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Production and optimization of dengue virus- like particles for immunization

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Dengue virus (DENV) causes more than 100 million cases of dengue fever (DF) annually, making it the common and widespread viral infection of the humans and there are no protective vaccines available currently. Moreover, individuals exposed to DENV infections earlier appear to be more susceptible to complications after secondary infection, a phenomenon attributed to antibody-dependent enhancement (ADE). DENV serotypes can be readily produced in cell culture through the expression of the heterologous pre-membrane/membrane (prM/M) and envelope (E) structural proteins that contain the majority of immunogenic domains for antibodies against DENV, have recently gained considerable attention for studies of viral replication and as potential vaccine target. Baculovirus expression systems in insect cells have emerged as a highly efficient means of producing sub-unit vaccines that generate potent protective immune responses against a variety of viral pathogens. We made DENV-2 VLPs through heterologous expression of the full prM/M and E structural proteins in insect cell cultures such as Sf9 and high five cells. The prM/M and E genes were successfully synthesized as codon-optimized gene cassettes in recombinant baculoviruses, expressing combinations of DENV-2 prM/M and E proteins that packaged a DENV-2 replicon with a single poly prM/M-E gene and produced single-round VLP in culture. After recovery of recombinant VLP from cells, expression of DENV-2 proteins was checked by western blotting and later confirmed for VLP production through transmission electron microscopy. Studies are underway to test the efficacy and immunogenicity of these DNA constructs under the appropriate animal models. Vaccine antigens generated through this robust system may serve as a candidate DENV vaccines.

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S. aureus modulates host innate immune response by regulating the expression of type 1 IFN

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The complications due to infection bacterial and viral have a major impact on the clinical course of patients with chronic lymphocytic leukemia inspite of rapid development in therapeutic approaches to this disease and supportive care. Various infections are known to be common including during chemotherapy specifically *Staphylococcus aureus, Streptococcus pneumonia, Hemophilus influenza, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* are frequent isolates. The emergence of *S. aureus* is becoming major concern due to emergence of antibiotic resistant strains. The recent developments from our lab had shown that it is major cause of dysregulation of systematic innate immune response. In the current study we report the intracellular survival and its role in innate immune response.The *S. aureus* infected HEK 293 T cells gradually induced type 1 IFN after 6hr infection of *S. aureus* showed decreased almost 90% after 24 hr *S. aureus* infection. The HEK 293 T cells were infected with *S. aureus* for 0hr, 4hr, 6hr and 24hr. The expression of type-1(IFNα) induction was checked through RT-PCR. The gradual increase found in IFNα induction. Another study showed that the stressed *S. aureus* cells at 95°C induce type 1IFN rather than given treatment of 65°C in host cells.The HEK 293 T cells were infected with *S. aureus* and transfected with MITA, MAVS, NLRX1 and IKKε the molecules involved in induction of type 1 IFN. The western blot analysis showed that IFN was more induced by MAVS in comparison of MITA and NLRX1 at 6 hr infection. Another analysis showed that type 1 IFN less induced in IKKε transfected HEK 293T cells taken MAVS as control. The truncated region of all the molecules sh-MITA, sh-MAVS, sh-NLRX1 and shIKKε associated with type 1 IFN induction via different pathway were checked for INF induction associated with % C.F.U concludes *S. aureus* infection in host cells. It is observed that sh-MAVS increased the C.F.U. 130% in comparison of sh-MITA and shNLRX1 showed decreased 70% and 40% in C.F.U. count. The count of *S. aureus* cells found almost same in shIKKε transfected HEK 293 T cells when compared with shNLRX1 taken sh-MAVS as control. The study here strongly suggests that *S. aureus* can survive intra-cellularly and may be cause of chronic inflammatory conditions. This may have important implication during hematological malignancies. The long term goal will be tackle the host innate immune response to tackle *S. aureus* infection during these malignancies.

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