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Inhibition of natural killer cell activity by dietary lipids

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1. Summary

Natural killer (NK) cells are a subset of cytotoxic lymphocytes found mainly in blood and the spleen. NK cells play a role in natural immunity to microbes, viruses and tumor cells and are involved in the rejection of grafts. The present study investigated the effects of diets containing oils rich in saturated fatty acids, mono-unsaturated fatty acids, *n*-6-poly-unsaturated fatty acids (PUFA) or *n*-3-PUFA on the NK cell activity and on the lymphokine-activated killer (LAK) cell activity of rat spleen lymphocytes. Weanling rats were fed for 10 weeks on a low-fat (LF) diet (~ 2% fat by weight) or on 1 of 5 high-fat (HF) diets, which contained 20% (by weight) hydrogenated coconut oil (HCO), olive oil (OO), safflower oil (SO), evening primrose oil (EPO) or menhaden (fish) oil (MO). Each of the HF diets suppressed the NK activity of freshly prepared spleen lymphocytes compared with the LF diet; cells from the MO-fed rats exhibited the lowest NK activity. Culture with IFNα for 3 h increased the NK activity of spleen lymphocytes from all animals, except those fed the OO diet; the increase in NK activity varied from 20% (LF) to 50% (MO). Although feeding the OO, EPO or MO diets resulted in lower IFNα-stimulated NK activity than that obtained by feeding the LF diet, the only consistent significant difference was the lower activity of the cells from the OO-fed rats compared with those from the LF-fed rats. LAK cells were generated by culture of spleen lymphocytes with ConA for 24 h followed by IL-2 for 72 h. All of the HF diets decreased LAK cell activity; statistically significant effects were observed for lymphocytes from rats fed the MO diet compared with those from rats fed the LF-fat diet. These observations show that dietary PUFA, particularly those of the *n*-3 family inhibit NK cell activity. This may be useful in prolonging graft survival, but may also decrease the ability of the host to destroy invasive microbes, viruses and tumor cells.

2. Introduction

One of the most important mechanisms by which the immune system deals with foreign cells is to damage or destroy them. Typical target cells include malignant cells, normal cells of the host that are infected with viruses or other microorganisms and normal cells from individuals unrelated to the responding host. Natural killer (NK) cells are a subset of lymphocytes found mainly in blood and the spleen [1]. They are derived from the bone marrow, but are neither T cells nor B cells and they do not undergo thymic maturation. NK cells appear as large lymphocytes with numerous cytoplasmic granules and have often been described as 'large granular lymphocytes' [1,2]. These cells lack several properties characteristic of cytotoxic T lymphocytes; they lack the T-cell receptor for antigen recognition, they do not undergo immunoglobulin or T-cell receptor gene rearrangements and they do not express CD3 molecules [3]. Killing by NK cells is part of natural rather than specific immunity, since it is not induced by a specific antigen and is not restricted by MHC molecules.

IL-2 has been shown to stimulate both the proliferation and cytotoxicity of NK cells; NK cells that have been cultured for short periods with highly purified IL-2 are described as lymphokine-activated killer (LAK) cells and display cytotoxicity towards a variety of autologous, allogeneic and xenogeneic tumours [1]. There is good evidence that the phenotype of LAK cells is compatible with the phenotype of NK cells, but is

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Abbreviations: EPO, evening primrose oil; HCO, hydrogenated coconut oil; LF, low fat; HF, high fat; MO, menhaden oil; OO, olive oil; PUFA, polyunsaturated fatty acids; SO, safflower oil.

divergent from that of T lymphocytes [1]; in other words, IL-2 specifically stimulates the cytotoxic activities of NK cells. LAK activity is inducible in vivo and has been observed following intravenous injection of IL-2 into cancer patients and in the lymph nodes of experimentally immunised mice [4], which suggests that LAK cell activity may be part of a normal immune response.

Poly-unsaturated fatty acids (PUFA) have been shown to suppress T-cell functions in vitro. For example, it has been shown that fatty acids inhibit T-cell proliferation [5–8], IL-2 production [6,7] and expression of activation markers on the cell surface [6]. Furthermore, supplementation of the diet with oils containing PUFA, particularly fish oils containing *n*-3-PUFA, results in suppression of T-lymphocyte proliferation in animals [9] and in man [10] and IL-2 production in man [10]. These suppressive effects suggest that PUFA could be useful in the therapy of autoimmune and inflammatory diseases, which involve inappropriate activation of the immune system.

It has been demonstrated that a decrease in the total fat content of the diet of healthy men results in an increase in NK activity [11], indicating that dietary fat could potentially modulate NK cell activity. Yamashita et al. [12] have shown that addition of triacylglycerols containing the *n*-3-PUFA, eicosapentaenoic or docosahexaenoic acids, to human PBMC in vitro significantly inhibits NK activity. Furthermore, infusion of the eicosapentaenoic acid-containing triacylglycerol into healthy volunteers lowered NK activity measured in vitro 24 h later [12]. There have, however, been few studies investigating the relationship between different dietary lipids and NK cell activity. Three studies have compared the effects of fish oil and corn oil feeding to mice on NK activity [13–15]. Each of these studies demonstrated that fish oil results in decreased NK activity compared with corn oil. The effects of diets containing oils other than fish or corn oil on NK cell activity have not previously been investigated. The present study investigates, for the first time, the effects of a range of dietary lipids on the NK cell activity and LAK cell activity in the rat. Since NK cells are involved in tumor cell killing [16] and graft rejection [17], modulatory effects of fatty acids on NK cell activity could be useful in the reduction of tumor growth or in the care of patients undergoing organ transplantation.

3. Materials and Methods

3.1. Materials

The sources of chemicals were as described previously [5–7]. In addition, IFNα was obtained from Sigma (Poole, Dorset, UK) and ^{51}Cr was obtained from Amersham International (Amersham, Bucks, UK). IL-2 (Cetus) was a gift from Dr. M. Dallman, Nuffield Department of Surgery, John Radcliffe Hospital, Oxford, and the YAC-1 cell line was a gift from Dr. K. Ohlen, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford.

3.2. Animals and diets

Weanling male Lewis rats (aged 3 weeks, weighing between 65 and 85 g) were obtained from Harlan-Olac (Bicester, Oxon, UK). The animals were housed individually in the Department of Biochemistry for a period of 10 weeks prior to death, during which time they were allowed access, ad libitum, to water and to 1 of 6 experimental diets. Animals were fed either a LF diet (rat and mouse No. 1 diet, provided by SDS, Witham, Essex, UK) or 1 of 5 HF diets (custom diets, provided

TABLE 1
FATTY ACID COMPOSITION OF THE DIETS

Lipid was extracted from diet (0.05 g) using chloroform/methanol (2:1 v/v). Fatty acids were prepared by saponification in methanolic KOH and extracted to ethyl acetate. Fatty acids were methylated with diazomethane and analysed by gas chromatography, as described elsewhere [18]. Data are the mean of 2 determinations.

Diet	Fatty acid (mol%)										
	10:0	12:0	14:0	16:0	16:1 (*n* 7)	18:0	18:1 (*n* 9)	18:2 (*n* 6)	18:3	20:5 (*n* 3)	22:6 (*n* 3)
LF	–	2.2	2.4	31.6	–	7.4	21.1	31.5	–	–	–
HCO	5.0	47.3	18.2	10.5	2.2	11.0	2.4	3.5	–	–	–
OO	–	3.0	1.5	11.1	–	3.9	69.9	15.3	–	–	–
SO	–	2.2	0.4	11.8	–	4.4	20.8	60.4	–	–	–
EPO	–	–	–	10.3	–	3.2	15.8	68.4	5.0 [a]	–	–
MO	–	–	10.9	24.7	16.9	4.4	13.8	5.5	2.3 [b]	11.7	5.2

[a] Represents γ-linolenic acid (18:3*n*-6).
[b] Represents α-linolenic acid (18:3*n*-3).

by ICN Biomedicals, High Wycombe, Bucks, UK), all of which were in pelleted form. The LF diet contained approximately 2.4% lipid by weight and the HF diets contained 20% by weight of the lipid under study, plus 1% corn oil to prevent essential fatty acid deficiency. The HF diets contained either 20% hydrogenated coconut oil (HCO), 20% olive oil (OO), 20% safflower oil (SO), 20% evening primrose oil (EPO) or 20% menhaden (fish) oil (MO). The fatty acid composition of the diets was analysed by gas chromatography performed as described elsewhere [18] and is shown in Table I.

3.3. Lymphocyte preparation

Spleens were dissected and gently ground into sterile phosphate-buffered saline (PBS). The suspension was filtered through lens tissue and lymphocytes were collected by centrifugation ($1000 \times g$, 10 min), resuspended in sterile PBS and purified by centrifugation on Lymphoprep ($1500 \times g$, 20 min). Lymphocytes were washed once in sterile PBS and resuspended in HEPES-buffered RPMI.

3.4. Measurement of natural killer cell activity

3.4.1. Preparation of effector cells

Spleen lymphocytes were prepared as described above. To assay the NK activity of freshly prepared cells they were resuspended at a concentration of 2×10^7 cells/ml in HEPES-buffered RPMI supplemented with 2 mM glutamine and antibiotics. To assay the NK activity of IFNα-stimulated cells, spleen lymphocytes were cultured for 3 h, at a density of 5×10^6 cells/ml, at 37°C in an air/CO_2 (19:1) atmosphere in HEPES-buffered RPMI supplemented with 2 mM glutamine, 2.5% (v/v) autologous serum, 1000 U/ml human recombinant IFNα and antibiotics. After incubation, cells were collected by centrifugation, washed 3 times with RPMI and resuspended, at a concentration of 2×10^7 cells/ml, in HEPES-buffered RPMI supplemented with 2 mM glutamine and antibiotics.

To prepare LAK cells, spleen lymphocytes were cultured for 24 h, at a density of 5×10^6 cells/ml, at 37°C in an air/CO_2 (19:1) atmosphere in HEPES-buffered RPMI supplemented with 2 mM glutamine, 2.5% (v/v) autologous serum, 5 mg/ml ConA and antibiotics. After the incubation period, cells were collected by centrifugation, washed 3 times with RPMI and then cultured for a further 72 h in HEPES-buffered RPMI supplemented with 2 mM glutamine, 2.5% (v/v) autologous serum, 100 U/ml human recombinant IL-2 and antibiotics. At the end of this incubation period, cells were collected by centrifugation, washed 3 times with RPMI and resuspended, at a concentration of 2×10^7 cells/ml, in HEPES-buffered RPMI supplemented with 2 mM glutamine and antibiotics.

3.4.2. Preparation of target cells

NK activity was assessed using the YAC-1 murine lymphoma cell line as the target cell in a ^{51}Cr release assay. YAC-1 cells were maintained at a density of 10^5 cells/ml in HEPES-buffered RPMI supplemented with 10% (v/v) fetal calf serum (FCS), 2 mM glutamine and antibiotics. Cells were collected by centrifugation; 2×10^6 cells were resuspended in 100 ml of ^{51}Cr (4 μCi) dissolved in isotonic NaCl and incubated at 37°C for 1 h. The YAC-1 cells were then washed twice with 10% (v/v) FCS in PBS and incubated at 37°C for 30 min in 1 ml of HEPES-buffered RPMI supplemented with 10% (v/v) FCS, 2 mM glutamine and antibiotics; this incubation ensures that spontaneous release of ^{51}Cr during the assay is minimal. The YAC-1 cells were finally collected by centrifugation and resuspended, at a concentration of 2×10^5 cells/ml, in HEPES-buffered RPMI supplemented with 10% (v/v) FCS, 2 mM glutamine and antibiotics.

3.4.3. The NK assay

Effector cells and ^{51}Cr-loaded YAC-1 cells (2×10^5 cells/ml) were added, in triplicate, to each well of a 96-well flat-bottomed microtitre plate at effector/target cell ratios of 100:1, 50:1, 25:1 and 12.5:1 (final volume: 200 μl/well). Maximal ^{51}Cr release was determined by incubating YAC-1 cells with 2.5% (v/v) Triton X-100 in RPMI, instead of the effector cells. Spontaneous ^{51}Cr release was determined by incubating YAC-1 cells with glutamine- and antibiotic-supplemented RPMI in place of the effector cells. Plates were centrifuged at 300 rpm for 3 min to bring effector and target cells into contact and were then incubated at 37°C in an air/CO_2 (19:1) atmosphere for 4 h. After incubation, the plates were centrifuged at 500 rpm for 5 min. The amount of ^{51}Cr released was determined by placing 100 μl of medium in a gamma-counter. Percentage cytolysis was calculated as

$$\% \text{ Cytolysis} = \frac{\text{Experimental cpm} - \text{Spontaneous cpm}}{\text{Maximum cpm} - \text{Spontaneous cpm}} \times 100$$

Total release of ^{51}Cr was usually approximately 40 000 cpm/2×10^5 YAC-1 cells; spontaneous release of ^{51}Cr was always approximately 10% of the total release.

3.5. Data presentation

Data are mean ± SEM of cell preparations from 4 animals fed each of the diets, prepared on 4 separate

days; measurements were made in triplicate on each of the 4 days. Statistical significance was determined using the Student's t test with Dunnet's tables for multiple comparisons [19].

4. Results

4.1. Food intake, energy intake and body weight gain

Food intake of the LF-fed animals (1.55 ± 0.03 kg/10 weeks) was significantly greater than that of the animals fed on the HF diets (1.18 ± 0.01 kg/10 weeks). Total energy intake was the same for animals fed on the LF diet (5425 ± 105 cal/10 weeks) and for those fed on the HF diets (5390 ± 85 cal/10 weeks) [20]. Animals fed the different diets had identical rates of weight gain up to 5 weeks; after this period of rapid growth, the increase in weight was much slower in all groups, but particularly in the LF-fed animals. The LF-fed rats had a lower total weight gain and smaller final weights than those fed on the HF diets [20]. Animals fed on the MO diet had a greater weight gain than those fed on the OO or EPO diets and their final weights were greater [20].

4.2. The effect of dietary lipid manipulation on NK cell activity

The absence of NK cells from the thymus and lymph nodes was confirmed using the ^{51}Cr release assay (data not shown). The effect of dietary lipid manipulation on the NK activity of freshly prepared spleen lymphocytes at various effector/target cell ratios is illustrated by Fig. 1 and the results from 4 separate cell preparations are shown in Table II. At all ratios of lymphocyte/target cells, all of the HF diets suppressed NK activity compared with the LF diet; statistical significance was observed for spleen lymphocytes from rats fed the OO, EPO or MO diets at all lymphocyte/target cell ratios (Table II). Cells from the MO-fed rats exhibited the lowest NK activity. This was lower than the activity of cells from rats fed the HCO, SO and EPO diets, although not all of these differences were statistically significant at the lowest lymphocyte/target cell ratios (Table II).

4.3. The effect of dietary lipid manipulation on IFNα-stimulated NK cell acivity

Culture with IFNα for 3 h increased the NK activity of spleen lymphocytes from all animals, except those fed the OO diet (Table III). At a lymphocyte/target cell ratio of 100:1 this increase in NK activity was

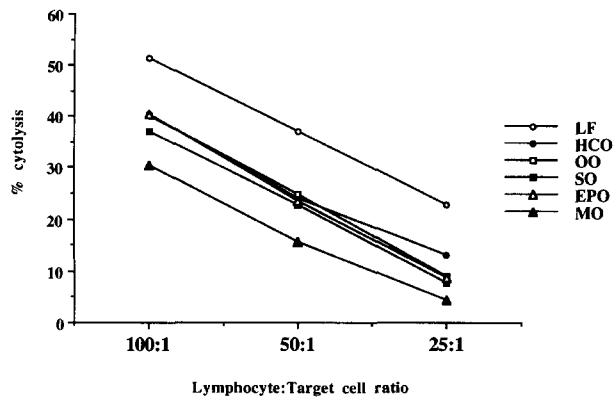

Fig. 1. The effect of dietary lipid manipulation on the NK activity of freshly prepared lymphocytes. Lymphocytes were prepared as described in Materials and Methods and resuspended in HEPES-buffered RPMI supplemented with glutamine and antibiotics. YAC-1 (target) cells were incubated with ^{51}Cr for 1 h at 37°C, washed and reincubated for 30 min and then resuspended in HEPES-buffered RPMI supplemented with FCS, glutamine and antibiotics. Effector and target cells were incubated together at various ratios at 37°C in 96-well plates for 4 h; the amount of ^{51}Cr released during the incubation was determined by counting an aliquot of the medium in a gamma-counter. Results are expressed as percent cytolysis. Data are for 1 representative cell preparation from each dietary group.

statistically significant for the cells from rats fed the HCO, SO, EPO and MO diets. At this ratio of lymphocyte/target cells, the increase in NK activity varied from 20% (LF) to 50% (MO). Although feeding the

TABLE 2
THE EFFECT OF DIETARY LIPID MANIPULATION ON NK ACTIVITY IN FRESHLY PREPARED SPLEEN LYMPHOCYTES

Lymphocytes were prepared as described in Materials and Methods and resuspended in HEPES-buffered RPMI supplemented with glutamine and antibiotics. YAC-1 (target) cells were incubated with ^{51}Cr for 1 h at 37°C, washed and reincubated for 30 min and then resuspended in HEPES-buffered RPMI supplemented with FCS, glutamine and antibiotics. Effector and target cells were incubated together at various ratios at 37°C in 96-well plates for 4 h; the amount of ^{51}Cr released during the incubation was determined by counting an aliquot of the medium in a gamma-counter. Results are expressed as percent cytolysis. Data are mean ± SEM of cell preparations from 4 animals fed each of the diets, prepared on 4 separate days; measurements were made in triplicate on each of the 4 days.

Diet	% Cytolysis (L/T ratio)			
	100:1	50:1	25:1	12.5:1
LF	46.5 ± 2.0^{cef}	29.7 ± 2.8^{cef}	17.7 ± 2.1^{bcdef}	7.4 ± 1.2^{cef}
HCO	40.4 ± 2.4^{f}	22.9 ± 1.5^{f}	11.5 ± 0.6^{afe}	4.5 ± 0.8
OO	36.4 ± 2.8^{a}	20.8 ± 1.7^{af}	9.3 ± 0.9^{af}	3.4 ± 0.4^{a}
SO	42.1 ± 1.5^{f}	24.2 ± 1.3^{f}	11.2 ± 1.1^{af}	3.9 ± 0.7
EPO	40.7 ± 0.7^{af}	19.1 ± 2.6^{a}	8.9 ± 0.2^{abf}	3.2 ± 0.4^{a}
MO	29.4 ± 2.1^{abde}	13.6 ± 0.7^{abcd}	5.0 ± 0.3^{abcde}	3.1 ± 0.8^{a}

Statistical significance (Student's t test with Dunnet's tables for multiple comparisons) for $P < 0.05$ at least is indicated as follows: [a] vs. LF, [b] vs. HCO, [c] vs. OO, [d] vs. SO, [e] vs. EPO, [f] vs. MO.

OO, EPO or MO diets resulted in lower IFNα-stimulated NK activity than that obtained by feeding the LF diet, the only consistent significant difference was the low activity of the cells from the OO-fed rats compared with the LF-fed (and in some cases the HCO-fed) rats (Table III).

4.4. The effect of dietary lipid manipulation on LAK cell activity

LAK cells were generated by culture of spleen lymphocytes with ConA for 24 h and then IL-2 for 72 h. LAK cell activity was much lower than the NK activity of lymphocytes from the same animals (Table IV). This may mean that YAC-1 cells are not good targets for measuring LAK activity or it may simply be that only a small proportion of lymphocytes are transformed to LAK cells during culture with IL-2. All of the HF diets decreased LAK cell activity. The greatest effects were observed with LAK cells derived from animals fed the OO, SO and MO diets, although statistically significant effects were observed only for lymphocytes from rats fed the MO diet compared with those from rats fed the LF diet (Table IV).

TABLE 3
THE EFFECT OF DIETARY LIPID MANIPULATION ON NK ACTIVITY IN IFNα-STIMULATED SPLEEN LYMPHOCYTES

Lymphocytes were prepared as described in Materials and Methods and cultured for 3 h in HEPES-buffered RPMI supplemented with IFNα, glutamine, autologous serum, ConA and antibiotics. Cells were washed and resuspended in HEPES-buffered RPMI supplemented with glutamine and antibiotics. YAC-1 (target) cells were incubated with ^{51}Cr for 1 h at 37°C, washed and reincubated for 30 min and then resuspended in HEPES-buffered RPMI supplemented with FCS, glutamine and antibiotics. Effector and target cells were incubated together at various ratios at 37°C in 96-well plates for 4 h; the amount of ^{51}Cr released during the incubation was determined by counting an aliquot of the medium in a gamma-counter. Results are expressed as percent cytolysis. Data are mean ± SEM of cell preparations from 4 animals fed each of the diets, prepared on 4 separate days; measurements were made in triplicate on each of the 4 days.

Diet	% Cytolysis (L/T ratio)			
	100:1	50:1	25:1	12.5:1
LF	55.4 ± 3.8 [c]	37.7 ± 3.1 [cf]	16.1 ± 1.1 [c]	7.9 ± 1.4
HCO	55.6 ± 3.8 [c]	38.0 ± 1.7 [cf]	15.4 ± 1.7	8.8 ± 1.6
OO	32.7 ± 2.9 [abde]	24.5 ± 3.1 [ab]	12.5 ± 0.7 [a]	5.8 ± 1.1
SO	51.7 ± 3.9 [c]	32.3 ± 1.3	13.9 ± 1.2	7.1 ± 0.7
EPO	50.8 ± 3.6 [c]	28.2 ± 2.2	12.2 ± 1.6	4.9 ± 1.1
MO	44.3 ± 5.5	27.1 ± 1.4 [ab]	11.1 ± 1.7	5.0 ± 1.4

Statistical significance (Student's t test with Dunnet's tables for multiple comparisons) for $P < 0.05$ at least is indicated as follows: [a] vs. LF, [b] vs. HCO, [c] vs. OO, [d] vs. SO, [e] vs. EPO, [f] vs. MO.

TABLE 4
THE EFFECT OF DIETARY LIPID MANIPULATION ON THE LAK ACTIVITY OF SPLEEN LYMPHOCYTES

Lymphocytes were prepared as described in Materials and Methods and cultured for 24 h in HEPES-buffered RPMI supplemented with glutamine, autologous serum, ConA and antibiotics and then for 72 h in HEPES-buffered RPMI supplemented with glutamine, autologous serum, IL-2 and antibiotics. Cells were washed and resuspended in HEPES-buffered RPMI supplemented with glutamine and antibiotics. YAC-1 (target) cells were incubated with ^{51}Cr for 1 h at 37°C, washed and reincubated for 30 min and then resuspended in HEPES-buffered RPMI supplemented with FCS, glutamine and antibiotics. Effector and target cells were incubated together at various ratios at 37°C in 96-well plates for 4 h; the amount of ^{51}Cr released during the incubation was determined by counting an aliquot of the medium in a gamma-counter. Results are expressed as percent cytolysis. Data are mean ± SEM of cell preparations from 4 animals fed each of the diets, prepared on 4 separate days; measurements were made in triplicate on each of the 4 days.

Diet	% Cytolysis (L/T ratio)			
	100:1	50:1	25:1	12.5:1
LF	18.0 ± 1.2 [cd]	13.6 ± 3.3 [d]	6.8 ± 2.7	2.5 ± 0.9
HCO	13.2 ± 3.6 [d]	10.7 ± 3.8	5.7 ± 2.9	1.8 ± 0.9
OO	10.9 ± 4.1	6.7 ± 3.9	3.2 ± 2.8	1.5 ± 1.3
SO	5.5 ± 4.1 [a]	4.1 ± 3.5	2.4 ± 2.1	1.1 ± 0.9
EPO	13.1 ± 5.7	13.9 ± 7.4	10.9 ± 5.4	5.6 ± 3.3
MO	1.7 ± 1.0 [ab]	2.0 ± 1.7 [a]	1.7 ± 1.4	0.8 ± 0.6

Statistical significance (Student's t test with Dunnet's tables for multiple comparisons) for $P < 0.05$ at least is indicated as follows: [a] vs. LF, [b] vs. HCO, [c] vs. SO, [d] vs. MO.

5. Discussion

This study investigated, for the first time, the effect of HF diets, rich in particular types of fatty acids, on NK cell activity; in addition, a LF diet was included in the study. Dietary lipid manipulation had profound effects on the NK activity of rat spleen lymphocytes; the most consistent effects were seen with cells from animals fed the OO, EPO and MO diets. These diets resulted in significant inhibition of NK activity in freshly prepared lymphocytes; the MO diet was particularly inhibitory (Table II). In confirmation of previous studies with mice [13–15], it was shown that fish oil suppresses NK cell activity compared with oil rich in linoleic acid (corn or safflower oil).

NK cells can be activated to increase their ability to lyse target cells by treatment with IFN; each of the 3 types (α, β, γ) of IFN can be employed, although the most commonly used is IFNα [1,21,22]. The HF diets resulted in lower NK activity of IFNα-stimulated spleen lymphocytes than the LF diet, although this was statistically significant only for lymphocytes from rats fed the OO diet (Table III).

LAK cell activity was also affected by dietary lipid manipulation; the effect of dietary lipids on this activity

has not previously been investigated. At high ratios of lymphocyte/target cells, each of the HF diets resulted in lower LAK cell activity than the LF diet (Table IV); the greatest inhibition of LAK activity was observed with cells from rats fed the MO diet. The overall LAK cell activity was rather low; in all cases this activity was lower than that of NK cell activity in freshly prepared, unstimulated lymphocytes (Tables II and IV). This suggests that the YAC-1 cell line may not be a good target for the measurement of LAK cell activity.

Thus, feeding animals certain dietary lipids results in significant inhibition of NK and LAK cell activity. The potent inhibitory effect of the fish oil-containing diet on NK activity has been suggested by the in vitro study of Yamashita et al. [12], but the inhibitory effect of olive oil is surprising. The mechanism by which dietary lipids containing different types of fatty acids exert differential effects on NK cell activity is not known. One possibility is that the effects may be mediated through the production of eicosanoids, for which arachidonic and eicosapentaenoic acids are precursors. Indeed, NK activity appears to be under negative control by PGE_2, for which arachidonic acid is a precursor. Roder and Klein [23] have shown that PGE_2 inhibits, while indomethacin (a cyclooxygenase inhibitor) enhances, murine NK activity against YAC-1 target cells. The inhibition was entirely at the level of the NK cells and not the target cells. In contrast, leukotriene B_4, a 5-lipoxygenase product of arachidonic acid metabolism, has been shown to augment human NK activity [24]. However, although Yamashita et al. [12] have demonstrated that lipoxygenase inhibitors can also inhibit NK activity, the effect of the inhibition could not be reversed by adding back exogenous leukotrienes. Studies investigating the effects of eicosanoids on NK activity have so far been restricted to the use of those of the 2 series prostaglandins and 4 series leukotrienes, i.e., those derived from arachidonic acid. While these eicosanoids will have been derived from the linoleic acid present in corn oil, safflower oil and evening primrose oil, the fish oil diet had a greater effect on NK activity than these oils in this study (Tables II–IV) and in other studies [13–15]. This may be due to the formation of eicosanoids derived from eicosapentaenoic acid, but there has been no investigation of the effects of the 3 and 5 series eicosanoids on NK activity. Of equal interest is the fact that the EPO diet, which contains a similar proportion of linoleic acid to the SO diet (and an almost identical overall fatty acid composition), had profound effects on NK activity (Tables II and III), which suggests that the small amount of γ-linolenic acid present in this diet (Table I) is very potent in its effects on this activity.

Since NK cells play a major role in the rejection of grafts following transplantation surgery [17], it is possible that the inhibition of NK cell activity by dietary PUFA may be useful in prolonging graft survival. Several studies have shown beneficial effects of *n*-6 or *n*-3 fatty acids on transplant survival in both animals [24–26] and in man [27]. It could be speculated that these effects may be exerted through NK cells, although the experiments carried out in the present study are xenogeneic and may not be directly applicable to allogeneic transplant models.

While maintenance of graft survival represents a potential positive use of the suppression of NK cell activity by dietary oils, a possible negative aspect may be diminished host response to microbes, viruses and tumor cells. This is suggested by a study in which the mortality rate in mice fed fish oil (20% by weight) for 4 weeks was higher after a peroral challenge with *Salmonella typhimurium* compared with those fed diets containing 20% corn oil or hydrogenated coconut oil or fed a LF chow diet [28]. The spleens from the mice fed the fish oil diet also presented the highest number of bacteria 7 days after intraperitoneal infection with this bacterial strain [28]. Although the authors of this study could not define the cause for these observations, it is not unreasonable to speculate that they may have been at least partially brought about by impaired NK cell activity.

There is no doubt that some dietary fatty acids have profound effects on NK cell activity in the rat. The mechanism for these effects is as yet unknown, but may involve the synthesis of eicosanoids. The clinical implications of the observations reported in the present study are still speculative and warrant further investigation.

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