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Development of protective immunity against many pathogens requires fine orchestration of both humoral as well as cell mediated immunity. While, circulating antibodies play crucial role in the elimination of extra cellular infections, majority of intracellular infections (e.g. malaria, leishmaniasis, candidiasis etc.) require generation of CD8⁺ cytotoxic T-cells (CTL) in conjunction to strong CD4⁺ T – helper cells (Th) because such pathogens adapt intracellular parasitism as a strategy to avoid recognition by antibodies. Besides, some pathogens (e.g. Palsmodium sp, Chlamydia sp, HIV) introduce substantial antigenic variations, which further complicate the process of vaccine designing. Ironically, a typical protocol followed in immunization with soluble antigens leads to the induction of humoral immune response mainly. In contrast activation of the cell mediated immune response, upon administration of soluble antigens has remained an uphill task. This requires strategies to expose antigens to the proteasome machinery, a multifunctional protease complex in the cytosolic proteolytic system forms the cardinal step involved in the induction of cytotoxic T lymphocytes (CTLs). Therefore to generate a CD8⁺ T- lymphocyte response, this is a prerequisite to delivery antigens into the cytosol of the APCs, which is further followed by its processing and presentation along with class I major histocompatibility complex (MHC I) molecules. The present study has been planned to fulfill the requirement of eliciting desired immune responses against various intracellular infections and has been presented in two parts.

In the first part of the study we have developed novel fusogenic liposomes made up of lipids from S. cerevisiae (saccharosomes). These liposomes have been shown to undergo membrane membrane fusion with cytoplasmic membrane of target cell including professional antigen presenting cells. The study demonstrates that antigen encapsulated in saccharosome could be successfully delivered simultaneously as well as endosomal processing pathways of antigen presenting cells, leading to the generation of both CD4⁺ T- helper and CD8⁺ cytotoxic T cell response. In contrast, encapsulation of same antigen I egg PC liposomes, just like Antigen- Incomplete Freund's Adjuvant (IFA) complex, has in efficient access to the cytosolic pathway of MHC I dependent antigen presentation and failed to generate antigen specific CD8⁺ cytotoxic T cell response. However, both egg PC liposomes as well as saccharosomes encapsulated antigen elicited strong humoral immune response in immunized animals but antibody titre was significantly higher in the group of animals immunized with
saccharosomes encapsulated antigen. Furthermore, antigen, antigen entrapped in saccharosomes stimulates antigen specific CD4^+ T cell proliferation and also enhances the level of IL-2, IFN-γ and IL-4 in the immunized animals. These results imply usage of liposome based adjuvant as potential candidate vaccine capable of eliciting both cell mediated as well as humoral immune responses.

In the second part, we evaluated the (A) saccharosomes and (B) niosome as an antigen delivery system in the immunization studies against blood stage infection of lethal *Plasmodium yoelii* (MDR) in BALB/c mice. Today, malaria is considered one of the most devastating and deadly disease that claim about 1.4 to 2.7 million deaths annually. Furthermore, the recent development of resistance of *Plasmodium sp.* to the chemotherapeutic agents and of its vector to DDT, alarm us to opt for alternative weapons for its control. This has led to choose prophylactic measures as complementary tools for controlling this dreadful disease, which in turn requires understanding of the basic immunological complexities involved in the resolution of the disease. However, in spite of the numerous efforts made for developing malaria vaccine, no effective malaria vaccine is available today.

It has been shown that elimination of liver stage *Plasmodium* infection require involvement of CD8^+ CTL response, while immunity against blood stages of malaria is dependent on IFN-γ secreting CD4^+ Th - cells and IgG2a isotype of antibodies. In the study, soluble blood stage antigens of *P. yoelii* (sAg) were encapsulated in saccharosome and niosome or EPC/chol liposomes and BALB/c mice were immunized at various days for performing protection as well as immunological studies. Result from the study revealed that immunization with saccharosome and niosome entrapped sAg induced strong protective immune responses that successfully suppressed drug resistant *P. yoelii* infection, whereas other formulation of sAg such as EPC/chol liposome entrapped sAg, or sAg with incomplete Freund, s Adjuvant (IFA) failed to impart significant levels of protection. Among all the sAg formulations used in the study, saccharosomes and niosomes based vaccine elicited strongest humoral as well as cell mediated immune responses in immunized BALB/c mice.

Saccharosome – sAg and nio-sAg mediated protection was found to be associated with enhanced antigen specific CD4^+ and CD8^+ T cell populations. Furthermore, activation of Th- cells requires not only TCR occupancy by presented
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MHC-antigen complex, but also a set of other co-stimulatory signals provided by APCs. In this concern, co-stimulatory molecules (CD80 and CD86) provide mutation signals to T-lymphocytes, leading to their proliferation cytokine production and development of effector function. Higher expression of CD80 and CD86 on the macrophages incubated with saccharosome-sAg or nio-sAg again supports the data representing the expansion of Th cells. Analysis of cytokine profiles in immunized animals revealed that the saccharosome mediated delivery of sAg was associated with the induction of a mixed Th1/Th2 (IFN-γ and IL-4) cytokine response. Moreover, vaccination with saccharosome and niosome entrapped sAg elicited high IgG1 and IgG2a isotype responses that played important role in imparting protection against blood stage infection of Plasmodium yoelii (MDR) in BALB/c mice.

In the final part of study we have evaluated potential of combination therapy comprising immunomodulator picroliv and antimalarial chloroquine against experimental Plasmodium yoelii infection in BALB/c mice. The immunomodulatory potential of picroliv was established by immunizing animals with model antigen along with picroliv. Immune response was assessed using T-cell proliferation assay and also by determining the antibody isotype-profile developed in the immunized mice.

In the next set of experiment, co-administration of picroliv in combination with chloroquine was used against treatment of P.yoelii (MDR) infection in BALB/c mice. The development of full blown malaria suggests non-effectiveness of parasite specific antibodies and T-cells develop by the host during the course of establishment of infection. This in turn suggests that the use of immunomodulator picroliv in combination with CHQ could be a useful choice in revoking immune system of the host ensuing in successful elimination of pathogen. The immunomodulatory role of picroliv was established by its potential to induce humoral (antibody production) as well as cell mediated immunity (T cell proliferation) in the host. As evident from data of the present study pretreatment with picroliv helps in induction of strong immune response in the animals upon their exposure to OVA. Interestingly, picroliv was found to help in induction of IgG2a isotype of antibody. This indirectly suggests that the immunomodulator helps in skewing of immune response in favor of Th1 subtype of T-helper cells. Co-administration of picroliv enhances efficacy of CHQ against experimental murine infection. The combination therapy resists the establishment of infection and helps in maintaining low multiplication rate.