Abstract

Rotavirus is the major cause of infectious diarrhea that claims over 600,000 lives annually in children below 5 years of age, with majority of the deaths occurring in developing countries. It is estimated that India alone accounts for 122,000 to 153,000 deaths due to rotavirus and spends Rs. 2.0 to 3.4 billion in medical cost for treatment of rotavirus diarrhea. Recently, two new rotavirus vaccines have been licensed globally for intervention of the disease. However, the low (39.3-48.3%) efficacy of these vaccines in developing countries accentuates the need for alternate and adjunct strategies for the management of rotavirus infection. Oral delivery of specific neutralizing immunoglobulins that provide passive immunity and fast acting treatment for rotavirus diarrhea may carry valued significance to reduce the disease burden in economically weaker nations. Because of the evolutionarily unique biochemical properties, egg yolk antibodies are receiving considerable attention in human and veterinary medicine. The use of antigen specific egg yolk antibodies (IgY) has been emphasized against important enteric infections, dental carries, gastritis, systemic vascular disorders and cancer.

The beneficial effect of purified IgY antibodies specifically generated against rotaviruses has previously been described. However, earlier studies have rarely included the production and evaluation of IgYs against globally prevalent human rotavirus (HRV) serotypes, G1-G4 and G9, essentially required for practicability of the approach. Further, the effect of orally supplied rotavirus specific IgY antibodies on the intestinal viral load and cellular pathological changes has not been examined to date.

The present study reports the production of IgYs against common HRV serotypes and their evaluation detailing the influence on the virological/histopathological consequences. Prototype strains (HRV-1, KU/G1P[8]; HRV-2, S2/G2P[4]; HRV-3, YO/G3P[8]; HRV-4, ST-3/G4P[6] and HRV-9, F-45/G9P[8]) of common HRV serotypes propagated in MA104 cells and purified on 20-60% continuous sucrose gradient were utilized independently with adjuvant to immunize specific pathogen free hens for the generation of anti-HRV IgY. The anti-HRV IgY responses in serum and IgY preparations purified from the egg yolk of immunized hens were monitored by an indirect ELISA. The anti-HRV IgY titers in the serum samples increased rapidly after 2nd dose of
immunization, peaked (geometric mean titer value 1:144915.8/1:256000) after 4th dose (at 35 days of 1st immunization) and remained at plateau. The anti-HRV IgY titers in the egg yolks prepared against all five types of HRV also remained high (1:64000-1:512000) throughout the experimental period (11-12 weeks) with a peak titer value of 1:256000/1:512000 at 40-60 day of immunization.

The in-vitro neutralizing activity of the anti-HRV IgYs was tested against both homotypic and heterotypic rotavirus infections in a cell culture based neutralization assay. Each of the anti-HRV IgY preparations showed the presence of multiserotypic neutralizing activity with high (1:1600-≥1:6400) homologous and low (≤1:50-1:800) heterologous titers against two or more serotypes of rotavirus apart from the serotype involved in its own production. Anti-HRV-3 IgY neutralized all of the serotypes tested in the study indicating broader in-vitro neutralizing activity. The multiserotypic neutralization response was observed to be independent of doses used for immunization and probably developed slowly against the conserved epitopes on the VP7 and VP4 capsid proteins across the rotavirus serotypes used in the study.

A murine model of HRV gastroenteritis of G3 origin was developed during the study to evaluate the homologous in-vivo protective efficacy of anti-HRV-3 IgY. Although mouse is the established and most extensively used animal species to study various aspects of rotavirus infection, experimental studies of human rotavirus infections in mice are limited and there is lack of information on the quantitative assessment of rotaviral replication and its relationship with histological changes. In the present study, consequences of human rotavirus strain, YO induced gastroenteritis in infant BALB/c mice were analyzed for the occurrence of clinical symptoms, histopathology and virological events. The infected animals developed diarrhea and dehydration and showed accumulation of vacuolated enterocytes with lodging of the rotavirus antigens and shortening of villi in the intestine over a period of 5 days. The ileum was identified as the most susceptible and supportive part of small intestine for perpetuation of rotavirus infection in mice. Rotaviral antigen/RNA in stool and RNA in intestine were detected throughout the clinical disease period. The diarrhea was at peak level at 48-72 hours post inoculation (hpi) of virus affecting 90-95% of the animals with increase in the load of viral RNA and intense pathological lesions in intestine suggesting it as the critical time point in the course of infection. The rising titers of anti-rotavirus neutralizing antibodies ascertained the replication of human
rotavirus YO in mice. These data may contribute to the understanding of pathophysiological, immunological and virological characteristics of rotavirus infections in mice.

The efficacy of pre and post infection treatment of anti-HRV-3IgY was assessed in the infant BALB/c mouse model of HRV-3 infection by monitoring percent diarrhea, severity and duration of diarrhea, intestinal viral load and histopathology. Post exposure treatment with anti-HRV-3IgY significantly reduced the extent of diarrhea and intestinal virus load and inhibited histopathological changes whereas pre exposure anti-HRV-3IgY treatment imparted immediate protection from development of rotavirus gastroenteritis in the mice. The effect of anti-HRV-3IgY was dose dependent. The 12 hourly regimen of post infection treatment with 1.25 mg/ml, 0.625 mg/ml and 0.3125 mg/ml of anti-HRV-3IgY resulted in the decrease of percent diarrhea respectively by 58%/81%; 53%/62% and 26%/35% on day 2/3 as compared to that noted in the infected but untreated mice. There was clearance of the virus from intestine and normalization of the pathological lesions at the end of the course of treatment (96 hpi) with the highest dose whereas viral load and vacuolization of enterocytes in the intestine were detected to be reduced with lower doses in comparison with untreated (control) group of mice. On the other hand, treatment of the mice with anti-HRV-3IgY prior to infection did not develop diarrhea and were devoid of rotaviral RNA at 72 hpi with clear intestinal histology. Thus, the oral administration of anti-HRV1IgY decreased morbidity and disease incidences in mice infected with HRV suggesting its potential implication in prophylactic and therapeutic usage to reduce rotavirus disease burden in human.