Effect of age, malnutrition and renutrition on free secretory component and IgA in secretions\textsuperscript{1,2}

Ronald R Watson, David N McMurray, Phyllis Martin and Marco A Reyes

ABSTRACT The development of free secretory component (FSC) was studied in the tears of normal infants, children and adults. The level of FSC in tears was higher in older adults than in children. Free secretory component was also measured in the tears of normal, moderately and severely malnourished Colombian children. Children suffering from kwashiorkor, combined protein–calorie malnutrition or marasmus were studied before and after renutrition. No change was detected in the concentration of FSC in tears of moderately malnourished (Grade I and II) children. There was a significant difference between normal and severely malnourished children which improved with renutrition. The levels of tear IgA were decreased in the moderately malnourished children. These results indicate that reduction in secretory IgA levels in moderate malnutrition may not be explained by a lack of available free secretory component in tears, but that severe malnutrition may impair the S-IgA system by significantly reducing the availability of free secretory component. Am J Clin Nutr 1985;42:281–288.

KEY WORDS Secretory component, IgA, age, kwashiorkor, marasmus, tears, saliva

Introduction

Protein–calorie malnutrition (PCM) is a major contributing factor facilitating infection in malnourished populations in the developing areas of the world (1). Increased incidence and prolongation of diarrhea and other infections of mucosal surfaces are routinely associated with malnutrition in children (1, 2, 3). The mucosal surfaces of children with PCM are particularly susceptible to increased colonization or invasion by microorganisms. These surfaces, including the eye, are protected in part by the secretory immune system (4). Suppression of secretory IgA (S-IgA) by malnutrition has been observed in human nasal secretions (5, 6), human (7, 8) and guinea pig (9, 10) tears, guinea pig vaginal secretions (9) and mouse intestinal secretions (11, 12), but not in human intestinal secretions where the S-IgA levels were increased (13). Reduced concentrations of secretory IgA would enhance the ability of mucosal pathogens to attach to epithelial surfaces and replicate (14).

A theory proposed to explain the altered IgA levels observed in the secretions of malnourished individuals is based upon reduced transport of IgA from submucosal lymphoid tissue across the epithelial barrier into the lumen of the secretory organ (14). Secretory component, a protein produced and secreted by epithelial cells, is necessary for IgA transport from tissues into secretions (4). Secretory component may be found bound to the epithelial cell surface, as an integral part of the S-IgA in secretions, or in a free, unbound form. Studies of the role of secretory component in the mechanism of suppression of S-IgA by malnutrition were performed by measuring free secretory component (FSC) in tears. To control for the effect of age alone on maturation of the secretory immune sys-

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tem, the development of FSC was measured for the first time in normal infants, children and older adults. In addition, the effects of mild nutrition, and severe malnutrition followed by renutrition, on immunoglobulins and FSC in tears are reported.

Patients and methods

Normal subjects

Normal US adults, teenagers and children without ocular disease or major illnesses attending outpatient clinics at Indiana University Medical School, graduate and medical students, as well as normal healthy US and Colombian children without evidence of malnutrition, were sampled measured for age-related changes in FSC. In addition, some newborn children at the University Medical Center, Jackson, MS were sampled.

Moderately malnourished children

The 64 children included in this portion of the study were participating in a 2-yr longitudinal study of the influence of malnutrition on immune responses. They were 1.5 to 2 yr old when sampled and clinical evaluations are described elsewhere in detail (8). All lived in the same neighborhood of Cali, Colombia and their families had a similar low socioeconomic background. Using the Gomez classification based on weight for age (15), 25 of the children were normal, 30 were grade I and 9 were grade II undernourished. None of the moderately malnourished children exhibited signs of marasmus or kwashiorkor, and none were clinically ill when these samples were taken.

Severely malnourished children

Forty-three severely malnourished children (22 females, 21 males; ages 18-60 mo) were recruited in the outpatient clinic and emergency ward of the Hospital Universitario del Valle, Cali, Colombia. Written informed consent was obtained from a parent or guardian of each child. A complete physical examination was performed and a detailed medical and nutritional history was taken. A diagnosis of Kwashiorkor was based upon clinical findings such as loss of subcutaneous tissue, diminished muscle mass with edema and normal serum albumin concentrations as described previously (17). Combined protein-caloric malnutrition was defined as the presence of one or more characteristics of both kwashiorkor and marasmus. Marasmus would be defined as body weight as less than the standard, healthy children (15) with diminished muscle mass without edema and normal serum albumin concentrations. Parasitic infestation, data on other infections, and the socioeconomic observations have been summarized previously (16).

The children were hospitalized and received a mixed diet (meat, milk, eggs, fruit, cereals) containing initially 1–2 g of protein and 120 cal/kg body weight/day. If a child was admitted with diarrhea, vomiting and dehydration, liquids were given intravenously or orally until the conditions improved. As soon as the child could tolerate the diet provided, usually within one week, the protein and energy content was increased to 4–5 g protein and 180 cal/kg/day. The children were treated with antibiotics and antihelminths when appropriate and most were given oral iron supplementation. The children remained in the hospital until the physical and biochemical signs of malnutrition had improved, usually 4–5 wk. At this time they were discharged and returned to their homes. Additional physical and biochemical characteristics as well as cellular immune changes have been reported elsewhere (17).

The nutritional status of each child was determined on the basis of anthropometric criteria (15), and clinical findings such as loss of subcutaneous fat and diminished muscle mass, with or without edema, and serum albumin concentration. A mixed form, combined protein-calorie malnutrition, was defined as the presence of one or more characteristics of both kwashiorkor and marasmus.

Saliva collection

Whole saliva was collected by suction into a soft plastic dropper. Parotid saliva was collected by stimulation of saliva flow from Stenson’s duct with sour lemon candy. The saliva was collected through a cup placed over the duct, with saliva flow down a tube. The saliva was stored on ice until it could be centrifuged at 5000 × g for 10 min. The soluble materials were frozen at −20°C.

Tear collection

Tears were collected from both eyes by placing a 2 × 5 × 2 mm, ethylene oxide sterilized, sponge (Weckcel; E Weck and Co, NY) between the lower conjunctiva and the eyeball of each eye. After 30 to 60 s the tear-soaked sponges were removed and placed in a 0.5 ml polyethylene microsample tube (Bolab, Inc, NH) at 4°C. The tears were expressed from the sponge after cutting 4–6 mm off the bottom of the tube containing the sponge and placing this tube into another. The cut tube with tear-containing sponge was placed into an empty tube. Then the tears were expressed from the sponges by centrifugation at 3000 × g for 5 min. Tears removed by centrifugal force moved through the hole in the bottom of the sponge-containing tube into the collecting tube. Tears from both eyes were pooled for each subject and stored at −20°C.

Proteins in tears

Radial immunodiffusion was used to measure several proteins essentially as described previously (7, 18). Antiser was diluted in phosphate buffered saline buffer and mixed with melted agar. The latter was placed into plastic trays 6 × 18 cm, and allowed to gel. It was stored at 4°C until used. Then 2-mm wells were cut into the agar and 5 μl of fluid (tears or serum) were added and the tray was incubated in a humid container at 37°C for 48 h. The diameter of the precipitation ring was measured, compared to standards and the concentration determined as described in detail elsewhere (18). Free secretory component (FSC) was quantitated using the radial immunodiffusion technique (18) with monospecific antisera (Meloy Laboratories, VA). Purified free secretory component was a gracious gift of Dr Lamm (Case Western Reserve University). The monospecificity of the anti-FSC was confirmed by Ouchterlony immunodiffusion with newborn tears which are essentially IgA free (19).
serum which is FSC deficient, and adult human tears which contain FSC. S-IgA did not react with the antisera, which was specific for determinants on FSC, but not on bound SC. Serum IgA was quantitated using the radial immunodiffusion technique (18) with monospecific anti-alpha chain antisera and commercial standards (Meloy Laboratories, VA). Total protein in secretions was measured by the technique of Lowry et al. (20) modified to require only 1 μl of sample. Lysozyme (muramidase) activity in tears was measured based on hydrolysis of Micrococcus lysodeikticus in agar plates (21). Purified hen's egg lysozyme was used as a standard. Lysozyme values were multiplied by a factor of 2, as the specific activity of human lysozyme is almost twice that of hen's egg lysozyme which we used as the standard (21).

Hematology and serum biochemistry

Hematocrit, hemoglobin, total and differential white blood cell counts were performed using standard procedures in the clinical laboratory of the Hospital Universitario del Valle. Serum total protein was measured using a modification of the Lowry technique (20) while serum albumin was quantified by the Duomas procedure (22). Serum transferrin levels were measured using the radial immunodiffusion technique employing monospecific commercial antiserum and standards (Meloy Laboratories).

Results

Development of FSC with age

Figure 1 illustrates the changes in the concentration of FSC in tears with increasing age in residents of the United States. The levels of FSC in tears are very similar in young babies and teenagers. Although concentrations of FSC tended to be higher in adults, the differences were not statistically significant due to wide individual variation. There appeared to be no decrease in FSC levels in the tears of elderly subjects. The several adult groups together tended to be higher.

FSC and S-IgA in tears of moderately malnourished children

The concentration of S-IgA in the tears of moderately malnourished (Grade II) Colombian children was significantly lower (p < 0.05) than controls matched for age and socioeconomic conditions (Table 1). However, the level of FSC in grade II children's tears, although lower, was not significantly different from that of the normal children. Lysozyme, an enzyme with known antibacterial activity, was also found in reduced concentrations in Grade II children. The total protein content of the tears was not significantly influenced by nutritional status.

Serum immunoglobulin levels in severe malnutrition

As shown in Table 2, the serum IgA levels decreased upon renutrition significantly (p < 0.05) in children with kwashiorkor and marasmus. While serum IgG tended to higher values following therapy, these changes were not significant except in children with kwash-

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Free secretory component and IgA in tears of marginally malnourished young children (2 yr of age)</strong></td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
</tr>
<tr>
<td>Tear protein*</td>
</tr>
<tr>
<td>Free secretory component (mg/dL/g of protein)</td>
</tr>
<tr>
<td>S-IgA (mg/dL/g of protein)</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
</tr>
<tr>
<td>Lysozyme (mg/dL/g of protein)</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Significantly lower than normal children (p < 0.05).
TABLE 2
Changes in serum immunoglobulins of severely malnourished children following renutrition

<table>
<thead>
<tr>
<th>Serum protein*</th>
<th>Kwasioork (n = 21)</th>
<th>Protein–calorie malnutrition (n = 11)</th>
<th>Marasmus (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission</td>
<td>Renourished</td>
<td>Admission</td>
</tr>
<tr>
<td>IgA</td>
<td>215 ± 34</td>
<td>135 ± 24†</td>
<td>145 ± 24</td>
</tr>
<tr>
<td>IgG</td>
<td>1046 ± 62</td>
<td>1763 ± 185†</td>
<td>1264 ± 157</td>
</tr>
<tr>
<td>IgM</td>
<td>173 ± 32</td>
<td>187 ± 17</td>
<td>192 ± 18</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Significantly different from admission (p < 0.05).

IgA and lysozyme in saliva of severely malnourished children

A subsample of the Colombian children was examined for the effects of renutrition on parotid saliva (Table 4). The concentration of secretory IgA was significantly higher in renourished children. The salivary flow rate was also increased, but wide variation in values obtained from children at discharge prevented this difference from reaching statistical significance. The combined effect of increased IgA and flow rate in parotid saliva resulted in significantly more total IgA secreted in renourished (0.694 ± 0.046 μg/min) as opposed to malnourished (0.164 ± 0.007 μg/min) children.

Table 5 illustrates the influence of renutrition on concentrations of IgA and lysozyme in the whole saliva of children admitted with kwashiorkor, PCM or marasmus. Although renourished children in all three groups tended to have increased levels of IgA, the differences were not statistically significant. Nutritional status appeared to have no consistent effect on lysozyme concentration in whole saliva.

TABLE 3
Effects of renutrition on tear proteins in severely malnourished children

<table>
<thead>
<tr>
<th>Serum protein*</th>
<th>Kwasioork (n = 21)</th>
<th>Protein–calorie malnutrition (n = 11)</th>
<th>Marasmus (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission</td>
<td>Renourished</td>
<td>Admission</td>
</tr>
<tr>
<td>IgA</td>
<td>19.9 ± 4.8</td>
<td>17.4 ± 2.6</td>
<td>27.5 ± 7.0</td>
</tr>
<tr>
<td>IgG</td>
<td>20.9 ± 5.8</td>
<td>16.1 ± 3.3</td>
<td>19.1 ± 5.6</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>107 ± 34</td>
<td>210 ± 39†</td>
<td>59 ± 16</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Significantly different from admission (p < 0.05).
TABLE 4
Effects of severe malnutrition and renutrition on parotid saliva in Colombian children

<table>
<thead>
<tr>
<th>Salivary measurement</th>
<th>Admission &lt;0.05</th>
<th>Renourished</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA (mg/dL)</td>
<td>0.25 ± 0.12†</td>
<td>0.78 ± 0.22‡</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>0.40 ± 0.18</td>
<td>0.45 ± 0.19</td>
</tr>
<tr>
<td>Lysozyme (mg/dL)</td>
<td>0.79 ± 0.51†</td>
<td>0.84 ± 0.47</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.064 ± 0.006</td>
<td>0.089 ± 0.021</td>
</tr>
</tbody>
</table>
* Includes children with kwashiorkor, marasmus or combined PCM.
† Mean ± SEM.
‡ Significantly different from admission (p < 0.05).

Discussion

Little is known about the effects of various types of nutritional stress on secretory host defenses. Protein undernutrition has been shown to suppress significantly the secretion of lysozyme into tears (7, 8). In the present study, lysozyme levels in tears were significantly lower (about 50% less) in moderately malnourished grade II children than in normal children (Table 1). The synthesis and secretion into tears and parotid saliva of another locally produced protein, S-IgA, was also impaired. Reduced concentrations of S-IgA and, to a lesser extent, lysozyme, are potentially important factors in the defense of mucosal surfaces. The lower levels of these two secretory proteins differed significantly from those serum proteins, such as IgG, aminopeptidase, and albumin, the tear levels of which were found not to be influenced by nutritional status (7, 8, 23). This indicates that the reduced levels of S-IgA, and lysozyme were not due to reduced volume of secretion in malnourished children, as has been suggested by experimental studies with saliva in malnourished rats (24). It appears that the mechanism involves an impairment in the local synthesis and/or secretion of these proteins (14, 25). These observations have been confirmed into the present study by examining parotid saliva during the renutrition of children with kwashiorkor, PCM, or marasmic malnutrition. Saliva flow rate increased about 50% during 4 wk of renutrition with a high-protein diet. Therefore, the non-specific immunity due to the washing effects of secretions was significantly increased, as was the specific immunity provided by IgA.

The FSC level in tears beginning at 2 days of age did not change significantly until 20 yr of age in normal individuals (Fig 1). The relatively constant levels of FSC in tears of newborn and young children indicate that FSC does not increase significantly with age after birth. However, the variation in samples was sometimes large in the normal as well as malnourished children. Some proteins in tears, such as aminopeptidases, are found at adult levels at birth, while others, including lysozyme and S-IgA, have been shown to increase during the first few years of life (1, 7). The elevated levels of FSC in tears of the normal adults, both the middle-aged and advanced-aged ones, may be associated with increased production of FSC or a reduced tear flow (1). The flow rate of salivary secretions and the levels of individual component proteins are inversely related.

Both S-IgA and lysozyme levels were reduced in the moderately malnourished children’s tears to a greater extent than FSC levels. To determine the effects of nutritional stress on FSC in tears, we measured the levels in severely malnourished children before, during and after renutrition. Tear concentrations of FSC were significantly reduced on admission compared to normal values, but increased dramatically to attain levels ap-

TABLE 5
Effects of severe malnutrition and renutrition on IgA and lysozyme in whole saliva in Colombian children

<table>
<thead>
<tr>
<th>Salivary protein</th>
<th>Kwashiorkor (n = 10)</th>
<th>Protein-calorie malnutrition (n = 11)</th>
<th>Marasmus (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Admission</td>
<td>Renourished</td>
<td>Admission</td>
</tr>
<tr>
<td>mg/dL*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>2.8 ± 1.1</td>
<td>3.3 ± 0.7</td>
<td>2.8 ± 0.9</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.32 ± 0.14</td>
<td>0.257 ± 0.21</td>
<td>0.20 ± 0.04</td>
</tr>
</tbody>
</table>
* Mean ± SEM.
† Significantly different from admission (p < 0.05).
proaching these of normal children after re

Since the levels of lysozyme and S-IgA increase with maturation of the child, malnutrition could delay attainment of adult levels and protection (1). We have shown such a maturation delay in S-IgA levels in intestinal secretions of moderately protein malnourished mice (11). Reduced S-IgA in secretions and elevated serum IgA measured in some but not all studies of malnourished people or animals could be explained by: a) altered maturation of FSC synthesis or impaired production, b) lower IgA synthesis, c) less efficient binding of membrane associated FSC to IgA, resulting in reduced IgA transport into secretions. Since the secretion rate of FSC does not seem to correlate with maturation of other components of the secretory immune system, it appears unlikely that retarded maturation of secretion of FSC explains the high-serum and low-secretory IgA in the malnourished. However this does not exclude the possibility that alterations in maturation and/or function of the secretory system at the epithelial cell membrane level may be occurring that would not result in a change in the amount of FSC secreted or the FSC concentration in tears. Others have shown that the absence or a large reduction in FSC due to a genetic defect results in the absence of S-IgA in the gut (26). Our data suggest that impaired secretion of IgA in the tears of severely malnourished children is due at least in part to reduced concentrations of FSC. As FSC levels increase with improving nutritional status, so do levels of S-IgA.

In severely protein-undernourished Thai (27), Indian (26), and Colombian (7, 8) children, the level of S-IgA in secretions was found to be significantly reduced (35–50%). In malnourished humans this seems to be a general phenomenon, with low levels of S-IgA, but not IgG or albumin found in nasopharyngeal secretions (6), tears (7), or saliva (1). Reduced levels of S-IgA were not found in whole saliva from malnourished adults (28) or in intestinal secretions of humans (13) but were observed in mice (12). In the present study, the concentration of IgA in tears and saliva from moderately malnourished children was decreased compared with normal children living under the same con

ditions. These results confirm a similar observation made in nasopharyngeal washings from older, more severely malnourished children (6), and indicate that even moderate forms of malnutrition may have a profound effect upon the secretory immune system (8).

Lysozyme and FSC or IgA are all produced in mucosal tissues. However, very different types and transport mechanisms are involved. So unless a common parameter is involved they may not change similarly. That is, if changes involved permanent damage to the thymus, synthesis of IgA/S-IgA may never be normal, while lysozyme might return to normal. The explanation for the differences between IgA levels in tears and saliva after renutrition is not clear. However, S-IgA is produced by two very different organ systems in that one produces 1–2000 ml of fluids/day while the other produces 0.1–0.2 ml/day.

Reduced levels of S-IgA were observed in parotid, but not whole saliva from severely malnourished children (Tables 4 and 5). Cheatham and colleagues (28) also failed to detect significant alterations in the S-IgA content in whole saliva collected from malnourished, hospitalized adults. Whole saliva may contain IgA from sources other than local secretory surfaces, since the presence of heavily inflamed gingiva could result in the leakage of IgA into the mouth from the serum. However, this would result in elevated levels of IgG and albumin, which were not observed. A better measure of the influence of malnutrition on IgA in saliva was obtained by studying secretions taken directly from the parotid duct.

The reduction of S-IgA in malnourished patients also may be the result of selective depression of IgA synthesis in the submucosa. This seems unlikely, as much of the serum IgA is synthesized in this area and the serum IgA levels in malnourished children were elevated (Table 2). Elevated serum IgA levels due to malnutrition have been seen before in other studies (3, 5, 6, 7, 8). Extremely little is known about the effects of malnutrition on the number of lymphocytes producing immunoglobulin for secretion (1, 29). Jejunal biopsies taken from severely protein-malnourished children revealed fewer IgA- and IgG-containing cells, but the differences were
not statistically different when compared to the normal children. When the number of cells in the malnourished children were compared with that of African children with gastroenteritis, there was a highly significant decrease in IgA-, but not IgG- or IgM-bearing cells (29–31). Beatty and colleagues (30) measured production of S-IgA by in vitro cultures of duodenal biopsies from children with kwashiorkor and found enhanced S-IgA synthesis.

A selective decrease of S-IgA could occur in malnutrition as a result of increased catabolism of S-IgA. There is no evidence either to support or reject the possibility of increased breakdown of S-IgA. Several lines of evidence suggest the S-IgA system in humans is slow to develop, taking several years to reach maturity. The reduced numbers of IgA-containing cells, with normal numbers of IgM-containing cells, in protein malnutrition may indicate that the maturation process of the S-IgA system is retarded (29).

Impaired mucosal immunity may contribute to the increased frequency and severity of mucosal infection which often accompany nutritional stress (31). The small amounts of S-IgA may fail to prevent mucosal binding of bacteria and enterotoxin, a prerequisite of functional and metabolic abnormalities caused by several enteropathogenic organisms. Systemic spread of pathogens may also occur more easily because of the reduced efficiency of the mucosal immunity in preventing pathogenic organisms from penetrating into submucosal tissues. Other macromolecules, such as dietary proteins, pollen, and other allergens, may also get across the mucous membrane more readily. Food antibodies of IgG and IgA classes are frequently found in high titer in malnourished children (32).

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