Abstract

Chandipura virus (CHPV) belongs to family Rhabdovoridae and has emerged as an important encephalitis causing pathogen with high mortality (55-75%) among pediatric population (aged between 2months to 15 years) from three Indian states. Epidemic causing potentiality and rapid onset of disease (death between 24 hours to 48 of hospitalization) are some of the important attributes of CHPV. Till date there is no vaccine available and the treatment is only symptomatic. So far, the disease is prevalent in restricted areas of the country and vaccination of children may prove beneficial in the control / elimination of this life-threatening and rapidly progressing disease killing large proportion of encephalitis cases.

Considering the well-documented immunological efficacy of Glycoprotein in other closely related viruses i.e. Rabies, Vescular stomatitis virus etc. the recombinant Glycoprotein (rGp) was the protein of choice in our study.

The entire G-gene of CHPV (CIN0327R strain from Andhrapradesh epidemic, 2003) was cloned and expressed by Baculovirus expression system. The expression of rGp was confirmed by immunoblot analysis. It was pleasing to note that the protein was predominantly present in the supernatant of the SF9 cells. The rGp was purified by HPLC and used for mice immunization, 3 doses, and 4 weeks apart. The sero-conversion was immunogen (rGp) concentration dependent. As low as 100ng rGp was able to induce IgG-anti-CHPV antibodies and neutralizing antibodies in 40 and 20% of the immunized mice and 1µg of rGp was found to be optimum. Sero-conversion was observed as early as 2nd week by detecting anti CHPV IgG antibodies by both virus neutralization test (NT) and in house developed ELISA. Antibody titers were immunogen-concentration dependent. Intracerebral challenge of the immunized mice with 100 LD$_{50}$ of the homologous strain, demonstrated 90% protection. In in-vitro, neutralizing antibodies from the immunized mice were able to neutralize heterologous viruses. Anti-CHPV titers are almost constant till 6 months indicating probable long-lasting immunity. In this family of viruses, though it is not well defined that the protection is based on cell-mediated immune response, we have
observed 60% of T-cell proliferation against rGp in immunized mice. The study shows that rGp induces both arms of immune response and represents an ideal vaccine candidate for further evaluations.

Although an ELISA based on the use of complete virus as the antigen was developed earlier, since we had a recombinant protein we attempted to develop an ELISA. Our aim was to explore the possibility of rGp in the development of ELISA for diagnosis (IgM-anti-CHPV antibodies) and surveillance (IgG-anti-CHPV antibodies). The first priority was IgM test. The protein did not recognize IgM antibodies and therefore could not be used. However, a highly sensitive and specific ELISA is developed for the detection of IgG-anti-CHP antibodies. In this ELISA a panel of human (n=490) and swine (n=248) sera tested earlier employing in-vitro neutralization test was screened. From 468 NT positives, 464 were ELISA-positive (99.1% sensitivity) and 25/270 NT-negatives were ELISA positive (90.7% concordance).

The important point to note here is that the serum-free supernatant of SF9 cells without any purification step could be used for coating the solid phase. The protocol required 3 hours and 45 minutes to complete, including coating of the antigen for 2 hours and a further incubation for 30 minutes for blocking the uncoated sites. However the true status of NT negative, ELISA positive serum samples needs to be worked out.

As the disease is predominantly rural with poor nutritional status, to ensure maximum coverage for Chandipura vaccine, an attempt was made to evaluate combination of rGp and a commercially available DPT vaccine (CHP-DPT). When CHP-DPT was used for immunization of mice, 90% seroconversion against rGp with high antibody titers (1:1200 by ELISA and 1:320 by neutralization test) was observed and did not differ from mice immunized with rGp alone (p>0.05). Similarly seroconversions and antibody titers against DPT were comparable in mice immunized with DPT alone or in combination with rGp. Seroconversions and antibody titers ranged from 90-100% and 1:1200 to 1:2400 respectively. Intracerebral challenge with homologous CHPV strain resulted in 90% survival in both rGp alone and CHP-DPT groups. Lymphocyte proliferative responses were also comparable.
Thus, neither components of the candidate combination vaccine inhibited immune response to the other component. Isotype analysis of antibodies generated by rGp alone or in combination with DPT clearly showed the predominance of Th2 type of response with both formulations. A combination vaccine seems to be feasible for use in a restricted area where the disease is endemic and should be subjected to additional studies required for future use in humans.

After generating a convincing data, we strongly feel that both formats of the vaccine i.e., Chandipura alone (rGp) and as a combination with DPT must undergo pre-clinical and clinical trials on priority and used in the affected areas to save lives of a large number of children.