

PAPERS AND ORIGINALS

Reduced Secretory Antibody Response to Live Attenuated Measles and Poliovirus Vaccines in Malnourished Children*

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Summary

Serum and nasopharyngeal IgA antibody levels were estimated in 20 malnourished children and 20 matched healthy controls after immunization with a single dose of live attenuated measles or poliovirus vaccine. Seroprevalence and serum neutralizing antibody titres were comparable in the two groups. Secretory IgA antibody was detected significantly less often in undernourished children; the time of its first appearance was delayed and its maximum level was significantly lower. Impaired secretory antibody response in malnourished children may contribute to slow inadequate recovery from viral and enterobacterial infections and predispose to life-threatening complications.

Introduction

Malnutrition and infection often coexist and augment each other. In undernourished people the immune defence mechanisms are significantly impaired;¹ they may have lymphopenia,² depressed cell-mediated immunity,²⁻⁵ low levels of serum siderophilin,⁶ polymorphonuclear leucocyte dysfunction,⁷⁻¹⁰ reduced carbon clearance by macrophages,¹¹ and complement defects.^{2 12 13}

For many infections which often affect malnourished children and are associated with much morbidity, severe complications, and high mortality—such as viral and enterobacterial diseases—recovery and immunity to infection on subsequent exposure depend largely on antibody response in external secretions.

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Moreover, the effectiveness of certain vaccines, both viral and bacterial, depends mainly on inducing local mucosal response rather than serum antibody production. There are no reports of the assessment of local immunity in malnutrition.

Patients and Methods

In 20 boys, aged 1-4 years, malnourishment was diagnosed on the basis of a history of reduced dietary intake, loss of subcutaneous tissue, and hair changes. Weight and height were 50-70% of the mean on reference growth charts. No child had significant infection at the time of immunization. Twenty age- and sex matched healthy children served as controls.

Immunization.—A single dose of trivalent live attenuated poliovirus vaccine (Haffkine Institute) containing 10^5 median tissue-culture infective dose (T.C.I.D.) of the virus was given by mouth to 10 seronegative children in each group. Another 10 malnourished patients and 10 healthy controls received one dose of live attenuated measles virus vaccine (Merck, Sharp, and Dohme) subcutaneously.

Specimens.—Samples of serum and nasopharyngeal secretions were obtained each week in the first month after immunization and every two weeks in the second month. Nasopharyngeal secretions were obtained by instilling 5 ml of sterile 0.9% saline into each naris and immediate suction with a polyethylene nasal catheter attached to an aspirator bulb. Samples were kept at -20°C and processed by the method of Ogra *et al.*¹⁴

Antibody Tests.—Neutralizing antibody titres were estimated by standard endpoint tube-dilution methods in monkey-kidney cell cultures using about 100 T.C.I.D.₅₀ of the virus with incubation for 60 minutes at room temperature.¹⁵ Antibody activity in various immunoglobulin classes was measured by radioimmunoassay and autoradiography. Antisera (Behringwerke) were absorbed and tested to be monospecific against heavy-chain determinants. The antiserum for IgA antibody estimation in nasopharyngeal washings was specific for the secretory component. Virus grown in primary rhesus-monkey kidney cell cultures was radiolabelled with ^{32}P -sodium phosphate, harvested, and concentrated by ultracentrifugation. Ainsbender *et al.*'s¹⁶ method as modified by Ogra *et al.*¹⁴ was used for the immunoglobulin class-specific antibody activity.

Proteins and Immunoglobulins.—Total protein was determined by the biuret method. Albumin and IgA, IgG, and IgM were measured by radial immunodiffusion using monospecific antisera raised in rabbits. The antiserum against IgA was specific for the secretory component.

Statistical Analysis.—Differences between the data for malnourished and healthy groups were analysed by Student's *t* test for antibody titres and by the χ^2 test for seroconversion.

Results

Total protein and albumin concentrations were slightly lower in malnourished children (table I) but the differences from control values were not statistically significant. IgA was significantly reduced in the nasopharyngeal secretions of malnourished patients.

TABLE I—Mean Levels of Total Proteins, Albumin, and IgA in Nasopharyngeal Secretions

| | Total Proteins (g/l) | Albumin (g/l) | IgA (g/l) |
|-----------------|----------------------|---------------|---------------|
| Malnourished .. | 0.887 ± 0.098 | 0.121 ± 0.016 | 0.156 ± 0.019 |
| Healthy .. | 1.078 ± 0.163 | 0.152 ± 0.027 | 0.281 ± 0.027 |
| P | >0.10 | >0.10 | <0.05 |

Seroconversion after poliovirus vaccine was achieved in eight out of 10 malnourished children and in all the healthy controls (table II). There was a slight difference in the serum antibody levels attained by the two groups, but this was statistically insignificant ($P>0.05$). Only six out of 10 malnourished patients had detectable levels of specific IgA antibody in nasopharyngeal washings (fig. 1), and the titres were significantly lower than those in the controls ($P<0.01$). The first appearance of the secretory antibody was delayed by one to three weeks compared with findings in the control group. Two patients had nasopharyngeal IgG antibody to poliovirus in low titres (1/1). All 10 healthy children showed only IgA class of secretory antibodies.

TABLE II—Mean Serum Neutralizing Antibody Titres (\log_2) to Poliovirus Type 1 after Single Oral Dose of Live Attenuated Vaccine in Malnourished and Healthy Children. Figures in Parentheses Show Number Positive for Antibody out of Total Number Tested

| | Interval after Immunization (Weeks) | | | | | | |
|--------------|-------------------------------------|--------|--------|----------|----------|----------|----------|
| | 0 | 1 | 2 | 3 | 4 | 6 | 8 |
| Malnourished | <2(0/10) | 2(3/9) | 3(5/9) | 4.5(7/9) | 6(8/10) | 7(8/9) | 6.5(8/9) |
| Healthy .. | <2(0/10) | 3(7/8) | 4(6/6) | 5(7/7) | 7(10/10) | 7.5(7/7) | 7(9/9) |

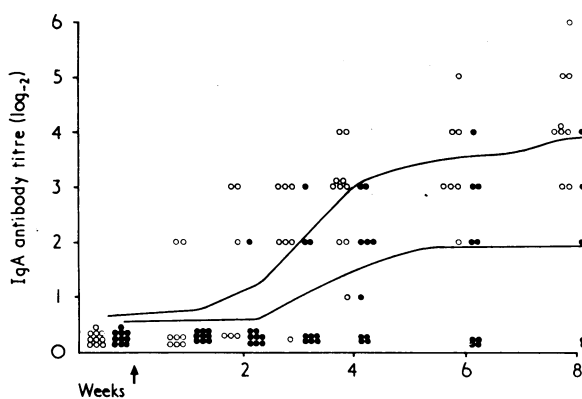


FIG. 1—Level of specific IgA antibody to poliovirus type 1 before and after single oral dose of live attenuated trivalent poliovirus vaccine. ○ = Healthy children. ● = Malnourished children.

Measles neutralizing antibodies were detected in the sera of all the malnourished and healthy children (table III). Antibody levels were comparable in the two groups ($P>0.1$). There was a significant difference in the frequency of detection, time of first appearance, and titre of nasopharyngeal IgA antibody to measles virus (fig. 2). Secretory antibody was found in samples of five out of 10 undernourished patients and in seven out of eight controls. The antibody titre at all test periods after immunization was

significantly lower in those with malnutrition ($P<0.01$). Three patients showed nasopharyngeal antibody activity in the IgG class in low titres (maximum 1/2).

TABLE III—Mean Serum Neutralizing Measles Antibody Titres (\log_2) after Single Subcutaneous Dose of Live Attenuated Vaccine in Malnourished and Healthy Children. Figures in Parentheses Show Number Positive for Antibody out of Total Number Tested

| | Interval After Immunization (Weeks) | | | | | | |
|--------------|-------------------------------------|----------|----------|--------|----------|--------|----------|
| | 0 | 1 | 2 | 3 | 4 | 6 | 8 |
| Malnourished | <3(0/10) | 3.5(8/9) | 5(8/8) | 6(9/9) | 6(10/10) | 7(7/7) | 7.5(9/9) |
| Healthy .. | <3(0/10) | 3.5(8/8) | 4.5(7/7) | 6(5/5) | 7(8/8) | 8(8/8) | 7.5(8/8) |

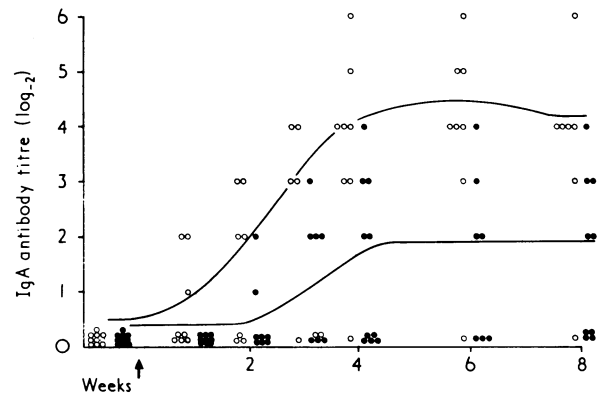


FIG. 2—Pre-immunization and post-immunization measles IgA antibody titres. ○ = Healthy children. ● = Malnourished children.

Discussion

The data show that secretory antibody response to live attenuated measles and poliovirus vaccines is reduced in malnourished children. Together with impairment of other immune reactions it may explain the frequency and severity of infections in undernourished populations. These observations may have wide biological and clinical implications.

The reduction in nasopharyngeal IgA and impaired secretory antibody response seem mostly to be selective since total protein and albumin concentrations in the nasal washings from malnourished children did not differ significantly from values in healthy children. This would also point against the possibility of rapid general proteolysis by enzymes in the secretions or an altered flow rate. There was no evidence of reabsorption of secretory IgA since the serum did not react with antiserum raised against secretory component.

The origin, characteristics, and functions of secretory antibodies have been extensively studied.¹⁷ Mucosal IgA antibody response correlates with protection and resistance in various infections, and in some diseases it may be more important than serum antibodies. The impaired secretory antibody induction after immunization suggests that immunity after infections such as measles and gastrointestinal infections may be inadequate in malnourished children. Recovery would be slower and incomplete, which would permit a severer illness. Measles is particularly devastating in malnourished people and is associated with high mortality. Furthermore, the lack of adequate local response may permit more prolonged replication and shedding of the virus, thereby increasing the period of contagiousness. Preliminary data suggest that malnourished children who develop measles may continue to have the virus in nasopharyngeal secretions for up to six weeks.

Impaired clearance of the infective agent may lead to its continued replication in the nasopharyngeal tissue. Reduced mucosal immunity may also permit systemic spread. This may partly explain the frequent occurrence of Gram-negative

septicaemia in these patients. Bacteraemia occurring commonly in malnourished children with impaired mucosal immunity would stimulate systemic lymphoid tissues with resultant hyperimmunoglobulinaemia, a common finding in such patients.

Poor secretory antibody response with good humoral response may predispose to severe reactions when subjects are exposed to the virus again. Administration of inactivated measles vaccine, which elicits a poor antibody activity in nasopharyngeal secretions, when followed by natural infection or administration of live attenuated vaccine has resulted in severe local and systemic reactions.¹⁸ It has been postulated that antigenaemia in the presence of high titres of serum antibody would induce immune-complex formation. This may occur locally at the site of the injection of vaccine or the lungs in the case of natural infection or systemically. Some malnourished children who have fulminant fatal measles may have had a subclinical episode earlier, which may have predisposed them to a severe illness via the above pathogenetic mechanism. This may also be true of other infections.

There is a high frequency of serum antibodies to common food proteins in malnourished children.¹⁹ Several factors may contribute to the genesis of such food antibodies: malnutrition is associated with a gross atrophy of the gut wall, the villous height is reduced, and there is inflammatory cell infiltration in the lamina propria; the permeability of the gut wall is increased; and the pancreatic and other digestive enzymes are impaired. These factors, together with reduced secretory antibody response, may allow free passage of food proteins intact or partially digested. The impaired function of the hepatic reticulo-endothelial system would allow such antigens absorbed through the portal circulation to bypass the phagocytic filter of the liver and thus reach systemic lymphoid structures, which are stimulated to form antibodies.

Immunodeficiency, especially IgA deficiency, is associated with a high frequency of autoimmune disease and atopy. Impaired exclusion of antigens at the mucosal level may possibly lead to overstimulation of IgE-producing cells. In the offspring of reaginic parents transient IgA deficiency is associated with atopic disease in the child within one year of birth.^{20 21} Adults positive on skin tests to *Dermatophagoides farinae* and Timothy grass pollen had more IgE antibody and less IgA antibody to the respective allergens compared with controls negative on the skin test.²² It remains to be investigated whether children who are malnourished early in life, including

those with low birth weights,^{23 24} and have poor secretory IgA response are more susceptible to atopic and autoimmune diseases, though the design of such a study which would have to control many other variables is obviously formidable.

Finally, our observations are relevant to the effectiveness of immunoprophylaxis programmes. In underprivileged populations with high incidence of malnutrition protection may be inadequate. It remains to be seen whether malnourished children given measles vaccine have any reactions when they encounter natural virus later.

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Cardiovascular Control in Diabetes Mellitus

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Summary

Heart rate variability and the changes in heart rate and blood pressure which occur on standing were measured in 21 diabetics. These simple measures distinguished four groups of patients, with loss of parasympathetic activity being commoner than loss of sympathetic activity.

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Introduction

The integrity of the autonomic control of the cardiovascular system in diabetics can be studied by observing the heart rate variability¹ and the effects of standing on heart rate and arterial blood pressure.² In early accounts of diabetic autonomic neuropathy patients showed falls in systolic arterial blood pressure accompanied by dramatic tachycardia.³ Since then postural hypotension with variable degrees of tachycardia⁴⁻⁶ or postural hypotension with no change in heart rate⁷ have been described.

No study of diabetics has related postural changes in blood pressure to changes in heart rate over a period of time or described variability in heart rate at rest and changes of heart rate and systolic blood pressure induced by standing. We report here our observations in diabetic patients, which help to clarify some of the apparently contradictory earlier reports.