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Confocal Fluorescence Microscopy for Studying Signal Transduction in Mast Cells and Basophils

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The release of histamine and other inflammatory mediators from mast cells and basophils is the primary event in a variety of acute allergic and inflammatory conditions. The release of granules from these cells is an energy- and calcium-dependent process which is initiated by the receptor crosslinking with antigen. However, it has not been determined yet how the receptor crosslinking induces signal transduction in mast cells and basophils.

Then, we have studied here receptor-mediated calcium signals in rat basophilic leukemia cells (RBL-2H3) and rat mast cells by a confocal fluorescence microscope with an argon ion laser and a He-Cd laser (Nakato et al. 1992, Teshima et al. 1994, Horikoshi et al. 1994). Confocal fluorescence images of fluo-3 loaded RBL-2H3 cells, excited by an argon ion laser (488 nm), became much brighter and more nonhomogeneous than those before antigen stimulation (Fig. 1). Time-dependent fluorescence changes in intensities were abrupt and quite similar to the patterns of the intracellular calcium ion concentration $[Ca^{2+}]_i$ observed by a conventional fluorescence microscope using fura-2. From the morphological patterns of the calcium images, the parts of the bright fluorescence seemed to belong to the nucleus in RBL-2H3 cells. To confirm the above events we measured confocal fluorescence images of the nuclei in RBL-2H3 cells. From the fluorescence images of co-loaded Hoechst 33342 (a DNA-specific fluorescent probe), which was excited by a He-Cd laser (325 nm), the brighter part of the fluo-3 fluorescence intensity was identified to the nucleus in the cell (Fig. 1). These results suggested the possibility that the receptor-mediated calcium signals may transfer not only to the cytoplasm but also to the nucleus (Nakato et al. 1992, Horikoshi et al. 1994). Similar kinds of nuclear calcium signals were also observed in the activation of B lymphocytes, T lymphocytes and mast cells (Yamada et al. 1991, Furuno et al. 1993, Okamoto et al. 1995).

It was said that the nucleus contains an autonomous phosphoinositide signaling system every bit as complex as that at the plasma membrane (Berridge 1993). Then, we have studied the mechanism of the intranuclear calcium signaling pathways using Ba^{2+} and Mn^{2+} instead of Ca^{2+}, because Ba^{2+} and Mn^{2+} entered into these cells through Ca^{2+} channels (Kwan and Putney 1990). We found that Ba^{2+} and Mn^{2+} entered into the cells through Ca^{2+} channels and quenched the fluo-3 fluorescence both in the nucleus and in the cytoplasm. This showed that the increased intranuclear calcium ions after antigen stimulation may come from the cytoplasm not from the nuclear calcium stores (Okamoto et al. 1995).

Next, a confocal fluorescence microscope was used to study the exocytotic secretory processes of mast cells in combination with an fluorescent molecular rotor, 9-(dicyanovinyl) julolidine (DCVJ) (Furuno et al. 1992). DCVJ is known to be a unique fluorescent dye which increases its quantum yield with decreasing intramolecular rotation (Kung and Reed 1989, Iwaki et al. 1993). Here, DCVJ-loaded rat mast cells were stimulated with compound 48/80 and lecithins (Furuno et al. 1992, Nishimura et al. 1994), and their fluorescence images were

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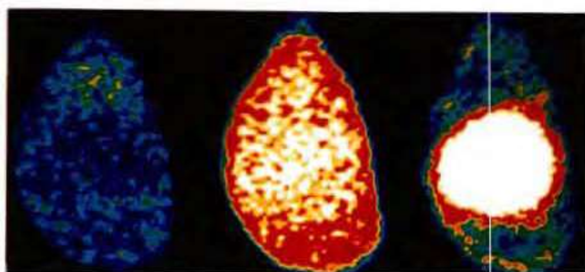

Fig. 1. Confocal fluorescence images of a fluo-3 and Hoechst 33342-loaded RBL-2H3 cell. (left) A fluo-3-fluorescence pseudo-images just before antigen stimulation. (middle) A fluo-3-flyorescence pseduo-images after antigen stimulation at 37°C. (right) A confocal fluorescence iamge of DNA-specific fluorescent probe (Hoeschst 33342)-loaded RBL-2H3 cell which was excited by a He-Cd laser (325 nm).

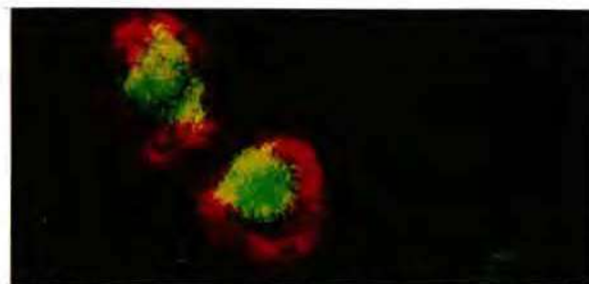

Fig. 2. Confocal fluorescence images of a DCVJ-loaded mast cell after the addition of compound 48/80. DCVJ fluorescence formed a ring-like structure (red color) around the nucleus (yellow color).

Fig. 3. Confocal fluorescence images of the surface expression of CD63 antigen in RBL-2H3 cells. The expression of CD63 antigen was visualized using anti-CD63 antibody and FITC-la-beled anti-mouse antibody on the cell surfaces (yellow color).

compared with fluorescence calcium images of fluo-3-loaded mast cells. Subsequent to transient increases in intracellular free calcium ion concentration, DCVJ fluorescence increased dramatically in the cytoplasm and formed a ring-like structure around the nucleus (Fig. 2), suggesting the possibility that the dye bound to the proteins composing the cytoskeletal architecture. Furthermore, the increases of DCVJ fluorescence intensities were mostly blocked in the presence of cytochalasin D. However, fluo-3 fluorescence intensities still increased after addition of compound 48/80. This suggested that the dye bound to the proteins composing the cytoskeletal architecture and that the molecular movements of proteins and their assembly occurred before the histamine secretion (Furuno et al. 1992, Nishimura et al. 1994).

Lastly, we have investigated the surface expression of CD63 antigen in RBL-2H3 cells and rat mast cells after stimulation of antigen and compound 48/80, respectively. CD63 antigen is located on the basophilic granule membranes in resting basophils, mast cells and platelets (Metzelaar et al. 1991). Activation of basophils and mast cells is thought to induce the fusion of cytoplasmic granules with plasma membranes and to induce the successive release of inflammatory mediators, such as histamine. The surface expression of CD63 antigen was visualized by confocal fluorescence microscopy using anti-CD63 antibody and FITC-labeled anti-mouse antibody (Fig. 3). After antigen stimulation FITC-fluorescence intensities of the membrane surfaces in RBL-2H3 cells became much brighter than those before stimulation. This indicated that the amounts of surface expression of CD63 antigen in RBL-2H3 cells increased after antigen stimulation. The expression of CD63 antigen was maximal between 10 and 20 mins after antigen stimulation (Fig. 3). Time-courses of the surface expression of CD63 antigen were well consistent with the time-courses of the histamine secretion in RBL-2H3 cells. For comparison, we stimulated RBL-2H3 cells with thapsigargin, an in-hibitor of intracellular Ca^{2+} pump. Thapsigargin neither induced the surface expression of CD63 antigen nor the secretion of histamine in RBL- 2H3 cells, although thapsigargin increased intracellular free calcium ion concentration in RBL- 2H3 cells. These result indicated that the surface expression of CD63 antigen is closely related to the histamine secretion in RBL-2H3 cells.

All of the above findings elucidated new insights of the dynamic cellular functions of exocytotic processes in mast cells and basophils.

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