Impaired Humoral Immunity in PNG Highlanders

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Immunological function was investigated in 42 adult Papua New Guinea villagers and 38 adult males who had been inmates of the Goroka prison for more than three months.

Serum IgG, IgA, IgM and IgE levels were all significantly elevated in both Papua New Guinea groups when compared to Australians.

Antibody responses to tetanus toxoid immunization were greatly impaired in both Papua New Guinea (PNG) groups. The impairment was greater in the villagers. Antibody responses to immunization with typhoid vaccine were also impaired in both P.N.G. groups compared with Australians. Some evidence was obtained to indicate that unresponsiveness was associated with lower serum albumin levels, suggesting a relationship to protein deprivation.

Antibody response after first immunization was predominantly in the IgM class for both tetanus and typhoid vaccines, with conversion to the IgG class on re-immunization. This indicates that most P.N.G. highlanders have not been exposed to these antigens previously.

The prevalence of autoantibodies (mitochondrial, gastric parietal cell and antinuclear) was similar to that of a normal Australian population. The prevalence of smooth muscle antibodies, although higher, was probably not remarkable.

Australia antigen was found in 9%, compared with less than half of 1% in the Australian population.

Cellular immune function, as measured by delayed hypersensitivity reactions, was not impaired.

The implications of these findings for mass immunization programmes are discussed.

Mass immunization campaigns are a major weapon in the control of communicable disease in the tropics. The effectiveness of such campaigns depends upon the ability of those immunized to produce an effective immunological response. This study was undertaken to assess humoral and cellular immune competence in Highland Papua New Guineans.

SUBJECTS AND METHODS

The study was carried out in the Eastern Highlands District of Papua New Guinea (PNG). Two groups of Papua New Guineans were investigated and the results compared with healthy adult Australians.
PNG Group 1:

Forty-two adult members of Kefaiu village via Kwong in the upper Asaro Valley were studied. The village was located at an altitude of 2,400 metres in mountainous terrain, 55 km from the District Headquarters, Goroka. The staple foodstuff was sweet potato, with an occasional admixture of meat during pig feasts. Despite the cool climate, most villagers wore only the scanty traditional clothing. They lived in smoke-filled huts. Upper and lower respiratory tract infections were common. Only minimal medical care was available.

PNG Group 2:

Thirty-eight adult males who had been inmates of the Goroka prison for more than three months were studied. The prison, at an altitude of 1,600 metres, was located several kilometres from Goroka. They received a more nutritious diet, including daily meat, wore more clothing, lived in better housing, and were more accessible to medical care. Respiratory tract infections were not prominent.

Malaria is uncommon in the Eastern Highlands District.

Serum levels of immunoglobulins (Ig) G, A, and M, were measured with Behringwerke immunodiffusion plates. Standard solutions were obtained from Behringwerke. Serum IgD and IgE levels were measured by the radioactive single radial diffusion method (Rowe, 1969). IgE standard was from a batch of pooled human serum 69/204, supplied by W.H.O. Serum albumin levels were measured using Behringwerke immunodiffusion plates and standard.

Subjects were immunized with tetanus toxoid (Commonwealth Serum Laboratories 0.5 ml s.c. and typhoid vaccine (C.S.L.) 0.1 ml s.c. Blood was collected at the time of immunization and two weeks later. Twenty-eight subjects in Group 2 were re-immunized approximately one month after the first immunization. Haemagglutinating antibodies to tetanus toxoid and precipitating antibodies to S. typhi H antigen were measured as previously described (Forbes, 1971).

Some sera were treated with 0.2M mercaptoethanol in Dulbecco buffer for 1 hour at 37°C, dialysed against Dulbecco buffer, then antibody titres measured. Mercaptoethanol-sensitive antibody was defined by a fourfold drop in titre. Mercaptoethanol-sensitive antibody was assumed to be in the IgM class, while resistant antibody was assumed to be in the IgG class (Rowley, Wistar and MacKay, 1972).

Mitochondrial, smooth muscle and gastric parietal cell autoantibodies and antinuclear factors were measured by the indirect fluorescent technique (Taylor, Roitt, Doniaich, Couchman and Shapland, 1962) using rat liver and stomach, and horse antihuman immunoglobulin (Roboz Surgical Instrument Co., Washington).

Delayed hypersensitivity (DHS) reactions to intradermal injections of 0.1 ml Candida albicans 0.5% (Bencard, Brentford), Mumps Skin Test Antigen (Eli Lilly, Indianapolis) and Streptokinase-Streptodornase (“Varidase”, Lederle, New York) diluted in 0.9% saline to streptokinase 10 units and streptodornase 2.5 units/ml were measured at 48 hours. Reactions were also measured to phytohaemagglutinin (PHA) Reagent Grade (Burroughs Wellcome, Beckenham), diluted 1:20 in 0.9% saline. An area of induration of 5 mm or more in diameter was considered positive. DHS reactions were measured only in PNG Group 1.

Australia Antigen was measured with the radioimmunoassay kit, “Ausriva” (Abbott, North Chicago).

RESULTS

Immunoglobulin Levels

Serum immunoglobulin levels are shown in Table 1. IgG, IgA, IgM and IgE are all significantly elevated in both PNG groups when compared with Australians. There were no differences between the two PNG groups for these parameters, but IgD levels in Group 1 were significantly greater than those in Group 2 (p<0.005, “t” test).

Antibody Response

Antibody responses to tetanus toxoid immunization (Figs. 1 and 2) were greatly impaired in both PNG groups compared with Australians (p<0.0001, Fisher’s Exact Test). Tetanus haemagglutinating antibodies were not detectable two weeks
Table 1. Immunoglobulin Levels

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<th>PNG Group 1</th>
<th>PNG Group 2</th>
<th>Australians</th>
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<tbody>
<tr>
<td>IgG</td>
<td>No</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>3340</td>
<td>1,080 - 5,600</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>3240</td>
<td>300 - 6,200</td>
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<tr>
<td></td>
<td>80</td>
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<td>Range</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>240</td>
<td>100 - 380</td>
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<tr>
<td></td>
<td>38</td>
<td>230</td>
<td>120 - 340</td>
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<tr>
<td></td>
<td>80</td>
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<td>50 - 550</td>
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<tr>
<td>IgM*</td>
<td>No</td>
<td>Mean</td>
<td>Range</td>
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<tr>
<td></td>
<td>42</td>
<td>200</td>
<td>100 - 415</td>
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<tr>
<td></td>
<td>38</td>
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<td>80</td>
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<td>50 - 350</td>
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<tr>
<td>IgD*</td>
<td>No</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>27</td>
<td>2 - 240</td>
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<tr>
<td></td>
<td>38</td>
<td>10</td>
<td>1 - 120</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>16</td>
<td>1 - 270</td>
</tr>
<tr>
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<td>No</td>
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<td></td>
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<td>2250</td>
<td>200 - 26,000</td>
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<tr>
<td></td>
<td>38</td>
<td>2100</td>
<td>200 - 26,000</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>120</td>
<td>25 - 500</td>
</tr>
</tbody>
</table>

* Geometric Mean
** Compared with Australians: Students “t” test
IgG, IgA, IgM: mg/100 ml
IgD, IgE: units/ml

after immunization in 34 of 42 (81%) subjects in PNG Group 1, 22 of 37 (59%) of subjects in PNG Group 2 and none of 59 (0%) Australians. There was a significantly greater impairment of antibody responsiveness in PNG Group 1 than PNG Group 2 (p < 0.025, Fisher’s Exact Test). There were no differences in immunoglobulin levels between tetanus responders and tetanus non-responders. There was a slow decline in tetanus antibody titre in those patients who did respond to immunization and were measured at both two and six weeks after immunization. Twenty-eight patients in Group 2 were re-immunized (Fig. 3). Fifteen of these had failed to respond on first immunization, and nine (60%) failed to respond on repeat immunization. Eleven of the thirteen who had responded on first immunization, had increased titres after the second immunization.

Antibody responses to immunization with typhoid vaccine (Fig. 4) were also impaired in both PNG groups compared with Australians (p < 0.0005, X², Brandt and Snedcor’s Formula). Titres were lower in PNG Group 1 than in PNG Group 2 but not at a statistically significant level.

Three patients in Group 2 who failed to respond to immunization with typhoid vaccine were re-immunized. All responded to repeat immunization. Sera of 9 subjects

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who responded to immunization, were treated with mercapto-ethanol and antibody titres measured. Five subjects had an IgM response after the initial tetanus immunization, while four had a predominantly IgG response. Re-immunization produced an IgG response in all except two subjects. All subjects had a predominantly IgM response after initial typhoid immunization, while re-immunization was associated with a mixed IgG and IgM response.

**Table 2. Albumin Levels**

<table>
<thead>
<tr>
<th></th>
<th>No</th>
<th>Mean</th>
<th>S.D.</th>
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<tbody>
<tr>
<td>PNG Group 1</td>
<td>41</td>
<td>2.95</td>
<td>.52</td>
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<tr>
<td>PNG Group 2</td>
<td>38</td>
<td>3.47</td>
<td>.55</td>
</tr>
<tr>
<td>Australians</td>
<td>24</td>
<td>3.68</td>
<td>.46</td>
</tr>
<tr>
<td>Tetanus Responders</td>
<td>21</td>
<td>3.37</td>
<td>.58</td>
</tr>
<tr>
<td>Tetanus Non Responders</td>
<td>57</td>
<td>3.12</td>
<td>.62</td>
</tr>
<tr>
<td>Typhoid Titres $&gt;1:40$</td>
<td>52</td>
<td>3.30</td>
<td>.56</td>
</tr>
<tr>
<td>Typhoid Titres $&lt;1:20$</td>
<td>27</td>
<td>3.00</td>
<td>.68</td>
</tr>
</tbody>
</table>

*gm/100 ml

**Albumin Levels**

Serum albumin levels (Table 2) were reduced in both PNG groups compared with Australians, but the reduction was greater in Group 1 than Group 2 ($P<0.001$, "t" test). Tetanus non-responders had a lower mean serum albumin, but not at a statistically significant level. Subjects who developed a typhoid titre of 1:20 or less had a significantly lower mean serum albumin level than those who developed a titre of 1:40 or more ($p<0.05$, "t" test).
Autoantibodies

Autoantibodies were measured in 68 subjects. Mitochondrial and gastric parietal cell antibodies were not detected, antinuclear factor was found in one subject and smooth muscle antibodies in seven subjects (10%). These figures do not differ from the normal Australian population apart from smooth muscle antibodies which are found in about 5% of the population.

DHS Reactions

The prevalence of DHS reactions to candida, mumps and streptococcal antigens was similar to that found in the Australian population (Fig. 5). Only 2% of subjects failed to react to one of the three antigens which is comparable to the figure of 1% for Australians. Reactions to PHA were similar to those seen in Australians. No subject failed to react to at least one of the three antigens or PHA.

Australia Antigen

Australian antigen was found in seven of 76 sera (9%) compared with less than...
half of one percent in the Australian population.

MARKED DEPRESSION OF HUMORAL IMMUNITY IN PAPUA NEW GUINEANS

DISCUSSION

Marked depression of humoral immunity has been found in Papua New Guinea Highlanders, as compared with healthy Australians. This has been shown by substantially impaired capacity to make antibodies to tetanus and S. typhi H antigens. In contrast, cellular immunity as assessed by DHS skin reactions was intact. Similar, but less marked findings were reported from the Gambia where 50% of normal controls failed to respond to tetanus immunization, although normal responses were found to S. typhi H antigen and DHS skin tests (Greenwood, Bradley-Moore, Palit and Bryceson, 1972; Greenwood, Whittle and Molyneux, 1973).

Raised serum levels of IgG, IgA and IgM have been reported from lowland regions of Papua New Guinea (Wells, 1970), while Crane, Pitney, Hobbs and Gunn (1971) reported raised levels of IgG and IgM but normal levels of IgA compared with healthy Australians. Frequent exposure to the antigenic stimulus of helminths, protozoa, fungi, bacteria and viruses is the most likely cause of the high immunoglobulin levels seen in the tropics (Wells, 1968). Such exposure may also impair antibody responses to tetanus and typhoid immunization by "antigenic competition". This phenomenon occurs when antibody response to one antigen is reduced by prior contact with a second unrelated antigen and is observed in many species and with a wide variety of antigens (Michaelis, 1902; Barr and Llewellyn-Jones, 1955; Adler, 1964).

Impaired antibody responsiveness has been shown in chronic infections (Lee, 1971; Forbes, 1971), including malaria (McGregor and Barr, 1962). Chronic respiratory, gastrointestinal and cutaneous infections are frequent in the population studied, but malaria is not common. Depression of humoral immunity has been shown in mildly protein-deprived animals (Jose and Good, 1972). Gross protein deficiency, as in kwashiorkor, may lead to depression of cell-mediated immunity (Smythe, Schonland, Brereton-Stiles, Covadil, Grace, Loening, Mafoyane, Parent and Vos, 1971). Kwashiorkor was not seen here. The depression of antibody response in Papua New Guineans was greater in villagers than in prisoners. Serum albumin levels were less in Papua New Guineans, than in Australians, the reduction being most marked in the villagers. This parameter also, may reflect a poor protein diet or chronic infection. It seems likely that impaired antibody responsiveness is multifactorial in origin, resulting from the interplay of diet, clothing, housing, chronic infection and adequacy of medical care, these factors being less favourable for villagers than prisoners.

Antibody response after first immunization was predominantly in the IgM class for both tetanus and typhoid vaccines, with a conversion to the IgG class on re-immunization. IgG antibody after the first exposure presumably reflects previous exposure, whether naturally or as a result of mass immunization campaigns. Re-immunization with tetanus toxoid induced a detectable antibody response in about half the subjects who had failed to respond to the first immunization. This is comparable with the results obtained when Australians
suffering from chronic infections and who failed to respond to tetanus immunization were re-immunized (Forbes, 1971).

The high prevalence of Australia antigen in the serum confirms the observations of others (Woodfield, 1973). It may be another marker of impaired immunity. The high prevalence, nearly 10%, suggests that Australia antigenaemia may be prolonged. While this may in part be due to increased exposure, it may also indicate defective immunological handling of the virus, as immunodeficiency has been suggested as an explanation for the high prevalence of Australia antigenaemia in a variety of disease states such as Leprosy, Leukaemia and Down's Syndrome (Campion, 1973).

This study has important implications for mass immunization campaigns. It cannot be assumed that a procedure which has shown to be effective in a temperate environment in a developed country will necessarily be effective in a developing country in the tropics. It would seem worthwhile to carry out pilot studies in such countries to assess the efficacy of a proposed programme. Should the result be less than desired, further investigation with increased doses of antigen and different schedules of immunization is warranted. Such manipulations, in association with improved standards of living and hygiene, may lead to improved effectiveness of mass immunization campaigns.

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REFERENCES


