature.

Results

Phagocytosis under Patch–Clamp Conditions

The morphology of 77% of cells under giga seal conditions (n=103) was unaffected by the presence of the patch electrode on the cell membrane, as shown by the persistence of random pseudopod formation during measurements lasting up to 2 h. In the remaining cases the cell reacted to the presence of the patch electrode by a gradual withdrawal of pseudopods, which led to cell rounding. Such cell rounding was not associated with enhanced current activity.

After attainment of a giga seal, the latex particle was maneuvered toward the cell and presented at a membrane site some distance from the patch electrode (Fig. 1 A, frames 2 and 3). Attachment of the particle to the cell membrane was marked by a cessation of the random movement of the particle in the bathing solution. Irrespective of the kind of particle coating, attachment of a particle to the cell membrane always led to ingestion, as observed by engulfment of the particle by pseudopods (Fig. 1 A, frames 4–6). Particles not accepted for phagocytosis by cells either had no effect on cell morphology or led to pseudopod retraction and cell rounding. Of the 46 cells tested, 25 accepted a latex particle for phagocytosis. Of these 46 cells, 9 out of 12 (75%) phagocytosed HSA/IgG-coated latex particles, 13 out of 22 (59%) uncoated particles, and 3 out of 12 (33%) HSA-coated particles. This illustrates the enhancing effect of IgG opsonization (18) as well as the inhibitory effect of HSA coating of particles (1) on phagocytosis.

Ionic Channels during Phagocytosis

After attainment of a giga seal, the pipette was held for 5 min at a holding potential of 0 mV to allow stabilization of the patch to occur. Records were accepted if stabilized channel activity persisted for a further 5 min. Under these conditions no attempts were made to establish the presence of voltage-dependent ionic channels in the membrane patch, because it was found that application of voltage pulse protocols to the pipette affected subsequent channel activity at a holding potential of 0 mV (data not shown). Stabilized ionic channel activity consisted of occasional outward and/or inward channel currents sometimes occurring in brief bursts. In control experiments, records with a duration of up to 2 h showed stationary channel activity with no distinct patterns of changes associated with morphological alterations.

In 23 out of 25 cells in which giga-seal conditions were maintained and either an opsonized (HSA/IgG-coated) or a non-opsonized (uncoated or HSA-coated) latex particle was phagocytosed, attachment was followed first by a lag period and then by a strong increase in ionic channel currents. Attachment of a particle to the cell membrane had no immediate effect on channel activity in the membrane patch (Fig. 1 B, frame 3). The occurrence of inward as well as outward single channel currents during ingestion indicates the involvement of more than one class of ionic channels during phagocytosis. Single channel current amplitudes ranged up to 4 pA and the channel currents showed time-dependent behavior (Figs. 1 and 2). An example of a recording showing only inward channel currents during the sequences shown in Fig. 1 A is given in Fig. 1 B. An example of outward channel activity is illustrated in Fig. 2. In two cells the phagocytosis-associated channel activity consisted of a sequential activation of inward channels followed by outward channel currents.

Although the occurrence of phagocytosis was influenced by coating of the particle, not all of the particles were accepted by the cell for phagocytosis. When a particle offered for phagocytosis did not attach to the cell immediately, it was pressed against the cell again to make certain that contact with the cell membrane had occurred. Cells which still did not phagocytose the offered particle either failed to react to this stimulus or became round due to retraction of their pseudopods. Cells which did not phagocytose particles offered to them did not show enhanced channel currents, which indicates that the enhanced channel currents associated with phagocytosis were induced not by mechanical stimulation of the membrane but by the process of phagocytosis.

Since various types of K^+ channel have been described in macrophages (4, 6, 13, 23, 30), we looked for K^+ channels in patches that had shown outward channel currents during phagocytosis. Voltage pulses of various amplitudes were applied and channel current amplitudes were measured in cells that had completely internalized IgG-coated particles after showing outward channel activity during phagocytosis. Since current–voltage characteristics can be used to establish the reversal potential of ionic channels (reflecting ion selectivity of the channels) if the resting membrane potential of the attached cell is known, this resting membrane potential of cells containing IgG-coated latex particles was determined. For this purpose, cells were exposed to IgG-opsonized latex particles for 30 min at room temperature in the presence of the saline solution used throughout the study. Cells with internalized particles were then impaled by microelectrodes
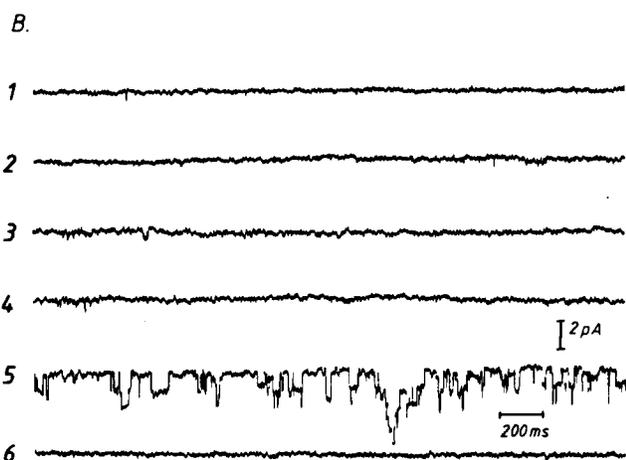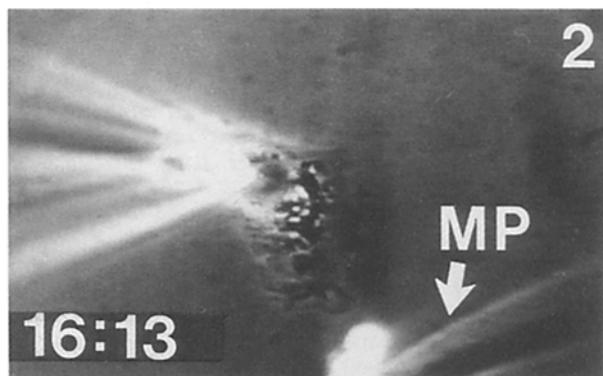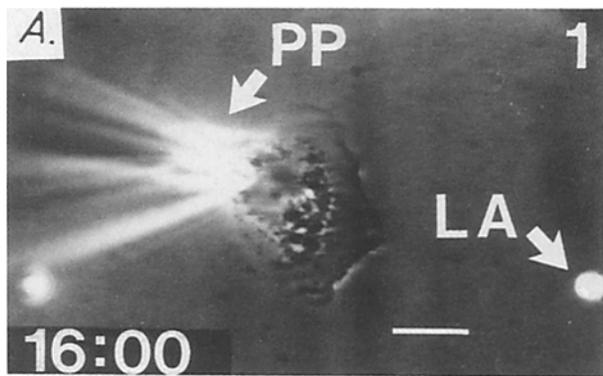
Figure 1. Single channel activity accompanying phagocytosis of a latex bead by a human macrophage (*A* and *B*). (*A*) Photographic sequence taken from a video time-lapse recording of a patch–clamp measurement during phagocytosis of a latex particle. Frame numbers (1–6) are indicated in the upper right corner and the time (hours:minutes) in the lower left corner of each frame. (*1*) A gigaseal has been established with a patch pipette (*PP*) in the cell-attached patch mode on a human macrophage situated in the vicinity of a latex particle (*LA*). (*2*) Use of a micropipette (*MP*) to manouver the latex particle toward the cell. (*3*) Attachment of the particle to the cell was indicated by cessation of the random movement of the particle. (*4–6*) After attachment, the particle is engulfed by the cell. In this example the latex particle was coated with HSA. (*B*) Single ionic channel current records associated with the frames shown in *A*. The corresponding frame number is indicated beside each trace. Attachment of the particle to the membrane did not lead to any detectable change in ionic channel current (trace 3). During phagocytosis, however, there was a transient increase of inward channel current which persisted for ∼5 min (trace 5) after which it returned to the original activity level (trace 6). Bar, 10 μm.
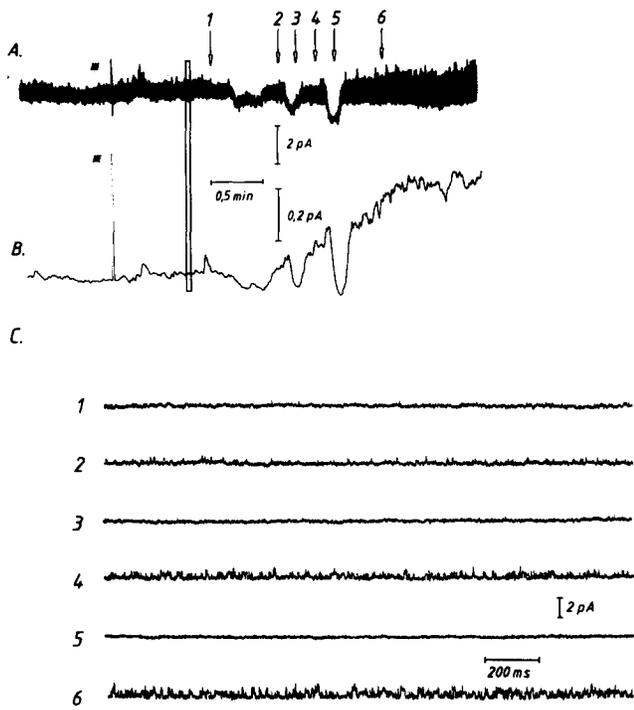
Figure 3. Current–voltage curves of three cells in which outward channel currents were observed during phagocytosis of IgG-coated particles. In one of the cells an additional channel type was active during the voltage pulse protocols, as can be seen from the divergent reversal potential of this channel (△,▲). This cell had shown both inward and outward channel currents during phagocytosis. These current–voltage characteristics have been corrected for the resting membrane potential (−40 mV) of human macrophages containing ingested IgG-coated latex particles. The reversal potentials of the channels in question are −85 mV (△), −80 mV (□), −70 mV (○) and −20 mV (▲). The channel conductances are indicated next to each current–voltage characteristic.

Figure 2. Enhanced outward single channel currents showing time-variant kinetics associated with phagocytosis of an HSA/IgG-coated particle (*A*, *B*, and *C*). (*A*) Current trace as recorded on a slow time scale after the current had passed through a low-pass filter shows three sequential and transient negative-going shifts in the background current after a lag time after the moment of particle attachment (indicated by the vertical bar). A 10-mV pulse was applied (moment indicated by an asterisk) to determine the value of the combined resistance of the seal and the attached patch, which in this case amounted to 40 giga ohms. (*B*) The root mean square (rms) of the signal in *A* after it had passed through a high-pass filter is used as a signal which gives a qualitative measure of the changes in ionic channels currents. High-pass filtering eliminates the contribution of the slow changes in the background current and low-pass filtering eliminates noise components which cannot be attributed to channel activity. (*C*) Current traces 1–6 recorded on a fast time scale and corresponding with those on the slow time scale in *A*, show that the transient changes in background current affect the channel currents.

and the resting membrane potential was measured as described elsewhere (11). For 60 human macrophages with phagocytosed particles, the average resting membrane potential amounted to −40.6 mV (SEM 4.2). This value was used to correct the current–voltage characteristics of the outward channels observed during the phagocytosis of IgG-coated particles (Fig. 3). In three cells showing outward channel current activity, the reversal potentials were −70, −80, and −85 mV. These values are close to the expected K$^+$ Nernst potential of −89 mV found for such cells on the basis of intracellular ion concentration measurements (10). In one of these cells both outward and inward channel activity occurred during phagocytosis, and a channel was identified from the current voltage characteristics with a reversal potential near −20 mV (Fig. 3), confirming the presence of more than one class of ionic channel during phagocytosis.
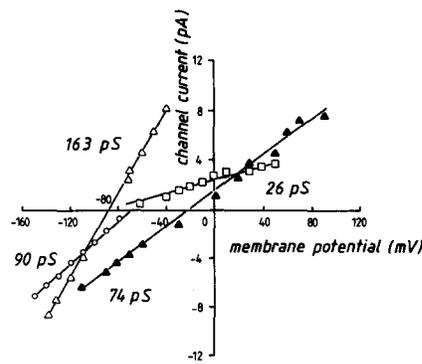
Slow Transient Changes in Background Current during Phagocytosis

Phagocytosis also led to slow transient changes in the background current, probably due to changes in the membrane potential of the attached cell. These transient changes were usually directed inward (22 out of 25 cells), reached amplitudes of up to 6 pA, and lasted from several seconds to several minutes. Background current changes occurred in the presence as well as in the absence of ionic channel activity. Three such successive transient changes in background current affecting the occurrence of single channel currents are shown in Fig. 2 A. In two other cells the amplitude of single channel currents active during a transient background change varied linearly with the amplitude of the background current (data not shown).

Effect of Opsonization on Ionic Channel Activity during Phagocytosis

To find out whether the enhanced ionic channel activity is related to the phagocytic process itself or to the surface properties of the latex particles, we investigated the effect of opsonization on the electrophysiological changes observed during phagocytosis. Both inward and outward channel currents accompanied phagocytosis of HSA/IgG-coated, HSA-coated, or uncoated particles and no evidence was found for a relationship between specific classes of ionic channels and the coating of the latex particles used for phagocytosis. Particle acceptance by the phagocytes was, however, distinctly enhanced when the particles were coated with HSA/IgG. Furthermore, video recordings showed that particles opsonized with IgG/HSA were ingested more rapidly than uncoated or HSA-coated particles. This difference was reflected in shorter lag times between particle attachment and the onset of both ionic channel currents and background current changes

occurring during Fc-receptor-mediated phagocytosis. That lag intervals between particle attachment and enhanced channel activity are shorter during Fc-receptor-mediated phagocytosis is illustrated by comparison of rms records of band-passed currents with coinciding enhanced rms levels and enhanced channel activity (Fig. 2 *B*) during phagocytosis of HSA/IgG-coated and non-opsonized particles (Fig. 4). Lag times between particle attachment and changes in background current were also shorter for phagocytosis of HSA/IgG-opsonized particles (mean 1.1 min, SEM 0.3, $n = 9$) than for uncoated (mean 3.4 min, SEM 0.9, $n = 12$) or HSA-coated (mean 12.9 min, SEM 8.8, $n = 3$) particles.

Discussion

The results of the present study show that during the phagocytosis of latex particles by human macrophages, changes in ionic channel currents and background currents occur after a lag time after particle attachment. This lag time was shortest during Fc-mediated phagocytosis, longer during the phagocytosis of uncoated particles, and longest during the phagocytosis of albumin-coated particles. The finding of both inward- and outward-flowing channel currents during phagocytosis in the cell-attached patch configuration indicated the involvement of at least two classes of ionic channel in phagocytosis. The conclusion that the outward channels observed during phagocytosis are probably K^+ channels is indicated by the current-voltage characteristics reported here. This conclusion is supported by three observations: phagocytosis by rat peritoneal macrophages is associated with membrane hyperpolarizations (2, 15), membrane hyperpolarization due to the activation of a calcium-dependent K^+ conductance (4, 9, 10, 12, 13, 21, 22) in intact human macrophages is associated with outward channel currents (13), and both membrane hyperpolarization in and phagocytosis by human macrophages are reversibly blocked by quinine (cf. 13), an ubiquitous K^+ channel blocker. The iden-

tity of the inward-directed channels is less certain, although their presence was suggested by earlier reports concerning action potentials in human monocyte-derived macrophages (18). Chloride channels described in macrophages (23, 24) could also account for the inward currents seen during phagocytosis. Although the foregoing is mainly concerned with voltage-activated channels, it is conceivable that the phagocytosis-associated channels are activated by second messengers. Further studies will be needed to identify the inducers of these channel currents.

The slow changes in background current found in the present study to accompany phagocytosis are probably caused by changes in the membrane potential of the attached cell. Lines of evidence supporting this view include: (*a*) the linear variation of the amplitude of channel currents active during slow transient changes in background current with the amplitude of the background current; (*b*) as would be expected from the effect of membrane potential changes on voltage-activated channels, changes in background current affected the occurrence of ionic channel activity (Fig. 2); and (*c*) the recent finding that membrane potential changes can affect the level of background current in the cell-attached patch configuration (3). However, the possibility that these changes in background current are caused by the action of extremely small channels cannot be excluded.

Phagocytosis and the ionic channel activity measured in the cell-attached patch occurred at different sites on the cell membrane. The attachment of particles to the cell membrane seems to be a local process, since its occurrence was not detected by a patch electrode elsewhere on the cell membrane. During the ingestion of a particle, however, an electrophysiological response occurs at distant sites. This indicates that the process of phagocytosis affects other parts of the cell membrane besides the site of ingestion. The dependence of the lag time on the type of particle coating suggests that local particle recognition plays a role in the appearance of ionic channel currents throughout the cell membrane.
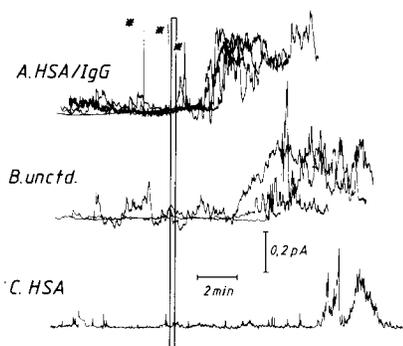

Figure 4. Effect of particle coating on ionic channel currents associated with phagocytosis. The records give the rms value of the ionic channel currents as a function of time. (*A*) Four examples of the rms signal of band-passed current records of cells phagocytosing an HSA/IgG-coated particle. The moment of particle attachment is indicated by the vertical bar. Three examples recorded during phagocytosis of an uncoated particle (*B*; unctd) and one of an HSA-coated particle (*C*). Comparison of the traces in *A* with those in *B* and *C* shows that the lag time between particle attachment and the onset of channel activity is shorter for particles coated with IgG.

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