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Ribonucleic acid efflux from isolated mouse liver nuclei is altered by diet and genotypically determined change in nuclear envelope composition

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Differences in immunological abnormalities like autoimmunity, abnormal T cell proliferative disorders and accelerated ageing occur between MRL/Mp-lpr/lpr(lpr/lpr) and MRL/Mp-+/+(+/+) mice as a consequence of one gene. The present study was designed to assess the effect of these differences in genotype and diet on the composition and function of the liver nuclear envelope. Mice of both strains were fed nutritionally adequate diets differing only in fatty acid composition for 4 weeks. Phospholipid fatty acid composition of the liver nuclear envelope was determined and the effect of altering the lipid composition of the nuclear membrane on nucleoside-triphosphatase (NTPase) activity, ribonucleic acid (RNA) efflux and binding of L-triiodothyronine (L-T_3) was determined. Strain of mouse and level of dietary linoleic acid exhibited significant effects on the phospholipid fatty acid composition of the nuclear envelope. Levels of 18:1($n-9$) and 18:2($n-6$) were lower and 20:4($n-6$) content was higher in nuclear envelope phospholipids of lpr/lpr mice compared with mice of the +/+ strain. Mice fed the high linoleic acid diet exhibited higher levels of 18:0, 18:2($n-6$) and 20:4($n-6$) and lower levels of 16:0 and 18:1($n-9$) in liver nuclear envelope phospholipids, compared with mice fed the low linoleic acid diet. These changes in membrane composition were reflected in alteration of NTPase activity and efflux of RNA from isolated mouse liver nuclei. Nucleoside triphosphatase activity and efflux of ribonucleic acid from isolated nuclei were significantly higher in livers of the lpr/lpr strain. NTPase activity and RNA efflux from isolated nuclei were higher in the high linoleic acid fed group compared with the low linoleic acid group. A single class of binding sites for L-T_3 was present in liver nuclear envelopes of these mice and K_d values were not influenced by strain or dietary linoleic acid levels. Nuclear envelopes prepared from +/+ animals exhibited a significantly higher number of binding sites for L-T_3 compared with the lpr/lpr group. These observations indicate that the single gene difference characterizing lpr/lpr mice from +/+ mice results in alterations in the composition and function of the nuclear envelope. This genetic difference also alters the response of this membrane to dietary factors known to modulate characteristics and functions of the nuclear envelope.

Abbreviations: NTPase, nucleoside-triphosphatase, EC 3.6.1.15; T_3, triiodothyronine.

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Introduction

The nuclear envelope is unique among intracellular membranes in that it is a double membrane structure with both membranes intercon-

nected by nuclear pore complexes. The nuclear envelope is also considered to control nucleocytoplasmic exchange of macromolecules in eukaryotic cells [1]. Nucleotide triphosphatase is localized near the nuclear pore complex [2] and provides energy for nucleocytoplasmic translocation of ribonucleic acid. Several ATPases are reported to be lipid-dependent [3,4]. The lipid dependency of nucleoside triphosphatase activity and efflux of RNA from isolated nuclei is controversial [1,5,6]. The nuclear envelope possesses binding sites for steroid and thyroid hormones and may be involved in regulating entry of these hormones into the nucleus, thereby mediating hormone action [7,8]. Binding of L-T_3 to nuclear envelopes is also dependent on the lipid environment in terms of number of binding sites [9]. Thus, it is likely that the structural composition of this membrane is directly associated with control and expression of nuclear activity integral to the cell's function.

Previous work with C57 Black 6 mice indicated that the phospholipid fatty acid profile of the nuclear envelope in liver can be altered by dietary lipid composition, resulting in altered functions of this membrane [9]. Recent work with the lpr/lpr strain of mice, characterized by an abnormal T cell proliferative disorder and accelerated ageing, suggested that genetic background and level of dietary linoleic acid could modulate membrane phospholipid fatty acid profile in T and B cells and thus the function of these cells [10,11]. The present investigation was undertaken to assess whether the single gene difference characteristic of MRL/Mp mice would also influence nuclear envelope phospholipid fatty acid composition and thus the function of the liver nuclear envelope. In this paper we report that a specific gene difference alters the nuclear envelope phospholipid fatty acid profile, nuclear envelope nucleoside triphosphatase activity, RNA efflux from isolated nuclei, and binding of L-triiodothyronine to liver nuclear envelopes of MRL/Mp mice.

Materials and Methods

Animals and diets. To assess the effect of strain of mouse, 6-week-old male lpr/lpr and +/+ mice purchased from Jackson Laboratories were fed nutritionally adequate semipurified diets containing 20% (w/w) fat of high or low linoleic acid content (Table I). The fat in the high linoleic diet was based on soybean oil and provided approx. 22% of calories as $18:2(n-6)$, whereas the fat in the low linoleic acid diet was based on a blend of beef tallow and sunflower oil to provide approx. 2% of calories as $18:2(n-6)$. Mice were fed these diets for a period of 4 weeks and were then killed by cervical dislocation, and the livers were collected on ice. Liver nuclear envelopes were isolated and analysed for phospholipid fatty acid composition, nucleoside triphosphatase activity, binding of L-triiodothyronine and ribonucleic acid efflux from nuclei isolated from mouse liver. Due to the limited availability of lpr/lpr mice, the basic assay conditions for RNA efflux measurements and L-T_3 binding were established using 8-week-old C57 Black 6 mice.

Isolation of nuclear envelopes. Liver nuclei were isolated by modifications of the procedure of Ref.

TABLE I

COMPOSITION OF THE DIETS

Diets used in the present study contained a high linoleic acid diet providing 22% of calories as $18:2(n-6)$ or a low linoleic acid diet containing 2.2% of total calories as $18:2(n-6)$. The fatty acid composition (percent w/w) of the high linoleic acid was 56% $18:2(n-6)$, 5% $18:3(n-3)$, 10% 16:0, 2% 18:0 and 23% 18:1 and the low linoleic acid diet had 5.5% $18:2(n-6)$, 0.5% $18:3(n-3)$, 22% 16:0, 37% 18:0 and 35% 18:1.

Ingredient	g/kg diet
Fat	200
Starch	200
Casein	270
Glucose	212.65
Non-nutritive fiber	50
Vitamin Mix [a]	10
Mineral Mix [b]	50.85
L-Methionine	2.5
Choline	2.75
Inositol	1.25

[a] A.O.A.C. vitamin mix (Tekland Test diets, Madison, WI) provided the following, per kg of complete diet: 20000 IU vitamin A; 2000 IU vitamin D; 100 mg vitamin E; 5 mg menadione; 5 mg thiamin-HCl; 8 mg riboflavin; 40 mg pyridoxine-HCl; 40 mg niacin; 40 mg pantothenic acid; 2000 mg choline; 100 mg *myo*-inositol; 100 mg *p*-aminobenzoic acid; 0.4 mg biotin; 2 mg folic acid and 30 mg vitamin B-12.

[b] Bernhart Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH) was modified to provide 77.8 mg of Mn^{2+} and 0.06 mg Se^{2+}/kg complete diet.

12. Liver nuclear membranes were prepared by extensive digestion of purified nuclei with deoxyribonuclease [13]. After digestion with deoxyribonuclease at room temperature for 20 min, the crude nuclear envelopes were purified by layering on a discontinuous sucrose gradient and centrifuging at $100\,000 \times g$ for 90 min using an SW28 swinging-bucket rotor. The major band of purified nuclear envelopes was removed from the gradient and washed twice in 10 mM Tris-HCl (pH 7.4) at $33\,000 \times g$ for 10 min. Marker enzymes were measured [14] to determine purity of nuclear envelope preparations.

Extraction of lipid and purification of nuclear membrane phospholipids. Lipids were extracted from membranes using 2-ethoxyquin as antioxidant [15] and extracts were dried under nitrogen. Lipid samples were subjected to thin-layer chromatography on high-performance thin-layer plates (LHPK-HPT2C) for separation of phospholipids. The solvent system chloroform/methanol/2-propanol/0.25% (w/v) potassium chloride/triethylamine (30 : 9 : 25 : 6 : 18 v/v) was used [16]. Individual phospholipids were visualized by spraying plates with 1% (w/v) 8-anilinonaphthalene-sulfonic acid and viewed under ultraviolet light. Phospholipid spots were scraped directly into tubes into which hexane was added. Phospholipid fatty acids were methylated with boron trifluoride methanol reagent [17]. Methyl esters were analysed by automated gas-liquid chromatography using a fused silica capillary column [18]. Fatty acids analysed included all saturated and unsaturated fatty acids of 14 to 24 carbons in chain length.

Protein determination. Protein content in the nuclei and nuclear envelopes was determined by the method of Lowry et al. [19].

NTPase assay. NTPase activity was assessed as suggested by Schroder [20]. The reaction mixture to determine NTPase activity was composed of 25 mM Tris-HCl buffer (pH 8.0) containing 2.5 mM $MgCl_2$, 2.5 mM adenosine triphosphate, 130 mM sucrose and 400 μl of nuclear envelope suspension. Incubation was performed at varying temperatures and times at different concentrations of protein. The reaction was arrested with 50% (w/v) trichloroacetic acid and the P_i released was measured [21].

Binding of L-T_3 to nuclear envelopes. The L-T_3 binding assay has been described [22]. Nuclear envelopes were incubated in a final volume of 0.25 ml of 20 mM Tris-HCl (pH 7.4) containing [^{125}I]T_3 (50 000 cpm/tube) for 2 h at room temperature. For Scatchard analyses, tubes contained varying amounts of unlabelled L-T_3. To identical tubes, 10 μM L-T_3 was added to determine nonspecific binding. The reaction was terminated by centrifugation in a microfuge and removal of the supernatant. The pellet was washed twice with 0.25 ml of 20 mM Tris-HCl (pH 7.4). Radioactivity in the pellet was assayed by cutting the tip of the tube containing the pellet and counting in a Beckman LS-5801 after adding 5 ml aquasol.

Preparation of labelled mouse liver nuclei and cytosol for measurement of RNA efflux.

Nuclei: Nuclear RNA was prelabeled in vivo by an intraperitoneal injection of 25 μCi of [^{14}C]orotic acid to mice. Mice were killed by cervical dislocation 1 h after injection. Liver nuclei were prepared by homogenizing the livers in 15 vol. of 2.3 M sucrose/3.3 mM calcium acetate and sedimenting the nuclei at $35\,000 \times g$ at 4 °C for 60 min. The pellet was resuspended in a buffer containing 1.0 M sucrose/1.0 mM calcium acetate, centrifuged at $2000 \times g$ for 5 min and resuspended in the same buffer [23].

Cytosol: For preparation of the cytosol fraction, livers of mice were perfused with 0.25 M sucrose in 50 mM Tris-HCl buffer (pH 7.85)/2.5 mM $MgCl_2$/25 mM KCl, homogenized in the same buffer and centrifuged at $20\,000 \times g$ for 10 min; the supernatant was then centrifuged again at $145\,000 \times g$ for 2 h. The supernatant was dialysed overnight against perfusion buffer without sucrose [23].

Measurement of RNA efflux from isolated nuclei. Labelled liver nuclei obtained from mice were incubated in a cell-free system [24] to measure RNA efflux. The standard system contained 40 mM Tris-HCl buffer (pH 7.85), 20 mM KCl, 2.0 mM $MgCl_2$, 0.55 mM $CaCl_2$, 0.27 mM $MnCl_2$, 4.5 mM NaCl, 4.5 mM spermidine, 1.0 mM dithiothreitol, 2.3 mM Na_2HPO_4, 275 μg/ml yeast RNA (to suppress RNAase activity), 170 mM sucrose, 2.0 mM ATP, 3.3 mM phospho*enol*-

pyruvate, 0.3 mM GTP, 0.41 mg/ml methionine, 9.0 units/ml pyruvate kinase, labelled nuclei (at different concentrations of protein) and cytosol (8 mg protein). Incubations were carried out for the time specified and at temperatures indicated in the results. Tubes containing the incubation mixture were centrifuged at 1 000 × *g* for 10 min at 4 ° C to remove nuclei. The supernatant was precipitated with 10% trichloroacetic acid and the trichloroacetic acid pellets were processed for scintillation counting by solubilizing with 0.5 ml protosol prior to addition of 5 ml of aquasol. Radioactivity was determined by counting the samples in a Beckman LS-5801. Counting efficiency was approx. 95%.

Results

Nuclear envelopes prepared were of high purity. Succinic dehydrogenase activity could not be detected in these preparations, indicating that membrane preparations were free of mitochondrial contamination. Very low levels of acid phosphatase, 5′-nucleotidase and arylesterase were detected in nuclear envelopes, indicating that contamination by lysosomes, plasma membranes and endoplasmic reticulum was also very low. Electron microscopic examination indicated that the nuclear envelope preparations were intact, with inner and outer membranes well preserved.

Effect of strain and level of dietary linoleic acid on the phospholipid fatty acid composition of nuclear envelopes

Strain and level of linoleic acid in the diet have significant effects on the phospholipid fatty acid composition of nuclear envelopes isolated from livers of lpr/lpr and +/+ mice (Table II). Nuclear envelopes prepared from livers of lpr/lpr mice generally had significantly higher levels of $20:4(n-6)$ with the exception of phosphatidylinositol, and lower levels of $18:1(n-9)$ and $18:2(n-6)$ in the phospholipid fractions compared with the +/+ group. Levels of 16 : 0 and 18 : 0 did not differ in nuclear envelopes prepared from the two strains, with the exception of a higher 16 : 0 content in phosphatidylinositol and lysophosphatidylcholine fractions and higher levels of 18 : 0 in lysophosphatidylcholine and sphingomyelin fractions of nuclear envelopes from lpr/lpr mice compared with the +/+ mice. The fatty acid content of the phosphatidylinositol fraction of liver nuclear envelopes was similar for the two strains.

Feeding the high linoleic acid diet significantly increased incorporation of $18:2(n-6)$ (except in lysophosphatidylcholine and sphingomyelin) and $20:4(n-6)$ into phospholipid fractions (except in phosphatidylethanolamine and sphingomyelin) of liver nuclear envelopes and decreased levels of 16 : 0 and $18:1(n-9)$, compared with nuclear envelopes isolated from livers of animals fed the low linoleic acid diet.

Significant interaction between strain of mouse and effect of diet treatment was observed for the fatty acid content of phosphatidylcholine, polyunsaturated fatty acids of phosphatidylethanolamine, saturates and for the $18:2(n-6)$ content of phosphatidylserine and for all fatty acids except $18:2(n-6)$ in the lysophosphatidylcholine and sphingomyelin fractions of liver nuclear envelopes.

Effect of strain and high or low dietary linoleic acid on the nucleoside triphosphatase activity in mouse liver nuclear envelopes

Nuclear envelope associated NTPase was magnesium-dependent and activity was optimum at 37 ° C in the presence of 2.5 mM magnesium chloride and 50 μg ribonucleic acid. NTPase activity increased with protein up to 30 μg protein and with time up to 60 min. Activity of NTPase was significantly higher in liver nuclear envelopes of lpr/lpr mice compared with +/+ mice. Nuclear envelopes prepared from livers of mice fed the high linoleic acid diet exhibited significantly higher activity of NTPase compared with the group fed low linoleic acid diet (Fig. 1). Apparently, change in the fatty acid composition of nuclear envelopes is related to differences in NTPase activity.

Effect of strain and dietary high or low linoleic acid on ribonucleic acid efflux from isolated nuclei

Efflux of RNA from prelabelled mouse liver nuclei was dependent on concentration of protein, temperature and duration of incubation (Tables III–V). RNA efflux from isolated nuclei increased linearly with increase in protein concentration (Table III). The rate of efflux of RNA was higher

for animals fed the high linoleic acid diet compared with the low linoleic acid fed group. Variations in the in vivo prelabelling time could alter the pulse-labelled RNA populations released in vitro. Labelled tRNA is transported predominantly during the first 30 min and labelled mRNA at 30–120 min. Efflux of RNA from isolated nuclei in reconstituted cell-free systems has been

TABLE II

EFFECT OF STRAIN AND LEVEL OF DIETARY LINOLEIC ACID ON THE PHOSPHOLIPID FATTY ACID COMPOSITION OF MOUSE LIVER NUCLEAR ENVELOPES

Statistical analysis was conducted by analysis of variance with main effects and interactions tested (replicates of four independent determinations). Values are %(w/w) fatty acid. Those without a common superscript are significantly different and represent the mean ± S.D. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. = not significant.

Strain:	lpr/lpr		+/+		Main effects		Two-way interaction
Diet:	high linoleic acid	low linoleic acid	high linoleic acid	low linoleic acid	strain	diet	
Phosphatidylcholine							
16:0	26.9±0.2 ^{b}	26.6:0.5 ^{ab}	26.0±0.7 ^{a}	28.1±0.2 ^{c}	n.s.	**	***
18:0	18.7±0.2 ^{b}	18.8±0.8 ^{b}	20.1±0.5 ^{c}	17.9±0.0 ^{a}	n.s.	**	***
18:1($n-9$)	5.7±0.2 ^{a}	14.2±0.5 ^{b}	5.7±0.3 ^{a}	16.6±0.4 ^{c}	***	***	***
18:2($n-6$)	20.3±0.3 ^{c}	12.4±0.3 ^{a}	21.5±0.2 ^{d}	14.2±0.3 ^{b}	***	***	*
20:4($n-6$)	17.9±0.1 ^{b}	17.6±0.6 ^{b}	18.0±0.3 ^{b}	13.3±0.5 ^{a}	***	***	***
Phosphatidylethanolamine							
16:0	25.2±0.9 ^{a}	30.3±3.0 ^{b}	25.0±3.6 ^{a}	31.7±2.0 ^{b}	n.s.	***	n.s.
18:0	28.9±0.4 ^{b}	23.3±0.0 ^{a}	27.3±4.9 ^{b}	26.1±0.0 ^{ab}	n.s.	*	n.s.
18:1($n-9$)	10.8±1.0 ^{c}	3.5±0.5 ^{b}	2.6±0.9 ^{b}	1.3±0.7 ^{a}	***	***	***
18:2($n-6$)	12.5±1.0 ^{b}	8.3±1.0 ^{a}	17.3±1.9 ^{c}	7.6±3.2 ^{a}	n.s.	***	*
20:4($n-6$)	27.0±5.4	28.7±5.8	29.8±3.6	24.7±5.8	*	n.s.	*
Phosphatidylserine							
16:0	29.1±4.2 ^{b}	13.4±2.5 ^{a}	18.8±6.3 ^{a}	19.3±4.4 ^{a}	n.s.	**	**
18:0	26.2±9.2 ^{ab}	32.0±3.3 ^{b}	31.2±3.9 ^{b}	21.9±1.4 ^{a}	n.s.	n.s.	*
18:1($n-9$)	5.3±1.0 ^{a}	9.7±2.3 ^{ab}	5.4±1.0 ^{a}	13.5±5.0 ^{b}	n.s.	***	n.s.
18:2($n-6$)	5.5±0.2 ^{a}	6.0±1.0 ^{a}	10.3±1.4 ^{b}	4.8±0.2 ^{a}	***	***	***
20:4($n-6$)	30.3±0.4 ^{c}	20.2±0.6 ^{b}	22.0±1.2 ^{b}	10.8±0.6 ^{a}	***	***	n.s.
Phosphatidylinositol							
16:0	7.8±0.1 ^{a}	11.2±2.5 ^{b}	12.9±1.4 ^{b}	16.7±1.0 ^{c}	***	***	n.s.
18:0	43.5±0.6 ^{c}	26.9±1.4 ^{a}	37.4±2.3 ^{b}	29.6±5.8 ^{a}	n.s.	***	***
18:1($n-9$)	3.6±0.3 ^{a}	9.2±4.1 ^{bc}	5.4±1.6 ^{ab}	10.4±3.1 ^{c}	n.s.	**	n.s.
18:2($n-6$)	5.7±0.3 ^{b}	4.5±1.1 ^{ab}	5.8±1.1 ^{b}	4.0±0.8 ^{a}	n.s.	**	n.s.
20:4($n-6$)	33.5±1.2 ^{b}	24.6±1.2 ^{a}	29.6±2.9 ^{ab}	28.0±7.5 ^{ab}	n.s.	*	n.s.
Lysophosphatidylcholine							
16:0	30.6±4.8 ^{b}	14.7±0.8 ^{a}	25.7±2.9 ^{b}	27.0±2.5 ^{b}	*	***	***
18:0	27.2±2.7 ^{a}	31.7±3.8 ^{a}	25.2±5.0 ^{a}	20.0±0.5 ^{b}	**	n.s.	**
18:1($n-9$)	11.8±3.0 ^{b}	8.6±1.3 ^{a}	11.0±1.5 ^{ab}	20.2±0.7 ^{c}	***	**	***
18:2($n-6$)	8.7±1.4	7.6±2.5	11.4±2.1	12.4±4.9	*	n.s.	n.s.
20:4($n-6$)	9.0±0.4 ^{c}	7.0±1.0 ^{b}	8.5±1.2 ^{c}	2.8±0.4 ^{a}	***	***	***
Sphingomyelin							
16:0	31.2±4.1	25.7±2.3	29.7±3.9	28.7±3.1	n.s.	n.s.	n.s.
18:0	33.7±1.6 ^{b}	21.4±1.2 ^{a}	21.9±2.2 ^{a}	20.7±2.4 ^{a}	***	***	***
18:1($n-9$)	7.1±1.9 ^{a}	11.3±2.1 ^{b}	7.8±0.3 ^{a}	17.3±1.1 ^{c}	***	***	**
18:2($n-6$)	8.5±1.3 ^{a}	7.7±2.2 ^{a}	13.4±4.7 ^{b}	13.6±1.5 ^{b}	**	n.s.	n.s.
20:4($n-6$)	4.6±0.9 ^{a}	12.3±2.2 ^{c}	7.3±1.6 ^{b}	3.0±1.2 ^{a}	***	*	***

reported to be dependent upon a 35 000 molecular weight cytosolic protein [24], while work from other groups does not support this view. Distinct cytosolic proteins (M_r 30 000–35 000 and 50 000–58 000) stimulate mRNA efflux largely by stimulating nuclear envelope NTPase in the presence of adenylated messengers [25]. Evidence that nuclear envelope NTPase is stimulated by poly(A)-rich mRNA and inhibited by microtubulin protein further suggests a possible role of NTPase and microtubulin in the regulation of efflux of mRNA [25]. RNA efflux increased with temperature and was similar at 15 and 20 °C, but was markedly higher at 30 °C for animals fed the high linoleic acid diet (Table IV). Efflux of prelabelled nuclear RNA increased with time (Table V). RNA efflux is also significantly influenced by strain as well as level of dietary linoleic acid fed (Fig. 2). Percent of labelled RNA efflux was significantly higher in the lpr/lpr mouse liver nuclei compared with the +/+ group. Animals fed the high linoleic acid diet exhibited a significantly higher level of mRNA efflux compared with the group fed low linoleic acid. Higher activity of nucleoside triphosphatase activity was noted in the groups in which the RNA efflux was high (Figs. 1 and 2).

Effect of strain and diet on binding of L-*triiodothyronine to nuclear envelopes*

Optimal conditions for binding of L-T_3 to

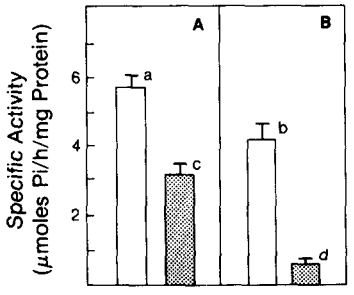

Fig. 1. Effect of strain and level of dietary linoleic acid on the specific activity of NTPase in mouse liver nuclear envelopes. Enzyme activity measured at 37 °C for 15 min using 25 μg nuclear envelope protein. (A) lpr/lpr mice; (B) +/+ mice; □, high linoleic acid diet; ▨, low linoleic acid diet. Statistical significance: main effects: diet: high linoleic acid vs. low linoleic acid group = $P < 0.001$; strain: lpr/lpr vs. +/+ = $P < 0.001$. Values without a common superscript are significantly different at $P < 0.05$.

TABLE III

EFFECT OF PROTEIN CONCENTRATION ON THE RNA EFFLUX FROM PRELABELLED NUCLEI

Mouse liver nuclei prelabelled in vivo for 30 min were isolated and incubated with 10 mg cytosolic protein for 30 min at 30 °C and the labelled nuclear RNA efflux was measured. Values are mean ± S.D. of three determinations. Values without a common superscript are significantly different at $P < 0.05$.

Concentration of nuclear protein (μg)	Efflux of labelled RNA (% nuclear cpm)	
	high linoleic acid diet	low linoleic acid diet
125	0.9 ± 0.18 [a]	0.6 ± 0.01 [a]
250	4.9 ± 0.25 [c]	2.3 ± 0.84 [b]
500	8.2 ± 0.31 [d]	3.0 ± 0.98 [b]

TABLE IV

EFFECT OF INCUBATION TEMPERATURE ON THE RNA EFFLUX FROM PRELABELLED NUCLEI

Mouse liver nuclei prelabelled in vivo for 30 min were isolated and incubated with 10 mg cytosolic protein for 30 min at specified temperatures and the nuclear RNA efflux was measured. Values represent the mean ± S.D. of three determinations. Values without a common superscript are significantly different at $P < 0.05$.

Temperature (°C)	RNA efflux (% nuclear cpm)	
	high linoleic acid diet	low linoleic acid diet
15	2.4 ± 0.74 [a]	2.6 ± 0.41 [a]
24	2.5 ± 0.29 [a]	2.8 ± 0.25 [a]
30	8.2 ± 0.37 [b]	3.0 ± 0.38 [a]

TABLE V

EFFECT OF INCUBATION TIME ON THE RNA EFFLUX FROM PRELABELLED NUCLEI

Lpr/lpr mouse (500 μg protein) liver nuclei prelabelled in vivo for 30 min were isolated and incubated with 7 mg cytosolic protein for specified time at 30 °C and the nuclear RNA efflux was measured. Values are the mean ± S.D. of five determinations. Values without a common superscript are significantly different.

Incubation time (min)	RNA efflux (% nuclear cpm)	
	high linoleic acid diet	low linoleic acid diet
15	6.9 ± 1.9 [a]	7.0 ± 2.1 [a]
30	10.8 ± 3.8 [b]	8.8 ± 1.4 [ab]
45	9.4 ± 1.5 [ab]	9.0 ± 1.1 [ab]

TABLE VI

INFLUENCE OF STRAIN AND LEVEL OF DIETARY LINOLEIC ACID ON THE BINDING OF L-TRIIODOTHYRONINE TO MOUSE LIVER NUCLEAR ENVELOPES

Scatchard analysis was based on the mean ± S.D. of three independent determinations. 5 μg nuclear envelope protein was incubated with 0.5 to 300 nM unlabelled L-T_3 in the presence of 0.07 nM L-[^{125}I]T_3 for 2 h at 22 °C. Specific binding was calculated from the difference in binding in the presence and absence of 10 μM L-T_3. The data were analysed according to Scatchard [36]. * $P < 0.05$; n.s. = not significant. Values without a common superscript are significantly different ($P < 0.05$).

Strain:	lpr/lpr		+/+		Main effects		Two-way interaction
diet:	high linoleic acid	low linoleic acid	high linoleic acid	low linoleic acid	strain	diet	
K_d (nM)	124.0 ± 3.0 [a]	114.0 ± 27.0 [a]	92.3 ± 18.5 [a]	130.0 ± 36.0 [a]	n.s.	n.s.	n.s.
Binding capacity/ (pmol/mg protein)	126.3 ± 14.5 [ab]	81.0 ± 13.0 [a]	160.6 ± 59.0 [ab]	183.0 ± 57.0 [b]	*	n.s.	n.s.
Correlation coefficient [1]	0.84	0.63	0.81	0.81			

[1] Correlation coefficient of bound L-T_3 vs. bound/free L-T_3.

nuclear envelopes were examined. Binding increased linearly for the first 30 min and attained equilibrium after the first 90 min (Fig. 3a). Binding of L-T_3 to mouse liver nuclear envelopes was dependent on protein concentration (Fig. 3b). Binding of L-T_3 was linear up to 10 μg protein and increasing protein concentration above 20 μg did not increase binding. Unlabelled L-T_3 competed for binding of [^{125}I]T_3 to mouse liver nuclear envelopes (Fig. 4).

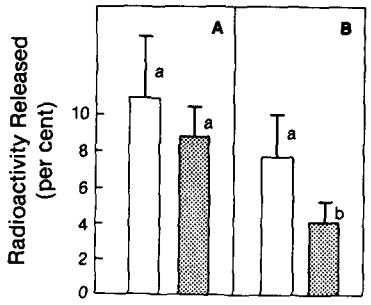

Fig. 2. Effect of strain and level of dietary linoleic acid on the RNA effluxed from prelabelled nuclei. 500 μg prelabelled nuclear protein was incubated with 7 mg cytosolic protein at 30 °C for 30 min. Values are mean of five independent determinations. RNA efflux from prelabelled nuclei was measured by measuring radioactivity in the RNA effluxed and expressed as percent of nuclear RNA effluxed. (A) Lpr/lpr mice; (B) +/+ mice; ▭, high linoleic acid diet; ▨, low linoleic acid diet; Statistical significance: main effects: diet: high vs. low linoleic acid = $P < 0.05$; strain: lpr/lpr vs. +/+ = $P < 0.001$. Values without a common superscript are significantly different at $P < 0.05$.

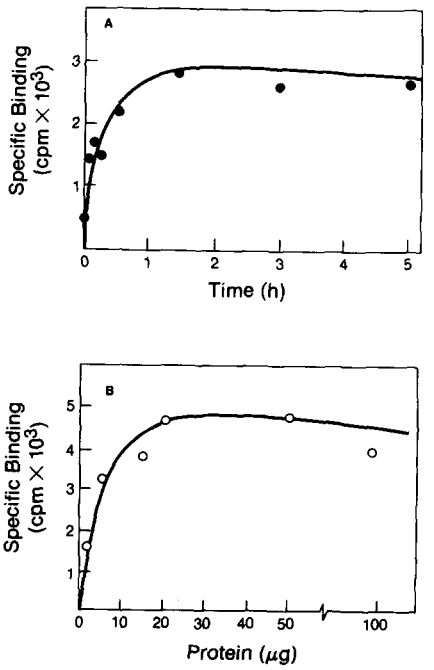

Fig. 3. (A) Time-course of binding L-T_3 to nuclear envelopes. Mouse liver nuclear envelopes (5 μg protein/tube) were incubated with 0.07 nM L-[^{125}I]T_3 and 0.5 nM unlabelled L-T_3 in the presence and absence of 10000 nM L-T_3 at 22 °C. Nonspecific binding was subtracted. Values are replicates of three determinations. (B) Effect of increasing concentration of protein on binding of L-T_3 to nuclear envelopes. Mouse liver nuclear envelope proteins were incubated with 0.07 nM L-[^{125}I]T_3 and 0.5 nM unlabelled L-T_3 in the presence and absence of 10000 nM L-T_3 at 22 °C for 2 h. Nonspecific binding was subtracted. Values shown are the mean of three determinations.

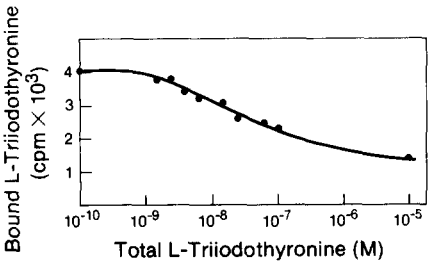

Fig. 4. Competition of [^{125}I]T_3 and unlabelled T_3 for binding to nuclear envelopes. Nuclear envelopes (5 μg protein/tube) were incubated with 0.07 nM L-[^{125}I]T_3 at 22°C for 2 h with 0.1 to 10000 nM unlabelled L-T_3. Nonspecific binding was not subtracted. Replicates of three determinations.

Scatchard analysis of the data indicated a single class of binding sites on mouse liver nuclear envelope for L-T_3 with the affinity site having a K_d of 124 ± 3.0 and 114 ± 27 nM for the lpr/lpr group fed diets of high and low linoleic acid, and 92 ± 19 and 130 ± 36 (nM) for the +/+ mice fed diets of high or low linoleic acid content, respectively (Table VI). Although affinity was not significantly influenced by strain or dietary level of linoleic acid, binding capacity was significantly affected by strain of animal. Nuclear envelopes prepared from +/+ animals exhibited a significantly higher number of binding sites compared to lpr/lpr animals. Analysing the data by Duncan's multiple range test indicated that nuclear envelopes of lpr/lpr mice fed the low linoleic acid diet also exhibited a significantly lower number of binding sites and +/+ mice fed the low linoleic acid diet had a significantly higher number of binding sites compared with the other groups.

Discussion

The present study indicates that genetic background and level of dietary linoleic acid are important determinants of the phospholipid fatty acid composition of mouse liver nuclear envelopes. The lpr/lpr autoimmune mice used in the present study develop immunologic abnormalities such as autoantibodies, immune complexes, lymphoproliferative disorders and accelerated ageing. Our earlier work with lpr/lpr mice suggested that genetic background and dietary linoleic acid could alter membrane fatty acid composition of immunologic cells, thereby altering the immunologic functions of these cells [10,11]. In the present study we have extended these observations by assessing whether or not genetically determined changes in the composition of the nuclear envelope could alter nuclear envelope functions and thus the expression of nuclear activity in the liver.

In this regard, lpr/lpr mice exhibited significantly higher levels of $20:4(n-6)$ and lower levels of $18:1(n-9)$ and $18:2(n-6)$ in phospholipids of liver nuclear envelopes compared to +/+ mice. High dietary linoleic acid levels increased incorporation of $18:2(n-6)$ and $20:4(n-6)$ into phospholipids of liver nuclear envelopes compared with animals fed the low linoleic acid diet. Diet- and strain-induced changes in the phospholipid fatty acid composition of nuclear envelopes were reflected in changes in the function of the nuclear envelope. Lpr/lpr mice exhibited significantly higher NTPase activity and the RNA efflux from isolated nuclei was higher in these animals when compared with the +/+ strain. Animals fed the high linoleic acid diet exhibited higher NTPase activity and higher RNA efflux compared with animals fed the low linoleic acid diet. Thus, genetically induced changes in the composition and function of the nuclear envelope were reflected in changes in the expression of nuclear activity in the liver.

Membrane protein–lipid interactions depend on the nature of the physicochemical make-up of the membrane lipid, which depends in part on dietary intake of fat [26]. Membrane-dependent functions are modulated by interactions of membrane components with catalytic proteins. Thus changes in NTPase activity and RNA efflux from isolated nuclei observed could be explained in terms of alterations in physical characteristics of the nuclear envelope due to alteration in phospholipid fatty acid profile. Phospholipid fatty acid composition has previously been reported to be altered in the nuclei and nuclear envelopes prepared from hepatoma cells [27], suggesting that 'lipid dedifferentiation' found in membranes of tumor cells also extends to nuclear envelopes. In the case of Ehrlich ascite tumor bearing mice, the fatty acid composition of cells is also markedly altered by feeding diets containing different fats [28]. These observations support the notion that change in the com-

position of nuclear envelope alters expression of nuclear activity.

The ($Mg^2 + Ca^2$)-ATPases are lipid-dependent enzymes and both polar and nonpolar regions of the phospholipid molecule are important for enzyme activity [4]. Nuclear envelope NTPase is known to exert considerable influence on rate of nucleocytoplasmic ribonucleic acid efflux in vivo [6]. Ribonucleic acid efflux is rate controlled, probably at the level of nuclear pore complexes and NTPase activity is also associated with the nuclear pore complexes [2]. In *Tetrahymena,* nucleocytoplasmic transport of ribonucleic acid proceeds exclusively through nuclear pore complexes [29] with lipid clustering modulating opening and closing of the nuclear pore complexes, thus regulating nucleocytoplasmic exchange of ribonucleic acid [30]. Evidence indicates that significant regulation of genetic expression occurs at the posttranscriptional nuclear level [31] and most posttranscriptional controls are operated within the nucleus or at the nuclear envelope to regulate qualitative and quantitative flow of ribonucleoproteins to the cytoplasm [31]. Phosphorylation of nuclear envelope components has been reported to be involved in regulation of nucleocytoplasmic transport of macromolecules [32] and cell-cycle events [33]. Smith and Wells [34] have reported nuclear envelopes to actively metabolise polyphosphoinositides, and this metabolism may be important in regulation of nuclear envelope function, especially NTPase activity and thereby NTPase-modulated RNA efflux.

Thyroid hormones generally act by selective stimulation of gene transcription or pretranslational events [35]. Feeding C57 Black mice diets of high or low linoleic acid content altered the maximum binding capacity of L-T_3-binding sites [9]. Lpr/lpr mice possess a single class of binding sites for L-T_3 on the nuclear envelope and this strain of mice exhibited significantly lower numbers of binding sites compared with the +/+ mice. It is not yet known whether strain-induced change in L-T_3 binding to the nuclear envelope alters expression of specific gene products within the nucleus. These studies are continuing to assess whether expression of specific gene products that may be stimulated by hormonal interactions at the level of the nuclear envelope may be altered by changes in the lipid composition of the nuclear envelope.

It is evident from the present study that lpr/lpr mice not only possess a defective immune system but also possess subcellular abnormalities in the mechanisms controlling expression of nuclear activity. These mice have altered phospholipid fatty acid profiles in the nuclear envelope that increase nuclear envelope NTPase activity and ribonucleic acid efflux compared with control animals. Thus, both genetic background and diet fat composition are important factors influencing phospholipid fatty acid composition of the nuclear envelope, thereby altering the characteristics and function of this membrane in the liver and perhaps other tissues.

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