ABSTRACT

Mast cells are components of the immune system that liberate a wide variety of pharmacologically active mediators. The principle method of activating mast cells is through the high affinity receptor for IgE (FcεRI). This activation then culminates with the release of mediators. Phospholipase D (PLD) acts on phospholipids, hydrolyzing phosphatidylcholine to phosphatidic acid (PA) and choline. PLD is activated following stimulation via FcεRI and plays an important role in signal transduction in mast cells. PLD has two isoforms, PLD1 and PLD2, which are differentially expressed depending on the cell type where none, one or both may be expressed. RBL-2H3 cells, a mast cell line, transfected to super express catalytically active (CA) and inactive (CI) forms of PLD2 were used in the present study. The role of PLD2 was examined in these cells in order to clarify the action of PLD2 in the secretory process. Although the CA and CI cells posses a greater total β-hexosaminidase activity, when stimulated these cells release less β-hexosaminidase than cells transfected with empty vector or wild type RBL-2H3 cells. In all cell lines, PLD2 was dispersed throughout the cytoplasm with a concentration in the juxtanuclear region suggesting an association of PLD2 with the Golgi apparatus. Double labeling with anti-PLD2 and mAb AA4, which recognizes gangliosides derived from GD_{1b} on the plasma membrane, showed that PLD2 was not associated with the plasma membrane. When the cells were double labeled with anti-PLD2 and anti-GM130, which labels the cis-Golgi saccules, PLD2 does colocalize with the Golgi apparatus, especially in CI cells. Labeling with anti-GM130 alone as well as experiments employing transmission electron microscopy revealed that the Golgi apparatus is well organized in the CA cells, but is disorganized and dispersed in the cytoplasm in the CI cells. By Western Blotting, the CI cells also expressed less GM130 than the other cell lines. When the production of PA by PLD2 was inhibited by 1-Butanol, the Golgi apparatus of the CA

cells presented the same phenotypic characteristics as that of the CI cells. Conversely, incubation of the CI cells with PA resulted in the reorganization of the Golgi apparatus. The structural maintenance of the Golgi apparatus is also related to microtubules. In the CI cells, the microtubule organizing center was difficult to identify and the microtubules were disorganized in the cytoplasm as compared to the other cell lines. These results show that the production of PA by PLD2 is important in the arrangement of the microtubules and in maintaining the structure of the Golgi apparatus. Alterations in the distribution of the microtubules and the structure of the Golgi apparatus in the CI cells affect the secretory process in these cells, and such alterations may affect the secretory process in other cell types as well. The findings presented here may lead to new therapeutic strategies to control the production and release of mediators during allergic and inflammatory processes.