n−3 Polyunsaturated fatty acids inhibit the antigen-presenting function of human monocytes

David A Hughes and Andrew C Pinder

ABSTRACT Diets rich in n−3 polyunsaturated fatty acids (PUFAs) are associated with suppression of cell-mediated immune responses, but the mechanisms are unclear. We hypothesized that n−3 PUFAs can inhibit the function of human antigen-presenting cells. A prerequisite for this role of blood monocytes is the cell surface expression of major histocompatibility complex (MHC) class II molecules [human leukocyte antigen (HLA)-DR, -DP, and -DQ], aided by the presence of intercellular adhesion molecule-1 (ICAM-1) and leukocyte function associated antigens 1 and 3. We showed previously that the n−3 PUFA eicosapentaenoic acid (EPA) inhibits the expression of HLA-DR on unstimulated human monocytes in vitro, but that docosahexaenoic acid (DHA) enhances its expression. However, both n−3 PUFAs suppress the expression of HLA-DR, HLA-DP, and ICAM-1 on interferon-γ-activated monocytes. We also established that dietary fish-oil supplementation can inhibit the expression of these surface molecules on circulating human monocytes. We subsequently showed that when EPA and DHA were combined in the same ratio as is commonly found in fish-oil-supplement capsules (3:2), there was no significant effect in vitro on the expression of HLA-DR on unstimulated monocytes, but the expression on activated monocytes remained significantly inhibited. In the same in vitro system, the ability of activated monocytes to present antigen to autologous lymphocytes was significantly reduced after culture with the combined n−3 PUFAs. These findings provide one potential mechanism for the beneficial effect of fish oil in the treatment of rheumatoid arthritis, a disorder associated with elevated expression of MHC class II and adhesion molecules on monocytes present within affected joints. Am J Clin Nutr 2000;71(suppl):357S–60S.

KEY WORDS n−3 PUFAs, major histocompatibility complex class II molecules, MHC class II molecules, monocytes, humans, antigen presentation, n−3 polyunsaturated fatty acids, HLA-DR, HLA-DP, HLA-DQ, ICAM-1, fish oil, rheumatoid arthritis, eicosapentaenoic acid, EPA, docosahexaenoic acid, DHA

INTRODUCTION Diets rich in n−3 polyunsaturated fatty acids (PUFAs) are associated with suppression of the immune system (1). The results of several well-controlled dietary supplementation studies have shown that fish oils, which are rich in n−3 PUFAs, can ameliorate the conditions of patients with several disorders, such as rheumatoid arthritis, that involve overreactive immune responses (2).

Blood monocytes initiate cell-mediated immune responses by processing and subsequently expressing antigens on their surface membranes for recognition by the appropriate T cells (3). A prerequisite for this antigen-presenting cell function is the expression of major histocompatibility complex (MHC) class II antigens such as human lymphocyte antigen (HLA)-DR, -DP, and -DQ (4). It has been shown that the T cell–proliferative response to an antigen is proportional to the number of MHC class II molecules on the surface of antigen-presenting cells (5). Research has also shown that the percentage of MHC class II–positive cells and the density of these molecules on the cell surface can alter the degree of immune responsiveness of an individual (6). In addition, the interaction of adhesion molecules between monocytes and lymphocytes appears to be critical for the initiation of a primary immune response, not only by enhancing adhesion but also by providing an additional, distinct costimulatory signal. The binding of the adhesion molecule, leukocyte function associated antigen-1 (LFA-1), to its ligand, intercellular adhesion molecule-1 (ICAM-1), has been shown to be capable of costimulating an immune response (7).

We reported previously that the n−3 PUFA eicosapentaenoic acid (EPA) can inhibit the expression of HLA-DR and ICAM-1 on normal human monocytes in vitro in a dose-dependent manner (8). In contrast, significant increases in the expression of HLA-DR and HLA-DP on monocytes were observed after incubation with the other major n−3 PUFA found in fish oil, docosahexaenoic acid (DHA). Because it has been shown that synovial fluid monocytes obtained from patients with rheumatoid arthritis express elevated amounts of MHC class II molecules (9), we also examined the effect of n−3 PUFAs on activated monocytes. These monocytes were cultured in the presence of interferon γ (IFN-γ) to up-regulate the expression of

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MHC class II molecules on the monocytes. Both EPA and DHA significantly inhibited the expression of HLA-DR, HLA-DP, and ICAM-1 on the activated monocytes (8). We have also shown that dietary fish-oil supplementation can inhibit the expression of these molecules on human peripheral blood monocytes (10).

The aim of this study was to investigate the combined effect in vitro of EPA and DHA, when provided in the same ratio as is commonly found in fish-oil supplement capsules (3:2), on the expression of functionally associated surface molecules on human monocytes. In addition, an in vitro assay of antigen presentation was used to investigate whether changes in the expression of surface molecules were associated with an alteration in antigen-presenting function.

SUBJECTS AND METHODS

Subjects

The monocytes used in this study were obtained by venipuncture from healthy, nonsmoking, adult volunteers: n = 3 men and 6 women (mean age 34 y; range 23–44 y) in the immunofluorescence study, n = 2 men and 4 women (mean age 35 y; range 22–53 y), all of whom had been inculated with tetanus toxoid within the previous 10 y, in the functional study. No subjects were receiving medication and none had been consuming fish-oil supplements or were regular consumers of oily fish. The study was approved by the Institute of Food Research Ethical Committee.

n–3 Polyunsaturated fatty acids

EPA and DHA were obtained from Sigma (Poole, Dorset, United Kingdom) and were solubilized in 95% ethanol. A concentrated stock mixture of EPA and DHA, at a ratio of 3:2, was stored under nitrogen until immediately before use.

Antibodies for immunostaining

The following monoclonal antibodies were used to investigate the modulatory effects of the fatty acids on cell surface antigen expression: anti-HLA-DR, anti-HLA-DP, and anti-HLA-DQ (Becton Dickinson, Oxford, United Kingdom); and anti-CD54 (ICAM-1), anti-CD11a (LFA-1), and anti-CD58 (LFA-3) (Serotec, Oxford, United Kingdom). Fluorescein isothiocyanate (FITC)-labeled rabbit antimouse immunoglobulin F(ab')2 fragment (Dako, High Wycombe, United Kingdom) was used as a second-layer antibody to detect monoclonal antibody binding. This FITC-labeled antibody was also used alone to control for nonspecific binding. Anti-CD45 (Serotec), which is common to all leukocytes, was used as a positive control.

Purification of monocytes

Monocytes were acquired by density gradient centrifugation by using a method described previously in detail (11). The recovered cells were resuspended in 1 mL of culture medium (HEPES-buffered RPMI containing 5% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 1 × 10^{-5} U/L penicillin, 0.07 mmol/L streptomycin; Gibco BRL, Paisley, United Kingdom). The cells were then counted and adjusted to 5 × 10^9 cells/L. The monocytes were >95% viable, as assessed by trypan blue exclusion.

Purification of autologous lymphocytes

For use in the assays of antigen-presenting function, autologous lymphocytes were obtained from an additional 50-mL blood sample by using a method described previously in detail (12); the lymphocytes were then resuspended at 1.1 × 10^9 cells/L in culture medium.

Cell culture with n–3 PUFA

Monocytes were cultured in the presence or absence of a combination of EPA and DHA in 15-mL polypropylene tubes (Falcon 2057; Becton Dickinson, Lincoln Park, NJ). The stock solution of EPA and DHA (3:2) was diluted in culture medium immediately before use (to minimize oxidation) and was added to the cultures at a final concentration of 12 μg/mL EPA and 8 μg/mL DHA. Control cells were mixed with equivalent amounts of 95% ethanol to reach a final concentration of 0.1% ethanol. In addition, cultures in the presence or absence of the n–3 PUFA were performed with the further addition of IFN-γ (Genzyme, West Malling, United Kingdom) to up-regulate surface molecule expression, at a previously determined optimal concentration of 4 × 10^9 units/L. After incubating for 48 h at 37°C, the cells were adjusted to a concentration of 5 × 10^6 cells/L in minimal essential medium for staining before flow cytometry.

Immunofluorescence and analysis of surface marker expression by flow cytometry

The cells were stained with the various monoclonal antibodies by using the technique described by Parker and Haslam (13). The percentages of FITC-positive cells and the intensity of expression of the cell surface molecules were quantified by laser flow cytometry as described previously (8).

Assay of antigen-presenting function

To determine the effect of n–3 PUFA pretreatment on monocyte function, an in vitro assay of antigen presentation was performed as we have described previously (11). However, lymphoproliferation was assessed by adding bromodeoxyuridine in place of the ^3H-thymidine. Bromodeoxyuridine was added to the cultures 18 h before quantification of its uptake with an ELISA kit (Boehringer Mannheim, Lewes, United Kingdom). We used soluble tetanus toxoid with a limited flocculation (Lf) value of 2050 Lf/mL and a purity of 1073 Lf/mg (Medeva PLC, Leatherhead, United Kingdom). The results were expressed as mean absorbance (±SEM) of triplicate cultures.

Statistical analysis

Differences between pairs of n–3 PUFA–treated and untreated samples were analyzed with the paired t test (14).

RESULTS

Effect of n–3 PUFA on surface molecule expression by monocytes

Unstimulated monocytes

The reductions in the percentages of monocytes expressing the various surface molecules after incubation for 48 h in the presence of 12 μg/mL EPA and 8 μg/mL DHA are shown in Table 1. The reductions are expressed as percentage reduction compared with controls cultured in the absence of n–3 PUFA.
Table 1
Effect of n−3 polyunsaturated fatty acids on the expression of surface molecules on unstimulated human monocytes

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Positive monocytes (%)</th>
<th>Relative median intensity of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% reduction compared with control cells</td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>7.6 ± 7.2</td>
<td>8.6 ± 10.2</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>11.5 ± 9.6</td>
<td>16.5 ± 10.4</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>3.5 ± 13.0</td>
<td>9.2 ± 12.9</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>23.3 ± 8.9</td>
<td>25.1 ± 8.2</td>
</tr>
<tr>
<td>LFA-1</td>
<td>4.5 ± 10.5</td>
<td>8.5 ± 7.0</td>
</tr>
<tr>
<td>LFA-3</td>
<td>21.3 ± 9.8</td>
<td>21.4 ± 8.4</td>
</tr>
</tbody>
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1SEM; n = 9. HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule; LFA, leukocyte function associated antigen.
2Significantly different from control, P < 0.05 (paired t test).

Table 2
Effect of n−3 polyunsaturated fatty acids on the expression of surface molecules on interferon γ−stimulated human monocytes

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Positive monocytes (%)</th>
<th>Relative median intensity of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% reduction compared with control cells</td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>10.2 ± 3.9</td>
<td>14.8 ± 6.5</td>
</tr>
<tr>
<td>HLA-DP</td>
<td>16.4 ± 4.4</td>
<td>21.8 ± 5.3</td>
</tr>
<tr>
<td>HLA-DQ</td>
<td>4.1 ± 7.3</td>
<td>6.9 ± 10.9</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>10.1 ± 3.8</td>
<td>17.6 ± 4.4</td>
</tr>
<tr>
<td>LFA-1</td>
<td>1.0 ± 6.7</td>
<td>7.1 ± 4.6</td>
</tr>
<tr>
<td>LFA-3</td>
<td>29.3 ± 7.9</td>
<td>28.5 ± 6.8</td>
</tr>
</tbody>
</table>

1SEM; n = 9. HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule; LFA, leukocyte function associated antigen.
2Significantly different from control cells (paired t test): 2P < 0.05,
3P < 0.01, 4P < 0.001.

DISCUSSION

We have shown that the n−3 PUFAs EPA and DHA, when combined in the same ratio (3:2) as is commonly found in commercially available preparations of fish oil, can inhibit the expression of cell surface molecules known to be required for the process of antigen presentation. Variability in the expression of these functionally associated molecules is known to be capable of altering the degree of immune responsiveness of an individual to antigenic stimulation (6). In this study, we observed an associated reduction in the ability of IFN-γ-activated monocytes to present antigen to autologous lymphocytes following preincubation with the combined n−3 fatty acids. As Janeway et al (6) emphasized, functional assays are of central importance in indicating the potential effects in vivo.

The inhibitory effects observed in vitro were obtained with concentrations of EPA and DHA that can be achieved in blood plasma by consuming relatively low doses of fish oil (3 g/d) (15). In a human supplementation study, we reported that 3 g fish oil/d for 21 d reduced the expression of MHC class II molecules and adhesion molecules on peripheral blood monocytes (10). The supplements used in this study contained the same EPA-to-DHA ratio as was used in the present in vitro study.

The ability of n−3 PUFAs to inhibit the antigen-presenting function of activated monocytes supports the possibility that fish oil may be beneficial in the treatment of autoimmune disorders. A number of double-blind studies have reported that consumption of fish oil by patients with rheumatoid arthritis was associated with mild to moderate improvement in symptoms, including reductions in morning stiffness and the number of tender joints (2). The striking inhibition by EPA and DHA of MHC class II molecules and ICAM-1 expression on IFN-γ-stimulated monocytes seen in this study may be particularly relevant to rheumatoid arthritis, because patients with this disorder have been shown to have abnormally elevated expression of both MHC class II molecules (9) and ICAM-1 (16) in chronically inflamed joints. A corresponding reduction in antigen-presenting function might lead to reduced helper T cell activation, thus decreasing both the production of inflammatory cytokines and the production of antibodies by B cells at these localized sites of disease.

Several animal studies have shown that n−3 PUFAs can inhibit the expression of Ia molecules, the murine equivalent of human MHC class II molecules. Kelley et al (17) reported that dietary supplementation with fish oil suppressed autoimmune lupus in MRL−lpr mice and prevented an increase in macrophage surface Ia expression. Mosquera et al (18) showed that administering fish oil to mice and rats by esophageal gavage reduced the percentage of peritoneal macrophages that expressed Ia compared with saline-gavaged controls. In addition, Huang et al (19) showed that mice fed fish oil and then infected with Listeria monocytogenes had reduced expression of Ia on the surface of peritoneal macrophages compared with mice fed other fat sources. Dietary enrichment with EPA has been shown to inhibit the ability of spleen cells to present antigens to murine helper T cell clones, and in vitro pretreatment of splenocytes with EPA...
also resulted in inhibition of antigen-presenting cell function (20). Recently, it has been shown that dietary fish oil can diminish the ability of rat dendritic cells (another class of antigen-presenting cells) to present antigen to autologous spleen lymphocytes (21). Taken together, the results of these studies and those of the current study support the hypothesis that n-3 PUFAs suppress cell-mediated immune responses, at least in part by inhibiting the function of antigen-presenting cells.

REFERENCES