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Plutella xylostella serpin-1 homologues: molecular cloning and analysis of expression pattern after the immunization

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The previous EST database identified three types of cDNA clones that resemble the Manduca sexta and Mamestra configurata serpin-1 family. Two serpins, 1b and 1c, possess a common conserved serpin amind terminal scaffold domain but bear no similarity to any members of the previously reported gene family within the reactive center loop. The other member, denoted serpin-1a, is closely related to the M. Sexta serpin-1Z and M. configurata serpin-1a. The expression levels of serpin-1a, 1b and 1c were induced by the infections of both Gram positive and negative bacteria, but serpin-1 family was more sensitive against Gram negative bacteria than positive bacteria. And the expression portion of serpin-1a and 1b were 31 and 33% respectively, however, those of serpin-1c was only 1%. This fact suggests that the rest 35% would be filled with other type of serpin-1 isotype(s). Spatial expression pattern obtained by light microscopic and electron microscopic in situ hybridization show that P. xylostella serpins as a group were mainly expressed in the perivisceral fatbody and not in the peripheral fatbody after the infections. These temporal and spatial expressions analysis of serpin-1a, 1b and 1c provide important new insights into the molecular events that occur during the innate immune response in P. xylostella.

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Pathogenic Roles of Viral Immunoreceptor Tyrosine Activation Motif of Coxsackievirus VP2 by NF-κB Regulated Proinflammatory Cytokine Induction

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The immunoreceptor tyrosine-based activation motif (ITAM) [Yxx(L/I)x6-12Yxx(L/I)] sequences conserved in B-cell and T-cell receptors are necessary for the coupling of extracellular signals to intracellular signaling molecules, which result in cellular activation and proliferation. Interestingly, it has been known that some viral proteins encode ITAM sequences which may affect viral pathogenesis. However, detailed mechanism of pathogenic effect remains poorly understood. This study revolves around the identification of an ITAM sequences in the C-terminal of the coxsackievirus capsid protein VP2. The mutant viruses with phenylalanines substituted in two tyrosines in ITAM seem to be highly attenuated, such as improvement of mortality, reduction of viral titer and down-regulation of proinflammatory cytokines in infected mouse. Interestingly, wild type virus (WT) could induce higher IL-6 via nuclear factor kappa B (NF-κB) activation than that YYFF did in macrophage cells. Moreover, YYFF VP2 partially hindered NF-κB activation by agonist. All data speculated that some of the cytokine signaling cascades of the mutant viruses had been impeded, and that they have defective signaling pathways. Ultimately, mutant viruses could not induce the proinflammatory cytokines triggering the tissue injury via NF-κB activation. Taken together, this constitutes the first known revelation of the presence of an ITAM sequences in CVB, and provides direct evidence that viral ITAM affects viral pathogenesis via NF-κB activation.

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Nasal immunization of mice with rotavirus VP2 DNA vaccine induces cellular and humoral immunity

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DNA vaccination using a plasmid encoding the rotavirus inner capsid VP2 has been explored in the mouse model of rotavirus infection. BALB/c mice were immunized with a VP2 DNA vaccine by nasal route. The vaccination induced the production of anti-VP2 IgG antibodies in serum. Profiles of cytokines, IFN-γ and IL-4, specific cellular and humoral responses were detected in the mice intranasal administered with VP2 gene. Also, splenocyte proliferation against the rotavirus was detected by MTT test that was considerable in the mice received the vaccine. The intranasal vaccination showed both of cellular and humoral immune response to the rotavirus. This research represents a significant step towards the development of a rotavirus DNA vaccines derived from only the inner layer of the rotavirus capsid which can be administered intranasally.

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Kinetics and thermodynamics study of novel NADP$^+$-dependent alcohol dehydrogenases in Euglena gracilis Z.

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Euglena gracilis accumulates wax esters under anoxia, which are neutral lipids with considerable importance for pharmaceutical, cosmetic, dietetic and technical applications. E. gracilis grown on 1-hexanol produced two isoforms of NADP+-ADHs (ADH-I & ADH-II), which were involved in the wax ester metabolism. Here we report, for the first time about the active site residues identification, kinetic mechanism and thermodynamics of 1-hexanol oxidation by these enzymes. The pKa1 and pKa2 values indicated the presence of imidazol and phenol in ADH-I whereas α-amino group and phenol in ADH-II as ionizable groups of the active site residues. Arrhenius plots for energy of activation (Ea) of 1-hexanol oxidation by ADH-I and ADH-11 showed biphasic and monophasic behaviors, respectively. ADH-II followed compulsory order mechanism with NADP$^+$ as the first binding substrate and involved the formation of two ternary complexes. The Km values for NADP+ and 1-hexanol were 44 μM and 5.6 mM, respectively. Dixon' s saturation constant (Ks) for NADP+, Vmax of ADH-II and turn over (kcat) were 189 μM, 0.368 : moles mg-1 protein ml-1min-1 and 20 min-1, respectively at 55 ° C, pH 8.8. Thermodynamic parameters for 1-hexanol oxidation were as follows: ΔH* = 45.8 kJ mol-1, ΔG* = 83.7 kJ mol-1, ΔS* = -116 Jmol-1K-1. Accordingly, the NADP+-dependent ADHs, which were located in the cytosol and active towards the mid and long-chain fatty alcohols, participate in the assimilation of fatty alcohols formed from wax esters under aerobic conditions, which are synthesized in anoxia (wax ester fermentation).

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Inhibition of LPS-induced toll-like receptor 4 homodimerization by gold compound

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Toll-like receptors (TLRs), which are activated by invading microorganisms or endogenous molecules, evoke immune and inflammatory responses. TLR activation is closely linked to the development of many chronic inflammatory diseases including rheumatoid arthritis. Auranofin, an Au(I) compound, is a well-known and long-used anti-rheumatic drug. However, the mechanism as to how auranofin relieves the symptom of rheumatoid arthritis has not been fully clarified. Our results demonstrated that auranofin suppressed TLR4-mediated activation of transcription factors, NF-kB and IRF3 and expression of COX-2, a pro-inflammatory enzyme. This suppression was well correlated with the inhibitory effect of auranofin on the homodimerization of TLR4 induced by an agonist. Furthermore, auranofin inhibited NF-kB activation induced by MyD88-dependent downstream signaling components of TLR4, MyD88, IKKb, and p65. IRF3 activation induced by MyD88-independent signaling components, TRIF and TBK1, was also downregulated by auranofin. Our results first demonstrate that auranofin suppresses the multiple steps in TLR4 signaling, especially the homodimerization of TLR4. The results suggest that the suppression of TLR4 activity by auranofin may be the molecular mechanism through which auranofin exerts anti-rheumatic activity.

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Inhibition of LPS-induced toll-like receptor 4 homodimerization by curcumin

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Toll-like receptors play a key role in sensing microbial components and inducing innate immune responses. Ligand-induced dimerization of TLR4 and TLR2 is required for the activation of downstream signaling pathways. Thus, the receptor dimerization may be one of the first lines of regulation in activating TLR-mediated signaling pathways and induction of subsequent immune responses. Here, we report biochemical evidence that curcumin and helenalin with a structural motif that can confer Michael-type addition, inhibits both ligand-induced and ligand-independent dimerization of TLR4. Therefore, all the data demonstrate that small molecules with non-microbial origin can directly inhibit TLRs-mediated signaling pathways at the receptor level. These results imply that the activation of TLRs and subsequent immune/inflammatory responses induced by endogenous molecules or chronic infection can be modulated by certain dietary phytochemicals we consume daily. This poster presentation was financially supported by Ottogi co., Ltd.