IMMUNE FUNCTION AND RENAL TRANSPLANTATION IN FABRY’S DISEASE


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Summary

A deficient leucocyte immunological function could cause the reported high rate of lethal infections following renal transplantation in patients affected by Fabry’s disease. We have studied humoral immunity, peripheral lymphocyte subsets, mitogenic lymphocyte response in vitro and granulocyte function in three patients with Fabry’s disease.

The immunological state appears to be quite similar to that of the uraemic population in general, not showing any specific impairment.

Introduction

Renal transplantation has been regarded as the first choice treatment of uraemia due to Fabry’s disease providing a correction of both uraemia and the enzymatic defect [1,2] but recently a high rate of life threatening infections has been reported after transplantation so that this treatment can no longer be recommended [3,4].

It has been suggested that the ceramide-trihexoside storage in leucocytes could alter their host defence function exposing the patients, when transplanted, to serious infectious complications [3].

In order to verify the hypothesis of a specific immunological defect, we performed a study of the immune function in these patients.

Patients and methods

Patients

Two male uraemic patients and one female without clinical nephropathy were studied. The diagnosis of Fabry’s disease was both clinical and biochemical in all. Their age ranged from 45–50 years and the two uraemic patients had undergone maintenance haemodialysis for 49 and 52 months respectively.
Control blood samples were obtained from six haemodialysis patients (5 male, 1 female) matched for age and length of treatment. All the patients were free from infectious episodes and had not received drugs affecting the immune system for at least two years.

**Methods**

Immunoglobulins A, G, M, and serum Complement fractions (C₃, C₄) were determined by immunonephelometry.

The total lymphocyte counts were determined from blood cells count and differential counts.

Peripheral mononuclear cells were prepared from heparinised blood by gradient (lymphoprep), washed three times and suspended at optimal concentrations. The T cell subsets were analysed by immunofluorescence staining with monoclonal antibodies (Ortho Diagnostic System). The total T cell population was quantified with OKT₃ antibody. The T helper cells with OKT₄, the suppressor/cytotoxic T cells with OKT₈ [5,6]. The B cells were estimated with surface Ig immunofluorescence and the Natural Killer cells with monoclonal antibody Leu 7 (Becton-Dickinson, Torino, Italy). At least 2,000 lymphocytes were scored for each determination.

Mitogenic lymphocyte response in vitro to phytohaemoagglutinin A, Concanavalin A, and pokeweed mitogen was measured. Lymphocytes were incubated in culture (RPMI-Gibco) supplemented with 10 per cent fetal calf serum. Mitogenic stimulations were carried out in triplicate in 96 well microtitre plates (Sterilin); 10⁵ lymphocytes per well with the medium alone and the mitogens at different concentrations were incubated at 37°C in humidified and five per cent CO₂ supplemented air. After 48 hours (phytohaemoagglutinin A and Concanavalin A) and 72 hours (pokeweed mitogen) 1μCi of ³H-metil-thymidine was added at each well then after a further 24 hours cultures were harvested (Sacrificator) and the incorporation of ³H-thymidine measured as counts per minute (β-counter Packard Tricarb).

Autologous E rosette formation was estimated with 5 x 10⁶ lymphocytes, previously incubated with RPMI medium supplemented with 20 per cent autologous serum to which 5μl of autologous erythrocytes at concentration of 280 x 10⁶/ml were added. After centrifugation and incubation for 24 hours, 200 elements were scored. Each test was carried out in duplicate.

Polymorphonuclear cells were prepared from heparinised blood by lymphoprep gradient at a concentration of 2.5 x 10⁶/ml (chemotaxis) and 10⁷/ml (phagocytosis). Chemotactic activity was studied with migration wells for chemotaxis through porous filters (‘blind well’ model). As chemotactic factor the synthetic peptide formil-metionil-leucil-phenil-alanine at concentration of 10⁻⁸M was used. Chemotactic activity was assessed as the number of cells per microscopic field (400x) reaching the last floor of the filter. Five fields were scored.

The morphological test for Candida albicans phagocytosis was performed. Candida, previously killed by heat, were brought at concentration of 10⁷/ml. Polymorphonuclear cells were incubated in autologous fresh serum then Candida
was added. The phagocytic properties were assessed as: a) number of cells phagocytizing at least one Candida (%Ph) and b) number of phagocytosed Candida per cell (Phagocytic index) per microscopic field (1000x). Both tests were carried out in duplicate. The polymorphonuclear cell tests were also performed on healthy volunteers to which the normal values mentioned in the text refer.

Results

Serum immunoglobulin and Complement fractions were in the normal range in all the patients.

The number of white blood cells (WBC), total peripheral lymphocytes, T₃, T₄, T₈, B lymphocytes and NK cells are shown in Table I. It is apparent that although the number of WBC varies from reduced to normal values, all the patients of both groups show absolute lymphocytopenia. The absolute number of T₃ and T₄ cells is also low when compared to the values usually obtained from a healthy population matched for age and sex but Fabry's patients do not show any notable difference from the control group. In particular T₄ cells are not relatively reduced and the T₄/T₈ ratio was over 1.7 in Fabry's patients. The absolute number of peripheral B lymphocytes and Natural Killer cells of patients affected by Fabry’s disease are not depressed.

**TABLE I. Absolute number of white blood cells (WBC), peripheral lymphocytes (L), T cell subsets, Natural Killers (NK), B lymphocytes and percentage of autologous E rosette (R) in Fabry’s (F) and control groups (C)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC</th>
<th>L</th>
<th>T₃</th>
<th>T₄</th>
<th>T₈</th>
<th>NK</th>
<th>B</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6000</td>
<td>1440</td>
<td>1008</td>
<td>734</td>
<td>274</td>
<td>158</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>F2</td>
<td>3600</td>
<td>1116</td>
<td>636</td>
<td>234</td>
<td>134</td>
<td>56</td>
<td>134</td>
<td>21</td>
</tr>
<tr>
<td>F3</td>
<td>6200</td>
<td>1612</td>
<td>967</td>
<td>500</td>
<td>177</td>
<td>338</td>
<td>81</td>
<td>15</td>
</tr>
<tr>
<td>C1</td>
<td>7400</td>
<td>1332</td>
<td>653</td>
<td>573</td>
<td>147</td>
<td>66</td>
<td>13</td>
<td>36</td>
</tr>
<tr>
<td>C2</td>
<td>7000</td>
<td>1190</td>
<td>797</td>
<td>512</td>
<td>226</td>
<td>131</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>C3</td>
<td>4800</td>
<td>1344</td>
<td>672</td>
<td>417</td>
<td>148</td>
<td>67</td>
<td>67</td>
<td>28</td>
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<tr>
<td>C4</td>
<td>4500</td>
<td>1125</td>
<td>652</td>
<td>394</td>
<td>112</td>
<td>123</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>C5</td>
<td>4900</td>
<td>1617</td>
<td>1100</td>
<td>728</td>
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<td>194</td>
<td>81</td>
<td>3</td>
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<tr>
<td>C6</td>
<td>6900</td>
<td>1242</td>
<td>534</td>
<td>149</td>
<td>149</td>
<td>62</td>
<td>62</td>
<td>11</td>
</tr>
</tbody>
</table>

**Mitogenic lymphocyte response.**

In Figure 1 the mitogenic lymphocyte responses in vitro to Concanavalin A (Con A), pokeweed mitogen (PWM), phytohaemagglutinin A (PHA) are shown.

*Con A* although a large variability in the responses among individual patients exists, Fabry’s patients response to Con A (T cell and T suppressor cells mitogen) is not lower than in the control group.
Figure 1. Mitogenic lymphocyte response in vitro to increasing concentrations of Con A, PWM, PHA in Fabry’s (—) and control group (---). On the zero points of the abscissas the spontaneous stimulation (when determined) with the medium alone is represented.
**PWM** in presence of very variable results, one patient affected by Fabry's disease appears to decrease his response notably with the higher concentrations of the mitogen.

**PHA** in the polyclonal stimulation with PHA the same patient shows a considerably depressed response at the lower concentrations of the mitogen.

Once again the very large distribution of the responses must be noted while it should be considered that the mean value obtainable from all the patients is lower than that found in a healthy population matched for age and sex in our laboratory.

**Autologous rosette**

Although the clinical significance of this test is still unclear, the percentage of rosetting cells of Fabry's patients (Table I) is to be considered normal.

**Polymorphonuclear cell functions (Table II)**

The chemotactic activity of polymorphonuclear cells was partly depressed in one patient with Fabry's disease and markedly reduced in three patients of the control group while another Fabry's showed an even higher value in respect to the normal subjects.

Polymorphonuclear cells obtained from patients affected by Fabry's disease did not show any defect in their phagocytic properties when compared to the uraemic and healthy subjects.

**TABLE II. Polymorphonuclear cell functions, Phagocytosis (%Ph, Ph.I, see text) and chemotaxis (Cht) in Fabry's (F) and control group (C). Normal values: %Ph: 50 ± 6, Ph.I: 0.85 ± 0.21, Cht: 68.2 ± 27**

<table>
<thead>
<tr>
<th>Patient</th>
<th>%Ph</th>
<th>Ph.I</th>
<th>Cht</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>55</td>
<td>0.75</td>
<td>62.1</td>
</tr>
<tr>
<td>F2</td>
<td>47.3</td>
<td>0.67</td>
<td>34.7</td>
</tr>
<tr>
<td>F3</td>
<td>52</td>
<td>0.77</td>
<td>118.6</td>
</tr>
<tr>
<td>C1</td>
<td>58.1</td>
<td>0.85</td>
<td>76.3</td>
</tr>
<tr>
<td>C2</td>
<td>51</td>
<td>0.69</td>
<td>30</td>
</tr>
<tr>
<td>C3</td>
<td>45.3</td>
<td>0.62</td>
<td>29.2</td>
</tr>
<tr>
<td>C4</td>
<td>53.5</td>
<td>0.73</td>
<td>48.6</td>
</tr>
<tr>
<td>C5</td>
<td>58</td>
<td>1.03</td>
<td>63.5</td>
</tr>
<tr>
<td>C6</td>
<td>39.7</td>
<td>0.56</td>
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</table>
Discussion

Uraemia is associated with impairment of the immune potential and our data are in agreement with those of others [7–9]. Patients affected by Fabry’s disease do not seem to differ from the uraemic pattern in our study. Lymphocytopenia [7], reduced the number of total T and helper T cells [7,9] and low mitogenic lymphocyte response in vitro [7,8] are the immunological defects which are frequently observed in uraemic patients undergoing long-term maintenance haemodialysis.

Humoral immunity as well as the polymorphonuclear cell functions appear to be preserved in Fabry’s disease. Our data do not show any specific immunological impairment in these patients which agrees with our previously reported clinical review of eight Fabry’s patients receiving 10 renal transplantations [10]. We recorded an incidence of 11 major infectious episodes none of which was lethal and all responded to medical treatment and no transplant nephrectomy was required.

The reason for the high rate of lethal septic complications [3] does not appear related to the Fabry’s disease. However, in spite of a lack of a qualitative immune function defect, one of our patients showed a more marked depressed response to stimulation with pokeweed mitogen and phytohaemagglutinin A. Although it can hardly be ascribed to the basic disease, it could lead us to suppose that, in some subjects, Fabry’s disease may make the uraemia induced immunodeficiency worse, probably related to a more severe involvement of the bone marrow and other organs of the immune system in this unusual systemic disease.

References

6 Reinherz EL, Kung PC, Goldstein G. Proc Natl Acad Sci USA 1979; 76: 4061

Open Discussion

VERROUST (Chairman) Do you think that there is immune deficiency in the disease? You first said that there was not but a later slide seemed to contradict this.

DONATI Yes, we have found a decreased response to stimulation with antigens in one patient. I think that we can hardly ascribe this to the patient’s disease. I think that one of the rarest complications of this disease is aplastic anaemia.
Probably there will be more severe involvement of the bone marrow and the other organs in some subjects. The best way is to evaluate the medullary reserve before subjecting the patients to transplantation. I don't think that clinically the Fabry's disease patients have any specific immunological failure. Maybe their basic disease makes the renal induced immune deficiency state worse probably depending on the degree of involvement of the bone marrow.