J. Korean Soc. Microbiol., Vol. 35, No. 5-6, 2000

Analysis of Cytokine-inducing Activity of Pneumolysin Produced by *Streptococcus pneumoniae*: an Essential Region of Pneumolysin for the Cytokine-inducing Activity is Different from that for the Membrane-lytic Activity

Ikuo Kawamura, Hisashi Baba, Chikara Kohda and Masao Mitsuyama

Department of Microbiology, Graduate School of Medicine, Kyoto University Yoshidakonoe-cho, Sakyo-ku, Kyoto, 606-8501, JAPAN

Pneumolysin (PLY) is a membrane-lytic toxin produced by *Streptococcus pneumoniae* and a member of thiol-activated cytolysins (TACYs). It has been shown that there is a significant homology between PLY and other member of the toxins in the entire amino acid sequence. These toxins bind commonly to cholesterol on the cell membrane followed by oligomerization of the toxin resulting in the pore formation. We previously demonstrated that listeriolysin O (LLO) produced by *Listeria monocytogenes*, one of the member of TACYs, had a strong cytokine-inducing activity as well as membrane-lytic activity. Administration of LLO to mice promotes generation of protective T cells by inducing endogenous cytokine productions, primarily IFN-γ that is a major cytokine involved in Th1 cell development. In the present study, we determined whether PLY has the activity to produce cytokines as LLO does. In order to test the activity, we have constructed recombinant PLY (rPLY) and various truncated forms of PLY. Contaminating LPS was extensively removed by several passages through polymyxin B-conjugated agarose column. Normal spleen cells of C3H/He mice were stimulated with rPLY and the truncated products, and IFN-γ in the culture supernatant was measured by EIA. Recombinant PLY exhibited the IFN-γ-inducing activity as well as cholesterol-binding and membrane-lytic activities. Comparing to those of rPLY, the PLY deleted for 21 amino acids of N-terminus showed the same level of cholesterol-binding and decreased level of membrane-lytic activities. However, it no longer induced IFN-γ production. On the other hand, the PLY deleted for 34 amino acids of C-terminus showed IFN-γ-inducing activity when cells were stimulated with a higher concentration of the product while it did not retain cholesterol-binding and membrane-lytic activities. These data indicated that the N-terminal region is critical for the cytokine-inducing activity and the C-terminal region that is essential for membrane-lytic activity appears to facilitate the cytokine production.