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Wortmannin inhibits transcytosis of dimeric IgA by the polymeric immunoglobulin receptor

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Abstract Phosphatidyl inositol 3-kinase (PI3K) plays an essential role in numerous signaling events, and increasingly has been implicated in regulation of certain membrane traffic events. The polymeric immunoglobulin receptor (pIgR) transcytoses dimeric IgA (dIgA) across epithelial cells and into external secretions, where the dIgA forms the first specific immunological defense against infection. We show here that wortmannin, a highly specific inhibitor of PI3K, inhibits transcytosis of dIgA by the pIgR. Instead, the dIgA is recycled back to the basolateral surface of the epithelial cell. PI3K therefore plays an essential role in regulating the transcytosis of dIgA, a key step in the mucosal immune response.

Key words: Regional immunity; Epithelial cell; Cell signaling; Signaling molecule; Phosphatidylinositol 3-kinase

1. Introduction

Phosphatidylinositol 3-kinase (PI3K) is a family of enzymes that catalyzes the phosphorylation of phosphatidylinositols on the 3′ position of the inositol ring [1]. This enzyme and its products have been implicated in a wide variety of intracellular signaling events. In the last two years there has been particular interest in the possible role(s) of PI3K in regulation of membrane traffic. The seminal observation in this field was the discovery that a PI3K plays a vital role in sorting of vacuolar enzymes in the yeast, *Saccharomyces cerevisiae*. Mutations of the *VPS*34 gene inhibit delivery of newly made vacuolar enzymes from the trans-golgi network (TGN) to an endosomal compartment and then to the vacuole [2–4]. The newly-made vacuolar enzymes are instead mistargeted and secreted from the cell. These mutations also cause a profound decrease in PI3K activity in yeast. The *VPS*34 gene was found to encode a protein with significant sequence similarity to the catalytic subunit (p110) of a mammalian PI3K.

The role of PI3K in various cellular events has been investigated using wortmannin, a highly specific inhibitor of PI3K [1]. Recently, wortmannin has been shown to inhibit the sorting of a newly made mammalian lysosomal enzyme (cathepsin D) from the TGN to endosomes and lysosomes, which are the mammalian equivalent of vacuoles [5,6]. Instead, the cathepsin D was efficiently secreted from the mammalian cells. This provided strong evidence that PI3K plays a similar role in sorting of lysosomal enzymes in both yeast and mammalian cells.

In polarized epithelial cells, sorting in the endosomal system plays a role the establishment and maintenance of cell polarity [7,8]. The plasma membrane of these cells contains an apical surface, which faces the lumen of an organ or gland, and a basolateral surface, facing other cells and connective tissue. The apical and basolateral plasma membranes have very different protein and lipid compositions. After endocytosis from either surface, a protein has at least three choices: recycling to the original plasma membrane domain, transfer to late endosomes and lysosomes, or transcytosis to the opposite surface. Transcytosis has at least two main functions [7]. First, many newly made proteins that will become permanent residents of the apical surface are delivered first to the basolateral surface, and then endocytosed and transcytosed to the apical surface. Transcytosis is the only route for delivery of membrane proteins to the apical surface that is universally found in all epithelial cells so far examined, and in some cells is the only route for delivery of membrane proteins to that surface.

Second, extracellular ligands can bind to a receptor at one surface and then be transcytosed to the opposite surface. The best studied example of ligand transcytosis is the transcytosis of dimeric IgA (dIgA) by the polymeric immunoglobulin receptor (pIgR) in many mucosal epithelial cells [9]. The pIgR is sent from the TGN to the basolateral surface, where it can bind dIgA. The pIgR is then endocytosed and delivered to basolateral early endosomes. The pIgR then passes through a specialized endosomal compartment, known as the apical recycling endosome, before finally being delivered to the apical surface. At the apical surface the extracellular, ligand binding domain of the pIgR is cleaved and released together with the dIgA into external secretions. This cleaved fragment is called secretory component. The transcytosed dIgA forms the first specific immunological defense against mucosal infectious agents. Transcytosis of dIgA is thus a key step in the mucosal immune response.

Like lysosomal enzyme sorting, transcytosis is a good model in which to study regulation of traffic in the endosomal system. Transcytosis of the pIgR is regulated by ligand binding and by several intracellular signaling pathways, including protein kinase C, the heterotrimeric Gs protein, cAMP, and protein kinase A [7,10]. Here we have used wortmannin to investigate a possible role for PI3K in regulation of transcytosis of pIgR and dIgA.

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Abbreviations: dIgA, dimeric IgA; pIgR, polymeric immunoglobulin receptor; PI3K, phosphatidylinositol 3-kinase; TGN, *trans*-Golgi network.

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2. Materials and methods

Virtually all of the procedures used, such as culture of MDCK cells expressing pIgR on Transwell filters, and the assay for transcytosis and recycling of radio-iodinated dIgA, were performed as described [10]. In our standard assay of transcytosis cells were allowed to internalize a cohort of radio-iodinated dIgA from the basolateral surface for 15 min, washed for 5 min, and delivery into the apical medium (transcytosis) or basolateral medium (recycling) was followed for 2 h. Transcytosis and recycling are expressed as a percentage of the total dIgA in all fractions, including remaining associated with the cells and filters at the end of the assay. Wortmannin was purchased from Kamiya Biochemicals, Thousand Oaks, CA and made up as a stock solution of 1 mM in DMSO. Wortmannin was included at the indicated concentrations in all steps, starting with the 15 min uptake. DMSO alone had no effect.

3. Results and discussion

As in our previous studies, we used MDCK cells that had been transfected with the cDNA for rabbit pIgR [10]. These cells were grown on permeable filter supports to form a tight monolayer. We have previously described an assay for transcytosis and recycling of a dIgA by the pIgR expressed in these cells. Cells were allowed to internalize a cohort of radio-iodinated dIgA from the basolateral surface. The transcytosis of this dIgA into the medium overlying the apical surface of the cells was then measured. Treatment of the cells with wortmannin resulted in a partial inhibition of transcytosis of the dIgA (Fig. 1). Inhibition of transcytosis increased with increasing concentration of wortmannin. The inhibition was most dramatic at earlier time points. Even the lowest dose utilized, 250 nM wortmannin, gave an approximately 75% inhibition of transcytosis at the earliest time point, 7.5 min. At later time points the inhibition was somewhat less, suggesting that the effect of wortmannin was to a considerable extent on the kinetics of transcytosis. However, even with 250 nM wortmannin the extent of transcytosis during the complete 2 h time course of the assay was significantly reduced.

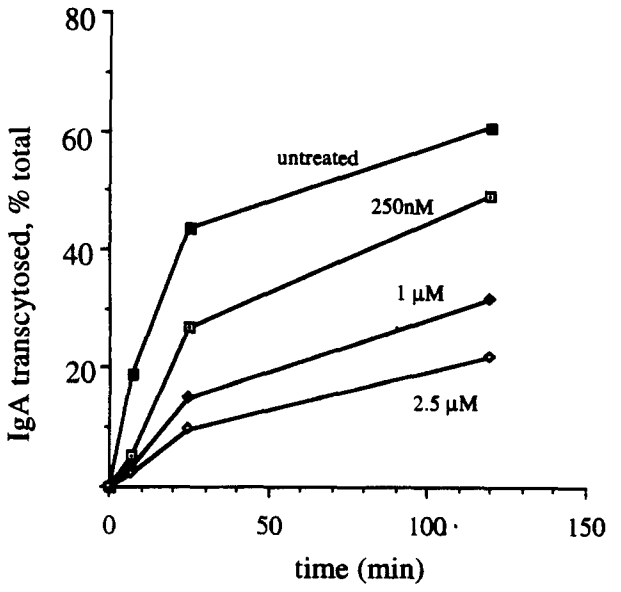

Fig. 1. Effect of wortmannin on transcytosis. Wortmannin was added at the indicated concentration. Transcytosis of a preinternalized cohort of radio-iodinated dIgA into the apical medium was measured over a 2 h time course. Data are means of 3 or 4 determinations. For clarity, error bars are not shown, but standard errors were less than 10% of the value.

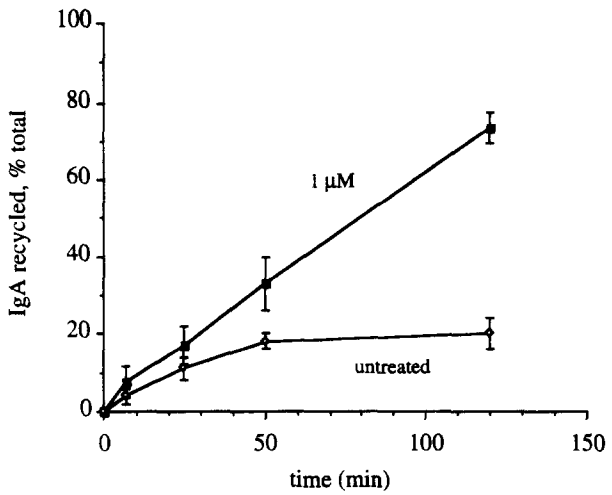

Fig. 2. Effect of wortmannin on recycling. Cells were either untreated or treated with 1 μM wortmannin. Recycling of a pre-internalized cohort of radio-iodinated dIgA into the basal medium was measured over a 2 h time course. Error bars are standard errors of 3 or 4 determinations.

As previously described, in the absence of wortmannin, about 20% of the dIgA recycles back to the basolateral medium [10,12]. However, in the presence of 1 μM wortmannin, recycling of dIgA to the basolateral medium was significantly increased (Fig. 2). The increase in recycling largely complemented the decrease in transcytosis, suggesting that the dIgA was redirected by the drug from the transcytotic to the recycling pathway. In all cases, degradation of dIgA was less then 5% of the total material, indicating that the dIgA was not mistargeted into the lysosomal pathway. Wortmannin did not alter the permeability characteristics of the tight MDCK monlayer, so our results were not an effect of dIgA leaking across the monolayer (not shown).

The total amount of dIgA internalized by the cells during the uptake period was not significantly affected by wortmannin (not shown), suggesting that initial delivery of pIgR from the TGN to the basolateral surface and endocytosis were not affected. This is in agreement with other studies that transport through the entire secretory pathway from the rough endoplasmic reticulum through the Golgi to the cell surface, as well as the actual step of internalization at the cell surface, are not affected by wortmannin [5,6]. Endocytosis of the platelet-derived growth factor is inhibited when the interaction of this receptor with PI3K is blocked [11,12]. However, this receptor is auto-phosphorylated on tyrosine residues, and thereby binds to an SH2 domain-containing PI3K. In contrast, the pIgR is not a tyrosine kinase and is not itself phosphorylated on tyrosine. Therefore it is highly unlikely that the pIgR interacts with a PI3K via an SH2 domain. The involvement of PI3K in trafficking of the pIgR is probably quite different from the role of PI3K in internalization of the platelet derived growth factor receptor.

In addition to its role in lysosomal enzyme trafficking, PI3K appears to play roles in antigen presentation [13], delivery of α factor to the yeast vacuole [14,15], insulin stimulated recruit-

ment of transferrin receptors and glucose transporters to the plasma membrane [16,17], sorting of lysosomal membrane glycoproteins (B. Reaves, pers. comm.) and several other processes [1]. Our results strongly suggest that PI3K plays a novel role in endosomal traffic in polarized epithelial cells, i.e. in transcytosis of pIgR from the basolateral to the apical surface. We do not know how this is related to the role of PI3K in delivery of lysosomal enzymes, but it may reflect some common underlying function of PI3K in regulating traffic in the endosomal system. In certain epithelial cells, such as Caco2 cells, high levels of lysosomal enzymes are found at the apical surface and in endosomes immediately underlying the apical surface [18], which may be related to the apical recycling endosomes through which pIgR passes on its way to the apical surface [19]. It is therefore plausible that delivery to the apical recycling compartment and/or apical plasma membrane have some aspects in common with delivery of lysosomal enzymes to lysosomes, including a similar role for PI3K. A more general hypothesis might be that many of the effects of wortmannin result from blocking exit of materials from endosomes, or at least certain pathways out of particular endosomal subcompartments. In the case of transcytosis, this might be on exit from some portion of the endosomal system into the transcytotic pathway.

In addition to VPS34 in yeast, at least four species of PI3K have been found in mammalian cells [1,20–26]. These differ in their susceptibility to wortmannin, and in their tissue and cell type distribution. Although some types of PI3K are inhibited by concentrations of wortmannin in the low nanomolar range, at least one type of PI3K (i.e. VPS34) requires a low micromolar concentration of wortmannin to be inhibited. For instance, sorting of cathepsin D in CHO or NRK cells required 1–3 μM wortmannin to demonstrate strong inhibition [6]. This is similar to the concentration range used here, suggesting that the PI3K involved in transcytosis of pIgR may be related to the PI3K involved in cathepsin D sorting in CHO or NRK cells.

Especially at concentrations at or above the low micromolar range, wortmannin can inhibit other enzymes, such as phosphatidylinositol 4-kinase, or protein kinases [1]. In the study using 1–3 μM wortmannin to inhibit cathepsin D sorting, it was concluded that the effect of wortmannin was specifically on PI3K, and not on some other enzyme [6]. It has previously been shown that both wide-spectrum inhibitors of protein kinases, such as staurospaurine, as well as more specific inhibitors of protein kinase C (e.g. H7) or of protein kinase A (e.g. H89), do not inhibit pIgR transcytosis [10,27]. Transcytosis of pIgR without dIgA bound depends on phosphorylation of the pIgR itself at Ser-664 [28]. Mutation of Ser-664 to Ala reduces transcytosis of pIgR molecules that do not have dIgA bound. However, dIgA bound to pIgR is transcytosed efficiently even in the absence of phosphorylation of residue 664 [29]. In the present study we measured the transcytosis of dIgA bound to pIgR, so it is quite unlikely that wortmannin exerts its effects by inhibiting phosphorylation of Ser-664.

The effects of wortmannin are strikingly similar to our previous observations on the effects of drugs that specifically inhibit calmodulin, such as W13 [30]. Both wortmannin and W13 block transcytosis of dIgA in a dose-dependent fashion and correspondingly increase recycling. It will be interesting to determine if calmodulin and PI3K work in the same pathway to regulate transcytosis and other membrane traffic events.

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