

The Influence of Human Plasma Lipoproteins and Fatty Acids in Immunological Reactions

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During recent years the biochemical aspects of immunological reactions have attracted considerable interest, and progress in this field is rapid. Among other things, research has concentrated on the biochemical role of fatty acids and lipoproteins in certain immune reactions. In this article we review the immunoregulatory effects of normal human lipoproteins and fatty acids.

Plasma lipoproteins are complex non-covalently organized particles composed of a variety of lipids and apoprotein chains. The lipoproteins in human plasma consists of four major lipoprotein classes: chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). For an extensive review see (40). Certain lipoprotein classes, as well as minor subsets of plasma lipoprotein classes, can regulate selected metabolic functions in a variety of cell types. Among the bioregulatory

properties of lipoproteins the first and most extensively documented has been the general ability of LDL to supply cholesterol and regulate cholesterol biosynthesis (2).

During the past decade knowledge of the regulatory properties of lipoproteins has been extended to involve immunoregulatory properties. These immunoregulatory properties of human plasma lipoproteins were first reported in detail by Curtiss & Edginton in 1976 (10). The importance of these properties lies in the fact that lipoproteins are strongly influenced by nutrition and to some extent by genetic factors.

Fatty acids are involved in various immunological reactions. The distribution of free fatty acids in plasma is greatly affected by nutrition. The immunoregulatory properties of fatty acids were first described by Resch & Ferber in 1975 (43).

Plasma lipoproteins and fatty acids also have profound effects on the immune system in

Abbreviations: Apo B, apoprotein B; Apo C, apoprotein C; Apo E, apoprotein E; C, cholesterol; CE, cholesterol ester; CM, chylomicrons; C567-INH, C567-inhibitor; Con A, concanavalin A; DMI, direct migration inhibition; EBV, Epstein-Barr virus; FFA, free fatty acids; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; Ig, immunoglobulins; iv, intravenous; LCAT, lecithin-cholesterol acyltransferase; LDL, low density lipoproteins; LDL-In, low density lipoprotein inhibitor; LL, lyso lecithin; LPL, lipoprotein lipase; LPS, lipopolysaccharide; PBM, peripheral blood mononucleated cells; PFC, plaque forming cells; Pg, prostaglandins; PHA, phytohaemagglutinin; PL, phospholipids; PPD, purified protein derivative of tubercle; PUFA, polyunsaturated fatty acids; PWM, poke weed mitogen; RIF, rosette inhibiting factor; sc, subcutaneous; SFA, saturated fatty acids; SRBC; sheep red blood cells; TG-FA, triglyceride-fatty acids; UFA, unsaturated fatty acids; VLDL, very low density lipoproteins.

Apo E suppresses the enhanced turnover of lymphocyte phospholipids, which is triggered by the mitogenic lectin PHA (1). Among the metabolism of the cellular phospholipids stimulated by PHA, the inositol-containing phospholipids are most characteristically inhibited by apo E. Apo E does not inhibit PHA-induced lymphocyte phospholipid turnover by preventing the PHA-lymphocyte interaction. The basic amino acid residues, lysine and arginine, are required for the lymphocyte-lipoprotein interaction, since chemical modifications of a limited number of these residues abolish both binding of and suppression by lipoproteins. The activity of this VLDL receptor may help to maintain the circulating blood lymphocytes in a non-proliferating state.

Effects of human VLDL in rodents

Human VLDL also affects the murine immune system (8). Human VLDL is a potent inhibitor of the primary humoral immune response to sheep red blood cells (SRBC) in mice. When 2.6 µg VLDL protein is administered i.v. 24 h prior to a primary inoculation of 1×10^7 SRBC, 50% inhibition of the day 5 haemagglutinin response is accomplished. Complete suppression could not be accomplished, since the VLDL appears to suppress, selectively or preferentially, a subset of antigen reactive cells. The primary and secondary response was equivalently reduced, suggesting that VLDL probably operates by direct inhibition of early inductive events such as antigen recognition or triggering, rather than by suppressing antibody synthesis or secretion.

Immunoregulatory effects of LDL

Human LDL inhibits human lymphocyte activation induced by the mitogens: PHA (23), Concanavalin A (Con A) and PWM (19). The target cell for this inhibition is the resting lymphocyte. This inhibition is caused by LDL suppression of primary inductive events: most notably enhanced Ca^{++} accumulation, suppression of cGMP synthesis (22) and phosphatidylinositol turnover, and by late events such as enhanced DNA synthesis (24), which occur as a result of

mitogen-triggered alterations of the lymphocyte membrane. This is shown by depression of $^{45}\text{Ca}^{++}$ accumulation in PHA-stimulated lymphocytes. The concentrations of LDL needed for suppression are within physiological concentrations. Smaller concentrations of LDL ($< 20 \mu\text{g}$ LDL protein/ 5×10^6 mice lymphocytes) have been shown to enhance the activity of PWM and lipopolysaccharide (LPS)-induced stimulation of lymphocytes (19). These mitogens are B-cell mitogens suggesting that LDL have a potentiating effect on B cells.

The LDL bind to lymphocytes via a specific receptor (immunoregulatory receptor) which binds the apo B and apo E in the LDL particles. The immunoregulatory potency of LDL correlates directly with the amount of LDL bound to the cell surface. The amount of bound LDL is not influenced by the concentration of PHA (3 µg/ml), which is optimally mitogenic. Surface binding sites for the LDL are saturated at LDL concentrations above 100 µg/ml. The corresponding lymphocyte concentration was 2×10^6 cells/ml. The LDL immunoregulatory receptor is distinct from the LDL high affinity receptor, which is important for cholesterol transport.

Membrane-bound LDL can be replaced by heparin and thereby protect the cells from immunosuppression by LDL. Heparin acts by displacing LDL from the cell surface, suggesting that membrane-associated LDL suppresses lymphocyte activation (23).

LDL linked covalently to Sepharose is as effective as native LDL in the inhibition of Ca^{++} -accumulation in PHA-activated lymphocytes, thereby suggesting that internalization of LDL is not required for immunosuppression (20).

In vivo LDL is a normal catabolic derivative of VLDL metabolism, and VLDL may therefore be considered as a potential progenitor of the immunoregulatory LDL, and the sub-species LDL-In and RIF (rosette inhibiting factor, found in the LDL fraction of some persons with malignant disease (6)). The importance of VLDL may extend beyond their own immunoregulatory properties and may involve their ability to generate the other immunoregulatory lipoproteins.

The effect of VLDL, LDL and IDL on EBV-transformed lymphocytes

Human plasma lipoproteins in physiological concentrations can suppress Epstein-Barr virus (EBV)-induced immortalization of adult human B lymphocytes (9). Several different mechanisms seem to be involved. LDL can directly reduce the ability of EBV to transform human B cells and it can selectively inhibit the EBV inducible step that occurs within 24 h of transformation. Secondly, VLDL, LDL and IDL all affect early inductive events rendering the B cell refractory to transforming signals from EBV. Finally, VLDL is capable of suppressing ongoing EBV-driven cellular proliferation. The plasma lipoproteins may therefore have an important immunoinhibitory effect, *in vivo*, in protecting against EBV-transformed malignant B cell clones. Taking the environmental aspects of VLDL and the genetic aspects of LDL into account, these lipoproteins may influence the heterogeneous distribution of Burkitt's lymphoma on world-wide basis.

Immunological activity of LDL-In

Human low density lipoprotein inhibitor, LDL-In, is a lipoprotein subfraction with immunosuppressive properties. LDL-In, which resides in the density range 1.006–1.019 $\mu\text{g/ml}$, is a minor subset of normal LDL and is also recovered in IDL (15).

In vitro LDL-In inhibits thymidine incorporation in PHA-stimulated lymphocyte cultures by 50% at a concentration of 6.4 μg protein and 16 μg lipid/ 10^5 lymphocytes (10) (see Fig. 2). This concentration is considerably less than the physiological concentration in LDL in serum. The inhibitory effect of LDL-In requires 24 h for induction of a stable suppressed state. If the induced suppression of lymphocytes is 50%, the inhibitory action of LDL-In is reversible provided that the lymphocytes are washed free of the lipoproteins. Completely inhibited cells do not recover, indicating that a relatively stable suppressed state can be evoked (18). The LDL-In inhibitory effect on lymphocytes does not persist beyond 19 h from stimulation, suggest-

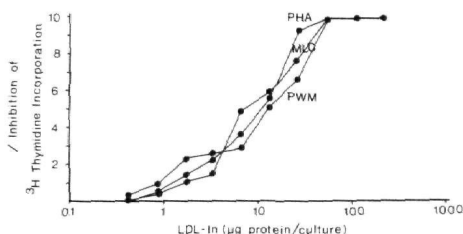


Fig. 2. Inhibition of thymidine incorporation of lectin and allogenic cell stimulation of lymphocytes by LDL-In. The mean incorporation of control cultures was PHA, 51,200 cpm; PWM, 18,801 cpm; and MLC, 17,388 cpm. Reproduced from (10) with the permission of Dr. L. K. Curtiss.

ing that LDL-In may influence metabolic events associated with the inductive phase of lymphocyte activation by lectins and allogenic cells (10). The inhibitory activity of LDL-In does not result from interaction with or neutralization of lectins.

The activity of LDL-In is macrophage-independent, since exposure of macrophages to LDL-In had no effect on subsequent *in vitro* lymphocyte responses to either PHA or allogenic cells (12). Human LDL-In was tested for its influence on E rosette formation and was found negative.

The immunoregulatory activity of LDL-In requires the presence of specific cell surface receptors for LDL-In. These receptors are distinct from the LDL immunoregulatory receptor on lymphocytes (13). Receptor activity involves binding of apo B and apo E in the LDL-In. The binding of LDL-In to lymphocytes is Ca^{++} dependent, temperature dependent and occurs with high affinity even at relatively low molar concentrations. The binding can be inhibited by proteolysis of the cell surface with trypsin and then regenerated to control levels, which implies that the LDL-In receptor involves a trypsin-sensitive surface protein.

Addition of human serum or foetal calf serum mitigates the suppressive activity of LDL-In. This suggests that other proteins or factors in serum play either an independent and facilitating role in the regulation of lymphocyte function or may directly interfere with

Table 1

Differential sensitivity of lymphocyte subpopulations to LDL-In exposure^{a,b}

Lymphocyte	Specific inhibitory activity ($\mu\text{g}/\text{ml}^c$)	Relative sensitivity
Total PBM	25	1.0
B	21	1.2
T helper	95	0.3
T suppressor	9	2.8

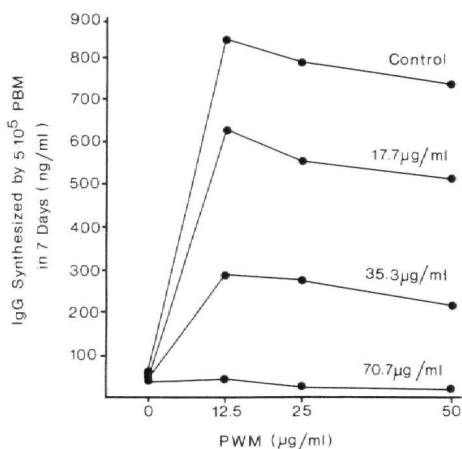
^a Reproduced from (14) with the permission of Dr. L. K. Curtiss.^b 24 h, 37°C.^c Amount of LDL-In (μg protein/ml) required for 50% inhibition of cell function.

Fig. 3. Influence of concentration of PWM on suppression of IgG synthesis by LDL-In (50% inhibition of PHA-stimulated ^3H thymidine uptake = 27.2 $\mu\text{g}/\text{ml}$ sp act). PBM ($5 \times 10^5/\text{ml}$) were precultured with LDL-In for 24 h before addition of PWM and the cultures were harvested 7 days later. 50% inhibition of IgG synthesis occurred at an LDL-In concentration of 27.7 $\mu\text{g}/\text{ml}$. Reproduced from (14) with the permission of Dr. L. K. Curtiss.

the mechanism of interaction of LDL-In with lymphocytes.

Selective suppression of lymphocyte subpopulation

Lymphocyte subpopulations appear to differ significantly in their sensitivity to suppression

by LDL-In. When lymphocyte subpopulations were incubated with LDL-In 24 h prior to PWM stimulation the sensitivities of lymphocyte subpopulations to LDL-In relative to PBW were 2.8, 1.2 and 0.3 for T suppressor cells, B cells and T-helper cells respectively (14) (see Table 1). Thus both B and T lymphocytes are sensitive to and can be regulated by LDL-In. When PBM are incubated with LDL-In for 24 h prior to PWM stimulation the IgG secretion on day 7 was suppressed (see Fig. 3). No Ig class switch occurs during this period. LDL-In is unable to inhibit ongoing IgG synthesis by committed PBM when added 20 h or more after PWM stimulation.

27.7 μg LDL-In protein/ml was required for 50% inhibition of PWM-stimulated IgG synthesis. This concentration of LDL-In is equivalent to the concentration of LDL-In required for 50% inhibition of ^3H -thymidine uptake in PHA-stimulated PBM. These values are within the physiological concentrations of LDL-In. Thus the inhibitory activity of LDL-In for ^3H -thymidine uptake and for IgG synthesis appears to be equivalent. The suppressive activity appears to be independent of mitogen concentration. Optimal inhibition by LDL-In requires at least 24 h of exposure before mitogen stimulation, suggesting that LDL-In suppresses early inductive events that must precede the proliferative signal to a resting lymphocyte.

Species independence of human LDL-In

Human LDL-In is not species specific and is capable of suppressing the *in vivo* mouse anti-sheep erythrocyte (SRBC) haemagglutination response by 88% (11). Maximal suppression occurred only when LDL-In was injected 24 to 48 h before antigen administration (see Fig. 4). Simultaneous or subsequent injection of LDL-In had no effect. The activity of LDL-In is influenced by the antigen dose and is maximal at low antigen doses. The addition of 100 μg of LDL-In to haemagglutination assays did not affect haemagglutination titres, thereby confirming that LDL-In did not directly interfere with the *in vitro* assay system. The number of splenic plaque-forming cells was also reduced, indicat-

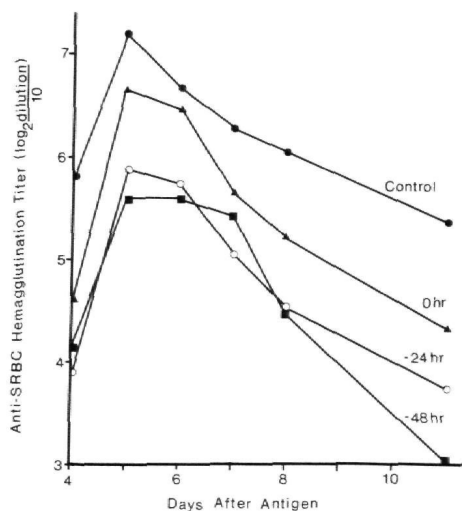


Fig. 4. Temporal aspects of LDL-In-mediated suppression of the humoral immune response. All C3H mice received SRBC IV and IP at time 0 and either PBS or 300 μ g LDL-In IV at the times indicated. Values are given as the mean anti-SRBC haemagglutination response. Reproduced from (11) with the permission of Dr. L. K. Curtiss.

Table 2
Influence of LDL-In on direct plaque-forming cells*

Group ^a	Days following antigen	Direct PFC/10 ⁵ lymphocytes ^b
LDL-In	3	2.6 $\pm$ 0.9
Control	3	28.0 $\pm$ 2.6
LDL-In	5	5.7 $\pm$ 0.8
Control	5	43.9 $\pm$ 3.9

* Reproduced from (11) with the permission of Dr. L. K. Curtiss.

^a Four mice per group; LDL-In, 700 μ g IV in PBS 24 h before a total dose of 1.0×10^7 SRBC, $\frac{1}{2}$ IV and $\frac{1}{2}$ IP; control, PBS IV 24 h before SRBC.

^b Recovery of $1.50 \times 10^8 \pm 0.18 \times 10^8$ cells/spleen.

ing a suppression of the clonal expansion of primary B cells to antibody secreting cells rather than only suppression of antibody synthesis by differentiated B cells (Table 2) (11).

Human LDL-In is also capable of inhibiting the mixed lymphocyte stimulation of either

human or murine cells *in vivo*. This occurs at a concentration of 10 μ g LDL-In protein/ 2×10^6 lymphocytes.

The active moiety of LDL-In

To investigate further whether the immunoregulatory activity resides within the lipid moiety or the protein moiety, LDL-In was depleted of more than 98% of its neutral lipid content (15). These delipidated particles were compared with native LDL-In with regard to charge, apoprotein content, suppressive activity for lymphocytes and specific binding to the lymphocyte LDL-In receptor. The cholesterol- and glyceride-depleted LDL-In retained all characteristics of the native LDL-In. The biologically active moiety of LDL-In resides in either a single or a molecular complex of the remaining constituents, the polar lipids and apoprotein chains. The lymphocyte surface receptor recognition of LDL-In is mediated by some or all of these same constituents.

Free fatty acids (FFA) have been demonstrated to suppress certain lymphocyte responses, including mitogen-stimulated H³-thymidine uptake. It was therefore investigated whether FFA associated with LDL-In particles are the biologically active constituents (16). However, the dose-dependence and the kinetics of LDL-In indicated that the suppression is distinctly different from those observed for FFA.

When more than 92% of the endogenous FFA of LDL-In were removed by incubation in the presence of delipidated albumin, no differences were observed between this FFA depleted LDL-In and native LDL-In regarding suppressive activity of lymphocyte stimulation or binding to the lymphocyte LDL-In receptors. Thus the FFA content or neutral lipid content of LDL-In is not responsible for the immunoregulatory activity of LDL-In.

Recent investigation of the protein moiety of LDL-In has revealed that the apo-E is responsible for the inhibitory activity of LDL-In (Dr. L. K. Curtiss, personal communication). Structural analysis of the active site of apo-E is in progress.

Immunoregulatory effects of HDL

It has been shown that human serum lipoproteins contain an active subset C567-inhibitor (C567-INH) which inhibits the attachment of the C567 complex of complement to bystander cells (29). The serum lipoproteins were responsible for approximately 25% of the serum C567-INH activity. The highest activity was revealed in the HDL fraction.

This inhibitory activity of HDL was counteracted by poly-L-lysine which also counteracted the inhibitory activity in serum. Only a partial characterization of C567-INH is available. Experiments designed to investigate the effect of lipoproteins on C56, C567 and C7 suggest that the C567-INH activity acts selectively on C567 complexes and not on C56 or C7. The counter effect of poly-L-lysine suggests an ionic interaction with the lipoprotein, whereas the binding of lipoproteins to C567 is presumably due to hydrophobic interactions.

Immunoregulatory properties of human lipoproteins

CM	→ Inhibits PHA-stimulation of human lymphocytes.
VLDL	→ Inhibits the PHA, PWM and allogenic-induced stimulation of human lymphocytes.
	→ Inhibits the primary humoral immune response to SRBC in mice.
IDL	→ Suppresses EBV-induced immortalization of adult human B lymphocytes.
LDL	→ In low doses LDL enhances mitogenic stimulation (B-cell mitogens) of mice lymphocytes.
	→ Suppresses PHA, Con A, PWM and allogenic-induced stimulation of human lymphocytes.
LDL-In	→ Shows selective immunosuppressive effects on human lymphocyte subpopulations.
	→ Suppresses antigenic, allogenic and PHA-induced stimulation of human lymphocytes.
	→ Suppresses the primary humoral immune response to SRBC in mice.
HDL	→ Inhibits the attachment of the C567 complex of complement to bystander cells.

Immunoregulatory properties of fatty acid

Unsaturated fatty acids (UFA) are capable of suppressing the PHA-induced lymphocyte transformation *in vitro* (38). It has been shown that fatty acids inhibit the incorporation of ^3H -thymidine and ^3H -uridine labelling in lymphocytes. This inhibition is not due to the toxicity of the fatty acids but may be due to changes in the

membrane fluidity, or it may be related to direct blocking of membrane-associated receptors.

Saturated fatty acids (SFA) have also been shown to inhibit PHA-stimulated lymphocyte transformation (45). These effects are also seen when fatty acids are bound to albumin. Human lymphocyte mixtures containing only SFA or UFA give considerable inhibition, while mixtures containing both SFA and UFA have a relatively small inhibitory effect. This phenomenon has been investigated with mouse, rat and pig lymphocytes. It was observed that effects of fatty acids are species-dependent (45). This inhibitory activity of UFA and SFA has also been confirmed by Mertin & Hughes (37).

The ability of fatty acids to suppress PHA-induced lymphocyte stimulation is also seen for lipoproteins. The suppressive effect of the lipoprotein is due to protein-receptor interaction, whereas the action of fatty acids may involve interference with the immunoregulatory receptor directly or indirectly by alterations of the membrane fluidity. This remains to be clarified.

In addition, Mertin & Hughes have reported that SFA (C16:0, C18:0) and UFA (C18:1, C18:2, C20:4) inhibits PPD-induced lymphocyte transformation at a fatty acid concentration of 80 μg fatty acids/ml 10% defibrinated blood. For the fatty acids tested there was a positive correlation between increase of unsaturation and increase in inhibitory effects (C20:4 > C18:2 > C18:1). Even though SFA, as well as UFA and PUFA, inhibit PHA- and PPD-induced lymphocyte transformation there is a clear difference in the effect when polyunsaturated fatty acids and the other fatty acids were tested. This difference suggests a non-specific suppression of the latter and a specific inhibitory action restricted to activated lymphocytes for PUFA (37). The mechanism of this specific inhibitory effect is not known, but the PUFA may act as prostaglandin (Pg) precursors.

PUFA-derived Pg of the E_1 and F_1 series have been shown to inhibit the antigen-induced immune response (38). These act by stimulation of the adenylate cyclase in the lymphocyte membrane, thus causing an increase in intracellular adenosine 3',5'-cyclic monophosphate

(cAMP). Thus the PUFA may act by stimulating the adenylate cyclase-cAMP system sharing the same Pg receptor, or as a Pg precursor. The PUFA do not act by altering cell viability or by altering the antigen/mitogen-cell interaction.

The PUFA concentration may therefore be an important component in the *in vivo* regulation of cell-mediated immunity, giving a decreased lymphocyte reactivity at high serum levels and an enhanced reactivity at low serum levels (0.1 µg to 5.0 µg/ml serum) (26, 34).

Immunoregulatory effects of FA in rodents

Inhibition of mitogen-induced lymphocyte transformation has also been shown in *in vivo* experiments in rats (28). Con A-induced transformation of spleen lymphocytes was significantly inhibited when lymphocytes from rats fed a high polyunsaturated-fat diet were compared with lymphocytes from rats fed a low-fat diet. This response was determined by ³H-thymidine uptake after 72 h of incubation in media supplemented with 10% serum. It was found that the responsiveness depends on the source of serum used in the experiments. Lymphocytes from rats fed a low-fat diet were suppressed in serum from rats fed a high-polyunsaturated fat diet. Alternatively, lymphocytes from rats on a high polyunsaturated-fat diet were more responsive in serum from low-fat fed rats compared with their response in autologous serum. One of the inhibitory factors in serum was the lipoprotein fraction; however, rats fed a high polyunsaturated-fat diet probably have additional inhibitors in their serum.

In addition, other investigators have confirmed that rats and mice fed a diet rich in polyunsaturated fat show a decreased antibody response, lymphocyte transformation, skin test reactivity, migration inhibition factor production and skin allograft rejection (30). Obesity in humans, mice and rats depresses immune functions, such as impairment of cell-mediated immune responses and reduction of intracellular bacterial killing by polymorphonuclear leukocytes (5, 39). For further details see (4).

Fatty acids and allograft survival

Prolonged allograft survival is an effect of suppression of the immune response which can occur after PUFA administration. Mice injected s.c. with PUFA (C18:2, C20:4) starting on the day of grafting (day 0) show a significant prolongation of graft survival, both in the primary and secondary allograft response (35). Prolongation of the skin allograft survival period not only occurs when PUFA is administered s.c. but also when PUFA is given orally. Prolonged treatment with C18:2 leads to destructive changes in the spleen (31).

After splenectomy prolongation of allograft survival in mice no longer occurred (36). Possible pathways of this action may include PUFA stimulation of suppressor cell generation in the spleen, excess biosynthesis of immunoinhibitory Pg by splenic macrophages, or generation of PUFA by enzymatic activity of splenic macrophages (27).

Influence of fatty acids on organ weight

S.c. injection of C20:4 depresses thymus weight (33). The effect is possibly due to a conversion of C20:4 to Pg, since it is significantly inhibited by indomethacin, a drug which blocks the conversion of C20:4 to Pg.

Effect of fatty acids on lymphocyte membranes

Fatty acids can affect the biochemical and physical properties of lymphocyte membranes in a number of ways (32). Some of these effects influence the immunological activity of the cells. The exact mechanism for these alterations in the cell membranes is not yet clarified.

Karnowsky (25) has shown that cis-unsaturated fatty acids disorder the membrane interior and inhibit capping of surface immunoglobulins (Ig). This effect was not seen with saturated fatty acids and with trans-unsaturated fatty acids. The cis-unsaturated fatty acids did not inhibit patch formation or receptor immobilization but inhibited the final energy dependent step in capping. The inhibition of capping could be reversed in a dose-dependent fashion

by the addition of extracellular Ca^{++} but not by Mg^{++} . The addition of Ca^{++} did not extract the fatty acids from the cell membrane. The inhibition of capping was not due to auto-oxidation of the fatty acids, nor to prostaglandin synthesis. Alteration of lymphocyte cell membranes (both murine and human) after addition of fatty acids has been confirmed by Santiago-Delfin et al. (44) who have shown that SFA, as well as UFA inhibits capping with anti-IgM. The exact mechanism of this effect is not clear.

Other important immunological reactions may be involved in cell membrane alteration by fatty acids, as proposed by Ferber & Resch (17) who suggest that changes in the fatty acid composition of the phospholipids occurring in the cell membrane shortly after lymphocyte stimulation may be the primary signal triggering the lymphocyte response.

Direct migration inhibition (DMI)

Human leukocytes *in vitro* respond to paramyxoviral antigens by direct migration inhibition (DMI) test (46). This response is significantly reduced by addition of C18:2 and C20:4 but not by C18:1. The concentration of fatty acids used in these experiments is in the physiological range found in blood as free fatty acids. The same results are obtained using PgE_1 and PgE_2 , suggesting that the fatty acids are acting as prostaglandin precursors. In healthy persons who display a vigorous DMI response to paramyxoviral antigens C18:2 significantly decreases DMI of leukocytes. Alternatively, leukocytes from persons with a decreased response to this antigen, showed an increased DMI activity.

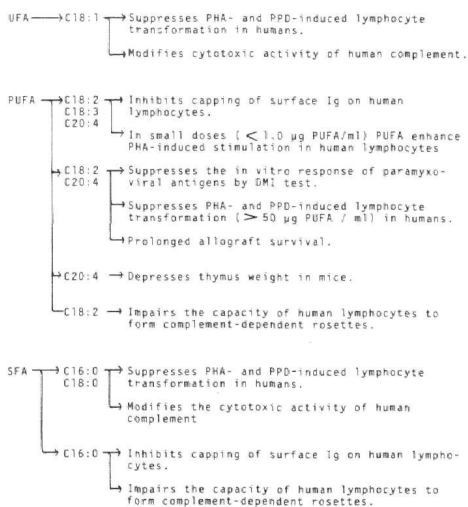
Fatty acids and complement

SFA can, when mixed with human complement prior to addition to antibody-sensitized line-10 guinea pig hepatoma cells, modify the cytotoxic activity of human complement (41). This effect is dependent on the specificity of the antibody used to sensitize the cells, the complement concentration and the fatty acid concentration. Larger and more complex lipids also influence

complement activity, but this action is not as antibody-dependent as the action of fatty acids. This suggests that fatty acids and complex lipids may affect complement in different ways. The complex lipids appear to be effective only in modifying activated fluid phase complement, while the fatty acids not only influence fluid-phase complement component, but, in addition, may interact with and modify the cell membrane. With regard to this, it has been shown that stimulation of incorporation of fatty acids into cellular lipids occurs within minutes after the addition of complement to antibody-coated cells and before detectable cell killing occurs (41). The mechanism by which lipids and fatty acids modify the cytotoxic action of complement is not clear. Kinetic experiments indicate that fatty acids and lipids are effective only when added concomitantly with the complement or within 1–3 min after the addition of complement to the antibody-sensitized cells.

Addition of fatty acids to human lymphocytes impairs their capacity to form complement-dependent rosettes, but has no effect on either spontaneous rosette formation with sheep erythrocytes or Fc γ -dependent rosette formation (42). Using murine lymphocytes, complement-dependent rosette formation was

Immunoregulatory properties of fatty acids



suppressed only after incubation with linoleic acid (C18:2) and showed no effect on Fcγ-dependent rosette formation.

The mechanism for this action remains to be elucidated, but, obviously, fatty acids do play an important immunological role in interacting with complement receptor activity.

Conclusion

The immunosuppressive activity of fatty acids and lipoprotein obviously has important immunoregulatory effects *in vivo*. The effects of lipoproteins are primarily based on protein-receptor interactions, whereas the effects of fatty acids may involve interaction with receptor activity and changes in the fluidity of the cell membranes.

In vivo the metabolism of lipoproteins and fatty acids is closely linked. Triglyceride lipases degrade CM and VLDL to lipoprotein particles ("remnants" containing less triglyceride) under liberation of fatty acids. The "remnants" can be further metabolised to LDL. An important aspect of the relation between fatty acids and lipoproteins *in vivo* is whether this relation has any immunoregulatory significance. This would be a subject for further investigation.

Another very important aspect is the well-known lipase activity of macrophages. Macrophages secrete various lipases, including lipoprotein lipase (3, 47). It has also been shown that macrophages have phospholipase activity. This enzyme is secreted during stimulation of macrophages (32). What, if any, is the immunological importance of these lipases?

Further investigations of the immunological role of fatty acids and lipoproteins may also help to clarify important biochemical aspects of immune reactions *in vivo*.

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