Endocytosis and vesicular trafficking of immune complexes and activation of phospholipase D by the human high-affinity IgG receptor requires distinct phosphoinositide 3-kinase activities

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FcγRI, the human high-affinity IgG receptor, is responsible for the internalization of immune complexes and their subsequent targetting to the lysosomes for degradation. We show here that aggregation of FcγRI by surface immune complexes in interferon-γ-primed U937 cells causes the transient appearance of swollen vacuolar structures, probably swollen late endosomes, which disappear as the immune complexes are degraded. Wortmannin and LY294002, specific inhibitors of phosphoinositide 3-kinases (PI 3-kinases), delay the disappearance of these structures and also correspondingly inhibit degradation of FcγRI-mediated immune complexes. In addition these inhibitors delay the initial phase of FcγRI-mediated endocytosis of immune complexes and block the activity of FcγRI-stimulated phospholipase D, an enzyme that has previously been implicated in membrane-trafficking events. p85 is the regulatory subunit of PI 3-kinase. A p85-dependent PI 3-kinase was shown to be involved in the initial phase of FcγRI-mediated endocytosis, but not in the trafficking of immune complexes for degradation or the activation of phospholipase D. The results presented here show a role for a p85-independent PI 3-kinase in regulating the trafficking of FcγRI-mediated immune complexes, either directly or as a result of the activation of phospholipase D, and a distinct role for a p85-dependent PI 3-kinase isoform in the initial phases of FcγRI-mediated internalization of immune complexes.

Key words: FcγRI, immunoreceptor, phosphatidylinositol 3-kinase, phospholipase D.

INTRODUCTION

Fc receptors (FcγRs) specific for binding the constant region of IgG are expressed on the surface of many different cell types of the immune system and play a pivotal role in linking the cellular and humoral arms of the immune response [1,2]. On leucocytes, aggregation of FcγRs leads to a number of cellular responses including the internalization of immune complexes by endocytosis, degranulation and the release of proteases, activation of the respiratory burst and the release of cytokines. As a result of these cellular responses, FcγRs can ultimately initiate targeted cell killing through antibody-directed cellular cytotoxicity [3], a process important for cancer surveillance and for the clearance of virus-infected cells [4].

Three classes of FcγRs have been identified and cloned in mammals (FcγRI, FcγRII and FcγRIII), each of which have different isoforms with different affinities for IgG and different tissue distributions [1,2]. FcγRI is the human high-affinity receptor [5] and, although its cytoplasmic tail contains no obvious signalling motif, it has been shown to interact with the γ-chain [6,7] which contains an immunoreceptor tyrosine based activation motif [8]. Aggregation of FcγRI leads to tyrosine-phosphorylation events [9–12], which in turn results in calcium transients [13] and initiates vesicular trafficking of internalized immune complexes for degradation [14]. Recently, downstream of tyrosine kinase activation, FcγRI in interferon-γ (IFN-γ)-primed cells has been shown to be functionally coupled to a novel intracellular signalling pathway involving the sequential activation of phospholipase D (PLD) and sphingosine kinase [12]. Inhibition of this pathway diminished both the rate of vesicular trafficking of immune complexes for degradation and calcium transients [12].

FcγRI has also been shown to activate phosphoinositide 3-kinases (PI 3-kinases) [15,16], and the inhibition of PI 3-kinases by wortmannin inhibits Fc-receptor-mediated phagocytosis of opsonized particles [15]. However, although PI 3-kinases have been implicated in regulating the internalization and trafficking of various tyrosine kinase linked receptors, little is known of the role of PI 3-kinase activation in FcγRI-mediated endocytosis and its relationship to the novel signalling pathway identified in these cells. p85 is the regulatory subunit of PI 3-kinase. Here we show that the activation of a p85-dependent PI 3-kinase by FcγRI in IFN-γ-primed cells plays a role in the initial endocytosis of immune complexes, but that a distinct p85-independent PI 3-kinase isoform is necessary for the vesicular trafficking of internalized immune complexes for degradation. Furthermore, this p85-independent PI 3-kinase activation is shown to be upstream of PLD activation.

Abbreviations used: FcγRI, high-affinity IgG receptor; IFN-γ, interferon-γ; IPTG, isopropyl β-D-thiogalactoside; PI 3-kinase, phosphoinositide 3-kinase; PtdBut, phosphatidylbutanol; PKC, protein kinase C; PLD, phospholipase D.

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MATERIALS AND METHODS

Materials

Human IgG and sheep anti-(human IgG) (γ-chain-specific) were obtained from Serotec Ltd (Kidlington, Oxford, U.K.). Wortmannin and LY294002 were obtained from Calbiochem. Cells were treated with inhibitors for 30 min at 37 °C prior to receptor aggregation or stimulation. Unless otherwise specified, wortmannin was used at a concentration of 50 nM and LY294002 was used at a concentration of 250 μM. Antisense oligonucleotides were purchased from Oswel DNA Services (Southampton, U.K.) and were designed as previously described [16]. Cells were incubated with 10 μM of oligonucleotide for 1 h before, and then for the duration of, culture with IFN-γ.

Cell culture

U937 cells were cultured in RPMI 1640 (Gibco) supplemented with 10% (v/v) fetal-calf serum (FCS), 2 mM glutamine, 10 units/ml penicillin and 10 μg/ml streptomycin at 37 °C, at 6.8% CO_2 in a water-saturated atmosphere. U937:Δp85 cells (generously provided by Dr. Len Stephens, The Babraham Institute, Cambridge, U.K.) were similarly cultured, but in addition were cultured in the presence of 0.6 mg/ml of G418 (the antibiotic Geneticin) and 0.1 mg/ml of hygromycin B (Calbiochem). Expression of Δp85 (a dominant negative form of p85) was induced with 15 mM isopropyl β-D-thiogalactoside (IPTG), 5 nM PMA, and 100 μM $ZnCl_2$ for 10 h. Cells were primed with 200 ng/ml IFN-γ (Bender Wein, Vienna, Austria) for 24 h prior to experimentation.

Measurement of endocytosis and rate of trafficking for degradation

IFN-γ-primed U937 (or U937:Δp85) cells were harvested and washed in RPMI/Hepes containing 5% FCS. The cells were then loaded with ^{125}I-labelled IgG as previously described [17]. Non-bound radiolabel was removed by dilution and centrifugation, cross-linking antibody was added and the cells were rolled at 37 °C for the given times. At each time point, triplicate aliquots of cells were transferred into ice-cold acidified PBS, pH 2.0, for 5 min to strip off cell-surface-bound radiolabelled immune complexes [23]. The cells were then harvested by centrifugation and the cell pellet counted for radioactivity in a Packard γ-radiation counter to yield the cell-associated radioactivity. At zero time, further aliquots of cells were harvested into ice-cold PBS, pH 7.4, to provide a measure of the total radioactivity bound to the cell surface. The cell-associated radioactivity for each time point was then expressed as the percentage of total radioactivity bound at time zero to provide a measure of the rate of internalization of the immune complexes.

To measure the rate of degradation of the radiolabelled immune complexes, at given times the supernatant of the cell culture was harvested [18]. Trichloroacetic acid (10% final concn.) was added to these cell-free supernatants and, after incubation on ice for 60 min, the trichloroacetic acid-insoluble radioactivity was removed by centrifugation (12000 ***g*** at 4 °C for 15 min) and the trichloroacetic acid-soluble radioactivity counted in a Packard γ-radiation counter. The trichloroacetic acid-soluble radioactivity was then expressed as a percentage of total radioactivity bound at time zero [14].

Electron microscopy

Electron microscopy of cells was carried out essentially as described by Harrison et al. [17]. Briefly, cells were fixed in suspension for 30 min in 2% glutaraldehyde in PBS, spun down and the pellets fixed for 15 min in the same buffer. Following three washes in PBS, cells were post-fixed in 1% osmium tetroxide in PBS for 60 min, and then washed for 1 h (with three changes of buffer) in 0.05 M malic acid, pH 5.2. Cells were bulk-stained in 0.5% uranyl acetate in maleate buffer overnight and then dehydrated through a graded series of alcohols before being embedded in Araldite CY212 resin. Sections of approx. 60 nm were stained with uranyl acetate and lead citrate and observed in a Philips EM300 electron microscope.

Measurement of PLD activity

PLD activity was measured in cell extracts by the transphosphatidylation assay as described previously [12].

RESULTS

Endocytosis of immune complexes is associated with the transient appearance of large vacuolar structures in the cytoplasm of U937 cells

Aggregation of FcγRI in IFN-γ primed cells resulted in the transient appearance of large vacuolar like structures in the cytoplasm (Figure 1A). These structures resembled swollen endosomes. Multiple large vacuolar-like structures were observed 15 min after receptor cross-linking and had begun to resolve after 30 min. By 60 min, the cell appearance had returned to that observed for resting cells and the large vacuolar structures had essentially disappeared.

The transient appearance of these structures correlates with the kinetics of FcγRI endocytosis and trafficking of internalized immune complexes for degradation [14,17] (Figures 2A, 2B and 2C). Thus all surface-bound radiolabelled IgG was internalized within 15 min of receptor cross-linking (Figures 2A and 2B). However, by 120 min, the amount of radiolabel trapped inside the cells had fallen to 42 ± 1.7% (mean $\pm$ S.D.) and this was matched by the appearance of trichloroacetic acid soluble radioactivity in the supernatant (Figure 2C); which can be taken as a measure of the rate of degradation of the immune complexes [18].

Initial internalization of immune complexes is delayed and the vesicular trafficking of immune complexes is inhibited by PI 3-kinase specific inhibitors, wortmannin and LY294002

As PI 3-kinases have been shown to regulate vesicular trafficking in a number of systems [19,20], the effect of inhibitors on FcγRI recycling, FcγRI-mediated endocytosis and immune complex trafficking was measured.

Treatment of cells with wortmannin (50 nM) or LY294002 (250 μM), both specific inhibitors of PI 3-kinases [21,22], had no effect on FcγRI recycling (results not shown). By contrast, wortmannin significantly delayed the rate of internalization of immune complexes by FcγRI in IFN-γ primed cells (Figure 2A). In addition, over longer time points, the rate of loss of cell-associated radioactivity was significantly inhibited following treatment with either wortmannin or LY294002 (Figure 2B). and the rate of appearance of trichloroacetic acid-soluble radioactivity in the supernatant was significantly delayed (Figure 2C). After 120 min incubation, only 12 ± 0.8% (mean $\pm$ S.D.) of the radiolabel appeared as trichloroacetic acid-soluble radioactivity in the culture media of cells treated with wortmannin, compared with 48 ± 0.8% (mean $\pm$ S.D.) for the control cells (Figure 2C). A similar inhibition was observed for cells treated with LY294002 (Figure 2C).

Consistent with these findings, electron microscopy revealed that wortmannin did not affect the appearance of the vacuolar-

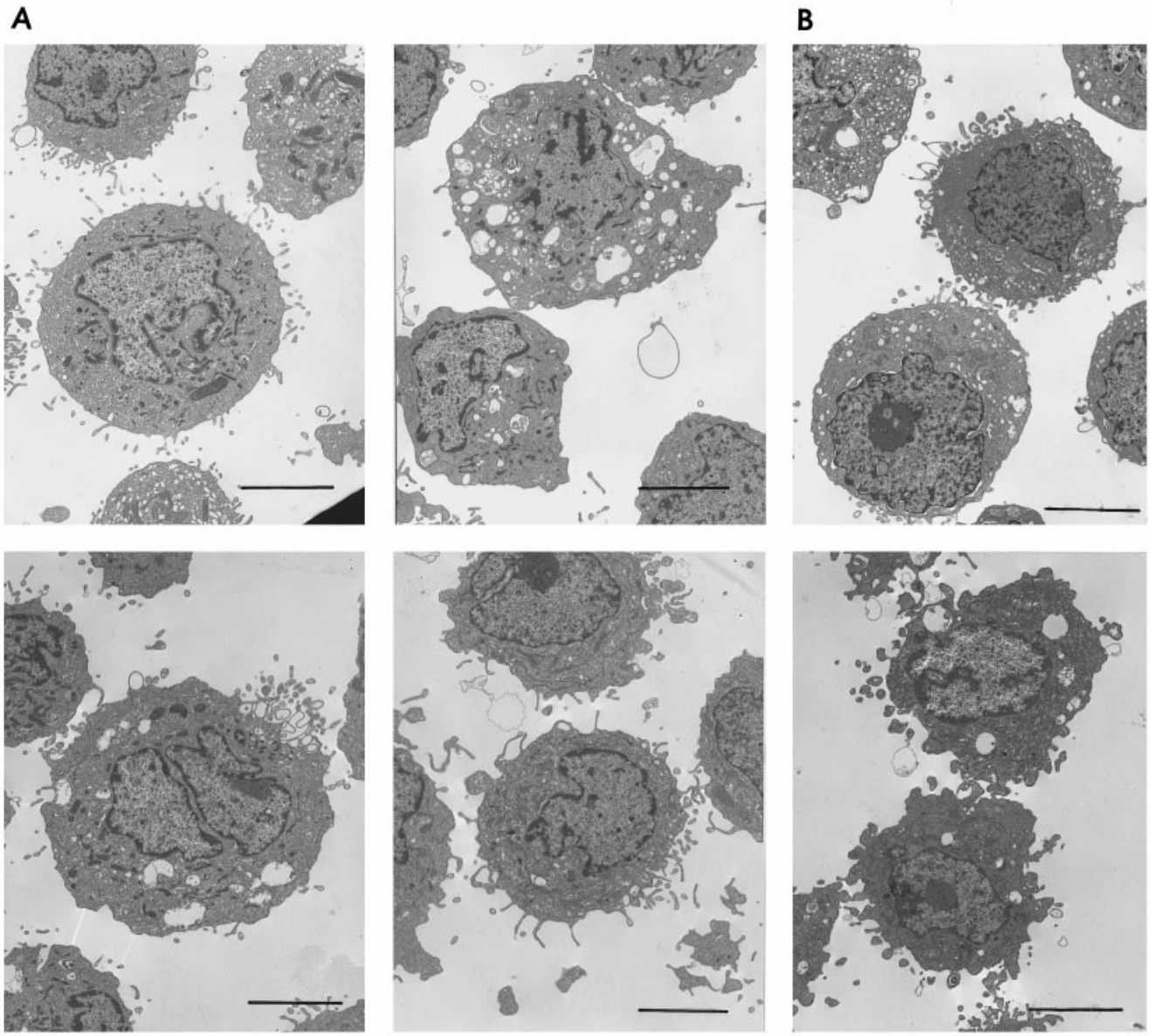

Figure 1 Endocytosis of FcγRI–IgG complexes results in the transient appearance of large cytoplasmic vacuoles in U937 cells

(**A**) Cells were loaded with IgG at 4 °C and then warmed to 37 °C in the presence of cross-linking antibody for 0, 15, 30, or 60 min (top left, top right, bottom left, bottom right respectively) prior to fixation and electron microscopy. The scale bar represents 5 μm. (**B**) Cells were pretreated with 50 nM wortmannin for 30 min prior to loading with IgG at 4 °C. Cells were then warmed to 37 °C in the presence of cross-linking antibody for 0 (top) or 60 min (bottom) prior to fixation and electron microscopy. The scale bar represents 5 μm.

like structures following FcγRI aggregation, but rather it inhibited their disappearance (Figure 1B). Thus 60 min after receptor aggregation, whilst the swollen vacuolar structures had disappeared in untreated cells, they were still clearly visible in the cells treated with wortmannin. The ultrastructure of the cells was not significantly altered by treatment of the cells with wortmannin alone in the absence of FcγRI activation.

The effects of wortmannin and LY294002 on the trafficking of FcγRI-mediated immune complexes were found to be dependent upon the concentration of inhibitor used (Figure 2D). The concentrations of these inhibitors that were found to be effective as inhibitors of vesicular trafficking were of the same order as those reported specific for PI 3-kinases [21,22].

Since we have shown previously that aggregation of FcγRI by surface immune complexes results in the activation of both p85-dependent and G-protein-βγ-subunit-coupled PI 3-kinase isoforms [16], it was decided to investigate whether either of these isoforms had a role in FcγRI-mediated endocytosis and/or in the trafficking. To investigate the role of the p85-dependent PI 3-kinase isoform in these events, a U937 cell line with an IPTG-inducible dominant-negative form of p85 (U937:Δp85) was used [23]. This dominant negative protein lacks the binding site for the p110 catalytic subunit of PI 3-kinase and when overexpressed oblates p85-mediated PI 3-kinase associations with other signalling components [23,24]. To investigate the role of the G-protein βγ subunit-dependent PI 3-kinase, antisense oligo-

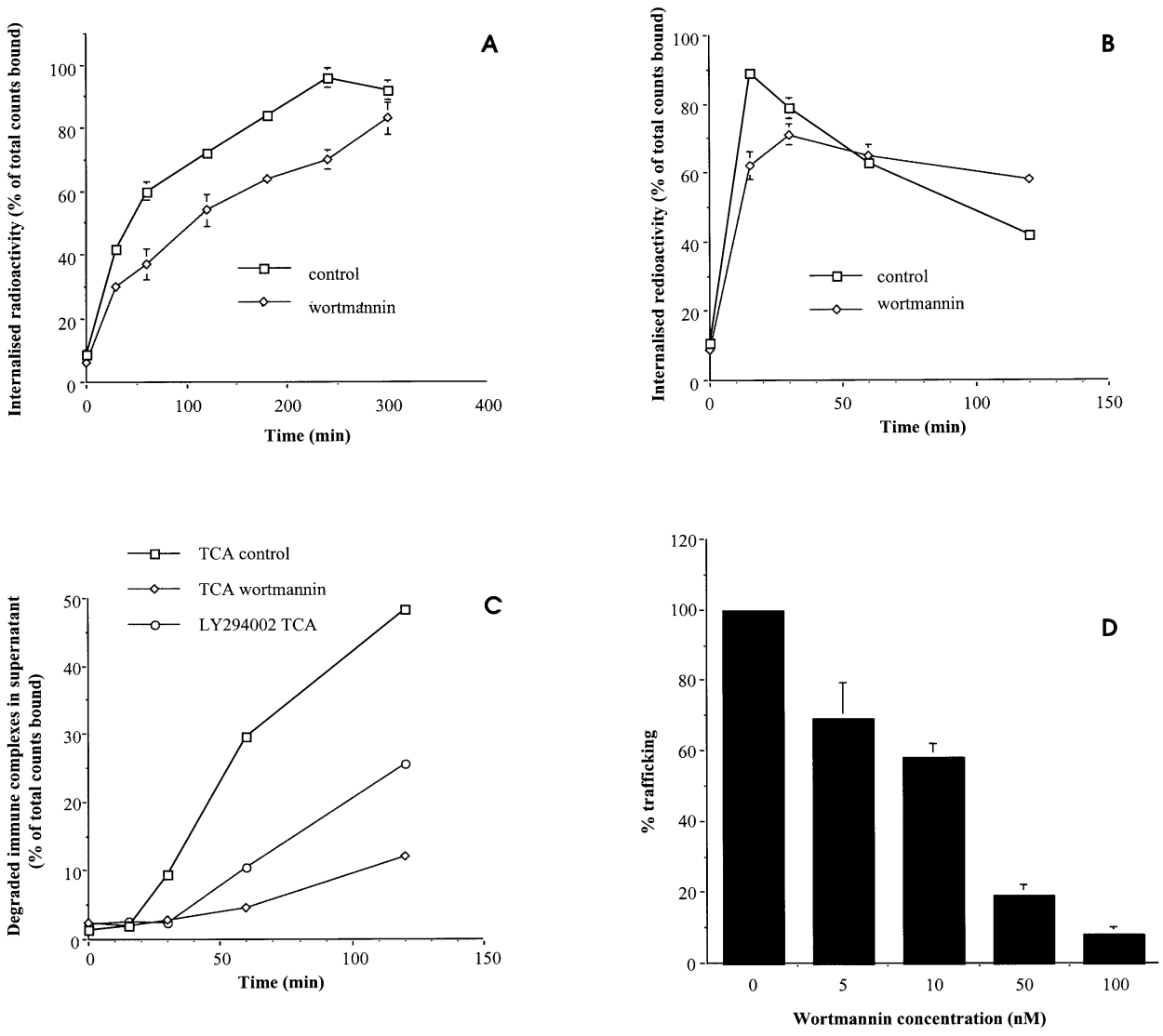

Figure 2 Initial internalization and vesicular trafficking of immune complexes by FcγRI is delayed by PI 3-kinase-specific inhibitors

(**A**) The initial rate of internalization of surface-bound ^{125}I-labelled IgG was inhibited by pretreatment of cells with wortmannin. The rate of internalization of surface-bound ^{125}I-labelled IgG following aggregation at 37 °C was measured by harvesting cells at the given time points and measuring the amount of radiolabel trapped inside the cells as non-acid releasable radioactivity. These are expressed as a percentage of the total radioactivity bound at zero time. Control, control cells; wortmannin, cells treated with wortmannin (50 nM). Results shown are the means ± S.D. for triplicate measurements at each time point. The results are typical of those from three separate experiments. (**B**) The rate of loss of internalized radioactivity was delayed by pretreatment of cells with wortmannin. The amount of internalized ^{125}I-labelled IgG was measured over prolonged time points by harvesting cells and measuring the amount of radiolabel trapped inside the cells as non-acid-releasable radioactivity. These are expressed as a percentage of the total radioactivity bound at zero time. Control, control cells; wortmannin, cells treated with wortmannin (50 nM). Results shown are the means ± S.D. for triplicate measurements at each time point. The results are typical of those from three separate experiments. (**C**) The rate of appearance of trichloroacetic acid (TCA)-soluble radioactivity in the supernatant was significantly delayed by pretreatment of cells with wortmannin or LY294002. Following internalization of ^{125}I-labelled IgG, the trichloroacetic acid soluble radioactivity in the cell supernatants were measured at the times indicated and expressed as a percentage of the total radioactivity bound at zero time. TCA control, control cells; TCA wortmannin, cells treated with wortmannin (50 nM); LY294002 TCA, cells treated with LY294002 (250 μM). The results shown are the means ± S.D. for triplicate measurements at each time point. The results shown are typical from three separate experiments. (**D**) The effect of wortmannin on trafficking of FcγRI-mediated immune complexes were dependent upon the concentration of the inhibitor. Cells were treated with wortmannin at the concentrations indicated for 30 min prior to loading with IgG. The rate of appearance of trichloroacetic acid-soluble radioactivity in the supernatant was measured 120 min after receptor cross-linking and incubation at 37 °C. Triplicate measurements were taken at times 0 and 120 min and the radioactivity in the incubation supernatant soluble in trichloroacetic acid at 120 min were calculated as a percentage of the total radioactivity bound at time zero. The extent of trafficking at each wortmannin concentration was expressed as a fraction of the loss of cell-associated radioactivity in untreated cells which were designated as '100% trafficking'.

nucleotides were used which have previously been shown to down-regulate significantly p110γ expression [16].

Overexpression of Δp85 resulted in a delay in the initial internalization of immune complexes by FcγRI, in a manner identical with that observed when U937 cells had been treated with wortmannin (Figure 3). In contrast with this, p110γ antisense oligonucleotides had no effect on the rate of FcγRI-mediated internalization of immune complexes (results not shown). Neither the overexpression of the Δp85 dominant negative protein or the use of p110γ antisense oligonucleotides inhibited either the rate of loss of cell-associated radioactivity (results not shown) or the rate of appearance of trichloro-

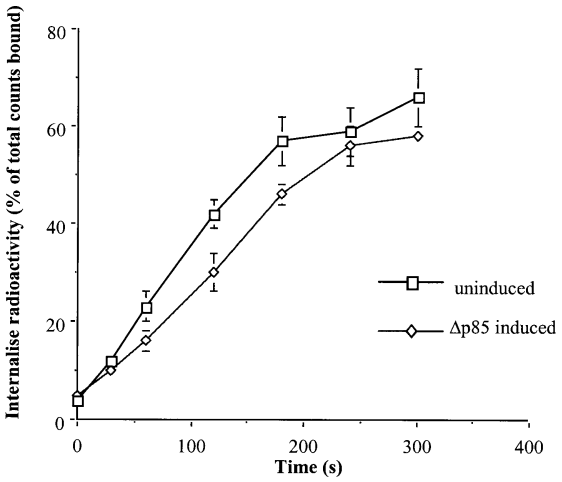

Figure 3 A p85-dependent PI 3-kinase is required for the initial internalization of immune complexes by FcγRI

Internalization of surface-bound ^{125}I-labelled IgG was delayed by expression of the dominant negative protein Δp85. Non-acid- releasable radioactivity was determined at the time points indicated and are expressed as a percentage of the total radioactivity bound at time zero. Uninduced, uninduced Δp85 expression; induced, induced Δp85 expression. Results shown are the means ± S.D. for triplicate measurements at each time points. The results are typical from those of three separate experiments.

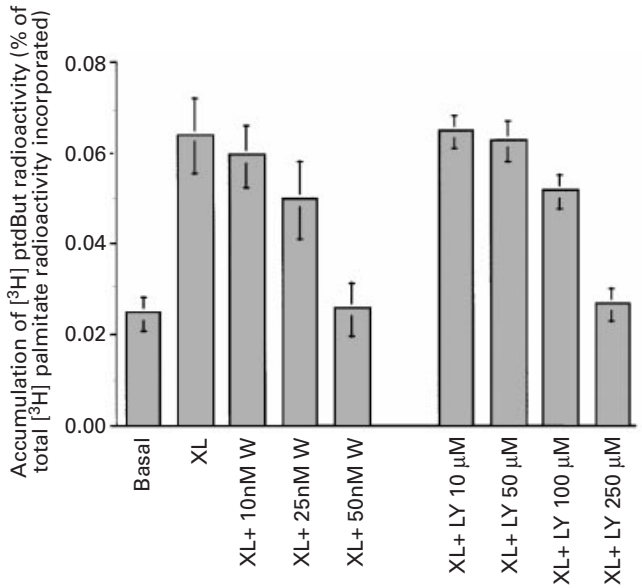

Figure 4 FcγRI activation of PLD requires PI 3-kinase

Cells were pretreated with wortmannin (W) or LY294002 (LY) at the concentrations indicated for 30 min prior to receptor cross-linking and measurement of PLD activity. PLD activity was measured by the accumulation of [^{3}H]phosphatidylbutanol (PtdBut) radioactivity. Results are means ± S.D. for triplicate measurements and are representative of the results from at least three separate experiments. XL, cross-linked.

acetic acid-soluble radioactivity in the supernatant (results not shown).

Taken together, these results indicate that a p85-dependent PI 3-kinase isoform is involved in the initial internalization of immune complexes by FcγRI and that a p85-independent PI 3-kinase isoform other than the G-protein-βγ-subunit-activated isoform is required for the trafficking of immune complexes from the early endosome for degradation.

FcγRI activation of PI 3-kinase is required to trigger PLD

FcγRI in IFN-γ primed cells is functionally coupled to a novel intracellular signalling pathway that involves tyrosine kinase activation of PLD and sphingosine kinase [12]. The relationship of PI 3-kinase to PLD activation was therefore investigated. Preincubation of IFN-γ-primed U937 cells with either wortmannin or LY294002 inhibited FcγRI activation of PLD in a concentration-dependent manner (Figure 4). A direct action of these inhibitors on PLD activity was excluded, as neither wortmannin (50 nM) nor LY294002 (250 μM) inhibited PLD activity after stimulation with the phorbol ester PMA (results not shown).

To investigate whether either p85-dependent or G-protein-βγ-subunit-dependent PI 3-kinase isoforms were involved in coupling FcγRI to PLD activation, the U937:Δp85 cell line and p110γ antisense oligonucleotides were used. Neither overexpression of the Δp85 dominant negative protein or treatment of cells with the antisense oligonucleotides altered PLD activation following FcγRI aggregation (results not shown). Taken together, these results imply that a PI 3-kinase isoform other than the p85-dependent or G-protein-βγ-subunit-dependent isoforms is involved in the signalling pathway which functionally couples FcγRI to the trafficking of immune complexes for degradation.

DISCUSSION

In the present study we have shown that endocytosis of FcγRI-mediated immune complexes results in the transient formation of large swollen vacuolar structures within the cytoplasm of IFN-γ-primed U937 cells, which disappear as the immune complexes are degraded. Wortmannin and LY294002, specific inhibitors of PI 3-kinases, delay the disappearance of these structures and the degradation of FcγRI-mediated immune complexes. These inhibitors delayed the initial internalization of immune complexes by FcγRI and blocked the FcγRI-stimulated activity of PLD, an enzyme previously implicated in membrane-trafficking events. The tyrosine kinase-regulated p85-dependent PI 3-kinase was shown to be involved in the initial phase of FcγRI-mediated endocytosis, but not in the trafficking of immune complexes for degradation or in the activation of PLD. Therefore, distinct PI 3-kinase isoforms have roles in internalization or trafficking of FcγRI-mediated immune complexes.

The appearance and disappearance of the large swollen vacuolar structures within the cytoplasm of IFN-γ-primed U937 cells correlates with the rate of internalization and disposal of immune complexes measured using ^{125}I-IgG. After internalization, endocytosed receptor-bound proteins are delivered to a common early endosomal compartment from which they are sorted and delivered to various intracellular destinations. These destinations include a putative recycling compartment, the *trans*-Golgi network, and from these destinations they can subsequently be diverted to a lysosomal degradative/storage compartment [24]. The mechanisms underlying the passage of ligands from early endosomes through deep-lying, late endosomes on the way to lysosome degradation is controversial as to whether it results from vesicular trafficking or endosome maturation [25,26]. The vacuolar structures observed following FcγRI aggregation are similar to those observed in newborn-rat kidney cells in which wortmannin was used to block traffic out of the endosomal system [27], and it is likely that these structures represent swollen

late-endosomal compartments, suggesting that these late-endosomal compartments become saturated and consequently swollen following FcγRI-internalized immune complexes. Subsequently, when these immune complexes are delivered to the lysosomes and degraded, the endosomes lose their swollen appearance.

Wortmannin and LY294002, both specific inhibitors of PI 3-kinases, delayed the rate of degradation of FcγRI-internalized immune complexes and the disappearance of the vacuolar structures following FcγRI aggregation. These results therefore suggest that PI 3-kinases are required for the trafficking of FcγRI-mediated immune complexes from the plasma membrane and early endosomes to the lysosomes, where the immune complexes are degraded.

A number of isoforms of PI 3-kinase have previously been implicated in vesicular trafficking. For example, mutants of the yeast *Saccharomyces* defective for the gene *vps34*, which encodes a homologue of a mammalian PI 3-kinase, are no longer able to direct proteins to the vacuole, the yeast equivalent of the lysosome [28]. Moreover, in the cellular slime mould *Dictyostelium discoidium*, inactivation of two genes encoding proteins related to mammalian p110 PI 3-kinases (DdPIK1 and DdPIK2) results in defects in endocytosis and lysosome to post-lysosomal transport [29]. A growing amount of work has indicated that wortmannin inhibits membrane traffic out of the endosomal system. This has been shown for insulin-dependent trafficking of the glucose transporter GLUT4 to the plasma membrane [20], transferrin receptor recycling [30–32], trafficking of the platelet-derived growth factor (PDGF) receptor to lysosomes for degradation [19], and lgp120, lgp110 and mannose 6-phosphate receptor recycling. Such work has led to the suggestion for a role for PI 3-kinase in regulating membrane traffic in the late-endocytic pathway.

The work presented here suggests that distinct PI 3-kinase isoforms have roles in the initial internalization of immune complexes by FcγRI and in the subsequent trafficking of these immune complexes to lysosomes. A p85-dependent PI 3-kinase is involved in the initial internalization step, whereas a p85-independent PI 3-kinase that is not the G-protein-$\beta\gamma$-subunit-dependent PI 3-kinase p110γ appears to be involved in trafficking to lysosomes. We have previously shown that both p85-dependent and G-protein-coupled PI 3-kinases are activated on FcγRI aggregation, as measured by phosphatidylinositol 3,4,5-trisphosphate production [16]. It, therefore, seems likely that a further PI 3-kinase isoform is activated on FcγRI aggregation. A likely candidate would be a class III PI 3-kinase isoform. These are phosphatidylinositol-specific and so would not be measured in previous assays [16] and which are homologous with yeast Vps34p, a PI 3-kinase which is responsible for the trafficking of proteins from the Golgi to the yeast vacuole [33,34].

In previous studies we have shown that FcγRI aggregation results in the sequential activation of tyrosine kinase(s), PLD and sphingosine kinase [12]. Here, we show that FcγRI-stimulated PLD activity requires PI 3-kinase activity. We have previously shown that butan-1-ol, which prevents the formation of the products of the PLD reaction, also delays FcγRI-mediated trafficking of immune complexes for degradation [12]. Recent studies have implicated a role for phosphatidic acid in the formation of certain types of transport vesicles [35]. It is therefore possible that the effect of wortmannin on the trafficking of FcγRI-internalized immune complexes observed here results from a failure to activate PLD rather than being directly due to a requirement for the products of the PI 3-kinase reaction for trafficking *per se*. Neither overexpression of the dominant negative Δp85 protein nor the use of p110γ antisense oligonucleotides blocked FcγRI stimulated PLD activity. These results are consistent with those for the trafficking of FcγRI-mediated immune complexes and suggests that the same p85-independent PI 3-kinase activity involved in the trafficking of the immune complexes is also necessary for the activation of PLD.

The mechanism by which PI 3-kinases might activate PLD is not clear. Some reports suggest that phosphoinositides can directly activate PLD [36]. PI 3-kinases have been shown to regulate the activity of the Ras-related low-molecular-mass GTPases Rab5 and Rab7, which have been shown to have roles in endocytosis and transport from early to late endocytic compartments [37,38]. In addition some isoforms of PI 3-kinase have binding sites for Ras-related GTPases [39], and Rab, Ras, Rho, Arf and other related low-molecular-mass GTPases have previously been shown to activate PLD isoforms [40–44]. It has also been suggested that there may be a role for isoforms of protein kinase C (PKC) in endocytosis and membrane trafficking [45]. Isoforms of PKC have been shown to be activated by, and to associate with, PI 3-kinases [46–48], and, in turn, PKC isoforms have been shown to activate PLD [36,49]. It is therefore conceivable that PI 3-kinase activates PLD in this system by the intermediate activation of low-molecular-mass GTPases and/or PKC isoforms.

The results presented here, and others presented previously [12], show that FcγRI activates a novel signalling pathway that is functionally coupled to the internalization and trafficking of internalized immune complexes for degradation. The internalization is dependent in part on the p85 form of PI 3-kinase, whereas a novel as yet unidentified PI 3-kinase is required for the activation of PLD and trafficking of immune complexes to lysosomes.

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