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$P2X_7$ Receptor and Polykarion Formation

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Cell fusion is a central phenomenon during the immune response that leads to formation of large elements called multinucleated giant cells (MGCs) of common occurrence at sites of granulomatous inflammation. We have previously reported on the involvement in this event of a novel receptor expressed to high level by mononuclear phagocytes, the purinergic $P2X_7$ receptor. Herein, we show that blockade of this receptor by a specific monoclonal antibody prevents fusion in vitro. In contrast, cell fusion is stimulated by addition of enzymes that destroy extracellular ATP (i.e., apyrase or hexokinase). Experiments performed with phagocytes selected for high ($P2X_7$ hyper) or low ($P2X_7$ hypo) $P2X_7$ expression show that fusion only occurs between $P2X_7$ hyper/$P2X_7$ hyper and not between $P2X_7$ hyper/$P2X_7$ hypo or $P2X_7$ hypo/$P2X_7$ hypo. During MGCs formation we detected activation of caspase 3, an enzyme that is powerfully stimulated by $P2X_7$. Finally, we observed that during MGCs formation, the $P2X_7$ receptor is preferentially localized at sites of cell-to-cell contact. These findings support the hypothesis originally put forward by our group that the $P2X_7$ receptor participates in multinucleated giant cell formation.

INTRODUCTION

During the immune response it is frequently observed that mononuclear phagocytes fuse to generate large elements called multinucleated giant cells (MGCs) (Fais *et al.*, 1997). It is common to find these polykarions at sites of chronic inflammation such as granulomas, whether due to bacterial pathogens or sterile foreign bodies, or as a consequence of viral infections such as acquired immunodeficiency syndrome (Cotran *et al.*, 1994). It is known that fusion requires macrophage activation by cytokines released by T lymphocytes, among which the most important appear to be interleukin (IL)-4, interferon-γ, and tumor necrosis factor-α, but the molecular mechanism involved is still mysterious. Multinucleation also occurs in the bone during osteoclast formation under the effect of locally released cytokines (Vignery, 1989; Suda *et al.*, 1995). Apart from osteoclasts that acquire an increased bone resorption capability after fusion, the physiological meaning of MGC formation is unknown, albeit a number of suggestions have been put forward (enhanced cytokine producing activity, a nonphagocytic pathway for antigen internalization, a mechanism for disposing infected or damaged monocytes). Likewise, the molecular mechanism that drives fusion is unknown. A few plasma membrane molecules probably involved have been identified such as intercellular adhesion molecule-1, leukocyte function–associated antigen-1, E-cadherins, CD98, CD44, or the newly cloned macrophage fusion receptor (Saginario *et al.*, 1998; Sterling *et al.*, 1998), but it appears that fusion does not depend on the engagement of a single receptor, but rather on the recruitement of several molecules mediating cell aggregation, establishment of close cell-to-cell contacts, and finally the actual fusion event.

Over the last years we have extensively characterized a plasma membrane receptor belonging to the subfamily of the P2X purinergic receptors, named $P2X_7$, that is expressed to a very high level by macrophage, microglial, and dendritic cells (Ferrari *et al.*, 1996; Chiozzi *et al.*, 1997; Coutinho-Silva *et al.*, 1999; Mutini *et al.*, 1999). $P2X_7$ is a ligand-gated receptor/channel formed by an unknown number of subunits each 595 amino acids long that upon sustained stimulation with ATP causes the formation of a nonselective pore permeable to low-molecular-weight aqueous solutes (Di Virgilio, 1995; Surprenant *et al.*, 1996; Rassendren *et al.*, 1997). This receptor was initially identified and characterized in immune cells (Di Virgilio *et al.*, 1995), and eventually cloned from a rat brain library (Surprenant *et al.*, 1996). Cloning from a brain library was not surprising because, although central neurons are basically negative for $P2X_7$, microglia is one of the cell types that expresses $P2X_7$ to the highest level.

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Abbreviations used: FSDC, fetal skin-derived dendritic cell; MGC, multinucleated giant cell; oATP, oxidized ATP; $P2X_7$ hyper, phagocytes hyperexpressing the $P2X_7$ receptor; $P2X_7$ hypo, phagocytes hypoexpressing the $P2X_7$ receptor.

In mononuclear phagocytes $P2X_7$ has been associated to cytotoxicity and to maturation and release of IL-1β (Ferrari *et al.*, 1997a; Di Virgilio *et al.*, 1998a), but it has been suggested that it also might participate in MGC formation (Chiozzi *et al.*, 1997). The first indication of a role for $P2X_7$ in macrophage fusion came from experiments performed in our laboratory showing that multinucleation of monocyte-derived human macrophages in vitro was efficiently prevented by the $P2X_7$ blocker oxidized ATP (oATP) (Falzoni *et al.*, 1995). Subsequently, we showed that macrophage cell clones expressing $P2X_7$ to a very high level ($P2X_7$ hyper) spontaneously fuse during in vitro culture, whereas clones selected for lack of $P2X_7$ ($P2X_7$ hypo) never do (Chiozzi *et al.*, 1997). The step at which $P2X_7$ takes part in cell fusion is at present unknown, but we speculate that it might be involved in the very last step of the process, the actual membrane fusion, with a mechanism reminiscent of that of the better known "fusion pore" putatively involved in the fusion of synaptic vesicles with the plasma membrane (Monck and Fernandez, 1996). In the present work we provide further evidence for the involvement of $P2X_7$ in MGC formation.

MATERIALS AND METHODS

Cells

J774 mouse macrophages and $P2X_7$ hyper and $P2X_7$ hypo clones were grown in Dulbecco modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 2 mM glutamine, 10% heat-inactivated horse serum, penicillin (100 units [U]/ml), and streptomycin (100 μg/ml). $P2X_7$ hyper and $P2X_7$ hypo variants were selected as described previously (Chiozzi *et al.*, 1996, 1997). Fetal skin-derived dendritic cells (FSDCs) were grown in Iscove medium (Sigma) containing 50 μM 2-mercaptoethanol, 2 mM glutamine, 10% heat-inactivated fetal calf serum (Life Technologies, Paisley, Scotland), penicillin (100 U/ml), and streptomycin (100 μg/ml) as previously described (Mutini *et al.*, 1999). Human monocytes were isolated from buffy coats by one-step Percoll gradient (Pharmacia Biotech Spa, Cologno Monzese, Italy) or by adherence on plastic Petri dishes as previously described (Falzoni *et al.*, 1995).

Antibodies

The antihuman $P2X_7$ monoclonal antibody (mAb) was previously characterized by Buell *et al.* (1998). The polyclonal anti-$P2X_7$ Ab was previously characterized by Solini *et al.* (1999). Fluorescein isothiocyanate (FITC)-conjugated Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Immunofluorescence

Cells were fixed in 2% paraformaldehyde/phosphate-buffered saline (PBS), pH 7.3, for 1 h at 4°C, and then rinsed three times with ice-cold PBS and incubated in 100 mM ammonium chloride for 20 min. At the end of this incubation, the monolayers were thoroughly rinsed with PBS, incubated with the 2.4G2 mAb (anti-Fc receptor) for a further 20 min at 4°C, and again rinsed with ice-cold PBS. Incubation with the polyclonal anti-$P2X_7$ Ab (Solini *et al.*, 1999) was performed overnight at 4°C at a dilution of 1:50. For immunofluorescence, the secondary Fluorescein isothiocyanate-conjugated Ab was used at a 1:200 dilution (40 min at 4°C).

Caspase Activation

Caspase 3 activation was measured fluorometrically with the Enz-Check Caspase 3 kit (Molecular Probes, Eugene, OR), as indicated by the manufacturer.

ATP Measurement

Monocyte-derived macrophages were seeded in microtiter plastic dishes in a total volume of culture medium of 100 μl in the presence or absence of concanavalin A (ConA) (10 μg/ml) and placed in a CO_2 incubator at 37° for 24 h. After this time, cells were rinsed and supplemented with 100 μl of diluent buffer (FireZyme, San Diego, CA) to stabilize extracellular ATP, and placed directly in the test chamber of a luminometer (FireZyme). Then 100 μl of a luciferin–luciferase solution (FireZyme) was added, and light emission was recorded.

Microscopy

Phase contrast and fluorescence photographs were taken with an inverted fluorescence microscope (Olympus IMT-2; Olympus Optical, Tokyo, Japan) equipped with 20 and 40× objectives and fluorescein and rhodamine filters. Some images also were taken with a Nikon Eclipse TE-300 fluorescence microscope (Nikon, Tokyo, Japan) equipped with 40, 63, and 100× (oil immersion) objectives.

RESULTS

We have observed that fusion spontaneously occurs in in vitro cultures of mononuclear phagocytes clones derived from J774 macrophages as well as dendritic cells derived from mouse skin (FSDCs) that express high levels of the $P2X_7$ receptor

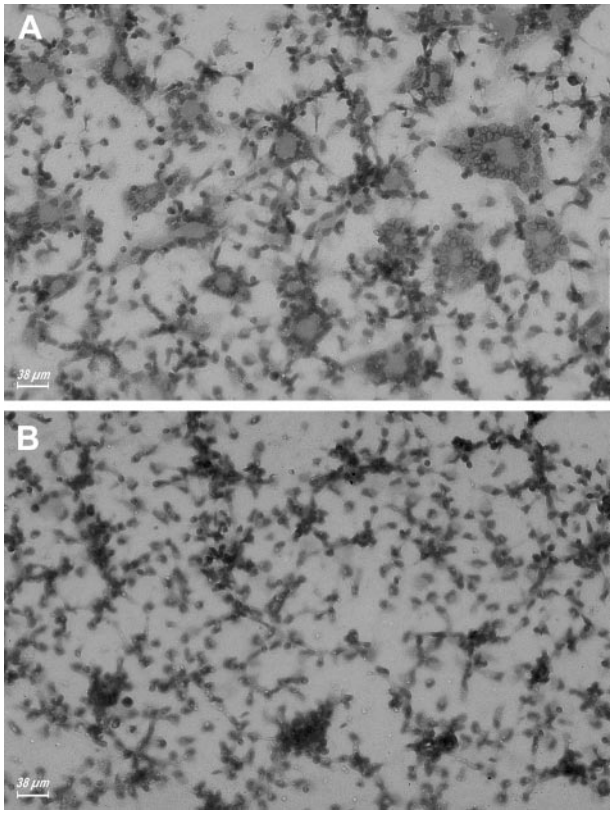

Figure 1. An anti-$P2X_7$ mAb blocks MGC formation. Monocytes were isolated from peripheral blood and plated as described in MATERIALS AND METHODS. ConA (10 μg/ml) was added soon after the plating and incubation carried on for 3 days. (B) Anti-$P2X_7$ mAb (10 μg/ml) was added together with ConA.

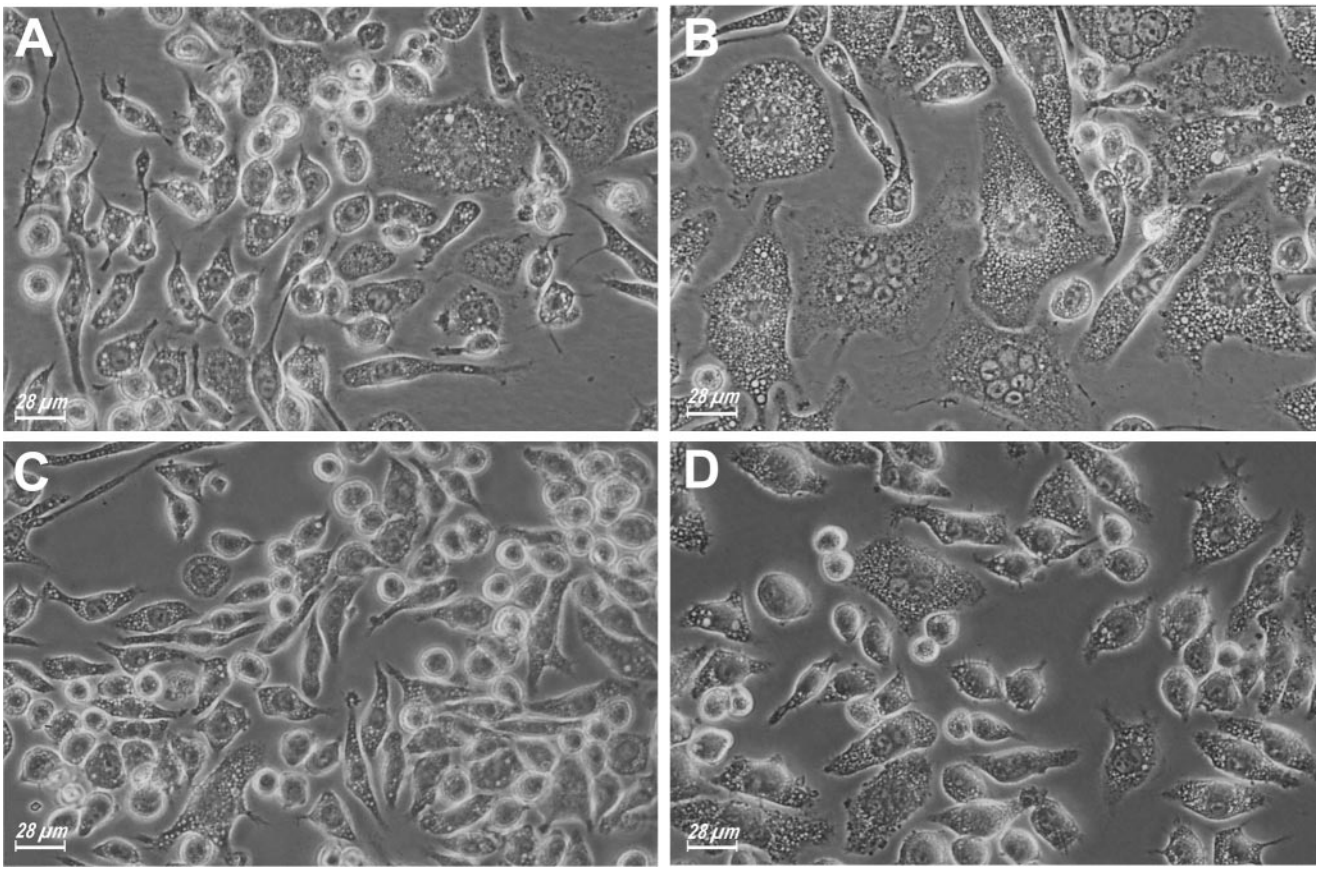

Figure 2. Enhanced MGC formation in macrophage monolayers incubated in the presence of ATP-hydrolyzing enzymes. $P2X_7$ hyper J774 macrophages were plated in 24-well plates and grown to confluence. At this time, hexokinase (100 μg/ml) (B) or apyrase (0.4 U/ml) (D) was added and the incubation further carried out for 24 h. A and C, parallel control cultures in the absence of the ATP-hydrolyzing enzymes.

($P2X_7$ hyper clones) (Chiozzi *et al.*, 1997; Chiozzi, Falzoni, and Di Virgilio, unpublished observations). Furthermore, fusion can be induced in primary cultures of human monocytes by incubation in the presence of ConA or phytohemagglutinin (PHA) (Takashima *et al.*, 1993; Falzoni *et al.*, 1995).

Recently, Buell *et al.* (1998) raised and fully characterized an inhibitory mAb directed against the outer domain of human $P2X_7$. Pretreatment with this mAb blocked several macrophage responses dependent on $P2X_7$ activation, including cytotoxicity and IL-1β release (Buell *et al.*, 1998). Our preliminary evidence suggested that this mAb also could prevent polykarion formation (Di Virgilio *et al.*, 1999). Thus, we tested more thoroughly the effect of the anti-$P2X_7$ mAb on ConA-stimulated fusion of monocyte-derived human macrophages. Figure 1 shows that this mAb almost completely blocked MGC formation, but very interestingly, did not prevent cell aggregation, suggesting that chemotaxis and surface molecule recognition were not affected. Fusion index of six different ConA-stimulated monocyte preparations ranged from 61 to 85%, and was not affected by incubation in the presence of irrelevant mouse IgG. Pretreatment with the anti-$P2X_7$ mAb brought the fusion index close to zero in all monolayers examined.

Blocking of fusion by a selective anti-$P2X_7$ mAb is a strong indication that this receptor participates in MGC formation. However, if a functional $P2X_7$ is needed for membrane fusion, one could expect that its activation by exogenous ATP facilitated fusion. We tested this hypothesis on J774 and FSDC $P2X_7$ hyper clones that spontaneously undergo fusion in culture, and found that this was not the case because incubation in the presence of a range of ATP concentrations sufficient to activate the $P2X_7$ receptor (1–5 mM) inhibited fusion. In contrast, the presence in the incubation medium of ATP-hydrolyzing enzymes such as hexokinase or apyrase powerfully enhanced MGC formation in the $P2X_7$ hyper clones (Figure 2). Boiling abolished apyrase and hexokinase fusogenic activity. In hindsight, the effect of apyrase and hexokinase was not totally unexpected because we and others have previously shown that in some cell types P2 receptors are chronically desensitized by the continuous leak of ATP into the pericellular medium, and that hexokinase or apyrase reestablish sensitivity to stimulation by ATP (Baricordi *et al.*, 1996; Buell *et al.*, 1996). Phagocytes release significant amounts of ATP into the pericellular milieu, thus it is likely that their $P2X_7$ receptor is exposed to high extracellular ATP concentrations, especially when they come in close contact before fusion. We measured bulk extracellular ATP before and during fusion in six different monocyte-derived macrophage monolayers, and found that it ranged from 250 to 592 pmol/10^6 cells in unstimulated cultures, and

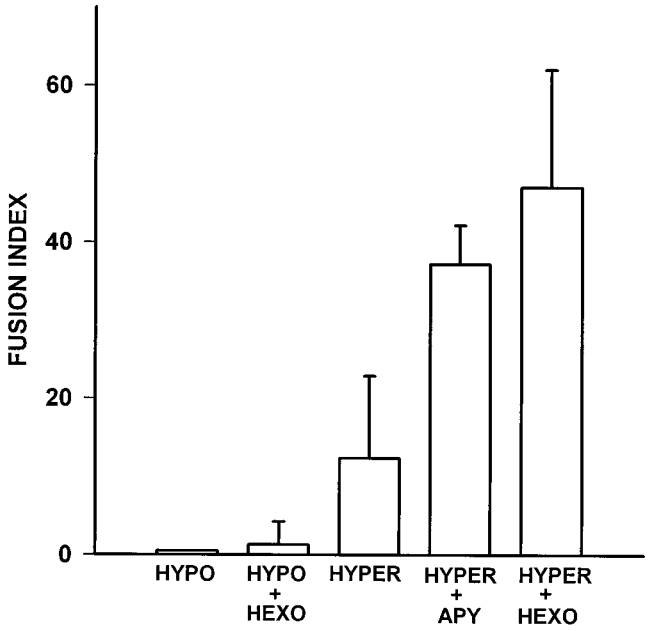

Figure 3. Fusion index of apyrase- or hexokinase-treated macrophage monolayers. The fusion index was determined by counting the (number of nuclei within MGCs)/(total number of nuclei counted) × 100. We identified as MGCs those cells that had more than two nuclei per cell. Three different microscopic fields were counted in three different MGC preparations. Data are average ± SD of nine determinations.

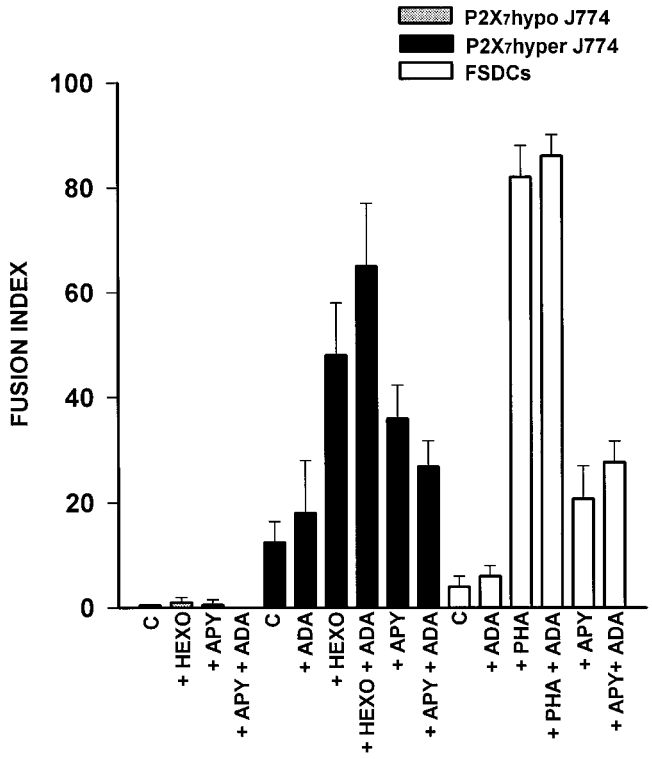

Figure 4. Effect of adenosine deaminase on MGC formation. The fusion index was determined as detailed in Figure 3. $P2X_7$ hyper and $P2X_7$ hypo J774 cells were treated with hexokinase (HEXO) or apyrase (APY) and processed as described in Figure 2. FSDCs were incubated in the presence of PHA (10 μg/ml) or apyrase (0.4 U/ml) for 2 d before determination of fused cells. Adenosine deaminase was added at a concentration of 2 U/ml. C, control. Data are average ± SD of six determinations.

from 410 to 1010 pmol/10^6 cells after 24 h of stimulation with ConA. Figure 3 reports the fusion index calculated in several $P2X_7$ hyper or $P2X_7$ hypo J774 monolayers incubated in the absence or presence of ATP-consuming enzymes. It is noteworthy that these agents did not trigger fusion in cells lacking $P2X_7$. Neither apyrase nor hexokinase could be used to enhance fusion of monocyte-derived macrophages because in the presence of ConA or PHA, these enzymes had a surprising toxic effect hallmarked by swelling, rounding, and vesiculation of the cells.

Hexokinase and apyrase, by increasing the rate of degradation of extracellular ATP, also might enhance accumulation of extracellular adenosine. Activation of A1 receptors has been shown to enhance MGC formation stimulated by phorbol 12-myristate 13-acetate in human monocyte cultures (Merril *et al.*, 1997). Under our experimental conditions, adenosine had no effect of cell fusion over a range of concentrations from 1 to 100 μM, in fact it was inhibitory at the higher dose. We then tested the effect of adenosine deaminase, an enzyme that destroys extracellular adenosine, on two fusion models: apyrase- or hexokinase-stimulated $P2X_7$ hyper J774 macrophages, and PHA- or apyrase-stimulated FSDCs (Figure 4). Adenosine deaminase slightly enhanced fusion by itself, and also potentiated MGC formation in the presence of the additional stimulants, but the increase never reached statistical significance, suggesting that albeit adenosine can stimulate fusion, it is not the main factor under our experimental conditions.

As shown in Figure 4 and by Chiozzi *et al.* (1997), $P2X_7$ hypo macrophage clones are unable to fuse in culture, in striking contrast to their $P2X_7$ hyper partners. We then asked whether expression of $P2X_7$ is needed on both partner cells undergoing fusion, or in other words, whether a $P2X_7$ hypo cell can fuse with a $P2X_7$ hyper, or fusion can only occur between $P2X_7$ hyper cells. To answer this question, we labeled $P2X_7$ hypo and $P2X_7$ hyper FSDCs with Texas Red and lucifer yellow, respectively, and then coincubated the two cell populations. Our anticipation was that if fusion occurred between $P2X_7$ hyper and $P2X_7$ hypo cells we should find MGC stained with both the red and the yellow/green stain, whereas if fusion only occurred between $P2X_7$ hyper we should only see MGC stained in yellow/green. Figure 5 shows that by mixing Texas Red-stained $P2X_7$ hypo and lucifer yellow-stained $P2X_7$ hyper FSDCs, we obtained the formation of MGCs that were almost exclusively stained in yellow/green. In >10 separate, similar experiments, we calculated that ~95–98% of MGCs were exclusively lucifer yellow positive. The residual small percentage of cells was positive for both stains due, we suggest, to the presence of some $P2X_7$-positive cells within the $P2X_7$ hypo population (see also Chiozzi *et al.*, 1997).

As of now, there are very few means to monitor activation of $P2X_7$, the best and most reliable being conductance (Surprenant *et al.*, 1996) or fluorescent dye uptake measurements (Steinberg *et al.*, 1987). However, it is very difficult to apply

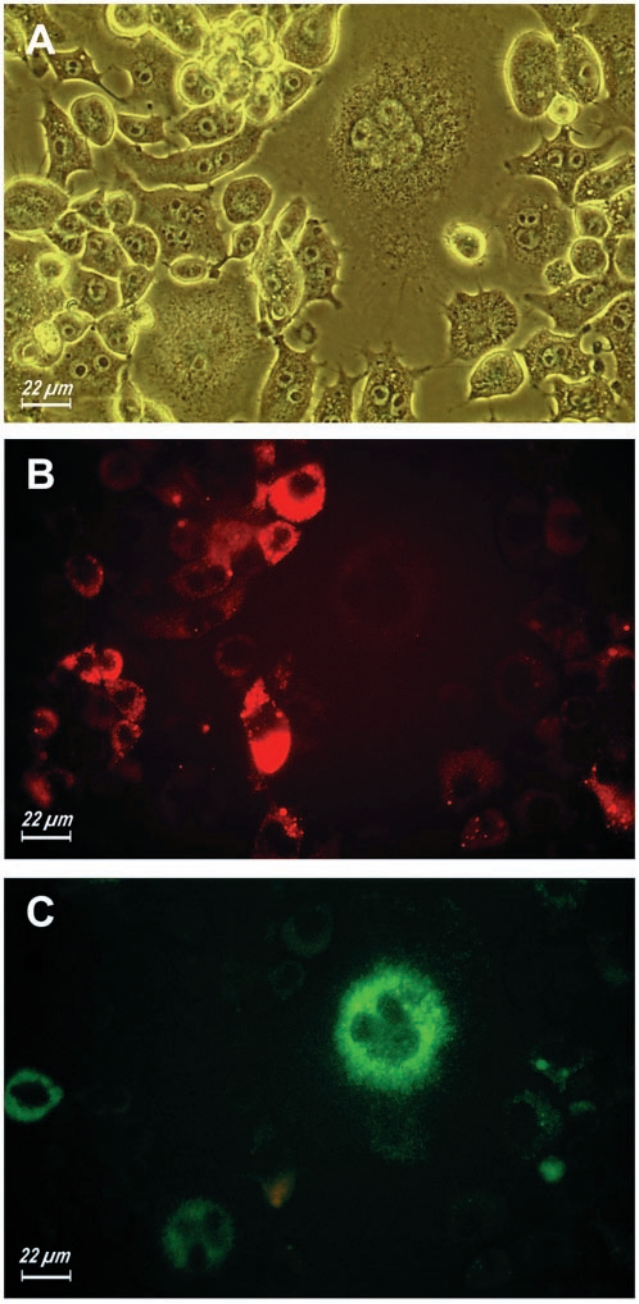

Figure 5. MGC formation requires $P2X_7$ receptor expression on all cells participating to fusion. $P2X_7$ hyper and $P2X_7$ hypo FSDCs were allowed to pinocytose lucifer yellow (1 mg/ml) or Texas Red (0.4 mg/ml), respectively, for 3 h. At the end of this incubation time, the two batches were mixed in the well of a 24-well culture dish, and further incubated for 3 days. (A) Phase. (B) Rhodamine filter. (C) Fluorescein filter.

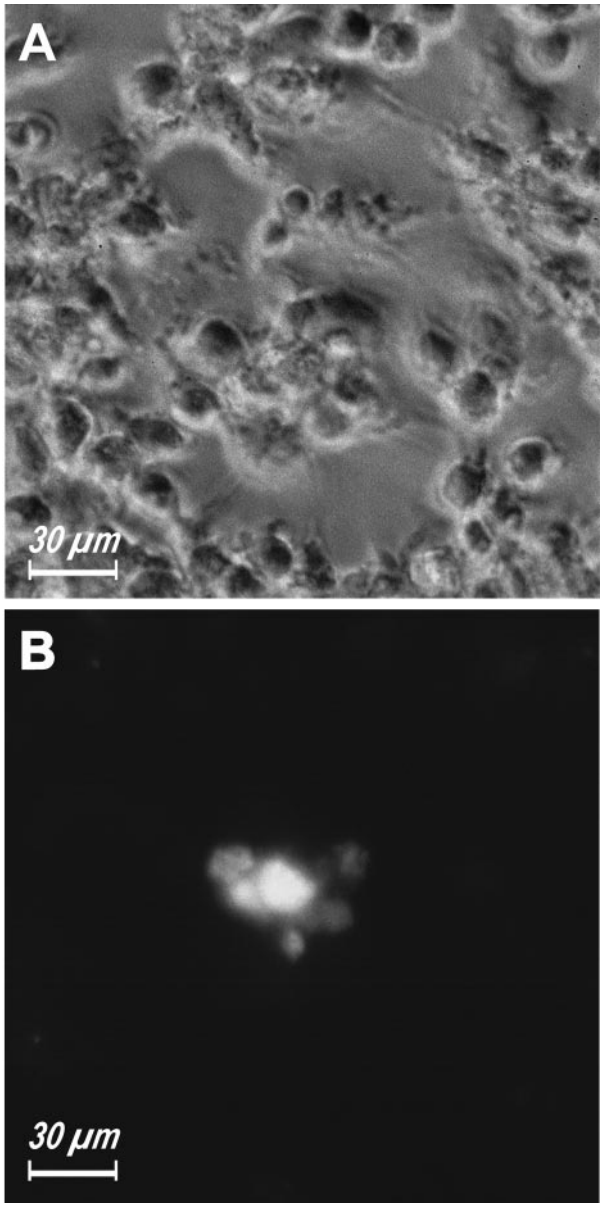

Figure 6. Spontaneous and selective lucifer yellow uptake by fusing monocyte-derived macrophages. Monocytes were isolated from peripheral blood and plated as described in MATERIALS AND METHODS. ConA (10 μg/ml) was added soon after the plating. The picture was taken after 24 h of incubation in the presence of ConA.

these techniques to the measurement of $P2X_7$ opening during cell fusion because patch clamp significantly perturbs the cellular microenvironment and dye uptake does not allow an easy quantification. We monitored lucifer yellow uptake in ConA-stimulated cultures of monocyte-derived macrophages and observed that cells involved in fusion showed an increased dye uptake (Figure 6, A and B). However, we also felt that dye uptake was an unsatisfactory assay for $P2X_7$ activation under these experimental conditions because by this means we might miss opening of those receptors located on the tightly juxtapposed plasma membranes of closely juxtaposed cells (i.e., the very cells that are about to fuse), and that presumably are segregated from the extracellular milieu. Ferrari *et al.* (1999) showed that stimulation of $P2X_7$ causes a large stimulation of caspase 3. We

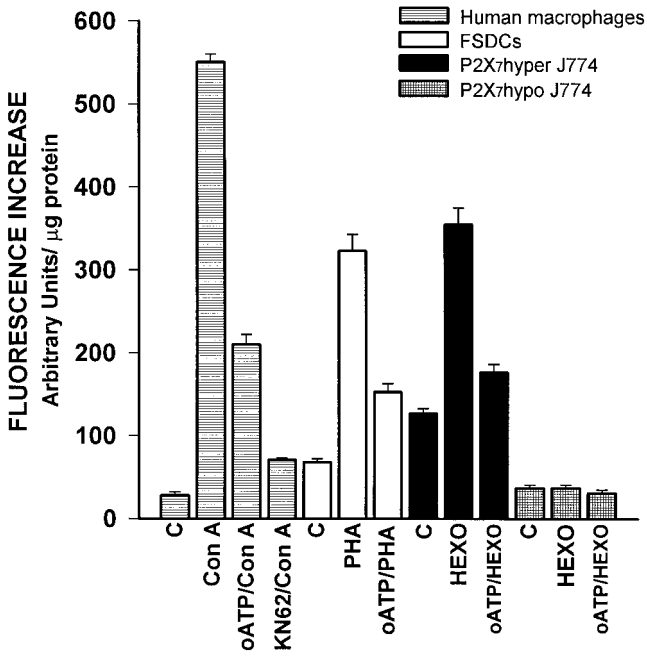

Figure 7. Caspase 3 activation during cell fusion. Cells were seeded in 24-well culture plates and incubated either in culture medium alone (C) or in culture medium supplemented with ConA (10 μg/ml) for human macrophages, PHA (10 μg/ml) for FSDCs, and hexokinase (HEXO) for J774 macrophages. In some experiments, oATP (300 μM) or KN-62 (50 nM) also were added. After 2 d, when MGC formation was maximal, the monolayers cells were rinsed several times, lysed in lysis buffer provided with the Enz-Check kit, and assayed for caspase 3 activation. Data are average ± SD of six determinations.

therefore asked whether this cystein protease is activated during macrophage fusion, and can thus be used as an indicator of $P2X_7$ opening. Caspase 3 activity was measured at peak time for fusion in four different cell types: human monocyte-derived macrophages, FSDCs, $P2X_7$ hyper, and $P2X_7$ hypo J774 macrophages (Figure 7). Monocyte-derived human macrophages in culture were stimulated to fuse with Con A, FSDCs with PHA, and $P2X_7$ hyper and $P2X_7$ hypo cells with hexokinase, and were then processed for caspase 3 activation measurement. In all cell models, with the exception of the $P2X_7$ hypo variant that is unable to fuse, there was a large caspase 3 stimulation, very likely due to $P2X_7$ activation because it 1) was inhibited by two specific $P2X_7$ blockers, oATP and 1-[*N*,*O*-bis(5-isoquinolinesulphonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) (Murgia *et al.*, 1993; Gargett and Wiley, 1997); and 2) did not occur in the $P2X_7$ hypo variant.

We then investigated the cellular distribution of $P2X_7$ during the fusion process, asking whether there was an increased localization of this receptor at sites of cell-to-cell contact during MGC formation in human monocyte-derived macrophage cultures induced to fuse with ConA. Figure 8A shows the stage at which the culture was fixed and stained with the anti-$P2X_7$ polyclonal Ab (after 1 day of culture in the presence of ConA): some MGCs were already formed and were growing in size by recruiting nearby macrophages. Podosomes projected from the cell body of the incoming macrophages (arrows) and established contact with the MGC plasma membrane. Staining with the anti-$P2X_7$ Ab revealed discrete patches at the level of the plasma membrane (Figure 8B) that at a higher magnification appeared to be more concentrated at sites of cell-to-cell interaction, especially at the tip of the podosome (Figure 8, C and D). Figure 8E shows a control experiment performed with preimmune rabbit serum.

DISCUSSION

There is increasing awareness that mononuclear phagocytes express plasma membrane receptors for extracellular nucleotides that are likely to have a mediator role during the inflammatory reaction (Di Virgilio, 1995; Ferrari *et al.*, 1996; Humphreys and Dubyak, 1996; Mutini *et al.*, 1999; Sikora *et al.*, 1999). These receptors belong to the P2Y (G-protein–coupled, seven-membrane–spanning receptors) or to the P2X (intrinsic ligand-gated ion channels) subfamilies (Burnstock, 1997; Ralevic and Burnstock, 1998). In particular, macrophage, microglial, and dendritic cells express to a high level the most peculiar member of the P2X subfamily, $P2X_7$. This is a bifunctional receptor that although upon transient stimulation with ATP behaves as a typical cation-selective ion channel permeable to K^+, Na^+, and Ca^{2+}, upon repetitive stimulation undergoes a transition into a nonselective pore that also allows transmembrane fluxes of low-molecular-mass hydrophylic molecules up to 900 Da. There is no clear-cut physiological function for such a receptor as yet, but it is clearly intriguing that it is up-regulated during monocyte-to-macrophage differentiation and by those stimuli that cause macrophage activation such as interferon-γ, and in some cases also bacterial endotoxin and tumor necrosis factor (Falzoni *et al.*, 1995; Humphreys and Dubyak, 1996; Di Virgilio *et al.*, 1998b). Due to the availability of a highly selective inhibitor of $P2X_7$, the mAb originally described by Buell *et al.* (1998), and used in the present work, we are now able to provide strong support to our original hypothesis on the involvement of this receptor in MGC formation. This mAb specifically recognizes an epitope located on the outer domain of the $P2X_7$ receptor, and the ability to almost completely prevent MGC formation is crucial evidence for the participation of this receptor in macrophage fusion. This is in keeping with the ability of this mAb to block other $P2X_7$-dependent responses such as transmembrane ion fluxes and IL-1β release (Buell *et al.*, 1998). The step in the fusion process in which $P2X_7$ takes part is however still uncertain. We think that because macrophage clustering in the presence of the mAb is not inhibited, $P2X_7$ does not act as a chemotactic or cell adhesion receptor, but rather intervenes in the very last phase of membrane fusion, maybe generating a "fusion pore" that establishes early bridges between the cytoplasm of the adjacent cells and drives the eventual fusion. In other words, it could be hypothesized that to form an efficient fusion pore it is necessary that at least two $P2X_7$ receptors on opposite plasma membranes come in contact via their extracellular domains, not dissimilarly from the mechanism whereby gap-junctional communication is established by the hemi-gap junctions expressed on the membrane of adjacent cells. The finding of an oATP and KN-62 inhibitable caspase 3 activation during fusion

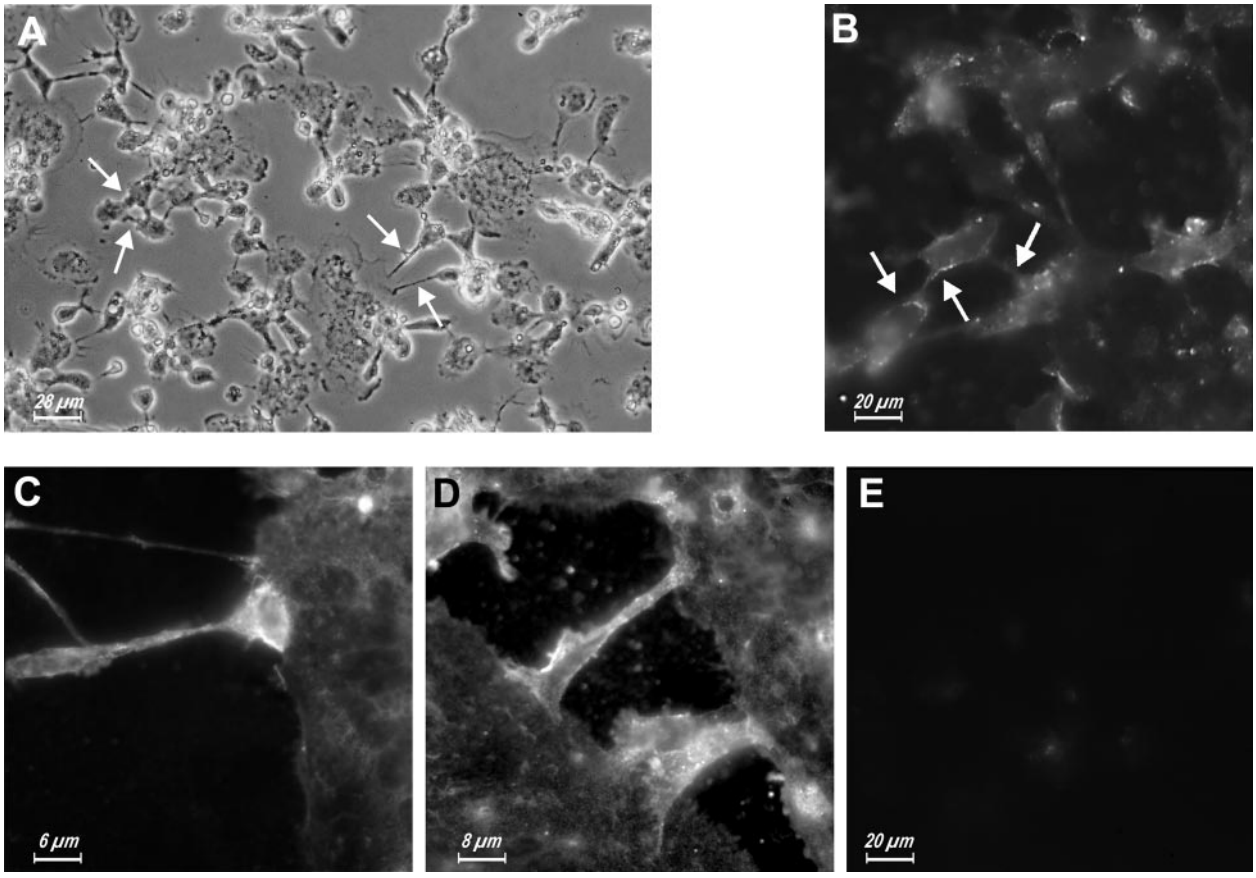

Figure 8. Immunolocalization of $P2X_7$ receptor during MGC formation. Monocyte-derived human macrophages were seeded in 24-well culture plates and stimulated with ConA (10 μg/ml). At the beginning of fusion (24 h after the seeding), cells were fixed and immunostained as described in MATERIALS AND METHODS. (A) Phase. (B–E) Fluorescence.

supports the hypothesis that $P2X_7$ transiently opens, but further experiments are needed to provide unequivocal evidence.

A related question is what turns on $P2X_7$ during fusion. We anticipated that the trigger could be the $P2X_7$ physiological ligand, i.e. extracellular ATP; however, this turned out not to be the case because addition of ATP to the macrophage monolayers, if anything, inhibited fusion. In hindsight, this was not entirely unexpected because it is clear that, in order to allow membrane fusion, opening of the $P2X_7$ pore must be strictly controlled and occur only when the opposing plasma membranes are tightly juxtapposed and ready to merge. In contrast, the mere addition of ATP to a macrophage monolayer very likely activates $P2X_7$ in an untimely manner, with an overall detrimental effect on cell fusion. Thus, we think that the trigger could be a surface molecule, maybe $P2X_7$ itself, on the opposing membrane. This interpretation received some support from the facilitating effect of apyrase and hexokinase. These two enzymes efficiently hydrolyze extracellular ATP, and have been shown to restore sensitivity of P2X receptors desensitized by the chronic leakage of ATP that is known to occur from many cell types, macrophages included (Baricordi *et al.*, 1996; Buell *et al.*, 1996; Ferrari *et al.*, 1997b). Thus, it is possible that under normal conditions $P2X_7$ receptors are partially desensitized by the continuous leakage of ATP, or even stably occupied by this nucleotide, and therefore unavailable for fusion. Removal of ATP by apyrase or hexokinase would reestablish $P2X_7$ sensitivity and thus accelerate fusion. Localization of $P2X_7$ during MGC formation supports our hypothesis. In resting macrophages and MGCs $P2X_7$ is uniformly distributed on the plasma membrane, but during fusion it concentrates in discrete membrane clusters at the site of cell-to-cell interaction.

In conclusion, our data support a role for $P2X_7$ as a novel plasma membrane receptor involved in macrophage fusion and MGC formation.

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