High-fat diets and the immune response of C57 Bl mice

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As a basis for studies of the influence of lipids on the immune response and health, adult C57 Bl mice were fed for 10 weeks or longer on one of the following diets: high (200 g/kg) polyunsaturated fatty acid, high (200 g/kg) saturated fatty acid and low (50 g/kg) polyunsaturated fatty acid purified diets and a standard commercial diet. The three test-fat diets were compounded to have approximately the same energy content and the mice of each group maintained similar body-weights. High-fat diets significantly reduced their subsequent delayed hypersensitivity response to challenge after sensitization with tuberculin. Immunoglobulin (Ig)M antibody formation against *Escherichia coli* lipopolysaccaride was transiently decreased, but IgG antibody against sheep erythrocytes and killed salmonella vaccine, IgG and IgE antibodies against ovalbumin remained unaffected. Total and differential blood counts revealed no differences between mice on high-fat and control diets in either the absolute numbers or the proportions of the types of leukocytes. Studies on peritoneal macrophages from mice of each group showed no difference in morphology and they ingested non-toxic and toxic particles releasing similar amounts of lactate dehydrogenase (*EC* 1.1.1.27) and β-glucuronidase (*EC* 3.2.1.31) for each substance, indicating that there were no differences in viability or phagocytic function. The present study shows that the C57 Bl mouse can provide a model for the investigation of some consequence of the reduced immunocompetence induced by high-fat diets.

High fat diet: Immune response: C57 Bl mice

The type of fat in the diet can influence the fatty acid profile of body lipids in both man (Widdowson *et al.* 1975) and the guinea-pig (Gurr *et al.* 1976). Past investigations indicated that feeding high levels of fat in diets can apparently depress immune responsiveness, as seen during in vivo challenge (McCoy *et al.* 1979; Medawar *et al.* 1979; Friend *et al.* 1980) or during in vitro tests on leukocytes from sensitized animals tested with antigen (Friend *et al.* 1980) or tested with mitogens (Kollmorgen *et al.* 1979; Friend *et al.* 1980; Ossman *et al.* 1980; Santiago-Delpin & Roman-Franco, 1980; De Deckere *et al.* 1988). Any reduction in immune competence might lead to an increased susceptibility of the host to infection. In order to investigate the effects of dietary fat on susceptibility to infection, sufficiently large numbers of animals are required to obtain significant results; thus, the mouse is an appropriate species. It was, therefore, necessary to establish if the immune response of the C57 Bl mouse was modified in the same manner as that of guinea-pigs fed on high-fat diets. Mice on the appropriate diets were tested for responsiveness to delayed hypersensitivity, for antibody formation, for any changes in blood count, and in vitro for macrophage function. The experiments reported much extend earlier work on the influence of fat on the immune response in mice and comprise the control tests for studies on resistance to infection. The quality of each batch of diet was checked before use by determining fat content and fatty acid composition to avoid the influence of artifacts.

Table 1. *Composition of the three experimental diets*

Constituent (g/kg)	High-PUFA diet	High-SFA diet	Low-PUFA diet
Wheat flour	366	366	581
Casein	254	254	216
Fat	200*	200†	50*
Vitamin B supplement	57	57	48
Solka floc	57	57	48
Jones Foster salt mix	38	38	33
p-aminobenzoate	9·5	9·5	8·2
Folic acid	9·5	9·5	8·2
Vitamins A, D, E	9·5	9·5	8·2
Liver extract (ml)	9·4	9·4	8·0
Vitamin E	0·1	0·1	0·1
Energy (kJ/kg) (Calculated)	2100	2100	1785

PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
* Safflower oil, cholesterol-free (Alfonal, Byfleet, Surrey).
† Coconut oil.

MATERIALS AND METHODS

Mice

C57 Bl mice were bred in our own colony. Male litter-mates from brother–sister matings were randomly allocated to each group at 6–8 weeks of age when they weighed about 20 g. Mice in each diet group were housed singly in cages grouped in sets of five. They received the appropriate diet and water *ad lib.*

Diets

The mice were fed one of the three experimental diets described in Table 1, or Porton combined diet (PCD; Special Diet Services Ltd). This diet is the standard mouse diet in our laboratory. A typical analysis of PCD by the manufacturer showed it to contain (g/kg): 106 moisture, 32 crude fat, 203 crude protein, 14 crude fibre, minerals and vitamins. The study was designed to compare the immune responsiveness of mice fed on high level polyunsaturated fat (PUFA) and saturated fat (SFA) with the low-fat purified diet. The PCD formed a baseline control for past experience of the mouse colony on that diet.

Each batch of diets was analysed for total fat and proportion of each fatty acid. No linoleic acid was added to the coconut-oil diet since the values indicated that it exceeded the minimum essential fatty acid requirement for mice as specified in *Nutrient Requirements of Laboratory Animals* (National Academy of Sciences, 1978).

Diets were prepared every 2 months and stored in sealed portions in the dark at −20° until use to avoid rancidity and, thus, keep them palatable. Each batch of diet was analysed before and at the end of its use. Fat content was determined by weighing after extraction with light petroleum (b.p. 40–60°) (Association of Official Agricultural Chemists, 1975). For fatty acid composition, the extracted oils were first esterified to give the methyl esters, which were then analysed by gas–liquid chromatography.

Table 2 shows the typical fatty acid composition of a batch of each of the four diets.

Assessment of immune competence

Tests of immune competence included delayed-type hypersensitivity (DTH) to tuberculin, and antibody formation to four antigens. In addition, macrophage function was evaluated

Table 2. *Fatty acid content of the three experimental diets and the Porton combined diet (PCD) stock diet**

Diet†	Total fat content (g/kg)	Fatty acid content by chain length (g/kg diet)									
		$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{18:3}/C_{20:1}$
High-PUFA	192	0	0	0·4	0·4	13	4·8	27	146	0·5	Tr
High-SFA	191	14	14	86	31	17·4	6·3	16	9	0	Tr
Low-fat purified	46	0	0	0·2	0·3	4·5	1·6	7·7	31	0·1	Tr
PCD	32	0	0	0	0·2	5·1	0·6	8·3	16·4	Tr	1·4

PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Tr, trace.
* Diets were fed to the mice for at least 10 weeks before any other treatment.
† For details of composition, see Table 1.

and peripheral blood counts done. All tests were made after the mice had been fed on the diets for at least 10 weeks. Macrophage tests were also made on mice fed on the high-fat diets for 6 months.

DTH. DTH was induced by one subcutaneous injection into the neck of 0·1 ml Freund's Complete Adjuvant H37 Ra (FCA; Difco, Detroit, USA). The mice were challenged 14 d later by injection into the ear of 5 μg purified protein derivation of tuberculin in 10 μl saline (9 g sodium chloride/l) (PPD; Ministry of Agriculture, Fisheries and Food, Weybridge, Surrey). The contralateral ear was injected with the same volume of saline and served as a control. Ear thickness was measured with a pocket micrometer accurate to 0·005 mm (Mitutoyo) immediately before and 6, 24 and 48 h after challenge. The degree of sensitization was assessed by comparing statistically, by Student's paired t-test, the difference in thickness between PPD- and saline-injected ears in FCA-treated and untreated mice.

Antibody tests and immunization procedures. Antibody formation to the T-independent antigen *Escherichia coli* lipopolysaccharide (LPS) (Sigma, Poole, Dorset) and the T-dependent antigen sheep erythrocytes (SRBC), *Salmonella typhimurium* vaccine (prepared in our laboratory) and ovalbumin (Sigma), were examined to test humoral immune competence.

SRBC. Mice were injected intraperitoneally with 1×10^8 washed cells on days 0, 14 and 21. Blood samples were taken from separate groups of five mice from each diet group 6 d and 28 d after the initial injection to measure the primary and tertiary responses respectively. In a separate experiment mice were injected with either 2·5 or 5×10^8 cells and bled 6 d later to determine the primary response to those doses.

LPS. LPS was injected intravenously (0·1 ml) into groups of five mice from each diet group. Blood samples were harvested from separate groups 1, 3, 5 and 7 d later.

Ovalbumin. Ovalbumin (10 μg) and aluminium hydroxide gel (1 μg) were injected intraperitoneally in 0·2 ml saline on days 0 and 28. Blood samples were taken from separate groups of mice from each diet group on day 14 for those injected on day 0 only and on day 35 for the remainder.

Salmonella vaccine. Mice received either nine or eleven injections of 0·2 ml of salmonella vaccine ($3{\cdot}4 \times 10^7$ cells) evenly spaced over a 3 or 4-week period respectively. They were bled 1 week after their last injection.

Antibody against SRBC was determined by haemagglutination tests, using 2 g washed SRBC/l.

The micro-enzyme-linked immunosorbent assay (ELISA) method (Voller *et al.* 1976)

Table 3. *Body-weights of mice fed on high-fat and control diets* after various time-periods on the diets*

(Mean values for ten mice/group)

Diet	Period on diet (weeks)...	Body-wts (g) 1	5	10	13
High-PUFA		19·9	23·3	27·4	29·4
High-SFA		20·9	24·5	28·4	30·1
Low-fat purified		19·6	24·3	27·0	28·4
Average Standard Error		0·43	0·63	0·57	0·57

PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
* For details of composition, see Tables 1 and 2.

was used to detect IgM and IgG antibodies to salmonella vaccine, ovalbumin (IgG) and LPS. In the case of LPS, the antigen was bound to the plastic surface using methylated bovine serum albumin. IgE antibody to ovalbumin was assayed by passive cutaneous anaphylaxis (PCA) in rats, using a 24 h challenge (Ovary *et al.* 1975). Identification of the PCA antibody is based on rats being receptive to mouse IgE but not IgG1 (Mota *et al.* 1969; Ovary *et al.* 1975) and inactivation of one sample heated at 56° for 60 min to which IgG1 is not susceptible (Mota *et al.* 1969).

Blood counts

After 10 weeks on the respective diet, mice were anaesthetized with halothane and blood collected by cardiac puncture into EDTA-treated tubes. Total leucocyte counts were obtained by counting stained cells in an improved Neubauer haemocytometer after dilution in leucocyte-diluting fluid (2% glacial acetic acid in distilled water; crystal violet added until deep blue). Differential counts were performed on blood films.

Macrophage function. Non-elicited peritoneal macrophages were incubated overnight (37°, 50 ml carbon monoxide/l air) in Eagle's Minimum Essential Medium (EMEM; Eagle, 1953) with 200 g calf serum/l, at a concentration of 2×10^6 cells/ml. The medium was then replaced by EMEM (without phenol red) supplemented with 100 g heat-treated calf serum/l (Gordon *et al.* 1979), either alone or containing quartz DQ12 or titanium dioxide. The cultures were then incubated for another 17 h. At the end of this period the proportions of lactate dehydrogenase (*EC* 1.1.1.27 (LDH); Wroblewski & Ladau, 1955) and β-glucuronidase (*EC* 3.2.1.31; Page *et al.* 1973) released into the medium were measured. For morphological studies, cells on coverslips were fixed and stained with haematoxylin and eosin.

RESULTS

Body-weights

Mice adjust their food intake according to the energy density of their diet. The experimental diets were, therefore, designed with identical nutrient densities (nutrients/420 kJ). The weights shown in Table 3 indicate that the mice had, as expected, a broadly similar energy intake and, therefore, nutrient intake (except for proportions of fat and carbohydrates). This was confirmed by monitoring the food intake of a separate group of mice (ten/diet group). Energy intakes were found to be 87 (SE 10·7), 85 (SE 11·6) and 76 (SE 7·2) kJ/mouse per d for animals on the high-PUFA, high-SFA and low-fat purified diets respectively. They also demonstrate that the animals were thriving equally well on the high- and low-fat diets, a finding confirmed by day-to-day observation.

Table 4. *Delayed hypersensitivity to tuberculin (PPD)* in mice fed on high-fat and control diets†*

(Forty mice/group were used for the experimental diets, but only thirty were measured at both 24 and 48 h after challenge; the values for the PCD group refer to fifty mice)

Diet	Mean difference in ear thickness (mm × 10^2) 24 h	48 h
High-PUFA	4·9[a]	7·8[a]
High-SFA	6·1[a, b]	9·8[a]
Low-fat purified diet	8·3[b]	13·6[b]
PCD (reference diet)‡	9·9	15·2
Average Standard Error	0·43	0·70
(df as for *F* value)	$F(2,115) = 5{\cdot}96$ $P = 0{\cdot}003$	$F(2,96) = 5{\cdot}58$ $P = 0{\cdot}005$

[a,b] Mean values with unlike superscript letters were significantly different.

PCD, Porton combined diet (Special Diet Services Ltd); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

* For details of procedures, see p. 19.

† For details of composition, see Tables 1 and 2.

‡ In this experiment the values for PCD animals are historical ones obtained over the same period and are given to illustrate their similarity to those of the low-fat purified diet group. They have not, therefore, been included in the statistical analysis.

DTH studies

When challenged by intradermal injection of antigen into the pinna of the ear, sensitized mice respond by an increase in ear thickness. The mean difference in thickness between antigen- and saline-injected ears was determined for animals in each dietary group (Table 4).

These results show that mice fed on the high-fat diets respond significantly less to sensitization with tuberculin than do mice fed on either the low-PUFA purified diet control or PCD. Mice fed on the high-PUFA diet exhibited the weakest reaction, which on average was 50% of that of the animals fed on the low-PUFA diet. The mice given the high-SFA diet showed a challenge response intermediate between that of the high- and low-PUFA groups.

Antibody studies

The IgM antibody response to LPS (Table 5) shows that the mice fed on a high-PUFA diet had less anti-LPS IgM than those on other diets, but only at days 3 and 5.

Table 6 summarizes the response to SRBC, salmonella vaccine and ovalbumin. There were no significant differences between diet groups following one, two or three injections of these antigens.

In the case of the SRBC, only the response to 1×10^8 cells after the first and third injections has been shown, but the pattern was similar when 2·5 or 5×10^8 cells were used to stimulate antibody formation. No IgM anti-ovalbumin was detected and the titres of IgE antibody by rat PCA showed no significant change.

Blood counts

Table 7 shows the mean total and differential blood counts of mice on the various diets. They revealed no significant difference between diet groups in either the total number or the proportions of the different types of leucocytes, with the exception of monocytes. The

Table 5. *Immunoglobulin M antibody response to* Escherichia coli *lipopolysaccharide*† *in mice fed on experimental diets and PCD*‡

(Values represent optical density at 400 nm; for days 1 and 3 assays, five mice/group were used; for days 5 and 7, ten mice/group)

Diet Period after immunization (d)...	1	3	5	7
High-PUFA	0·26	0·40	1·18*	3·25
High-SFA	0·29	0·51	1·60	3·07
Low-fat, purified	0·17	0·59	1·66	3·30
PCD	0·25	0·49	1·60	3·22
Average standard error	0·11	0·10	0·11	0·15

PCD, Porton combined diet (Special Diet Services Ltd); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

* Mean value was significantly different from that for low-fat purified-diet group ($P = 0·05$).

† For details of procedures, see p. 19

‡ For details of composition, see Tables 1 and 2.

Table 6. *Antibody response to sheep erythrocytes (SRBC), salmonella vaccine and ovalbumin* in mice fed on experimental diets and PCD*†

(Salmonella vaccine immunoglobulin (Ig)M and IgG and ovalbumin IgG levels were determined by enzyme-linked immunosorbent assay and are expressed as optical density at 400 nm units. Anti-SRBC antibody and anti-ovalbumin are expressed as the $\log_2$ of the haemagglutination and PCA titres respectively. Values are means from groups of five mice)

			Salmonella vaccine							
Antigen...	SRBC		IgM		IgG		IgG		IgE	
Diet	Day 6	Day 28	H	O	H	O	Day 14	Day 35	Day 14	Day 35
High-PUFA	5·0	9·2	0·098	0·61	0·96	1·56	0·15	0·46	5·0	5·4
High-SFA	5·4	6·4	0·13	0·58	0·98	1·82	0·15	0·38	4·8	6·2
Low-fat purified diet	5·4	7·0	0·16	0·57	0·94	1·48	0·18	0·42	4·4	5·4
PCD	ND	ND	0·13	0·62	0·75	1·76	0·15	0·37	4·6	5·6
Average standard error	0·2	0·27	0·02	0·25	0·04	0·10	0·03	0·05	0·24	0·27

PCD, Porton combined diet (Special Diet Services Ltd); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. ND, not done.

* For details of procedures, see p. 19.

† For details of composition, see Tables 1 and 2.

number of these cells was significantly higher in all three purified diet groups than in the PCD group. However, this is not considered biologically significant as all counts were within the normal range of mice of the colony.

Macrophage function

Morphological examination of cultured macrophages harvested from mice fed on high-fat diets showed that they had a very similar appearance to cells harvested from mice fed on control diets. Table 8 shows the response of macrophages from mice on each of the four diets to ingestion of a toxic (Quartz DQ12) and a non-toxic (TiO_2) particulate material, as assayed by release of LDH and β-glucuronidase. Release of these enzymes in large amounts indicates cell damage (β-glucuronidase) or death (LDH).

Table 7. *Total and differential blood counts† of mice fed on high-fat and control diets‡*

(Mean values for ten/group)

Diet	Leucocyte numbers ($\times 10^6$/ml)				
	Total	Neutrophils	Lymphocytes	Monocytes	Eosinophils
High-PUFA	6·2	0·4	5·0	0·7*	0·1
High-SFA	7·7	0·5	6·2	0·9*	0·09
Low-fat purified diet	8·2	0·5	6·8	0·8*	0·1
PCD	6·6	0·3	5·8	0·4	0·1
Average standard error	0·77	0·09	0·62	0·12	0·037
F values (df 3,35)	1·46 NS	1·41 NS	1·35 NS	3·24 $P = 0·034$	0·274 NS

PCD, Porton combined diet (Special Diet Services Ltd); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; NS, not significant.

* Mean values were significantly higher than that for the PCD group.

† For details of procedures, see p. 20.

‡ For details of composition, see Tables 1 and 2.

Table 8. *Release of enzymes from macrophages obtained from mice fed on high-fat and control diets* on ingestion of Quartz DQ12 or titanium dioxide†*

(Results are expressed as the percentage enzyme released by a treatment compared with the total released by lysis of cells with Triton X-100; values are means of four replicates per treatment)

Treatment...	Control		Quartz DQ12 (250 μg/ml)		TiO_2 (1000 μg/ml)	
Diet	LDH (%)	β-Glu (%)	LDH (%)	β-Glu (%)	LDH (%)	β-Glu (%)
High-PUFA	30	23	70	71	35	43
High-SFA	25	23	74	77	37	41
Low-fat purified diet	22	24	75	72	39	42
PCD	28	22	74	75	33	50
Average standard error	1·7	2·2	2·4	2·5	2·3	5·5

PCD, Porton combined diet (Special Diet Services Ltd); PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; LDH, lactate dehydrogenase (*EC* 1.1.1.27); β-Glu, β-glucuronidase (*EC* 3.2.1.31).

* For details of composition, see Tables 1 and 2.

† For details of procedures, see p. 20.

Macrophages from mice in each of the diet groups proved equally able to phagocytose particulate materials. Also, the diets of the cell donors did not cause any significant differences in enzyme release in vitro, whether spontaneous or following ingestion by macrophages of either a toxic (Quartz DQ12) or a non-toxic dust (TiO_2). Macrophage function was normal for those features in mice fed on the high-level fat diets for 6 months.

DISCUSSION

C57 B1 mice fed on large amounts of fat in the diet, particularly PUFA, were shown to have reduced responsiveness to DTH stimulation compared with that of mice fed on low, but sufficient, PUFA. Antibody formation to *E. coli* LPS was transiently depressed, but

antibody formation to sheep erythrocytes, killed *Salmonella typhimurium* vaccine and ovalbumin was unchanged. Blood leucocyte counts were normal and macrophage phagocytic function unchanged.

In order to avoid artifacts or aberrant results due to degeneration or oxidation of the fat, the diets were checked for both fatty acid content and composition both before storage at −20° and before use. The three experimental diets of high and low fat content were so balanced as to provide approximately the same energy per kg diet, and evidence that this was achieved is shown by the same growth rate and weights of mice in each group, as well as by measured food intakes. This is an important feature, because differences in mean weights of groups of mice may be associated with variations in immune responsiveness (W. E. Parish, unpublished results).

The decreased cell-mediated responsiveness in the mice fed on the 200 g fat/kg diets is consistent with an earlier study in this laboratory (Friend *et al.* 1980): in this it was shown that guinea-pigs fed on diets rich in maize oil (PUFA) or beef tallow (SFA) and sensitized to tuberculin PPD and to Keyhole limpet (*Megathura crenulata*) haemocyanin, reacted more weakly than controls fed on less fat on first challenge with these antigens. Lymphocyte responsiveness in vitro to antigen (Friend *et al.* 1980) and to mitogens (Kollmorgen *et al.* 1979; Friend *et al.* 1980; Ossman *et al.* 1980; Santiago-Delpin & Roman-Franco, 1980; De Deckere *et al.* 1988) is also decreased when cells are taken from animals fed on large amounts (up to 200 g/kg) of fat. The decreased response to stimulation appears to be due to inhibitory factors associated with fat chylomicrons in the serum (Curtiss & Edgington, 1979; Kollmorgen *et al.* 1979; Friend *et al.* 1980; De Deckere *et al.* 1988).

Other reports have claimed decreased responses in PUFA- but not SFA-fed animals (Erickson *et al.* 1980; Ossman *et al.* 1980), although this may well reflect less-sensitive detection systems, as most studies reporting diminished responsiveness in both PUFA- and SFA-fed animals showed PUFA to be the more potent. In one investigation (DeWille *et al.* 1981) DTH was unaltered by a fat-rich diet (250 g/kg), although essential fatty acid deficiency reduced lymphocyte responsiveness.

The influence of the larger concentrations of fat on antibody formation was more complex. The high PUFA diet significantly reduced IgM antibody formation to *E. coli* LPS on days 3 and 5 after immunization, though not on day 7. The high-SFA diet slightly reduced antibody formation compared with the low-PUFA control. However, neither high-fat diet altered the IgG response of our mice to T-cell-dependent antigens SRBC, *S. typhimurium* vaccine or ovalbumin, or to formation of IgE anti-ovalbumin.

This has similarities with other reports in which high proportions of dietary PUFA depressed primary IgM responses to SRBC but large amounts of SFA increased IgG responses (Erickson & Adams, 1983). Cells from fat-fed animals are reported to have reduced B-cell mitogenesis to LPS in vitro (Ossman *et al.* 1980), although the same laboratory in the same year (Erickson *et al.* 1980) also reported no effect on B-cell mitogenesis by LPS. However, a critical examination of their findings shows that if their radioscintillation counts per min results are expressed in the standard form of the stimulation index, there is reduced B-cell blastogenesis in SFA-fed rats. There are several divergent reports on the relationship between excess dietary fat, especially PUFA, and antibody formation, from no effect (DeWille *et al.* 1979) to decreased formation (McCoy *et al.* 1979; Friend *et al.* 1980). This could well be due, in part, to the time of serum sampling after antigenic stimulation. Friend *et al.* (1980) observed that only first challenge responses were less than those of controls in guinea-pigs fed on high-fat diets and they concluded that such diets do not depress immune responses permanently, but delay their maturation. In the study reported here the formation of antibody, except for LPS and

SRBC first injection, was assayed late after immunization with several injections of antigen. In this context it is interesting that formation of IgE, which occurs early during sensitization in the mouse (Mota *et al.* 1969), was unaffected by the amount of fat in the diet.

The mechanisms underlying the observed diminished responsiveness, particularly reduced DTH, are unknown. The monocyte count was significantly higher in animals fed on the experimental diets and this could conceivably affect immune responsiveness as they mature into macrophages. However, this cannot explain our results. There were no significant differences between the experimental-diet groups as regards monocyte number, yet the DTH responses were significantly higher in the low-fat purified diet group than in the high-fat groups. Furthermore, the values were well within the historical range for our colony.

Modulation of macrophage activity could, however, affect immune responsiveness because of this cell's antigen-presenting role. Also, macrophages are a major source of prostaglandins in the immune response. They are derived from linoleic acid and other PUFA and there is much evidence that they regulate immune responsiveness, acting as physiological inhibitors of lymphocyte function (Pelus & Strausser, 1977; Goodwin & Webb, 1980; Stenson & Parker, 1980).

The properties of resident peritoneal macrophages, assessed by their phagocytic capacity and the effects of toxic and non-toxic dusts, remained unaffected by the level of fat in the diet of the donors. These results suggest that impairment of macrophage function does not account for the diminished DTH response of PUFA-fed mice. However, the need to use calf serum in the cultures rather than autologous donor serum could have attenuated the effects of the diets by altering the fatty acid profile of the cells.

Consideration of reports of inhibitory substances for lymphocytes in the serum of animals fed a high proportion of fat (Curtiss & Edgington, 1979; Kollmorgen *et al.* 1979; Friend *et al.* 1980; De Deckere *et al.* 1988) will not contribute significantly to the context of this discussion, apart from the possible existence of a substance inhibiting membrane function or blocking receptors. Several groups of workers have identified a low-density lipoprotein (LDL) component in human serum which inhibits lymphocyte proliferation. Although termed LDL-In (inhibitory LDL), it was actually shown to belong to the intermediate-density lipoprotein class (IDL) by Pepe & Curtiss (1986) who identified its inhibitory moiety as apoprotein E. Apoprotein E is secreted by monocytes/macrophages and secretion is much increased in the presence of cholesterol in the serum, thereby decreasing lymphocyte responsiveness, but if the macrophages are stimulated, e.g. by immune complexes, apoprotein E secretion is greatly diminished and the lymphocyte responsiveness to stimulation increases. Thus, the lipoprotein-apoprotein E is an immunoregulatory process conditioned by the amount of lipid in the serum (Dyer *et al.* 1987).

The present study confirms that C57 B1 mice provide a suitable model for reduced immunocompetence associated with large amounts (200 g/kg) of fat in diets, and provides the control data for the investigations of any susceptibility to infection (J. V. Friend, R. W. R. Crevel, W. E. Parish, A. Humphreys, M. Carter and J. S. Crowther, unpublished results).

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