Introduction

The cytokine tumour necrosis factor (TNF) is one of a diverse range of molecules that play a major part in inducing and modulating inflammatory and immune processes. TNF, in common with other cytokines, is predominantly a product of the immune system; however, endothelial cells also have the capability of its production. Biologically, TNF acts as a trigger that activates a cascade of cytokine production. The molecule is released rapidly in response to inflammatory and infective agents and induces production of a wide range of cytokines, including interleukins 1 and 6 (IL-1 and IL-6) with which it has a number of actions in common. These include fever, free radical and acute phase protein production and up-regulation of adhesion molecules [1–3]. TNF may prolong the inflammatory process by inducing the chemokine IL-8 [4]. TNF can indirectly modulate immunological events by induction of IL-1, which stimulates IL-2 and IL-4 production. The latter two cytokines result in increased lymphocyte proliferation and switching of immunoglobulin classes respectively [4]. In addition to its important role as an early effector in inflammatory and immune processes, TNF is important in the killing of fungi and a number of viruses [5]. However, excessive or biologically inappropriate TNF production is closely associated with pathological events. Such events have been closely linked with mortality from cerebral malaria, endotoxic shock, sepsis and adult respiratory distress syndrome, and with pathology in a wide range of autoimmune disorders [1]. These include rheumatoid arthritis, inflammatory bowel disease, psoriasis and atherosclerosis (see [6]). A number of systems exist for limiting TNF production and effects. These have been reviewed elsewhere and include glucocorticoids, acute phase proteins, eicosanoids and soluble receptors [6]. External intervention is, however, necessary in situations where TNF production is disadvantageous to the host. Modulation can be achieved by nutrients or drugs. This paper deals with the influence of unsaturated fatty acids on the biology of TNF. Fats have been shown to be potent modulators of inflammation. In essence, fats rich in n-6 polyunsaturated fatty acids (PUFAs) enhance cytokine-mediated aspects of inflammation, and fats rich in n-3 PUFAs or n-9 monounsaturated fatty acids (MUFAs) have the opposite effect. Potentially, fats may exert their effects in a complex manner leading to modulation of production of TNF or of the actions of the cytokine upon target tissues [6].

Mechanisms whereby fats may modulate production and actions of TNF

Changes in membrane composition

Fats may influence cytokine biology predominantly by their effects upon the plasma membrane composition of cells capable of producing and responding to cytokines. Many studies have shown that membrane cholesterol content, and the fatty acid composition of the various classes of phospholipids in the membrane, can be modified by alterations in dietary fat. These changes may alter membrane fluidity and consequently the binding of both cytokines and cytokine-inducing agonists to receptors [7, 8]. They may also alter components of the signal transduction process that leads to cytokine production or effects. For example, fluidity changes may alter G-protein activity, thereby changing adenylyl cyclase, phospholipase A2 (PLA2) and phospholipase C (PLC) activity. This would result in changes in cyclic prostaglandin (PG) and leukotriene (LT), and diacylglycerol (DAG) production, respectively. All of these substances modulate cytokine production [10–12]. Alterations in membrane phospholipids will influence the synthesis of lipid-derived mediators such as the eicosanoids (PGs and LTs), and platelet-activating factor. Activation of PLA2 will release fatty acid from the sn-2 position of phospholipids that may be used in eicosanoid syn-
thesis. The sn-2 position is usually occupied by unsaturated fatty acids. Arachidonic acid (AA) is the parent compound of PGs and LTs of the 2 and 4 series respectively; eicosapentaenoic acid (EPA) is the precursor of the less potent PGs and LTs of the 3 and 5 series respectively. Eicosatrienoic (mead) acid (ETA) only acts as an eicosanoid substrate under conditions of essential fatty acid deficiency. Dietary fat composition will modulate the proportions of all three eicosanoid precursors. Linoleic acid (LA), an n-6 PUFA, is converted to AA, oleic acid (OA), an n-9 MUFA, will be converted to ETA, and e-linolenic acid (LNA), an n-3 PUFA, will be converted to EPA. Conversion of the n-3, n-6 and n-9 fatty acids to precursors of eicosanoids occurs after they have become attached to the sn-2 position of membrane phospholipids. The fatty acids compete for incorporation into the phospholipid structure. The affinity for incorporation is in the order LNA > LA > OA. Furthermore AA and EPA may be incorporated into phospholipids if present in the diet. EPA is incorporated with the highest affinity of all unsaturated fatty acids [7, 12, 13]. The fats consumed in diets contain widely differing unsaturated fatty acid contents. Saturated animal fats, such as those of beef, lamb and butter, contain low concentrations of LA. Although coconut oil is poor in LA, all other fats of plant origin are rich in LA. Butter, corn oil, palm oil and olive oil contain substantial quantities of OA. Oils from fatty fish are rich in EPA and docosahexaenoic acid (DHA; C22:n-3). Dietary LNA is derived from leafy vegetables, although none is particularly rich in this nutrient.

Changes in eicosanoid production
A number of studies have shown that PGE$_2$ can inhibit, and LTs enhance, production of IL-1 and TNF [11, 12, 15]. Moreover, inhibition of PGE$_2$ synthesis in phagocytic cells stimulated with lipopolysaccharide (LPS) enhanced production of TNF [16]. Thus alteration of eicosanoid production by dietary fat could modulate cytokine production. A number of investigators have studied the effects of fats on the ability to produce cytokines. Fats with a limited range of fatty acid compositions have been used. Most studies have examined the effects of fish oils, which are rich in unsaturated fatty acids of the n-3 series [17-20]. Fish oil might be expected to enhance TNF production by reducing PGE$_2$ production in response to an inflammatory stimulus. However, studies in humans [17, 19] and in rats [21] showed that feeding fish oil reduced the ability of macrophages to produce IL-1 and TNF. Studies in mice, on the other hand, have shown that fish oil can enhance IL-1 and TNF production by macrophages [18, 22]. Moreover, a diet rich in LNA fed to mice enhanced TNF production by resident and casein-induced peritoneal macrophages to a greater extent than in cells from animals fed with diets rich in LA [16]. PGE$_2$ production by cells from the latter dietary group was higher than in the former, as might be expected. Inclusion of indomethacin in the incubate resulted in enhancement of TNF production in resident cells from the group fed with LA to values similar to those in the corresponding group fed with LNA. In casein-induced cells, indomethacin enhanced production in both dietary groups but did not abolish the differences in TNF production between the two dietary groups. These results suggest that TNF production is also regulated by factors other than PGE$_2$. Studies in vitro, in which human peripheral blood mononuclear cells that were incubated with AA and EPA showed enhanced IL-1 production when incubated with LPS, likewise indicated that non-eicosanoid mechanisms might be important in influencing the effects of unsaturated fatty acids on cytokine production [23].

Changes in protein kinase C activity
Although much is known of how dietary fat influences the types of fatty acids at the sn-1 and sn-2 positions on the skeleton of the various classes of phospholipid, little is known of how dietary fat alters the proportions of the phospholipid classes in the membrane. Alterations in the proportions of phosphatidyserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and sphingomyelin (SP) may have functional significance in cytokine biology. Activation of protein kinase C (PKC) has been implicated in a wide range of macrophage functions, including immunoglobulin-mediated phagocytosis and production of IL-1, TNF and free radicals [24-28].

PKC is a ubiquitous protein that plays a key role in the transduction of extracellular signals, such as cytokines and cytokine-inducing agents, which result in turnover of plasma membrane phospholipids. During activation, phosphatidylinositol bis-phosphate is hydrolysed by PLC to yield two second messengers, namely inositol trisphosphate (IP$_3$) and DAG. IP$_3$ mobilizes intracellular calcium, causing cytosolic PKC to translocate to the plasma membrane, where it becomes activated by DAG. Activity also depends upon binding of PKC to PS, which is mostly located on the inner leaflet of the plasma membrane. Although PS is the major acti-
vating phospholipid for PKC, PE can activate the enzyme to a small extent [29]. A component of SP, sphingosine, may be able to inhibit PKC [30]. Thus phospholipids themselves, rather than eicosanoids derived from fatty acids released from them under the actions of phospholipases, have the ability to modulate cytokine biology. Non-esterified fatty acids may also play a part in the activation of PKC. The fatty acids may arise from the actions of PLA2 upon membrane phospholipids. A wide range of unsaturated fatty acids including OA, LA and AA can stimulate PKC activity in vitro in the presence of PS and DAG. Conversely, in the presence of the latter two substances DHA and EPA suppress PKC activity [31–34]. Peritoneal macrophages incubated with fatty acid–albumin complexes show an analogous effect. PKC was activated by LA and suppressed by DHA [35]. PKC activity in lymphocytes can be manipulated by feeding with a range of fats rich in unsaturated fatty acids. Decreased enzyme activity, IL-2 production and lymphocyte proliferation have been observed [36, 37].

The influence of n-6 PUFAs and total unsaturated fatty acid intake on membrane fluidity and cytokine production and actions

We examined the ability of TNF to induce IL-1 and IL-6 production from peritoneal macrophages of rats fed with a range of fats, representing the wide range of types encountered in human diets, for four and eight weeks. The fats studied were corn, olive, coconut and fish oils and butter. Chow-fed animals were included in the studies. Complex modulation occurred [21]. After four weeks of feeding, fish and olive oils suppressed IL-1 production (relative to chow-fed animals). However, after eight weeks of feeding, whereas fish and coconut oils suppressed IL-1 production, olive oil and corn oil enhanced production. After four weeks of feeding, IL-6 production was enhanced by fish and olive oils, and after eight weeks of feeding, fish, corn and olive oils and butter resulted in enhanced production. Coconut oil had no effect. However, despite the complexity of these effects, after eight weeks of receiving the diets, production of IL-1 related positively to the n-6 PUFA intake of the animals and IL-6 production with the total intake of unsaturated fatty acids (Figure 1).

The fluidity of cell membranes could be influenced by unsaturated fatty acid intake [7, 8]. We thus used the fluorescence recovery after photobleach (FRAP) technique to examine plasma membrane fluidity of macrophages from animals fed with the range of diets in our previous study. The effect of an inflammatory stimulus on macrophage membrane fluidity was also studied. The results are shown in Table 1. Fluidity was influenced in a complex manner by the type of fat in the diet, the period over which it was fed, and the presence, or absence, of an inflammatory stimulus in the form of LPS.

After a four-week feeding period, fluidity was lowest in animals fed with corn oil and highest in those fed with fish oil. Thus fats high in n-6 and n-3 PUFAs had contrasting effects upon membrane fluidity. Fluidity had decreased substantially in all dietary groups after eight weeks of feeding. The effect was most marked in the case of animals fed with fish oil and least marked for animals fed with corn oil. The phenomenon may be due to homoeoviscous adaptation. The cholesterol content...
Table I

<table>
<thead>
<tr>
<th>Oil/fat</th>
<th>4 weeks −LPS</th>
<th>4 weeks +LPS</th>
<th>8 weeks −LPS</th>
<th>8 weeks +LPS</th>
</tr>
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<tbody>
<tr>
<td>Corn</td>
<td>24.5 ± 1.2</td>
<td>28.2 ± 3.1</td>
<td>16.1 ± 1.4</td>
<td>14.3 ± 0.4</td>
</tr>
<tr>
<td>Olive</td>
<td>28.5 ± 3.1</td>
<td>18.4 ± 1.9</td>
<td>16.5 ± 2.1</td>
<td>14.7 ± 1.1</td>
</tr>
<tr>
<td>Coconut</td>
<td>29.8 ± 5.0</td>
<td>20.4 ± 2.4</td>
<td>17.6 ± 1.4</td>
<td>14.3 ± 1.1</td>
</tr>
<tr>
<td>Butter</td>
<td>31.2 ± 2.8</td>
<td>18.0 ± 1.8</td>
<td>19.6 ± 0.9</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td>Fish</td>
<td>36.0 ± 3.6</td>
<td>17.0 ± 1.9</td>
<td>15.2 ± 1.2</td>
<td>16.3 ± 0.9</td>
</tr>
</tbody>
</table>

may increase to offset the fluidizing influence of an increase of the proportions of PUFAs in membrane phospholipids, thereby reducing membrane fluidity.

The ability of macrophages to produce IL-1 and IL-6 showed no direct relation with membrane fluidity. Whereas after four weeks of dietary treatment a reduced ability of macrophages to produce IL-1 in the animals fed with fish oil was associated with high membrane fluidity, the reduced production at eight weeks was not. Furthermore, low membrane fluidity was associated with low and high production of IL-1 by cells from rats fed with fish oil, and corn and olive oil respectively, for eight weeks. No consistent relation between membrane fluidity and IL-6 production was noted after four and eight weeks of dietary administration.

When cells were incubated with LPS for 24 h, a substantial decrease in fluidity was noted in all dietary groups after four and eight weeks of feeding, with the exception of the dietary group whose cells exhibited the lowest fluidity at each time point. The latter dietary groups were those fed with corn oil for four weeks and fish oil for eight weeks. An increase in plasma membrane cholesterol may occur in macrophages of a similar nature to that seen in liver plasma membranes when cells are exposed to LPS.

Influence of dietary n-6 PUFA intake and endotoxin on liver plasma membrane phospholipid class distribution

To gain a better understanding of how inflammatory mediators and dietary fats influence the proportions of phospholipid classes in the plasma membrane, we fed rats with corn or olive oil or butter at three concentrations to achieve a wide range of unsaturated fatty acid intakes, before an injection of LPS. Liver and lung membrane composition was examined 24 h after injection. A number of physiological effects of LPS were also examined at this time; these are reported elsewhere [38]. In essence, enhanced rates of protein synthesis in liver, lung and kidney occurred in rats fed with corn oil or a high concentration of butter. Animals fed with low concentrations of butter, and all groups fed with olive oil, showed abrogated responses. Furthermore the magnitude of responses increased with the amount of corn oil in the diet. Major changes in plasma membrane composition occurred in response to LPS, which were modulated by the diet [39]. Data for liver are shown in Figure 2 and are expressed in relation to the LA intake of the dietary groups. Although most membrane constituents were unaffected by dietary LA intake, plasma membrane PS was inversely related to LA intake. In response to LPS, large decreases in membrane PI and PC occurred. The amounts of PS increased, as did cholesterol. A small decrease in PE and no change in SP occurred (data not shown). Similar changes occurred in lung membranes to those observed in liver, with the exception of the diet-mediated fall in PS (data not shown).

Activation of PKC requires the hydrolysis of PI and the presence of PS. The changes that occur in the composition studies described above indicate that an increase in both factors occurs in vivo in response to LPS, a potent stimulator of TNF production. Furthermore the magnitude of the responses increased with dietary LA intake. It is unknown whether changes occur in the plasma membrane of macrophages that are analogous to those observed in liver. Should much modulation be the case, then dietary modulation of PKC activity may, in part, play an important role in the modula-
Figure 2

Effect of LA intake and LPS on the proportions of phospholipid classes and cholesterol in liver cell plasma membranes of rats

Animals were fed with diets containing corn or olive oil or butter at concentrations (50, 100, 200 g/kg) to achieve a range of linoleic acid intakes, before an injection of LPS. Abbreviation used: prot, protein.

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Suppression of autoimmune disease by ω-3 fatty acids


†Arthritis Unit of the Medical Services and ‡Department of Pathology, Massachusetts General Hospital, the Departments of §Medicine and ¶Pathology, and ¶¶Center for Blood Research, Harvard Medical School, Boston, MA 02114, U.S.A.

Introduction

Dietary ω–6 and ω–3 polyunsaturated fatty acids are essential for normal health in humans and other mammals. A large body of evidence from epidemiological and experimental studies has documented that ω–3 fatty acids can modify a variety of cell functions and disease states. Incorporation of ω–3 fatty acids into tissue phospholipids is associated with the alleviation of some inflammatory diseases associated with autoimmunity. It has been proposed that the alteration of eicosanoid production by ω–3 fatty acid incorporation into phospholipids may account for at least some of the anti-inflammatory activity of ω–3 fatty acids, but not all of the anti-inflammatory effects of ω–3 fatty acids are readily accounted for by alterations of eicosanoids. Recent studies have documented that ω–3 fatty acids may suppress cytokine formation and it has been proposed that this action may modify a variety of inflammatory states (Endres et al., this colloquium).

Our laboratories have investigated the mechanisms of suppression of immune-induced inflammation by using autoimmune experimental animals and activated inflammatory cells ex vivo after dietary ω–3 fatty acid supplementation. We review here some studies of murine autoimmune strains as well as some recent investigations of the mechanism of inhibition of cytokine formation by ω–3 fatty acids.

Alleviation of autoimmune glomerulonephritis in inbred murine strains

All beneficial effects of ω–3 fatty acids on autoimmune disease were first demonstrated on several inbred strains of mice that develop spontaneous autoimmune disease, including glomerulonephritis.