学位論文抄録

Inhibition of mast cell functions by C4a – Elucidation of inhibitory mechanisms

（C4aによる肥満細胞機能抑制とその機序の解明）

謝 佩玉

Xie Peiyu

熊本大学大学院医学教育部博士課程医学専攻分子病理学

指導教員

山本 哲郎 教授

熊本大学大学院医学教育部博士課程医学専攻分子病理学

Abstract of the Thesis

Background and Purpose: Although complement component 4 (C4) deficiency but not C3 and C5 deficiencies occasionally associates with systemic lupus erythematosus, the detail mechanism is not fully understood. We previously found that C4-derived anaphylatoxin C4a inhibited the C5a anaphylatoxin-induced monocyte chemotaxis. We currently studied whether C4a would inhibit mast cells and whether the inhibition would encompass the secretary and respiratory burst reactions induced by downstream C3a and C5a in the complement cascades.

Methods: We prepared recombinant anaphylatoxins C3a, C4a, C5a and Leu72Gln mutant C5a and a conditioned medium of HMC-1 cells after shortly treated with C4a. Peripheral neutrophils, mast cell-like HMC-1 cells and peripheral blood $CD133^+$ cell-derived differentiated mast cells were used. Chemotaxis, histamine release, respiratory burst reaction, cytoplasmic Ca^{2+} influx, protein phosphorylations (MAPK phosphorylations) and the intracellular cyclic AMP concentration of leukocytes were assessed.

Results: C4a inhibited chemotaxis, histamine release, respiratory burst and cytoplasmic Ca^{2+} influx in mast cells that were induced by C5a or C3a. The conditioned medium also inhibited the anaphylatoxin-induced Ca^{2+} influx even after removal of C4a. Neither C4a nor the conditioned medium inhibited Ca^{2+} influx and respiratory burst in C5a- or C3a-stimulated peripheral neutrophils. C4a did not inhibit Ca^{2+}-independent Leu72Gln-C5a-stimulated chemotactic response. C4a treatment inhibited ERK1/2 phosphorylation in HMC-1 cells stimulated with other anaphylatoxins but did not inhibit p38MAPK phosphorylation in cells stimulated with Leu72Gln-C5a. The inhibitor secretion in the conditioned medium by C4a was prevented with pertussis toxin or with a phosphodiesterase inhibitor. Conversely, an adenylyl cyclase inhibitor reproduced the effect of C4a. C4a decreased the intracellular cyclic AMP concentration of HMC-1 cells.

Conclusions: The findings indicated that the effect of C4a is to liberate an autocrine inhibitor from the mast cells, and that peripheral neutrophils lack this inhibitory system. C4a elicited the Gi protein-adenylyl cyclase inhibition pathway in the autocrine inhibitor release, and the autocrine inhibitor then interrupted the Ca^{2+}-dependent intracellular signaling pathway elicited by C5a and C3a.