

FKCRRWQWRM), corresponding to residues 17-26 near the N-terminus of Lfcin-B, was the minimal sequence of Lfc-17/29 responsible for apoptosis induction in tumor cells.

[PB4-15] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Phospholipases D1 and D2 Regulate Different Phases of Exocytosis in Mast Cells**

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The rat mast cell line RBL-2H3 contains both phospholipase D (PLD)1 and PLD2. Previous studies with this cell line indicated that expressed PLD1 and PLD2 are both strongly activated by stimulants of secretion. We now show by use of PLDs tagged with enhanced green fluorescent protein that PLD1, which is largely associated with secretory granules, redistributes to the plasma membrane in stimulated cells by processes reminiscent of exocytosis and fusion of granules with the plasma membrane. These processes and secretion of granules are suppressed by expression of a catalytically inactive mutant of PLD1 or by the presence of 50 mM 1-butanol but not tert-butanol, an indication that these events are dependent on the catalytic activity of PLD1. Of note, cholera toxin induces translocation of PLD1-labeled granules to the plasma membrane but not fusion of granules with plasma membrane or secretion. Subsequent stimulation of calcium influx with Ag or thapsigargin leads to rapid redistribution of PLD1 to the plasma membrane and accelerated secretion. Also of note, PLD1 is recycled from plasma membrane back to granules within 4 h of stimulation. PLD2, in contrast, is largely confined to the plasma membrane, but it too participates in the secretory process, because expression of catalytically inactive PLD2 also blocks secretion. These data indicate a two-step process: translocation of granules to the cell periphery, regulated by granule-associated PLD1, and a calcium-dependent fusion of granules with the plasma membrane, regulated by plasma membrane-associated PLD2 and possibly PLD1.

[PB4-16] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Inhibition of tyrosine phosphatases blocks plasma membrane blebbing during Fas-induced apoptosis of Jurkat T cells without affecting the cytotoxicity of Fas-ligation**

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Plasma membrane blebs are observed in many types of apoptotic cells, but their processes of formation remain to be clarified. In the present study, we investigated whether there is a relationship between change of intracellular phosphotyrosine levels and biochemical apoptotic events in Jurkat T cells undergoing apoptosis by agonistic anti-Fas antibody. When Jurkat cells were treated with Fas-antibody in the presence or absence of pretreatment with sodium orthovanadate ( $\text{Na}_3\text{VO}_4$ ), a phosphotyrosine phosphatase (PTPase) inhibitor, membrane blebs disappeared in orthovanadate-treated cells. In contrast, DNA fragmentation and externalization of the membrane phosphatidylserine after the induction of apoptosis were not affected by the pretreatment of the phosphatase inhibitor. In addition, Fas-induced activation of caspases cascade also remained unaffected. These results suggest that orthovanadate has inhibitory effect on the formation of the plasma membrane blebbing and that blebbing of the plasma membrane may occur independently from other apoptotic changes.

[PB4-17] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Immunogenicity and protective effects of a novel reassortant influenza live virus, NC-22-8**

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