New target for asthma treatment: inhibition of cluster formation of lipid rafts

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Abstract

Introduction
Lipid rafts are microdomains of the plasma membrane that are enriched in cholesterol and sphingolipids and play an important role in the initiation of many pharmacological agent-induced signalling pathways. Basophils or mast cells play roles in the pathophysiology of allergic asthma. Cluster formation of lipid rafts with crosslinking of high-affinity IgE receptor contributes to the activation of basophils or mast cells and the process of granule exocytosis. Anti-asthmatic drugs, such as glucocorticoids and β2-agonists, inhibit cluster formation of lipid rafts, via mobility of the membrane and internalisation of β2-adrenergic receptors, respectively. This review highlights the recent findings on this new target of anti-asthma drugs through inhibition of the cluster formation of lipid rafts.

Conclusion
Future work will be required to determine whether long-acting muscarinic antagonists could inhibit cluster formation of lipid rafts on basophils or mast cells, as well as smooth muscle and gland cells.

Introduction
The term ‘lipid raft’ was first introduced by Simons and Ikonen to describe specialised liquid-ordered membrane microdomains that are enriched in cholesterol and sphingolipids. Lipid rafts have been defined as ‘small (10–200 nm) heterogeneous membrane domains, termed nanoclusters, that are involved in the compartmentalisation of various cellular processes’. Many surface receptors are constitutively or inducibly associated with lipid rafts, and it has been suggested that many multi-component signalling pathways are coordinated by colocalisation in lipid rafts, including the immunoglobulin E, T-cell antigen receptor, and G protein-coupled receptor (GPCR) signalling pathways. Downstream these signalling pathways lead to the induction of cytokines in lymphocytes and granule secretion, including histamine.

Asthma management is focused on achieving and maintaining asthma control. The international asthma management guidelines recommend an inhaled corticosteroid (ICS) as the first step for maintaining asthma control and a long-acting β2-agonist (LABA) as add-on therapy in patients with asthma. In this review, we will present evidence to support that the efficacy of asthma drugs, ICS and LABA occurs through raft-dependent exocytosis and endocytosis in basophils or mast cells, which are specialised secretory cells.

Discussion
The authors have referenced some of their own studies in this review. The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Lipid raft and exocytosis
Basophils or mast cells play an important role in allergic and autoimmune diseases. Lipid rafts contribute to the early phase of regulation of basophil or mast cell activation through crosslinking of IgE and triggering of granule exocytosis. Crosslinking of the plasma membrane high-affinity IgE receptors (FceRI) by antigens is required for basophil or mast cell activation and involves recruitment of receptor-associated tyrosine kinases to lipid rafts. Crosslinking of IgE induces Lyn phosphorylation. Flotillin-1, which is localised in lipid rafts, is involved in the process of phosphorylation of Lyn. Upon activation through the FcεRI, basophils or mast cells can release up to 100% of their content of preformed mediators from cytoplasmic secretory granules by compound exocytosis, through fusion with the plasma membrane. When purified human basophils were isolated from the blood of house dust mite (HDM) antigen-sensitive donors and stimulated with HDM, anti-IgE aggregated on the cells, and histamine was secreted via exocytosis (Figure 1). However, glucocorticoids (GCs) did not inhibit cluster formation of IgE crosslinking on HDM-treated basophils. After crosslinking of FcεRI, the process of granule exocytosis, including translocation of the vesicle, docking and fusion to the inner membrane, occurs successively on grounded lipid rafts. The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family of membrane fusion proteins, including synaptosomal-associated protein 23 (SNAP-23) and vesicle-associated membrane protein OA Immunology 2013 May 01;1(1):4.
proteins (VAMPs), plays a role in granule exocytosis. Recent work has uncovered a central role of SNARE proteins, which are present on both granules and the plasma membrane, in regulating the fusion of these granules with the plasma membrane of basophils or mast cells during exocytosis (Figure 1)\(^{12,13}\). Since SNARE proteins are partly localised in lipid rafts, inhibition of cluster formation of lipid rafts by GCs might prevent granule exocytosis.

**Lipid raft and non-genomic inhibitory effects of GCs**

GCs regulate various kinds of inflammatory cells through their anti-inflammatory effects via both genomic and non-genomic means. Among the non-genomic effects, GCs exhibit rapid onset and therefore occur through acute regulation of intracellular signaling cascades. Several studies\(^{11,14}\) have reported that non-genomic effects contribute to GC inhibition of granule exocytosis by basophils or mast cells. Because membrane-impermeable bovine serum albumin (BSA)-conjugated GC exhibits the same effect as non-conjugated GC, the inhibitory effects have been suggested to occur at least in part, through binding to membrane-bound GC receptors (mGRs).\(^{11}\) Non-genomic inhibitory effects of GCs through suppression of cluster formation of lipid rafts lead to inhibition of granule exocytosis from basophils or mast cells. Pretreatment with GCs inhibits the expression of mGR and GM1 gangliosides, which are enriched in lipid rafts and can be detected using a probe of cholera toxin-B (CT-b), in HDM-treated basophils from HDM-sensitive subjects (Figure 2). Non-genomic mechanisms are involved in the rapid inhibitory effect of GCs on cluster formation of lipid rafts, through binding to mGRs on the plasma membranes of activated basophils.\(^{11}\)

Caveolae are caveolin-1-enriched, smooth invaginations of the plasma membrane that form a subdomain of lipid rafts. Caveolae have been detected in the microvilli and intracellular vesicles in mast cells.\(^{15}\) Coimmunoprecipitation studies have identified interactions between mGRs and caveolin and have suggested that the activation function 1 domain within the mGR may support an interaction between mGR and caveolin.\(^{15}\)

The mechanisms by which GCs inhibit granule exocytosis are not well understood. Cholesterol serves as a spacer between the saturated chains of sphingolipids and is essential for maintaining the liquid-ordered phase

Figure 1: Images (left panel) and schema (right panel): Crosslinking of the plasma membrane high-affinity receptor for IgE (FcεRI), by antigens followed by granule exocytosis from basophils or mast cells. Purified human basophils were isolated from the blood of house dust mite antigen (HDM)-sensitive donors, and the response of the basophils to HDM was evaluated by measuring the expression of the activation marker, CD63, and anti-IgE crosslinking on the basophil surface. DIC: differential interference contrast.
of rafts and sequestering the embedded proteins from the rest of the membrane. Cholesterol and phospholipids play an important role in maintaining the proper fluidity and rigidity of plasma membranes. Cholesterol-rich microdomains exhibit slower mobility in the plasma membrane than non-raft regions. As small changes in the plasma membrane cholesterol content near the physiological set point may alter a variety of large biological responses, GCs might regulate the fluidity and rigidity of the plasma membrane.

Lipid raft and endocytosis of β2-adrenergic receptors

The principal action of β2-agonists is relaxation of airway smooth muscle through stimulation of β2-adrenergic receptors (β2ARs). This increases the intracellular messenger cyclic adenosine monophosphate (AMP) that is responsible for the control of smooth muscle tone. In contrast, secretory events in cells are generally accompanied by decreased levels of cyclic AMP. In mast cells, histamine release is associated with a fall in cAMP. Fenoterol, a LABA with a 12-h duration of action that was recently introduced to treat asthma, inhibits antigen-induced histamine release from basophils or mast cells in a dose-dependent fashion with concomitant increases in cAMP levels. Fenoterol also suppresses cluster formation of lipid rafts, thus inhibiting granule exocytosis from basophils or mast cells (Figure 3). β2-agonist activation of β2AR, which is a prototypical member of the GPCR family, leads to conformational changes that result in coupling to G protein, which, in turn, generates cAMP as a second messenger. The activated β2AR is then phosphorylated, resulting in the binding of β-arrestin that physically interdicts further G protein coupling leading to receptor desensitisation. The phosphorylated β2AR is internalised and undergoes resensitisation by dephosphorylation mediated by protein phosphatase 2A in the early endosomes. Among the mechanisms regulating heterotrimeric G protein internalisation, the majority of GPCRs are trafficked into clathrin-coated pits and internalised by endocytosis. Upon agonist binding, β2ARs are rapidly internalised by endocytosis into clathrin-coated pits, and they traffic into recycling endosomes. Lipid rafts and caveolae are also specialised membrane microdomains that have been implicated in regulating GPCR signalling cascades and the regulation of β2AR/Gα(s) signalling. Because Gαs is internalised with the raft-dependent endocytic pathway of CT-b, which has also been characterised as a dynamin-dependent, caveolar pathway, agonist-induced internalisation of Gαs appears to be dynamin 1-dependent through non-clathrin–mediated endocytosis (Figure 3). Pretreatment with β2-agonists inhibits the expression of fluorescent CT-b in HDM-treated basophils obtained from HDM-sensitive subjects. DIC: differential interference contrast.

Figure 2: Images (left panel) and schema (right panel): Non-genomic inhibitory effects of glucocorticoids (GCs) on suppressing cluster formation of lipid rafts in the process of inhibiting granule exocytosis from basophils or mast cells. Pretreatment with bovine serum albumin (BSA)-conjugated GC inhibits expression of membrane-bound glucocorticoids receptors (mGRs) and fluorescent cholera toxin B (CT-b), which is useful probe for detecting plasma membrane GM1 gangliosides, in house dust mite (HDM) antigen-treated basophils obtained from HDM-sensitive subjects. DIC: differential interference contrast.

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Figure 3: Images (left panel) and schema (right panel): Effects of a β2-agonist on suppressing cluster formation of lipid rafts in the process of internalisation of β2AR while inhibiting granule exocytosis from basophils or mast cells. Pretreatment with a β2-agonist inhibits expression of fluorescent cholera toxin-B (CT-b) in house dust mite (HDM) antigen-treated basophils obtained from HDM-sensitive subjects. DIC: differential interference contrast.

basophils obtained from HDM-sensitive subjects (Figure 3). The inhibition of cluster formation of CT-b implies internalisation of Gαs through the calveolar-dependent, clathrin-coated pit-independent pathway; consequently, inhibiting tracking Gαs into the cytoplasm might inhibit GTPase tubulin and microtubule dynamics leading to suppression of granule exocytosis.

Future of drug and lipid rafts
Cluster formation of lipid rafts with crosslinking of FcεRI contributes to the activation of basophils or mast cells during the process of exocytosis. Anti-asthmatic drugs, GCs and β2-agonists, do not inhibit cluster formation of IgE crosslinking. As the structure of GCs might stabilise the fluidity of the plasma membrane, a new GC drug could exploit the ability to stabilise the plasma membrane and inhibit granule exocytosis. Furthermore, internalisation of β2AR is also associated with clinical desensitisation, which implies that chronic treatment with β2-agonists may lead to deterioration in lung function. A new β2-agonist drug is needed in view of inhibiting desensitisation of Gαs. A variety of beta2-agonists with long half-lives, also called ultra long-acting beta2-agonists, are currently under development with the hope of achieving once-daily dosing. It has been hypothesised that the long duration of action of indacaterol may be related to its high affinity for the lipid raft domain of the plasma membrane.

Acetylcholine is involved in the control of airway smooth muscle constriction and in recruitment of inflammatory cells, via neuronal and paracrine effects on muscarinic type 3 (M3) receptors. Long-acting muscarinic antagonists (LAMAs) are well established in the guidelines for the treatment of chronic obstructive pulmonary disease (COPD) but are not currently licensed for use in asthma. M3 muscarinic receptors, as well as β2ARs, enter cells constitutively by clathrin-independent endocytosis and colocalise with markers of this endosomal pathway on recycling tubular endosomes. Muscarinic agonists promote histamine release via the M1-mediated pathway on basophils or mast cells.

Conclusion
Future work will be required to determine whether LAMAs could inhibit cluster formation of lipid rafts on basophils or mast cells, as well as smooth muscle and gland cells.
Abbreviations list
β2AR, β2-adrenergic receptor; BSA, bovine serum albumin; CT-b, cholera toxin-B; GC, glucocorticoid; GPCR, G protein-coupled receptor; HDM, house dust mite; ICS, inhaled corticosteroid; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

References