

autophagy pathway research. HCQ is a weak basic drug that accumulates in the acidic environment of cellular organelles to inhibit replication of different viruses by interfering with endosome/lysosome trafficking or viral protein maturation during virions maturation. We demonstrated that HCQ could inhibit four serotypes of dengue viruses (DENV1–4) infection in cells by immunofluorescence assays. RT-qPCR analysis of HCQ-treated cells showed induced expression of antiviral genes and cytokines such as interferon beta (IFN β), IFN-induced protein with tetratricopeptide repeats 3, C-X-C motif chemokine 10, melanoma differentiation-associated protein 5, mitochondrial antiviral signaling protein (MAVS) and tumor necrosis factor receptor-associated factor 3. The expression of inflammatory cytokines such as interleukin 6 (IL-6), IL-12 p19, IL-12 p40 and tumor necrosis factor α were also induced by HCQ. Mechanistic study suggested that HCQ activated the innate immune signaling pathways of IFN β , AP-1 and NF κ B via phosphorylation of IFN regulatory factor 3 and c-Jun and by increasing NF κ B p65 subunit nuclear translocation. Blocking type I IFN by antibody targeting IFN receptor or by inhibiting MAVS, TBK1 and IKK ϵ signaling reduced the efficiency of HCQ against DENV-2 infection. These results reveal an emerging role of HCQ activating the host innate immunity against virus infection.

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26 Murine melioidosis with CNS syndrome induced by CD11bCD62L cells harboring *Burkholderia pseudomallei*

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Backgrounds: As an infectious agent, *Burkholderia pseudomallei* is capable of persisting in CD11b phagocytes and escapes from innate immunity during subacute or chronic melioidosis. We hypothesized that those infected cells were used as a vehicle that facilitate the bacterial invading to CNS (central nerve system).

Materials and methods: The BALB/c and C57BL/6 respectively as subacute and chronic infectious models were used to evaluate the murine melioidosis with CNS syndromes via an intravenous injection of *B. pseudomallei* GFP or through an adoptively transferred by CD11b cells harboring *B. pseudomallei* GFP. Observation on occurrence of CNS melioidosis was performed by histological examination, cytokine analysis and bacterial loads in brain.

Results: We found that, during an initial 14 d-infection, by injecting *B. pseudomallei* GFP, the sL-selectin, sP-selectin, sICAM-1, MCP-1 and IFN-gamma were increased in blood and CSF specimens for the mice with melioidosis. After a 14 d-infection, the CD11b⁺ CD45⁺ subpopulation of brain infiltrating leukocytes (BILs) were significantly increased. Approximately 6.5% of BILs were harbored with *B. pseudomallei* GFP. After adoptive transfer of infected CD11b cells that collected from C57BL/6 WT (wild-type) with melioidosis, the bacterial colonization in the brain and neutrophil infiltration in meninges were obviously occurred while they did not occur in recipients after adoptively transferred if the donor cells were isolated from C57BL *sel*^{-/-} (selectin knock-out mice).

Conclusions/Significance: We suggest that *B. pseudomallei*-infected CD11b⁺CD62L⁺ cells play an important role in inducing melioidosis with meningitis that could be due to the migration of infected leukocyte involving in a selectin-dependent manner.

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27 IL-6 possibly modulates ghrelin expression through MEK1/p90RSK signaling cascade in pancreatic cell lines

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Aims: Ghrelin is a novel acylated 28-amino acid peptide involved in many physiological and biological roles, i.e. stimulating appetite and increasing food intake to exerting anti-inflammatory effects. The purpose of this study was to investigate the effect of pro-inflammatory cytokines on ghrelin expression, and to map the signaling transduction pathway undertaken by the cytokine to modulate ghrelin levels by using pancreatic cancer cell lines as model system.

Methods: AR42J pancreatic cell line (ATCC) was cultured in F-12 Ham (F12K) medium (Sigma Aldrich, USA) supplemented with 20% (v/v) of fetal bovine serum (FBS). Dose- and time-response tests with TNF- α , IL-1 β , IL-1 α , IFN- γ , and IL-6 were carried out, and the total RNA and protein were extracted. Real-time RT-PCR and western blot were then carried out to investigate the effect on the cytokines on ghrelin expression using Quantifast SYBR Green RT-PCR Kit (QIAGEN, Germany) and anti-ghrelin anti-

body (Santa Cruz Biotechnology, USA). The cells were stimulated in the presence or absence of inhibitor to specific signaling pathways prior to cytokine treatment to map the pathway undertaken by the cytokine to influence ghrelin expression by western blot using total and phosphorylated antibodies of Raf/MEK/ERK/p90RSK (Cell Signaling Technology Inc). β -actin was used as the housekeeping gene.

Result: TNF- α , IL-1 β IFN- γ , and IL-6, but not IL-1 α , down-regulated ghrelin expression significantly. Time course experiments showed that ghrelin was increased significantly in 2-h, but was reduced significantly at 16-h in the presence of IL-6. Out of the 9 inhibitors used to investigate the signaling pathway undertaken by IL-6, only rapamycin, U0126 and PD98059 abolished the IL-6 inhibitory effect on ghrelin expression suggesting the involvements of Akt/Raf/MEK1/ERK/p90RSK cascade. Further experiments confirmed that the phosphorylation of MEK1 at serine 298 and p90RSK at serine 380 play crucial roles in IL-6 regulation of ghrelin expression.

Conclusion: IL-6 affected the expression of ghrelin significantly in dose- and time-dependent manner, and may possibly exert its effects via the signaling cascade of Akt/Raf/MEK/ERK/p90RSK.

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28 Frequency of CD36 and CD109 deficiency on platelets: single institute study in Korea

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Background: CD36 deficiency could lead to CD36 iso-immunization. CD36 iso-antibody has been involved in platelet transfusion refractoriness, post-transfusion purpura, or neonatal immune thrombocytopenia. CD109 carries the biallelic codominant Gov alloantigen system. The relevance of the Gov system was demonstrated by the high incidence of anti-Gov alloantibodies in patients presenting with alloantibody-mediated platelet destruction syndrome. In this study, the frequency of CD36 and CD109 deficiency in Koreans was evaluated. We also investigated the correlation of CD expression level and degree of thrombocytopenia.

Methods: A total of 180 samples were randomly selected from subjects who requested CBC testing from June 2014 to September 2014. The expression levels of CD36 on platelets and CD109 were analyzed by flowcytometry using FITC-conjugated CD36 antibodies and PE-conjugated CD109 antibodies, respectively. Correlation between the median fluorescence intensity of CD36 and CD109 and the number of platelets was evaluated using Pearson's correlation coefficient.

Results: Lacking CD36 on platelets was present in 2.8%. The median fluorescence intensity (MFI) of CD36 did not show correlation with the count of platelets. The deficiency rate of CD109 was 1.4%. It also showed no correlation between MFI and count of platelets.

Conclusion: The frequency of CD36 and CD109 deficiency was similar to results from other studies. Studies to determine exact frequency of CD36 and CD109 deficiency in Koreans, including a larger population, should be conducted, and more case reports on patients immunized against CD36 and CD109 are also needed in order to elucidate the clinical importance and relevance of CD36 and CD109 deficiency testing and the transfusion of CD36-deficient platelets.

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29 Bioassay guided isolation for anti-inflammatory and antioxidant components from *Celastrus paniculatus* seeds

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Aim: *Celastrus* species are used as a folk remedy in India for the treatment of various inflammatory conditions. In order to identify the components responsible for pharmacological activity, in vivo anti-inflammatory activity of *Celastrus paniculatus* seeds were investigated using carrageenan induced rat paw edema as a screening model.

Methods: The methanolic extract of CP was fractionated sequentially with petroleum ether (PCP), chloroform (CCP), ethyl acetate (ECP) and n-butanol (BCP) solvents. Active PCP fraction was subjected to fractionation through successive column chromatography on silica gel, eluted with a gradient of petroleum ether: ethyl acetate to afford five sub-fractions (C₁–C₅). The activity of C₂ subfraction was evaluated against oxidative stress induced by carrageenan in rat paw tissues by studying the levels of antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT),