Ionic Channels in Murine Macrophages

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Abstract. In this paper we examine the different voltage or calcium-dependent currents present in murine peritoneal macrophages, and in a macrophage-like cell line, J774. Three of these are K currents while the fourth is carried by Cl. One K current, activated by hypopolarization, has all the characteristics of the inward rectifier found in egg or muscle cells. It appears in peritoneal macrophages only after several days in culture. A second K current, activated by depolarization, is a typical delayed rectifier. The amplitude of these currents and, as a consequence, the membrane potential of the cells, can be markedly changed by the movement of fluid around the cells. A third K current is activated by internal calcium levels in the micromolar range. It presents a low-voltage sensitivity and is blocked by 0.1–1 mM quinine. The Cl current flows through large-size channels (180–390 pS) that are active mainly in excised patches. These channels are unlikely to be half gap junctional channels, as suggested in former studies. The second goal of this paper is to examine if the activation of receptors for the Fc fragment of IgGs (Fc receptors) is associated with a change in the electrical properties of the membrane of macrophages. We have observed that the binding of multivalent ligands (the monoclonal antibody 2.4G2, aggregated IgGs, or sheep red blood cells coated with IgGs) to their Fc receptors on adherent macrophages did not trigger any change in resting potential. This is a surprising difference with former results obtained on non-adherent J774 cells (Young, J. D.-E., J. C. Unkeless, H. R. Kaback, and Z. A. Cohn, 1983, Proc. Natl. Acad. Sci. USA., 80:1357–1361) and on human alveolar macrophages (Nelson, D. J., E. R. Jacobs, J. M. Tang, J. M. Zeller and R. C. Bone, 1985, J. Clin. Invest., 76:500–507).
Table I. Composition of Solutions

|          | KCl | NaCl | Ca buffer | CaCl₂ | pCa | MgCl₂ | Heps | Junctional potential |
|----------|-----|------|-----------|------|-----|-------|------|---------------------|
| Bath     | 5   | 140  | 0         | 1    | 3   | 1     | 5    | 0                   |
| A        | 140 | 0    | 5.5       | 0.5  | 8   | 2     | 10   | +3.3                |
| B        | 100 | 0    | 20        | 10   | 7   | 0     | 10   | +3.7                |
| C        | 35  | 0    | 1.4       | 0.12 | 8   | 0.5   | 5    | +1.5                |
| D        | 5   | 135  | 5.5       | 0.5  | 8   | 2     | 10   | 0                   |
| E        | 100 | 0    | 20        | 5.5  | 6   | 0     | 10   | +3.7                |
| F        | 75  | 0    | 26.7      | 14.3 | 5.5 | 0.5   | 10   | +4.2                |

Pipette and bath solutions (mM), buffered at pH 7.2 with KOH. The Ca buffers were HEPES in solutions A–D, and HEDTA (1,2-hydroxyethyl) ethylenediamine-nN-triacetic acid) in solutions E and F. Solution C also contained 138 mM mannitol.

Materials and Methods

Preparations

Two cell types have been used in this study. The first one is a macrophage-like cell line, J774, derived from a mouse reticulum sarcoma (22). This cell line was grown in 50-ml bottles (C.E.B., Paris, France) in RPMI 1640 (Eu-robio, Paris, France) supplemented with 2% glutamine, 1% antibiotic-antimycotic solution (Gibco, Grand Island, NY), and 10% calf serum. Floating cells were plated in 35-mm dishes (C.E.B.) the day before the experiment. The size of these cells was variable: their capacitance was in the range of 10–100 picofarads (pF) with an average of 45 pF.

We also used mice peritoneal macrophages, obtained by abdominal injection of saline followed by a 2-min massage of the belly. The washed cells were plated in culture medium defined above, and the dishes washed 3 h later to remove the least adhering cells (lymphocytes and mast cells). The cells were studied 1–7 d after plating. They displayed a variety of irregular shapes including the well-known aspect of fried egg. The capacitance of these cells slightly increased from 24 pF on average after 1–2 d in culture (range: 10–54 pF) to 31 pF after 5 d in culture (range: 17–51 pF). In control experiments, we verified that these macrophages were able to phagocyte antibody-coated erythrocytes (see Results).

Recording

Patch-clamp experiments were done at room temperature (20–25°C), mainly in the whole-cell recording mode with an EPC7 amplifier (LIST Electronics, Darmstadt, Federal Republic of Germany). The compositions of the bath and of the pipette solutions are given in Table 1. When not otherwise stated, the pipette was filled with solution A. When needed, junctional potentials were corrected for, according to their experimental values as indicated in Table 1. The bath electrode was made with an AgCl pellet. The pipette tips were coated by dipping them in melted "sticky wax" (S.S. White, Harrow, England) before fire polishing. Current clamp mode was used to measure resting potentials within 30 s after obtaining of the whole-cell configuration. In voltage-clamp mode, the current transient induced by voltage jumps in the whole-cell configuration was systematically used to measure the series resistance of the pipette (3–10 MO) and the capacitance of the cell. With this circuit, an efficient analogical cancellation of capacitive transients was generally achieved. Remaining transients were blanked out when necessary. The current traces are shown without leak subtraction unless otherwise stated. Data were analyzed with a PDP 11/23 computer (Digital Equipment Corp., Marlboro, MA).

Drug Application

Two methods were used to modify the composition of the external fluid around the recorded cell. When large volumes of fluid were available (>3 ml), we used a perfusion system (24) based on a small glass U-tube pierced by a 100-μm hole. This system allows fast concentration changes (k<sub>0</sub> < 1 s<sup>−1</sup>) with limited mechanical perturbations. Quinine (Rhône-Poulenc, France) was applied in this way. For small volumes of external medium, we simply used a wide-opened pipette (with an opening of ~50 μm in diameter). The fluid in the pipette could move in either direction, depending on the pressure applied to the shaft. To make sure that the fluid was flowing out of the pipette, we generally checked for visible changes in the cell's appearance.

Immune Agents

Three types of ligands were used to bind to Fc receptors: (a) the monoclonal antibody 2.4G2 (27); (b) heat-aggregated immunoglobulin G (IgG); and (c) sheep red blood cells (SRBCs) coated with anti-sheep IgGs. All these ligands, and the J774 cell line were kindly provided by Dr. Daëron (Institut Curie, Paris).

Results

Inward Rectifier

This current is the largest one found in J774 cells. As shown by Gallin and Sheehy (7), this current has all the main properties of the inward rectifier found in muscle or egg cells (9, 12). We will refer to this K current activated by hyperpolarization as I<sub>Kh</sub>. Its properties are summarized as follows (Fig. 1). The voltage range where I<sub>Kh</sub> is activated changes with the external K concentration, so that these channels open mainly at potentials negative to the K equilibrium potential. As expected for an inward K current, I<sub>Kh</sub> is much larger in 140 mM external K (Fig. 1 b) than in 5 mM K (Fig. 1 a). Both the activation and the inactivation of I<sub>Kh</sub> are accelerated by hyperpolarization (Fig. 2, a and b). These kinetics are similar to those found in mammalian muscle (25). I<sub>Kh</sub> is blocked reversibly by 0.1 mM external barium (not shown). A small fraction of the channels is open at membrane potentials slightly less negative than the K equilibrium potential. This conductance does contribute to the resting potential of these cells: 0.5 mM Ba depolarizes the cells by ~20 mV (data not shown; see also reference 7).

1. Abbreviations used in this paper: HEDTA, 1,2-hydroxyethyl) ethylenediamine-nN-triacetic acid; SRBC, sheep red blood cell.
Figure 2. Kinetics of apparent activation and inactivation of \( I_{Kb} \) in two J774 cells, shown after leak subtraction. (a) Inward currents triggered by a series of voltage jumps from -70 to -170 mV (increment, 20 mV). The time constant of inactivation is plotted below as a function of membrane potential. (b) The activation of these currents are shown on a different time scale (the voltage jump to -190 mV was omitted in Fig. 2a for clarity). The time constant of apparent activation, measured without subtracting the inactivation phase, is plotted at the bottom as a function of membrane potential. (c) Current measured in a J774 cell during voltage jumps from -70 to -140 mV. The first current trace was obtained 1 min after obtaining the whole-cell recording. The second current trace was obtained 18 min later. The bottom image shows two traces superimposed, after scaling down the top trace.

The inward rectifier in J774 cells is a labile current which disappears completely after 10–30 min of whole-cell recording. A similar fade affects the delayed rectifier described below. Gallin and Sheehy (7) also reported that inward rectifier currents declined with time. However, this fade was only occasionally observed by these authors, whereas it was systematically present in our experiments. A several-fold reduction of the inward rectifier does not alter its kinetics, as shown in Fig. 2c. This indicates that the progressive disappearance of \( I_{Kb} \) is probably due to a loss of functional channels and not to a faster inactivation of these channels.

The inward rectifier was not found by Ypey and Clapham (33) in peritoneal macrophages cultured for up to 4 d. However, when tested on day 5 in culture, we found that these cells displayed a clear inward rectifier (Fig. 3). As in J774 cells, this current is very sensitive to the external K. When its concentration is increased 28 times (from 5 to 140 mM), the maximum slope conductance measured at the peak current increases by a factor 5.1 ± 1.0 (mean ± SD; \( n = 3 \)) which is close to the value expected (5.3) if this conductance varied with the square root of the external K, as observed in egg, muscle cells, and J774 cells (7, 9, 16).

Delayed Rectifier

In peritoneal macrophages and in J774 cells, a K current (\( I_{Kb} \)) is activated when the cell is depolarized beyond -40 mV (7, 33). We have not studied in details this well-known delayed rectifier.

In both types of cells this current is larger during the first few days in culture, and decreases later on (e.g., see Fig. 3). The outward current measured at 0 mV in peritoneal macrophages cultured for 1–2 d averaged 331 ± 349 pA (mean ± SD, \( n = 13 \)). Note that at this potential, the leakage current is null and only a K current can be outward. The dispersion of the values is due in part to the presence of a number of cells with a very small delayed rectifier. After 5–6 d in culture, the same current had an average of 19 ± 12 pA (\( n = 8 \)).

The absence of any inward current in these conditions showed that no voltage-dependent Na channel is present in these cells.

When most of the intracellular K was replaced by Na (solution D, composed so that the K ions are in equilibrium at 0 mV) and the bath contained either standard divalent ion concentration or 5 mM external barium, voltage jumps from -80 to 0 mV failed to evoke any time and voltage-dependent current. This suggests that these cells do not possess voltage-dependent Ca channels.

Ca-dependent K Current

Large-sized Ca-dependent K channels have been observed in blood monocytes (5), but whole-cell currents were not stud-
Figure 4. Effect of Ca on voltage-dependent currents in J774 cells (a–c) and in peritoneal macrophages (d–f). (a) A 41-pF cell with an internal pCa of 7 (solution B); (b) a 32-pF cell with an internal pCa of 6 (solution E); (c) current–voltage curve corresponding to a (●) and b (○); (d) a 17-pF cell with an internal pCa of 8 (solution A); (e) a 29-pF cell with an internal pCa of 5.5 (solution F); (f) current–voltage curves corresponding to d (●) and e (○).

ied under these conditions. Such currents have not yet been described in J774 cells or peritoneal macrophages.

As shown in Fig. 4, the currents measured in a J774 cell are much larger when Ca is buffered at 1 μM (b) than at 0.1 μM (a). The input conductance of these cells is on average nine times larger with a pCa of 6 than with a pCa of 8 (Fig. 5). A Ca-dependent conductance can also be observed in peritoneal macrophages (Fig. 4, d and e), but at slightly larger values of Ca, as shown in Fig. 5.

The value of the membrane potential measured under voltage clamp, corresponding to zero current, is identical to the resting potential of these cells if they had not been voltage clamped. This zero-current potential (resting potential) is high (~75 mV) in both cell types when the internal calcium is at 1 μM. As the Ca-dependent conductance is much larger than the other ones, with high internal Ca, the resting potential of the cell is pulled towards the equilibrium potential of the ions flowing through these Ca-dependent channels. All ion species have an equilibrium potential positive or null (and this is true also for a nonspecific leak), except K ions which are in equilibrium at -84 mV, with the solutions used. Therefore the only Ca-dependent conductance compatible with a resting potential of -75 mV is a K one. However, one cannot exclude the possibility that a minor component of the Ca-dependent conductance may have a different selectivity. Indeed, Lipton (17) has shown that a macrophage-like cell line possesses Ca-dependent cationic channels.

Quinine (0.1–0.5 mM) markedly reduced the Ca-dependent conductance and sometimes shifted the resting potential (zero-current potential) towards depolarized values (Fig. 6). This depolarization is probably due to the presence of the leak and of non-K ionic conductances, which are not counterbalanced by K conductances when the latter are blocked.

At the calcium concentrations which were tested, Ca-dependent currents did not present a marked voltage sensitivity: no marked relaxation appeared during voltage jumps, and the chord conductance was fairly independent of voltage, at least in the depolarizing direction.

Modulation of Voltage-dependent K Conductances

In the first experiments, where we applied IgGs to J774 cells, we frequently observed either a depolarization or a hyperpolarization of the cell. This was precisely what we were expecting (see references 20, 30). Unfortunately, when the same cells were subjected to a flow of plain mammalian saline without IgGs, the same various potential changes were observed. It thus appeared that a moving fluid environment was able to change some of the conductance systems of these cells (flow effect). In voltage clamp we observed that the depolarizations were generally due to a decrease in IKh, whereas the hyperpolarizations were accompanied by an increase of IKh. The mode of application of the fluid had some influence on whether one effect or the other was dominant. Two experiments where a positive pressure was applied to a wide-opened pipette filled with mammalian saline are shown in Fig. 7. The jet was strong enough to induce a clear deformation of the recorded cell. This perturbation provoked...
a marked reduction of I_{Kp}. When the jet was switched off, I_{Kp} rapidly recovered its initial value. In this experiment, the rate of inactivation of I_{Kp} was also increased during the jet but this phenomenon was not systematically observed. Fig. 7 b shows that the large inhibition of I_{Kp} would have resulted in a 50 mV depolarization of this cell since the zero-current membrane potential had been shifted by 50 mV. The reduction of I_{Kp} caused by a flow of fluid was not always as large as that shown in Fig. 7, a and b. Fig. 7 c illustrates a more common flow effect on I_{Kp}, accompanied by a depolarization of 5–10 mV. In this example, the flow also caused the activation of inward currents, possibly through Cl channels (see below). Their presence was clearly revealed by the increase of noise during the depolarization.

The delayed rectifier can be markedly increased when the macrophage is gently washed by saline flowing out of a U-tube perfusing system. In the example shown in Fig. 8, a and b, I_{Kh} of a J774 cell is approximately doubled in amplitude by a flow of saline which does not alter the shape of the cell, and does not affect I_{Kr} at all. A peculiarity of this flow phenomenon is its very slow reversibility. As shown in Fig. 8 a, 1 min after the end of the perfusion, I_{Kh} was still much larger than control.

In some cases, the increase of I_{Kh} provoked by a flow of fluid causes a hyperpolarization of the cell. This is shown in Fig. 8 c: this peritoneal macrophage presented voltage-dependent currents of very small amplitude. A flow of saline caused a clear increase of I_{Kh} (and a more subtle increase of the inward currents), and hyperpolarized the cell by 10 mV.

The modulation of K conductances by fluid movements could be of importance for two reasons: (a) experimentally, one has to be aware of this flow effect before interpreting any pharmacological effect; (b) physiologically, macrophages are subjected to fluid movements in vivo (see Discussion). Although we have not found out the basic mechanisms underlying these effects, we can at least rule out several possibilities.

Theoretically, both effects could result from the washing of K accumulated in unstirred layers around the cell. This explanation does not hold since (a) quantitatively, the amount of accumulated K would have to be much too large to be plausible; (b) this would affect simultaneously the two K currents, whereas it appears in Figs. 7 and 8 that one current can be largely modified and the other not.

Two intracellular molecules, ATP and protons, have been shown to block various K conductances (1, 26). None of them seems involved in the reduction of I_{Kh}. Thus, the effect of fluid movement on I_{Kh} still takes place when the pH of the internal solution is strongly buffered by 30 mM Hepes. Similarly, including 2–5 mM ATP in the internal solution does not seem to affect I_{Kh}: in J774 cells, the peak current at −140 mV was −264 ± 86 pA (mean ± SD, n = 6) for ATP loaded cells, and −422 ± 246 pA (n = 16) with ATP-free solutions. The difference is not significant (t test, 5% risk). Further, ATP-loaded cells displayed normal I_{Kh} reduction in response to fluid movement. Concerning the increase of the outward current induced by fluid movement, a participation of I_{Kh} could be ruled out, since the effect also takes place when the internal calcium concentration is strongly buffered at pH 8 by 20 mM EGTA. In these conditions an increase of C_{ci} above pCa 6 is improbable.

The flow effect severely limited the study of the pharmacology of K channels: 0.1 mM quinine (not shown) was observed to reduce I_{Kh}, but sometimes this inhibition was masked by the flow effect, and in the presence of the blocker a net increase of I_{Kh} was observed.

**Chloride Currents**

Large conductance Cl channels have been observed in the membrane of macrophages (23), with properties similar to those also described in Schwann cells (8), Madin–Darby canine kidney cells (13), and muscle cells (2). In these last three cell types, the channel had a conductance of 450–460 pS and a permeability ratio P_{Cl}/P_{K} of 0.2. It was active only at membrane potentials close to 0 mV.

We have also observed a large size channel (Fig. 9), with a conductance of 180–390 pS in both peritoneal macrophages and J774 cells. This channel was very rarely observed in whole-cell recording but was frequently active in cell-free patches. In inside- or outside-out patches with symmetrical chloride (see Fig. 9 a), the reversal potential of this current is near 0 mV, indicating either a chloride or a nonspecific cationic selectivity. In three outside-out patches, with solution...
C in the pipette, the reversal potential of this current was between -22 and -29 mV after correction for junction potentials. In these ionic conditions, the values of the equilibrium potentials for Cl and monovalent cations were -35.3 and +35.5 mV, respectively. This gives a permeability ratio \( P_{\text{cation}}/P_{\text{Cl}} \) between 0.07 and 0.19. This channel was frequently observed in outside-out patches (Fig. 9 b; pCa_i = 8) or in inside-out patches (Fig. 9 a; pCa_i = 3). The results of Fig. 9 suggest that the conductance and ionic selectivity of this channel are not strongly affected by a 10-fold change in the calcium concentration on its cytoplasmic side.

We have examined the voltage sensitivity of this channel in outside-out patches. The probability of opening of the large Cl channels measured during voltage ramps is shown in Fig. 9 c. It was high at positive potentials (up to +40 mV in the example shown in Fig. 9 c). It decreased when the patch was hyperpolarized or at highly positive potentials. Similar results were obtained both when the Cl concentration was symmetrical (solution A in the pipette) and when it was asymmetrical (solution C in the pipette as in Fig. 9 b). This voltage dependence is not exactly identical to those previously described in the various cell types mentioned above, where the channel had a tendency to close at potentials away from zero (by more than 10-20 mV).

In several of the papers mentioned above, it was suggested that these large size channels could represent halves of gap-junction channels. This is not very likely since gap-junction channels have a slightly higher permeability for cations than for anions (21). To test this point further, we have studied the effect of octanol, a well-known blocker of gap-
junction channels (II) on large-sized Cl channels. Octanol was used at a final concentration of 0.6 mM, in combination with 1.7 mM ethanol to solubilize it. The large Cl channels of J774 cells were totally unaffected by 0.6 mM octanol, in outside-out patches (n = 2) as well as in inside-out patches (n = 2). The same solution, applied to pairs of lacr myocytes under double whole-cell clamp, rapidly and reversibly closed the gap junction between these cells (Neyton, J., and A. Trautmann, unpublished data). These results indicate that the large Cl channels are unrelated to gap junction channels.

Stimulation of Fc Receptors

The aggregation of Fc receptors by multivalent ligands has been reported to create a structure controlling the entry of cations in J774 cells (30) and in alveolar macrophages (20). This channel has been purified and successfully reincorporated both in vesicles (31) and in planar bilayers (32). To examine this point further we applied either aggregated IgGs (100 μg/ml) or the monoclonal antibody 2.4G2 (directed against Fc receptors) at a concentration of 1 μg/ml. Stimulation was performed with a large-sized pipette on J774 cells under whole-cell recording. On 15 different cells, the only reproducible effects were an increase in I_Kd and/or a decrease in I_Kf which were also observed when the large pipette contained only mammalian saline. They represent the flow effects described above. In the absence of any specific effect of the above ligands, it was wondered whether, under our experimental conditions, the antibodies had properly attached to the cells. To visualize the binding of IgG on Fc receptors we then used SRBCs coated with IgG. Coated SRBCs attached rapidly and strongly to J774 cells or peritoneal macrophages, whereas control uncoated SRBCs could be easily washed out. Peritoneal macrophages exhibited a clear phagocytosis in the following minutes. This was observed at room temperature, in mammalian saline as well as at 37°C in culture medium. As judged visually, J774 cells were much less efficient than peritoneal macrophages in phagocytosing SRBCs.

When coated SRBCs were applied on macrophages under whole-cell recording, they failed to trigger any inward current. Perhaps the presence of the pipette before the application of SRBCs was a sufficient perturbation to prevent the predicted effect to develop. Therefore, we applied the coated SRBCs to macrophages first, and only after one or a few minutes the recording pipette was brought up to the cell giving the intact cell a chance to respond to the bound IgG. We measured the resting potential of these cells in order to detect...
bound IgG and in some cases started to phagocyte the SRBCs.

Figure 10. Membrane potential of peritoneal macrophages, measured under whole-cell current clamp recording conditions. The values measured in control conditions are shown at time 0 (○). Their mean is indicated by the dotted line. (•) Values measured at various times after the adhesion of coated SRBCs to the macrophages.

the presence of an inward current as expected from the results reviewed in reference 29: with input resistances >1 GΩ, currents as small as 10 pA would give a depolarization of >10 mV. Fig. 10 shows the resting potential of peritoneal macrophages measured either before or after the binding of coated SRBCs. It appears that although these cells have bound IgG and in some cases started to phagocyte the SRBCs, no significant change in resting potential can be detected. Similar results were obtained with J774 cells.

Discussion

Three lines of conclusion can be drawn from the present work. First, we show that different reports on four types of channels in macrophages do not correspond to differences between preparations, and that all current types, \(I_{Ks}, \ I_{Kd}, I_{K(Ca)}\) and \(I_{Ca}\), are present both in peritoneal macrophages after some days in culture, and in the cell line J774. These cells do not have any other obvious voltage-dependent currents and are particularly lacking Ca and Na currents. The resting potential of the macrophages that we have studied lies between -60 and -70 mV, i.e., less negative than \(E_K\) (-84 mV in our experimental conditions). The difference could be either due to the leak of the recording system, or to another minor conductance, that we have not determined.

\(I_{Kd}\) and \(I_{Ks}\) are not simply voltage-dependent currents, since they can be influenced by other modulations. The existence of an intracellular control of these currents is suggested by their progressive disappearance after the dialysis of the cells. This fade may be due to the loss of a cofactor, necessary for the normal functioning of these channels. A similar situation has been described for Ca channels (e.g., see reference 4).

Secondly, we have shown that a flow of saline can either cause a depolarization or a hyperpolarization of the cell, correlated with a decrease of \(I_{Kd}\) or an increase of \(I_{Ks}\), respectively. We cannot exclude the possibility that other conductances (e.g., the one selective for Cl ions) may also be influenced by streaming fluids. Such phenomena may well be experienced by adherent macrophages and even more by circulating cells such as monocytes, which also cross epithelia by diapedesis. It is thus conceivable that fluid movements may influence the resting potential of macrophages in vivo.

The large Cl channel already described in peritoneal macrophages (23) is also present in J774 cells. It seems unrelated to gap-junction channels since it is insensitive to octanol. Curiously, the properties of this Cl channel are very similar to those of the channel that Nelson et al. (20) have described as a cationic channel linked to the activation of Fc receptors. Both channels have similar full conductance, exhibit sub-states of conductance, and have an identical voltage-dependent probability of opening. Nelson et al. (20) do not present data which discriminate between a cationic and an anionic selectivity of this channel.

The last part of this work dealt with putative conductance changes controlled by Fc receptors. Under our experimental conditions, the binding of a multivalent ligand (SRBCs coated with IgG) to the receptors was sufficient to trigger a cascade of molecular events leading to phagocytosis without, however, any detectable change in the ionic conductances of macrophages. Although we cannot ascertain that there were no small, brief, and rare current changes associated with some step of the ingestion process itself, any inward current of a few pA lasting more than 10-30 s would have been detected. This is in apparent contradiction with other results (20, 29) which suggested that the aggregation of Fc receptors could lead to the opening of cationic channels, inducing a large sustained current. It is possible that this discrepancy is related to the state of the cells: we were working on cells fully spread on the bottom of the dish, whereas the former studies were done either on floating J774 cells (29) or on rounded alveolar macrophages (20). Spreading on a surface has many similarities with phagocytosis (3, 15), and may therefore be a major characteristic of the state of our cells.

We have shown that an immune phagocytosis can be triggered under conditions where no detectable cationic conductance is activated. In other words, the cationic flux controlled by Fc receptors in nonadherent J774 cells and in alveolar macrophages does not constitute a fundamental step in the cascade of events triggered by a specific binding to these Fc receptors. In line with this result, it has been recently reported that Fc receptor–mediated phagocytosis may occur in adherent peritoneal macrophages without an increase in average Ca (18), whereas in a previous study (28) an increase in Ca appeared to be associated with the binding of various ligands to Fc receptors of nonadherent J774 cells.

Are the modes of Fc receptor activation dependent upon the state of differentiation of the macrophage? Are there macrophage functions that can be altered by modulating their various K currents? These questions, raised by our results, should now be addressed.

We are grateful to M. DaCron and H. Fridman (Institut Curie, Paris) for helpful discussions and for kindly providing the J774 cell line, and the different ligands for Fc receptors. We wish to thank A. Marty for his comments on the manuscript.

This work was supported by grants from Centre National de la Recherche Scientifique (France), Institut National de la Santé et de la Recherche Medicale (France), and Université Pierre & Marie Curie.

Received for publication 29 September 1986, and in revised form 28 February 1987.

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