Effects of dietary supplementation on autoimmunity in the MRL/lpr mouse: a preliminary investigation

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SUMMARY The effects of dietary fatty acid supplementation on various disease parameters in the spontaneously autoimmune MRL-mp-lpr/lpr mouse model of systemic lupus erythematosus before onset of disease were investigated. A fat deficient diet was supplemented with the following oils: olive oil, sunflower oil, evening primrose oil (EPO), fish oil, and a fish oil/EPO mixture. The mice receiving a diet enriched with EPO showed an increase in survival, as did those receiving the fish oil/EPO mixture. These results, taken together with those of the other parameters monitored, suggest that EPO may be of benefit in alleviating the murine form of the disease.

Key words: murine systemic lupus erythematosus, fatty acid.

Mice of the MRL-mp-lpr/lpr (MRL) strain develop a T cell lymphoproliferative syndrome associated with the autoimmune disease of systemic lupus erythematosus (SLE).^{1,2} This spontaneous disease is characterised by massive lymph node enlargement, hypergammaglobulinaemia, and immune complex mediated glomerulonephritis, together with the production of multiple autoantibodies, including anti-DNA, antiglycoprotein 70, and rheumatoid factors.^{1,2} In addition, 15–25% of the MRL mice also display features analogous to those of human rheumatoid arthritis.^{1,2}

The results of a number of recent studies have suggested that controlled dietary intake in such animals may result in an improved prognosis as found in fish oil treatment of NZB/W mice³ and may indeed markedly increase their survival rate. For example, the implementation of protein calorie restriction on the autoimmune disease of the (NZB/NZW)F1 mouse strain facilitated the survival of these animals for up to twice their normal life span.^{4,5}

Manipulation of the polyunsaturated fatty acid

content of the diet is known to alter the composition of the cell membranes⁶ and raises the possibility of influencing the normal fatty acid derived inflammatory mediators. Thus it may be possible by dietary supplementation to reduce the levels of the very pro-inflammatory mediators such as leucotrienes of the four series by the much less active compounds of the five series^{7,8} and thereby markedly improve the prognosis (and possibly life span) for animals with SLE.

The present study was designed to investigate the effects of diet rich in polyunsaturated fatty acid in the MRL/lpr mouse by considering the survival rates, proteinuria, body weights, anti-double stranded deoxyribonucleic acid (anti-dsDNA) antibody, and heterophile rheumatoid factor levels together with the appearance of overt clinical manifestations of the disease.

Materials and methods

ANIMALS

MRL-mp-lpr/lpr mice were bred and maintained in the animal unit of the Department of Bioscience and Biotechnology at the University of Strathclyde. The mice were given dietary supplementation from the age of two months and were housed in groups of five

matched by age and sex, under standard conditions of light, heat, and humidity with food and water provided ad libitum.

Animals were monitored weekly for survival and fortnightly for proteinuria and autoantibodies and for the appearance of clinical manifestations of the autoimmune condition.

DIET

The diet used was a specially prepared powder (SDS; Special Diet Services Ltd, Witham, Essex) containing 0.5% (w/w) fat, 0.025% of which consisted of polyunsaturated fatty acids (PUFA). This diet, without additives, was sufficient to maintain the health of the mice (personal communication). Test oils, rich in specific, unsaturated fatty acids (Table 1), were added to the diet (5% w/w) together with butylated hydroxytoluene (0.054% w/w), which prevented the oxidation of the fatty acids.⁹

The experimental groups were designated as follows: (a) controls: fed with No 3 rat and mouse maintenance diet (SDS); (b) olive oil; (c) sunflower oil; (d) evening primrose oil (EPO); (e) fish oil; and (f) fish oil/EPO (4:1 ratio).

The diet thus prepared was supplied to the animals daily and the surplus stored at -20°C for up to seven days. The PUFAs in the diet have been shown, by gas-liquid chromatography, not to deteriorate under these conditions (unpublished data).

MEASUREMENTS

The weights of the mice were noted and the amount of protein excreted in the urine measured with Albus-tix (Amex Miles Labs). The detection of trace amounts of urinary protein from 0.03 (+) to over 20 (+++) mg protein/ml urine was possible by this

method. The value was considered to be an index of the severity of the immune complex mediated glomerulonephritis developed by these mice.

AUTOANTIBODIES

Plasma was collected from the tail vein into heparinised capillary tubes by removal of 1 mm thick tail sections under diethyl ether anaesthesia. After centrifugation the plasma was diluted (1/50) in 0.15 M saline solution and stored at -20°C until required.

Enzyme linked immunosorbent assay techniques, as described in full previously,¹⁰ were used to monitor plasma levels of anti-double stranded DNA antibody and heterophile rheumatoid factor.

CLINICAL SYMPTOMS

The mice were examined weekly and the appearance of overt clinical features noted. These included enlarged lymph nodes, vasculitis as evidenced by open sores swollen hind limbs, exophthalmia, and necrotic ears.

STATISTICAL ANALYSES

The survival data proved to be the only set of data suitable for statistical analysis as the numbers of mice in each group were small (i.e., less than or equal to five). The log rank test of Peto *et al*¹¹ was found to be appropriate.

Results and discussion

In this preliminary study the dietary regimens which appeared to impart most benefit were the fish oil and EPO supplemented diets. Statistical analysis showed that the survival of those mice receiving EPO increased significantly ($p<0.05$) compared with controls, while those being given the fish oil/EPO mixture had a similar increase in survival ($p<0.05$). There were no significant male:female differences (Table 2; Figs 1 and 2).

Olive oil acted as a 'negative control' as it contained up to 8% of essential fatty acids capable of being metabolised to arachidonic acid, whereas sunflower oil performed as a positive control as it contained 80% linoleic acid. It was expected that the inflammatory mediators produced would be maximal from the sunflower oil.

Evening primrose oil (EPO) consisted of 70% α -linoleic acid and 9% γ -linolenic acid and may contribute towards the production of prostaglandin E_1 . This has been reported to be produced in abnormally small amounts in patients with rheumatoid arthritis (personal communication), though the overall efficacy of EPO in such patients remains unresolved,^{12 13} despite its beneficial effects in the

Table 1 Fatty acid composition of oils

Fatty acid*	Trivial names	Weight (%)			
		Sunflower	Olive	EPO	Fish
14:0	Myristic	—	—	—	14
16:0	Palmitic	—	9-16	6	16
18:0	—	—	—	1	4
18:1	Oleic	7-42	65-85	8-12	16
18:2	Linoleic	55-81	4-12	70-75	1
18:3	γ -Linolenic	—	—	7.5-9	<1
20:4	Arachidonic	—	—	—	—
	Eicosapentaenoic? (EPA)	—	—	—	19
22:6	—	—	—	—	12
18:4 or	—	—	—	—	5
21:0	—	—	—	—	—
16:1	—	—	—	—	5

*Numbers show — No of carbon atoms; No of double bonds.

treatment of atherosclerosis.¹⁴ Fish oil contains a high proportion of eicosapentanoic acid from which leucotrienes (five series) and prostaglandins (three series) are produced.

The diets used were, as far as possible, isocaloric at 35–40 kJ ingested a day by each animal, while that eaten by control animals (i.e., those on No 3 rat and mouse maintenance diet (SDS)) was approximately

Table 2 Analysis of efficacy of dietary therapy on survival in MRL/lpr mice, initiated at 2 months of age, by the log rank test

Group comparisons	Olive	Sunflower	EPO	Fish	Fish/EPO	Control
Female mice ΣO/E* (χ ² =9.53; p<0.025; (d=5))*	1.72	0.927	0.703	1.617	0.807	2.111
Male mice ΣO/E (χ ² =16.46;p<0.01;(d=5))	0.726	1.111	0.736	0.219	1.451	4.23
Pairwise comparisons between experimental groups and control						
χ ²	3.66	3.58	9.50	2.84	4.71	
	p<0.05	p<0.1	p<0.05	p<0.1	p<0.05	
Comparison of male and female mice						
ΣO/E*	Male mice 1.254		Female mice 0.7996		χ ² 2.39	
	(d=1); not significant at p<0.1)					

*ΣO/E=summation of observed/expected values; d=degrees of freedom; χ²=significance obtained from table of values.

Fig. 1 Survival of MRL/lpr mice (aged 2 months).

65 kJ/day. Kubo *et al* reported that a decreased calorie intake prolonged life and delayed the onset of glomerulonephritis in the NZB/W mouse and that the influence of the restricted energy intake was greater than any effect of diet.¹⁵ This finding has

important ramifications in the context of this study as all of the diets employed were of a lower caloric value than the diet of the control animals. Hence an improvement in all groups would be expected if the observations of Kubo *et al* held for the MRL/lpr

Fig. 2 Survival rates of MRL/lpr mice (aged 2 months) continued.

Table 3 Heterophile rheumatoid factor monitored in MRL/lpr mice*

	Age (months)					
	2	4	6	8	10	12
Female mice						
Olive oil	25.0±2.53 (5)†	9.6±3.09 (5)	2.4±1.47 (4)	11.0 (3)	0.9 (2)	Dead
Sunflower oil	13.9±2.77 (5)	5.2±0.61 (5)	0.9±0.02 (5)	3.5±0.92 (3)	0.9 (2)	0.9 (1)
EPO	12.5±2.25 (5)	6.8±1.59 (5)	11.6±8.35 (5)	0.9±3.60 (5)	11.6±8.40 (3)	Dead
Fish oil	18.2±2.80 (5)	11.3±0.99 (5)	3.0±1.11 (5)	NS‡	NS	Dead
Fish/EPO	10.2±1.14 (5)	6.2±0.40 (5)	0.9 (5)	3.0±1.61 (5)	0.9 (2)	Dead
Control	8.6±1.10 (5)	16.7±4.70 (5)	13.0 (3)	NS	0.9 (1)	0.9 (1)
Male mice						
Olive oil	5.5±1.65 (5)	8.5±2.40 (5)	2.4±1.47 (5)	5.4±1.06 (5)	1.0±0.01 (3)	0.9 (2)
Sunflower oil	9.3±1.50 (5)	5.2±0.61 (5)	0.9±0.02 (5)	10.6±6.86 (4)	2.9±2.00 (4)	1.0 (2)
EPO	6.8±1.22 (5)	6.8±1.59 (5)	11.6±8.35 (5)	11.5±9.74 (5)	9.2±7.30 (3)	14.3 (2)
Fish oil	5.5±0.55 (5)	11.3±0.99 (5)	3.0±1.11 (4)	15.6±7.20 (4)	13.6±4.90 (4)	Dead
Fish/EPO	10.2±1.14 (5)	6.2±0.40 (5)	0.9±0.01 (5)	3.0±1.61 (4)	3.7±6.37 (3)	3.0 (2)
Control	10.2±1.22 (5)	16.7±4.70 (5)	18.0±0.01 (2)	Dead	—	—

*Values are expressed as $\bar{x} \pm \text{SEM}$.

†No of mice per group.

‡NS=no sample obtained.

Table 4 Anti-dsDNA antibody units monitored monthly in MRL/lpr mice*

		Age months					
		2	4	6	8	10	12
<i>Female mice</i>							
Olive oil	NS†		9.8±1.73 (5)‡	5.1±2.08 (4)	5.7±5.63 (3)	4.1 (2)	Dead
Sunflower oil	NS		4.2±0.33 (5)	4.8±2.18 (5)	6.4±1.73 (3)	11.1 (2)	<1 (1)
EPO	NS		2.4±1.49 (5)	3.9±0.99 (5)	0.9±0.01 (5)	2.3 (3)	2 (1)
Fish oil	NS		14.7±3.80 (5)	14.5±4.08 (5)	NS	2.5 (1)	Dead
Fish/EPO	NS		21.1±4.21 (5)	19.9±2.98 (5)	7.7±4.25 (5)	4.6±2.24 (3)	1.13 (2)
Control		6.2±2.68 (5)	0.9±0.01 (5)	19.0 (3)	NS	<1 (1)	NS
<i>Male mice</i>							
Olive oil	NS		3.33±1.04 (5)	6.84±5.10 (5)	4.56±2.19 (5)	1.40±0.30 (3)	1.35 (1)
Sunflower oil	NS		8.16±1.67 (5)	1.48±0.30 (4)	2.83±1.52 (4)	8.35±5.33 (4)	0.90 (2)
EPO	NS		4.98±3.75 (5)	3.98±0.84 (5)	0.96±2.54 (4)	3.18±1.04 (3)	0.90 (2)
Fish oil	NS		19.35±2.60 (5)	0.95±0.03 (4)	6.18±2.54 (4)	6.08±0.76 (4)	Dead
Fish/EPO	NS		2.78±0.80 (5)	3.20±0.84 (4)	5.30±1.13 (4)	9.90±4.13 (3)	15.15 (1)
Control		8.0±1.74 (5)	0.90±0.01 (5)	2.9 (2)	Dead	—	—

*Values are expressed as $\bar{x}$ ±SEM.

†NS=no sample obtained.

‡No of mice per group.

mouse.¹⁵ This was not the case as the 2 month old mice showed differential effects between groups and also the animals used by Kubo *et al*¹⁵ were of weaning age at the start of their study.

The levels of proteinuria found in all groups were variable and inconsistent with the progressively extensive renal damage that occurs in the disease, and group studies of large number may be necessary to elucidate the 'true' effects.

The variations in autoantibody titres (Tables 3 and 4) throughout the course of the investigation could be as a result of the hypergammaglobulinaemia that these animals develop.¹ It has been suggested that the monitoring of such antibody levels may be complicated by this extensive cross reactivity and low affinity and may explain the fluctuations found.¹⁶⁻¹⁸ Other workers have found that calorie restriction did not affect the levels of circulating immune complexes or the levels of anti-DNA antibodies in the MRL/lpr mouse,¹⁹ whereas both variables were found to be decreased in the NZB/W mice.¹⁵ This result correlates with our present results of consistently low levels of anti-dsDNA antibody in the control group. Fish oil supplements did appear to lower the levels of anti-dsDNA antibody present in the female mice.

In both male and female mice surviving at the age of 9 months it was found that those groups on diets supplemented with the fish oil or fish oil/EPO had the fewest signs of overt clinical features. Corroboration with histopathological parameters is currently being undertaken in an enlarged version of this study.

The overall conclusions drawn, considering all the parameters monitored, is that the diets enriched with EPO may be suitable for the administration to both male and female mice. Additionally, it appears that the fish oil/EPO mixture is at least as effective as EPO alone in improving the prognosis for male MRL/lpr mice.

The indications are that the survival data and their statistical analysis taken in tandem with the autoantibody results, proteinuria, and clinical features suggest that EPO treatment may be beneficial. Further dietary studies are under way to confirm the preliminary findings of this investigation, and it is apparent that precise metabolic studies into the mechanisms of action of the individual components of EPO are necessary in order to understand its role, both in healthy and diseased states.

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