Cellular Immune Function in Marasmic and Underweight Infants with Prolonged Diarrhoea

A. MORISON, D. W. BEATTY, M. D. BOWIE

SUMMARY

Lymphocytes from 10 marasmic and underweight infants with prolonged diarrhoea responded normally to phytohaemagglutinin stimulation. There was no gross deficiency in the total number of lymphocytes, serum immunoglobulins, or serum C3.

Defective cellular immunity does not appear to play a major role in the pathogenesis of diarrhoea in these children.


Chronic recurrent diarrhoea is common in malnourished children and may precipitate clinical signs of overt malnutrition. Pathogenic bacteria are infrequently isolated from stools, but intestinal flora are altered and a heavy overgrowth of Gram-negative bacteria is present in jejunal fluid. Depressed cellular immune responses are probably mainly responsible for the increased incidence of infection, including diarrhoea, in children with kwashiorkor and marasmic kwashiorkor. Cellular immune function studies in marasmic or underweight children with diarrhoea have, however, given inconsistent results.

In this article, studies of the immune responses of malnourished infants under 6 months of age with protracted diarrhoea are reported. These observations suggest that prolonged diarrhoea in young infants is not a consequence of either decreased circulating immunoglobulins or depressed cellular immune responses.

PATIENTS AND METHODS

Patients

Ten infants (8 girls and 2 boys) with prolonged diarrhoea were studied. They had failed to respond to at least 7 days of therapy, including intravenous fluid replacement. They were between 1 and 6 months of age. Five were marasmic and 5 were underweight for age. None had clinical signs of kwashiorkor, measles, tuberculosis or other overt clinical infection. At the time of investigation all were receiving intravenous fluid supplementation, all were well hydrated, and all had received milk feeds for at least 24 hours. They were treated with intravenous penicillin (30 000 U/kg/d), intravenous gentamicin (5 mg/kg/d), and oral potassium chloride (3 mEq/kg/d).

Methods

Full blood counts, bacteriological cultures of stool and urine specimens, and chest radiography were performed by routine methods. Total serum proteins were measured by the biuret method and albumin by quantitative densitometry on cellulose acetate micro-electrophoretic strips. Serum immunoglobulin concentrations were measured by radial immunodiffusion on commercial immunoplates with standards calibrated against the World Health Organization's reference preparation 69/67. Serum C3 was measured by radial immunodiffusion with commercial plates and standards.

Lymphocyte transformation. Lymphocytes were separated from defibrinated peripheral blood on a Ficoll gradient, as described by Boyum. Control lymphocytes were obtained from healthy adult personnel. Patients' serum was heat-inactivated at 56°C for 30 minutes, filtered through a 0.45-μm millipore filter and used immediately. Normal control serum was prepared from subjects with blood group AB, as previously described.

The lymphocytes were suspended in 0.025M tris-buffered Eagle's minimum essential medium containing 0.02M L-glutamine, 100 μg/ml penicillin and 100 μg/ml streptomycin.

Lymphocyte cultures were established in triplicate in the wells of sterile round-bottomed microtitre plates. Each well contained 200 000 lymphocytes, 12.5% serum and 0.5 μg purified phytohaemagglutinin (PHA) (Wellcome, UK) in a final volume of 200 μl. The plates were covered with sterile lids, sealed with a thin plastic film to prevent evaporation, and incubated for 72 hours at 37°C. Twenty-four hours before completion of the incubation period 0.075 μCi 3H-thymidine (specific activity 60 mCi/mmol) was added to each culture in 10-μl aliquots with a Hamilton repeating syringe. The radioactive lymphocytes were harvested on glassfibre filter paper discs, with a multiple automatic sample harvester. The filter papers were washed with an excess of water to remove free radioactivity. Liquid scintillation counting and calculation of results were performed as previously described.

RESULTS

The majority of infants responded to treatment within 2 weeks, but some required hospitalization for longer periods. Potential pathogens (2 salmonellae and 1 specific Escherichia coli) were isolated from stool specimens in 3
children. Two patients had minor radiological signs of pneumonia. Urine cultures were negative in all cases.

The main clinical and laboratory investigations are shown in Table I. Patients 1 and 3 had serum albumin levels below 30 g/l. Twenty days after admission patient 1 died of a Staphylococcus albus sepsicaemia. This child was the youngest in the study group and also had a subnormal serum C3 level. None had significantly low total peripheral blood lymphocyte counts and all had normal or raised serum immunoglobulin levels.

The results of the in vitro lymphocyte transformation experiments are shown in Table II.

In each experiment each patient's lymphocytes were cultured concurrently with lymphocytes from a normal adult control, in the patient's serum and in normal AB serum. For each variable, cultures were established with and without PHA stimulation.

Statistical analysis of the results with Friedman's two-way analysis of variance ($\chi^2 = 3.96, P>0.2$) and Wilcoxon's matched pairs, signed ranks test showed no difference between patients' and control lymphocytes when incubated either in patients' serum or in normal AB serum. Examination of the individual results in Table II shows that in patient 1 a definite depression of lymphocyte response is present in the patient's serum, and that in patient 7 the patient's lymphocytes responded suboptimally in both autologous and AB serum.

**DISCUSSION**

Peripheral blood lymphocyte counts were normal or elevated in our patients. In all but the 2 youngest infants serum C3 levels were normal. In patients with kwashiorkor, complement levels are usually low and in some cases lymphocyte counts are reduced.$^{6,8,9,17}$

Normal or raised levels of serum immunoglobulins are usual in children with malnutrition.$^{6,8,9,10}$ Our patients had very high serum IgM levels and relatively normal serum IgG and IgA levels, whereas in the abovementioned studies the most striking elevation was in the IgA class. The relatively low serum IgA concentration may be due to the age of our patients or may reflect depressed secretory IgA production, which has been proposed as a significant immunological defect in malnutrition.$^{10,19}$

Increased antigenic stimulation due to the diminished defences of a defective secretory IgA system, altered in-

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**TABLE I. CLINICAL AND LABORATORY RESULTS IN 10 MALNOURISHED INFANTS WITH PROLONGED DIARRHOEA**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (mo.)</th>
<th>Total protein (g/l)</th>
<th>Albumin (g/l)</th>
<th>IgG (g/l)</th>
<th>IgM (g/l)</th>
<th>IgA (g/l)</th>
<th>C3 (g/l)</th>
<th>Haemoglobin (g/100 ml)</th>
<th>Lymphocyte count (per μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>51</td>
<td>56</td>
<td>27</td>
<td>10,80</td>
<td>1,26</td>
<td>0,49</td>
<td>0,18</td>
<td>12,1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>57</td>
<td>60</td>
<td>37</td>
<td>9,00</td>
<td>2,35</td>
<td>0,69</td>
<td>0,40</td>
<td>10,0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>45</td>
<td>57</td>
<td>26</td>
<td>9,70</td>
<td>1,57</td>
<td>0,63</td>
<td>0,88</td>
<td>11,0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>78</td>
<td>53</td>
<td>31</td>
<td>7,80</td>
<td>1,35</td>
<td>1,12</td>
<td>0,83</td>
<td>10,1</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>65</td>
<td>63</td>
<td>40</td>
<td>11,20</td>
<td>2,55</td>
<td>0,90</td>
<td>0,53</td>
<td>10,6</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>78</td>
<td>61</td>
<td>36</td>
<td>6,80</td>
<td>1,53</td>
<td>0,53</td>
<td>0,57</td>
<td>8,2</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>62</td>
<td>58</td>
<td>35</td>
<td>7,90</td>
<td>1,42</td>
<td>0,57</td>
<td>0,53</td>
<td>13,5</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>80</td>
<td>56</td>
<td>32</td>
<td>5,80</td>
<td>0,78</td>
<td>0,98</td>
<td>0,88</td>
<td>6,7</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>51</td>
<td>67</td>
<td>39</td>
<td>12,90</td>
<td>1,10</td>
<td>0,85</td>
<td>0,88</td>
<td>9,3</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>47</td>
<td>67</td>
<td>38</td>
<td>14,20</td>
<td>2,97</td>
<td>0,73</td>
<td>0,67</td>
<td>10,4</td>
</tr>
<tr>
<td>Mean</td>
<td>3,7</td>
<td>61</td>
<td>59,8</td>
<td>34,1</td>
<td>9,61</td>
<td>1,67</td>
<td>0,75</td>
<td>0,61</td>
<td>10,6</td>
</tr>
</tbody>
</table>

Normal range for age 1 - 6 mo. = 2.72 - 11.25, 0.16 - 0.83, 0.16 - 0.93, 0.53 - 1.20

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**TABLE II. PHA-INDUCED TRANSFORMATION RESPONSES OF 2 X 10^5 LYMPHOCYTES FROM MALNOURISHED INFANTS AND CONTROL SUBJECTS, CULTURED IN PATIENTS' SERUM OR POOLED AB SERUM**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patients' serum</th>
<th>AB serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11 729 (19)</td>
<td>29 645 (28)</td>
</tr>
<tr>
<td>2</td>
<td>11 659 (46)</td>
<td>11 681 (73)</td>
</tr>
<tr>
<td>3</td>
<td>17 453 (49)</td>
<td>14 035 (63)</td>
</tr>
<tr>
<td>4</td>
<td>12 229 (159)</td>
<td>20 296 (221)</td>
</tr>
<tr>
<td>5</td>
<td>12 708 (51)</td>
<td>11 876 (54)</td>
</tr>
<tr>
<td>6</td>
<td>11 218 (109)</td>
<td>9 774 (80)</td>
</tr>
<tr>
<td>7</td>
<td>5 481 (39)</td>
<td>6 393 (18)</td>
</tr>
<tr>
<td>8</td>
<td>9 520 (93)</td>
<td>13 489 (89)</td>
</tr>
<tr>
<td>9</td>
<td>11 524 (1 116)</td>
<td>13 472 (70)</td>
</tr>
<tr>
<td>10</td>
<td>12 719 (200)</td>
<td>13 815 (164)</td>
</tr>
<tr>
<td>Average</td>
<td>11 624 (188)</td>
<td>14 447 (86)</td>
</tr>
</tbody>
</table>

Mean values of triplicate cultures expressed as disintegrations per minute of ^14C-thymidine. Unstimulated values are shown in parentheses.
Toxin flora and atrophied intestinal mucosa present in malnutrition may all or in part account for the high IgM response in our patients.

The lymphocyte transformation responses in these young malnourished infants with prolonged diarrhoea were normal.

 Serum suppressive activity was present in patient 1, who also had low serum albumin and C3 levels similar to but not as marked as those seen in patients with kwashiorkor. Slightly lower responses were also recorded in serum from patients 4, 8 and 9. This finding differs from observations in older children (aged 11 - 44 months) with clinical signs of kwashiorkor, in which serum factors have been shown to be significant in depressed lymphocyte responses.

Patient 7 showed subnormal lymphocyte responses in both autologous and AB serum when compared with its normal control. This patient also had the lowest peripheral blood lymphocyte count (4368/μl), which is, however, well above levels usually associated with cellular immune deficiency in patients with kwashiorkor. Retesting of this patient 1 week later showed a completely normal lymphocyte transformation response. Apart from patient 1, these children with minor abnormalities of their lymphocyte responses were similar to the other infants in all clinical and biochemical respects. Sellmeyer et al. measured lymphocyte transformation responses in 12 marasmic and underweight children with diarrhoea. These children had a wider age range (4 - 23 months) than those in the present series, many were dehydrated, and most had acute diarrhoea. They reported that all had significantly impaired lymphocyte responses to PHA, which seemed to be independent of nutritional status or state of hydration. These results conflict with our findings, but the groups are not strictly comparable. Schlesinger and Stekel found normal lymphocyte transformation values in 13 marasmic children. Of the 7 children in their series under 6 months of age, 4 had diarrhoea and are comparable to our patients. In contrast to normal lymphocyte transformation responses, they found defective dinitrochlorobenzene sensitization, and they postulated a failure of in vivo antigen processing or recognition. A recent study performed on marasmic adults reports similar findings of significant impairment of cutaneous delayed hypersensitivity responses, with normal in vitro lymphocyte responsiveness.

Delayed hypersensitivity skin testing with purified protein derivative (5 tuberculin units) and Candida antigen 1/100 was done in 5 of our children (results not shown). None responded to purified protein derivative and only 1 to Candida antigen, but this probably reflects the age of our patients and the fact that they had not undergone primary exposure to these antigens.

Our observations confirm that cellular immune responses are grossly intact in young (1 - 6-month-old) malnourished infants with prolonged diarrhoea, although we have not tested antigen recognition or processing with dinitrochlorobenzene sensitization. The well-established depression of cellular immunity as seen in kwashiorkor patients is not present, and defective local defence mechanisms, either specific or nonspecific, may be more important in the pathogenesis of prolonged diarrhoea in these children.

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REFERENCES