Old Herborn University Seminar Monograph 24:
Development of strategies to overcome barriers to effective
mucosal immunization of infants in developing countries.
Editors: Peter J. Heidt, Richard I. Walker, and Volker Rusch.
Old Herborn University Foundation, Herborn-Dill, Germany: 95-99 (2010).

EFFECTS OF MALNUTRITION AND MICRONUTRIENT DEFICIENCY ON HYPO-RESPONSIVENESS TO ORAL VACCINES: WHAT CAN BE DONE TO OVERCOME THIS?

FIRDAUSI QADRI

Immunology Unit, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh

SUMMARY

The purpose of this review is to bring to light, important factors that may contribute to lowered immune responses to vaccines in children in developing countries. Of the over 546 million under five year children in developing countries, 50 million are severely underweight. These children are also those most at risk of death due to malnutrition and infectious diseases, both of which follow a vicious cycle. Malnutrition includes both macronutrient and micronutrient deficiencies. Deficiencies in vitamins and trace elements are present in children in developing country settings because of the lack of access to food and also because of the lack of the knowledge for accessing these from available and affordable sources. Identifiable micronutrient deficiencies seen in countries that have been studied in the developing world include vitamin A and zinc. Natural infection studies show that zinc enhances innate and adaptive immune responses to enteric bacterial and parasitic infections. Vitamin A supplementation results in improved immune responses to both mucosal and parenteral vaccines. Additive effect of zinc and vitamin A has also been seen to oral cholera vaccine in children 2-5 years of age. Ways to implement such strategies to increase immune responses in younger children have been undertaken. Infants 10-18 months of age responded with increased vaccine specific immune responses while this effect was not seen in younger children. Overall, the analyses of data from different studies show that improvement of immunogenicity of natural infections and vaccines can be made with micronutrient interventions. The use of these strategies for improvement of vaccine efficacies and health of children is a challenge that will need to be met in order to improve lives of children in developing country settings and to meet the millennium development goals using available vaccines.

REVIEW AND DISCUSSION

The need for better understanding of the barriers that cause oral vaccines to be less effective in children in developing countries has become imperative. We ask ourselves time and again the reason as to why hypo-responsiveness to oral mucosal vaccines is being seen (*Sack* et al., 2008). The potential causes of hypo-responsiveness may include frequent breast-feeding practices in de-

veloping countries, pre-existing immunity, malnutrition, high parasitic load and also genetic and environmental factors. These factors may synergistically interact to cause the detrimental effect of lowered responses to oral vaccines.

The review is based on evidence obtained from different studies where Vitamin A and/or zinc have been used to improve immune responses in natural infection or vaccination. Vitamin A supplementation is known to decrease morbidity and mortality and also enhances the immune system. Zinc is a trace element and is needed for functional and structural integrity of proteins. Deficiency results in hampering of the immune system rapidly and extensively, more than it affects other organs and tissues. Deficiency also results in high rates of infectious diseases as well as a decrease of the humoral and cell mediated immune responses of the body. Zinc given as daily doses has been studied in children with natural bacterial infection in shigellosis, cholera and enterotoxigenic *E. coli* (ETEC) diarrhoea. It has also been used to study effect on enteric parasitic infections e.g. *Giardia lamblia* infections in children, *Entamoeba histolytica* associated diarrhoea and *Ascaris lumbricoides* infections (*Long* et al., 2007). In shigellosis, 14 days of supplementation with zinc resulted in increased B cells, proliferation of lymphocytes, plasma cells, shigellacidal killing activity and reduced duration of diarrhoea (*Raqib* et al., 2003, 2004, *Roy* et al., 2008). A randomized controlled trial was carried out to determine the benefit of zinc treatment (20 mg/day for 10 days), followed by zinc supplementation (10 mg/day for 3 months) on the clinical and immunological outcome of acute watery diarrhoea in children 6-24 months of age in a low income urban setting in Dhaka city in Bangladesh (*Larson* et al., 2010, *Sheikh* et al., 2010). The children were followed for 9 months after initiation of the study. In addition a sub-group of children with diarrhoea caused by ETEC were studied to determine the effect of this intervention on the innate and adaptive immune responses. Zinc supplementation followed by zinc treatment resulted in an additional 30% reduction in diarrhoeal incidence during the period of the intervention (*Larson* et al., 2010). It also provided an additional 20% reduction in acute diarrhoea and 12% reduction of the duration of diarrhoea over 9 months period of the study. There was no impact on acute respiratory infections in the children in this study. In children with ETEC diarrhoea in the cohort, an increase of complement C3 was seen on initiation of zinc treatment in comparison to the levels seen in children who were not given the interventions (*Sheikh* et al., 2010). The levels remained elevated in both the treatment and supplementation groups over the duration of the study. Increased phagocytic activity of granulocytes and monocytes were also observed. A reduction of reactive oxygen species in these cells was observed, suggesting that zinc decreased oxidative stress. A decrease in memory T cells and an increase of the naïve to memory T cell ratio was seen. The adaptive immune response to vaccine antigens, e.g. tetanus toxoid and diphtheria toxoid remained unchanged. There was no effect on the IgA and IgG antibody response to the heat labile toxin (LT) of ETEC contrary to that seen in the immune response in toddlers given Dukoral together with supplementation of zinc (*Qadri* et al., 2004).

Elegant studies in experimental models suggest that there is a role of the gut microbiota in altering the mechanism by which the immune re-

sponse becomes tuned to produce a less effective and appropriate response to pathogens, antigens and vaccination (*Sack* et al., 2008). The purpose of this review is to see what happens in the natural setting in developing countries and the factors that are responsible for lowered immune responses and determine ways to enhance these responses. Infants and children time and again are not responding to oral vaccines in rates seen in children in industrialized settings with high GDI/GDP (gross domestic product/gross domestic income). A major factor leading to such lowered responses in the children in developing countries is the high rates of malnutrition and micronutrient deficiency. The largest number of under-five children is in the developing countries of the world. About 90% of children are the developing countries including the less developed countries. About 50.6 million children are malnourished of whom 90% are in the developing world (*Faruque* et al., 2008). Major micronutrient deficiencies that have been identified include vitamin A and zinc and other trace elements. Malnutrition includes both macronutrient and micronutrient deficiencies. Mortality in severely malnourished children can be decreased by protocolized management interventions. Deficiencies in vitamins and trace elements exist due to lack of access to costly food and the knowledge to access it from affordable sources.

The role of micronutrients on the immune response to vaccines is reviewed. Hypo-responsiveness to many vaccines have been seen which include oral polio vaccine, the oral typhoid vaccine (Ty21A), Shigella vaccine (SC602), rotavirus vaccine (Rotarix, RotaTeq), cholera vaccines (Dukoral, CVD103HgR, Peru-15). It has not been studied whether this lowered immune response can also be observed after using parenteral vaccines and we do not know how this reflects on vaccination of adults living in developing countries.

The effect of vitamin A deficiency on the yellow fever vaccine (YFV) in adults is a question that was asked in a recent analysis (*Ahmad* et al., 2008). Adults with low vitamin A store were compared with those with high vitamin A stores. A distinct difference was seen in the response to YFV specific proliferation of peripheral blood mononuclear cells and TNF-α production which also correlated with whole body vitamin A stores.

In summary, vitamin A supplementation in Bangladeshi adults resulted in increased YFV and tetanus toxoid specific proliferative responses. It also resulted in increased YFV specific IL-5, IL-10 and TNF-α responses. The available results suggest that adults may also need to be targeted for micronutrient supplementation to achieve better responses. The response to oral vaccines has not been studied in this age group or in the elderly. The reasons for vaccine failures can be better targeted when all age groups have studied and compared.

Studies in toddlers (2-5 years of age) showed that daily dosing with 20 mg/day of zinc sulphate for three weeks prior to vaccination increases the innate immune response in natural disease, diarrhoeal diseases caused by bacterial or parasitic microbes.

An additive effect of zinc and vitamin A supplementation was seen when these two micronutrients are given together to 2-5 year old children and immunization with the oral cholera vaccine was carried out (*Albert* et al., 2003). When zinc was given prior and during oral cholera vaccination to younger infants and children, this resulted in an increased vaccine specific vibriocidal antibody response (*Ahmed*

et al., 2009). This effect was seen in children 2-5 years of age and in those 10-18 months of age but not in younger children 6-9 months of age. This is an important observation and caution needs to be taken when these results are extrapolated to immunization strategies for other vaccines and even younger age groups.

CONCLUSIONS

Overall, the analyses of the available data suggest that improvement of immunogenicity of natural infections and vaccines can be made with micronutrient interventions. The use of these strategies for improvement of vaccine efficacies and health of children is a challenge that will need to be met in order to improve lives of children in developing country settings and to meet the target of the millennium development goal towards improvement of lives and decreasing childhood deaths.

ACKNOWLEDGEMENTS

The work presented is from collaborative work of F. Qadri with scientists at the ICDDR,B (researchers and colleagues), University of Gothenburg (Ann-Mari Svennerholm) and at the Johns Hopkins University (David Sack). Funding for different aspects of the work is acknowledged to Sida, Bill and Melinda Gates Foundation and funds from the ICDDR,B.

LITERATURE

Ahmad, S.M., Haskell, M.J., Raqib, R., and Stephensen, C.B.: Men with low vitamin A stores respond adequately to primary yellow fever and secondary tetanus toxoid vaccination. J. Nutr. 138, 2276-2283 (2008).

Ahmed, T., Svennerholm, A.M., Al Tarique, A., Sultana, G.N., and Qadri, F.: Enhanced immunogenicity of an oral inactivated cholera vaccine in infants in Bangladesh obtained by zinc supplementation and by temporary withholding breast-feeding. Vaccine 27, 1433-1439 (2009).

Albert, M.J., Qadri, F., Wahed, M.A., Ahmed, T., Rahman, A.S., Ahmed, F., Bhuiyan, N.A., Zaman, K., Baqui, A.H., Clemens, J.D., and Black, R.E.: Supplementation with zinc, but not vitamin A, improves seroconversion to vibriocidal antibody in children given an oral cholera vaccine. J. Infect. Dis. 187, 909-913 (2003).

Larson, C.P., Nasrin, D., Saha, A., Chowdhury, M.I., and Qadri, F.: The added benefit of zinc supplementation after zinc treatment of acute childhood diarrhoea: A randomized, double-blind field trial. Trop. Med. Int. Health 15, 754-761 (2010).

Long, K.Z., Rosado, J.L., Montoya, Y., de Lourdes Solano, M., Hertzmark, E., DuPont, H.L., and Santos, J.I.: Effect of vitamin A and zinc supplementation on gastrointestinal parasitic infections among Mexican children. Pediatrics 120, e846-e855 (2007).

Faruque, A.S., Ahmed, A.M., Ahmed, T., Islam, M.M., Hossain, M.I., Roy, S.K., Alam, N., Kabir, I., and Sack, D.A.: Nutrition: basis for healthy children and mothers in Bangladesh. J. Health Popul. Nutr. 26, 325-339 (2008).

Qadri F, Ahmed T, Wahed MA, Ahmed, F., Bhuiyan, N.A., Rahman, A.S., Clemens, J.D., Black, R.E., and Albert, M.J.: Suppressive effect of zinc on antibody response to cholera toxin in children given the killed, B subunit-whole cell, oral cholera vaccine. Vaccine 22, 416-421 (2004).

Raqib, R., Moly, P.K., Sarker, P., Quadri, F., Alam, N.H., Mathan, M., and Andersson, J.: Persistence of mucosal mast cells and eosinophils in Shigella-infected children. Infect. Immun. 71, 2684-2692 (2003).

Raqib, R., Roy, S.K., Rahman, M.J., Azim, T., Ameer, S.S., Chisti, J., and Andersson, J.: Effect of zinc supplementation on immune and inflammatory responses in pediatric patients with shigellosis. Am. J. Clin. Nutr. 79, 444-450 (2004).

Roy, S.K., Raqib, R., Khatun, W., Azim, T., Chowdhury, R., Fuchs, G.J., and Sack, D.A.: Zinc supplementation in the management of shigellosis in malnourished children in Bangladesh. Eur. J. Clin. Nutr. 62, 849-855 (2008).

Sack, D.A., Qadri, F., and Svennerholm A.-M.: Determinants of Responses to Oral Vaccines in Developing Countries. Ann. Nestlé [Engl.] 66, 71-79 (2008).

Sheikh, A., Shamsuzzaman, S., Ahmad, S.M., Nasrin, D., Nahar, S., Alam, M.M., Al Tarique, A., Begum, Y.A., Quadri, S.S., Chowdhury, M.I., Saha, A., Larson, C.P., and Quadri, F.: Zinc influences innate immune responses in children with enterotoxigenic Escherichia coli-induced diarrhea. J. Nutr. 140, 1049-1056 (2010).