H-9

Biochemical characters and functions of novel pathogen-associated molecular patterns from Staphylococcus aureus

Chan-Hee Kim, Young Gerl Ma, Jin-Sung Ju, Hyeon-Hwa Lee, Kyoung Jin Ahn, Eun Kyoung Seo, Nam-Chul Ha, Myung-Shik Lee1, Bok Luel Lee

National Research Lab, College of Pharmacy, Pusan National University, Pusan 609-735, and 1Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710.

The innate immune system is a host defense mechanism involved in the recognition and control of the early stage of infection in all animals. The host recognizes bacterial components known as pathogen-associated molecular patterns (PAMPs) and regulates cellular immune responses. Staphylococcus aureus, a pathogenic Gram-positive bacteria, is a major source of mortality in medical facilities by shock. S. aureus family causes various infectious diseases, including sepsis, endocarditis and pneumonia. However, detailed information on molecular structures of S. aureus PAMPs are still unclear. In this study, we demonstrates that fractions obtained by heat-treated S. aureus lysate (HW1) except for peptidoglycan (PGN) and lipoteichoic acid (LTA) obtained from S. aureus functions as potent PAMPs. The purified HW1 components produced inflammatory cytokines, such as mouse interleukin-6 (mIL-6) and human interleukin-8 (hIL-8), on mouse bone marrow mast cells (mBMMC) and human mast cell-1 (HMC-1), respectively. Also, biochemical characterization of the purified HW1 components were analyzed by using Toll-like receptor 2 and 4 (TLR-2/4) knock-out BMMC. We will discuss the structural characters and the biological functions of newly purified PAMPs from S. aureus.

H-10

Bovine lactoferrin suppresses T cell activation induced by concanavalin A

Yung-Choon Yoo, Junglim Lee and Tae-Hyun Paik

Department of Microbiology, College of Medicine, Konyang University, Taejeon

Lactoferrin (LF), an iron-binding glycoprotein (M.W. 80kDa) secreted from neutrophils and epithelial cells in the mammary gland, has been shown to possess a variety of biological functions such as antibacterial activity and regulation of immune responses. Here we show immuno-regulatory effect of bovine lactoferrin (LF-B) on ConA-induced activation of splenic T cells in mice. The level of various cytokines such as IL-2, IL-4, IL-6 and IFN-Y in the supernatant of the cultures was measured by specific ELISA kits. Pre-treatment with LF-B before T cell activation markedly suppressed the production of cytokines, and inhibited DNA synthesis. Furthermore, T cells pre-incubated with LF-B showed significantly decreased expression of CD25 (IL-2Rα). Analysis of signal pathway related to the suppressive effect of LF-B revealed that LF-B resulted in down-regulation of tyrosine-dependent phosphorylation of PLC-Y1 during ConA-induced activation of T cells. Finally we found that LF-B inhibited cytolytic T lymphocyte (CTL) activity against allogenic tumor cells in vivo.

H-11

Cell permeable cytoplasmic domain of CTLA-4 inhibits T cell activation prevents allergic inflammation and hyper-responsiveness

Je-Min Choi[1], Mi-Hyun Ahn[2], Jung-Won Park[3], Choon-Sik Park[2], Sang-Kyou Lee[1,4,*]

[1]Department of Biotechnology, College of Engineering, Yonsei University, Seoul 120-749, and [2]Genome Research Center for Allergy and Respiratory disease, Soonchunhyang University Hospital, Bucheon, Republic of Korea

CTLA-4 is a negative regulator of T cell activation, and its inhibitory effects can be accomplished either via competition with CD28, or via the transmission of negative signals through its intracellular domain. In order to effectively utilize the cytoplasmic domain of CTLA-4 (ctCTLA-4) to suppress T cell activation and allergic diseases, we generated cell permeable ctCTLA-4 (Hph-1-ctCTLA-4) and its inhibitory effect on T cell activation was analyzed both in vitro and in vivo. Hph-1-ctCTLA-4 was determined to exert an inhibitory effect on the production of IL-2, and was also shown to down-regulate CD69 and CD25 at picomolar concentrations. Intracellular ctCTLA-4 inhibited NFAT and AP-1 promoter activity as well as proliferation of activated T cells. The cytoplasmic domain of CTLA-4, dephosphorylate ζ-chains, ZAP70, ERK, p38 and JNK, thereby inhibits T cell activation. The nasal administration of the Hph-1-ctCTLA-4 resulted in a marked reduction in the infiltration of inflammatory cells, the secretion of Th2-type cytokines, and airway hyper-responsiveness in an OVA-challenged asthma mouse model. These results indicated that Hph-1-ctCTLA-4 constitutes an effective immunosuppressive protein drug for use in the treatment of allergic asthma, via nasal administration.

H-12

Cisplatin induced auditory cell death involves the MAPK and NF-kB dependent secretion of pro-inflammatory cytokines

Hongseob So[1], HyungJin Kim[1], Jeong-Han Lee[1], Channy Park[1], Jae-Hyung Lee[1], Seong-Yeol Park[1], Yunha Kim[1], Eunsook Kim[1], Jin-Kyung Kim[1], Myung-Ja Youn[1], Se-Jin Kim[1], Jin-Bin Lee[1], JeHo Song[2], and Raekil Park[1]

[1]Vestibulocochlear Research Center & Department of Microbiology, [2]Department of Beauty Design, Wonkwang University School of Medicine, Jeonbuk, Korea 570-749

Cisplatin is a widely used chemotherapeutic agent that is also highly ototoxic. However, the roles of inflammatory cytokines in the pathogenesis of cisplatin ototoxicity have not been elucidated. Herein, we demonstrate that cisplatin increased the early immediate release and de novo synthesis of pro-inflammatory cytokines, including TNF-α, IL-1β and IL-6, through the activation of Mitogen-Activated Protein Kinases and NF-kB in HEI-OC1 auditory cells. We also observed a significant increase in the protein and mRNA levels of pro-inflammatory cytokines in both serum and cochlea of cisplatin-injected SD rats, which was suppressed by intraperitoneal injection of Etanercept, an inhibitor of TNF-α. Immunohistochemical studies revealed that TNF-α expression was located mainly in the spiral ligament, spiral limbus, and the organ of Corti in the cochleas of cisplatin-injected rats. NF-kB protein expression was very strong in specific regions of the cochlea, including the organ of Corti, spiral ligament and stria vascularis. NF-kB staining also overlapped with TUNEL-positive signals in cisplatin-injected mice. These results indicate that TNF-α plays a central role in the regulation of inflammatory mediators and the pathophysiology of sensory hair cell damage caused by cisplatin.