Fatty acid inhibition of lipopolysaccharide-stimulated B lymphocyte proliferation

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We have recently shown that saturated, monounsaturated and polyunsaturated fatty acids, including those of fish-oil origin, inhibit [3H]thymidine incorporation into DNA of concanavalin A (Con A)-stimulated rat lymph node lymphocytes [1, 2]. All nine fatty acids tested inhibited the response to mitogen, but the extent of inhibition was dependent upon the fatty acid concentration used, the time during culture of fatty acid addition and the duration of exposure of the cells to the fatty acid [2]. All fatty acids were inhibitory at concentrations of 50 μM or above; at lower concentrations some were inhibitory and some were stimulatory [1, 2]. Above 50 μM the inhibitory effect was concentration dependent [2]. Generally, unsaturated fatty acids were more inhibitory than saturated fatty acids; the greatest inhibition of proliferation was caused by 20:5, n = 3 and 20:4, n = 6 and the least inhibition was caused by 14:0 and 16:0 [1, 2]. These results confirm and extend a number of reports of the inhibitory effects of various fatty acids upon phytohaemagglutinin (PHA)-stimulated lymphocyte proliferation (see [1–3] for references). Although all of these studies have been performed with cell populations containing a mixture of T and B lymphocytes the specificity of Con A and PHA as T-cell mitogens [4] implies that the fatty acids are exerting their inhibitory effects on T lymphocyte proliferation. Bacterial lipopolysaccharide (LPS) induces B-cell proliferation but does not activate T cells [5]. There has been one previous report of the effect of fatty acids on LPS-stimulated B lymphocyte proliferation [6]; 16:0; 17:0; 18:0; 19:0 or 18:2, n = 6 inhibited LPS-stimulated [3H]thymidine incorporation into DNA in murine splenic lymphocytes, but the extent of inhibition caused by each fatty acid was less than the effect on PHA-stimulated proliferation [6]. This study did not investigate the effects of long-chain polyunsaturated fatty acids; therefore we decided to examine the effects of a wide range of fatty acids, including 20:4, n = 6 and two polyunsaturated fatty acids of fish-oil origin (20:5, n = 3 and 22:6, n = 3), on LPS-stimulated lymphocyte proliferation. We were particularly interested in the effects of the latter fatty acids, since it has been suggested that they may have immunosuppressive properties [7, 8] and may be useful in the treatment of inflammatory and autoimmune diseases [9, 10].

Cervical lymph nodes were collected from male rats (300 g), freed of adipose tissue and gently ground. Lymphocytes were collected by centrifugation and washed twice. The cells were cultured at 37°C in an air/CO2 (19:1) atmosphere at a density of 5 x 10^6 cells/well in RPMI medium supplemented with 10 mM-glucose, 2 mM-glutamine and 10% (v/v) foetal calf serum (dialysed for 48 h versus phosphate-buffered saline). The cell culture medium was also supplemented with Con A (5 μg/ml) or Salmonella typhimurium LPS (15 μg/ml) and bovine serum albumin (BSA)–fatty acid complexes (0.1 mM-fatty acid; BSA/fatty acid ratio = 1:1). The

Abbreviations used: Con A, concanavalin A; PHA, phytohaemagglutinin; LPS, lipopolysaccharide; BSA, bovine serum albumin.

Table 1. Effect of fatty acids upon LPS- and Con A-stimulated lymphocyte proliferation

Lymphocytes were obtained, cultured and harvested as described in the text. Proliferation was measured as LPS- or Con A-stimulated [3H]thymidine incorporation in the presence of 0.1 mM-fatty acids, added as BSA-fatty acid complexes. Data are means ± S.E.M. of eight observations for [3H]thymidine incorporation. Statistical significance (Student's t test) versus albumin-alone controls: *P < 0.05; **P < 0.01; ***P < 0.001. Results are also presented as percentage thymidine incorporation, taking the incorporation in the absence of fatty acid, but with albumin present, as 100%.

<table>
<thead>
<tr>
<th>Medium supplement</th>
<th>[3H]Thymidine incorporation (d.p.m.)</th>
<th>% of control</th>
<th>[3H]Thymidine incorporation (d.p.m.)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>80180 ± 3570</td>
<td>100</td>
<td>10710 ± 1350</td>
<td>100</td>
</tr>
<tr>
<td>+ myristate</td>
<td>68955 ± 2405*</td>
<td>86</td>
<td>9424 ± 535</td>
<td>88</td>
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<tr>
<td>+ palmitate</td>
<td>48108 ± 3207*</td>
<td>60</td>
<td>8996 ± 334*</td>
<td>84</td>
</tr>
<tr>
<td>+ stearate</td>
<td>31270 ± 1604*</td>
<td>39</td>
<td>7604 ± 128*</td>
<td>71</td>
</tr>
<tr>
<td>+ oleate</td>
<td>32574 ± 4009*</td>
<td>41</td>
<td>7282 ± 422*</td>
<td>68</td>
</tr>
<tr>
<td>+ linoleate</td>
<td>16036 ± 1651</td>
<td>32</td>
<td>5141 ± 189*</td>
<td>48</td>
</tr>
<tr>
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<td>40090 ± 1722*</td>
<td>53</td>
<td>7497 ± 433*</td>
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</tr>
<tr>
<td>+ arachidionate</td>
<td>22450 ± 1593*</td>
<td>28</td>
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<tr>
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<td>16838 ± 1488*</td>
<td>21</td>
<td>5783 ± 214*</td>
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</tr>
<tr>
<td>+ docosahexaenoate</td>
<td>36081 ± 1821*</td>
<td>45</td>
<td>6319 ± 118*</td>
<td>59</td>
</tr>
</tbody>
</table>
A longitudinal study of plasma \( n-3 \) fatty acid levels in a family with X-linked retinitis pigmentosa

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A number of years ago we identified a family affected by the X-linked form of retinitis pigmentosa (RP), a hereditary eye disease which primarily appears to involve the rod photoreceptor cells. This large kindred, the ‘G family’, with five affected males in two generations, was analysed for plasma fatty acid levels in a family with X-linked retinitis pigmentosa (Table 1), indicating that B lymphocytes are less susceptible to the inhibitory effects of fatty acids. This may result from differing abilities of the two cell types to incorporate or metabolize exogenously supplied fatty acids. Whatever the basis of the different susceptibilities of T and B cells, it is clear that fatty acids in general have an immunosuppressive effect but that the effect is greatest with polyunsaturated fatty acids.

It is possible that the fatty acid-induced inhibition of lymphocyte proliferation is due to modification of the fatty acid composition of membrane phospholipids, which could alter fluidity of the membrane. Such changes could effect many properties such as membrane potential, affinity of receptors or signal transduction. Whatever the mechanism involved, these results suggest that proliferation of both T and B lymphocytes, and hence an immune response, could be altered by dietary lipid manipulation.

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A number of years ago we identified a family affected by a X-linked form of retinitis pigmentosa (RP), a hereditary eye disease which primarily appears to involve the rod photoreceptor cells. This large kindred, the ‘G family’, with five affected males in two generations, was analysed for plasma lipoproteins and fatty acids. It was found that the affected men had lower levels of a particular fatty acid, docosahexaenoic acid (DHA) \((22:6, n-3)\), than did their non-affected relatives [1, 2].

DHA is interesting in that it comprises about 50% of the fatty acids in the rod outer segment membranes, the highest concentration in any tissue in the body [3]. It has been shown that animals deprived of DHA or its precursors in the \( n-3 \) synthetic pathway show reduced visual function [4–6]. Thus it is possible that the low levels of DHA in affected men in our X-linked family have an adverse effect on the retina, and contribute to the pathogenesis of the disease. Following on these observations, we have now shown that a number of other X-linked families and one autosomal dominant family also show low levels of DHA [2]. These results have been confirmed in an American study [7], and low DHA has also been described in a large Usher syndrome family (RP plus congenital deafness) [8].

It is possible, however, that the low levels of plasma DHA are due not to a biochemical defect but to some extrinsic factor, such as diet. For this reason we analysed the diet of our patients and their relatives over a period of 7 years, and a comparison of the analyses.

Fasting blood samples were obtained from family members, plasma prepared, lipids extracted using chloroform–methanol, and fatty acids methylated as previously described [2]. In the first three analyses, samples were analysed by packed column gas chromatography on a Silar 10C column, using a temperature programme of 200°C for 4