INHIBITION OF LYMPHOCYTE PROLIFERATION STIMULATED BY LECTINS AND ALLOGENEIC CELLS BY NORMAL PLASMA LIPOPROTEINS*

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It has recently been demonstrated that serum lipoproteins can regulate several aspects of lymphocyte function and metabolism. Chisari and Edgington (1) reported that a certain subfraction of serum low density lipoproteins (LDL)[1] from patients with viral hepatitis selectively inhibited the erythrocyte (E) rosette function of human T lymphocytes. In examining this rosette inhibitory factor further, Curtiss and Edgington (2) observed that a subspecies of LDL, apparently different from normal human serum, potently inhibited the proliferation of lymphocytes stimulated by phytohemagglutinin (PHA), pokeweed mitogen, and allogeneic cells. More recently, inhibition of lymphocyte stimulation by lectins and by allogeneic cells has been observed with plasma from patients with type IV or type V hyperlipoproteinemia (3). The inhibitory effect was found to reside in the chylomicron and very low density lipoprotein (VLDL) fractions obtained from these plasma samples. Human lymphocytes have also been shown to have high affinity cell surface receptors for LDL that vary in number depending upon the concentration of LDL to which the cells are exposed, and that play an important role in the regulation of cholesterol and lipoprotein metabolism by the cells (4).

None of the previous studies has systematically compared the effects of all the different classes of normal lipoproteins on stimulated lymphocytes. We now report the comparative effects of each of the lipoprotein classes, VLDL, intermediate density lipoproteins (IDL), LDL, and high density lipoproteins (HDL), on lymphocytes stimulated by PHA, concanavalin A (Con A), and allogeneic cells in lipoprotein-deficient plasma (LPDP).

Materials and Methods

Lipoprotein Preparation. Lipoproteins were isolated from fresh plasma and separated from whole blood collected in 0.1% EDTA as an anticoagulant from normal healthy subjects. Lipoproteins were separated by sequential ultracentrifugation of plasma at densities of 1.006, 1.019, 1.063, and 1.21, according to standard techniques (5); the lipoprotein fractionations were carried out in a Beckman model L2-65B ultracentrifuge utilizing the Beckman 50 Ti rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 40,000 rpm and 4°C for 16–40 h. The resulting lipoprotein fractions included the VLDL ($d < 1.006$ g/ml), IDL ($d = 1.006$–1.019), LDL ($d = 1.019$–1.063) and

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; E, erythrocyte; HDL, high density lipoprotein; IDL, intermediate density lipoproteins; LDL, low density lipoprotein; LDL-In, a subfraction of low density lipoprotein; LPDP, lipoprotein-deficient plasma; MLR, mixed lymphocyte reaction; PHA, phytohemagglutinin; VLDL, very low density lipoprotein.

HDL (d = 1.063–1.21), and the LPDP fraction ($d > 1.21$). The isolated lipoproteins and the LPDP were dialyzed extensively against phosphate-buffered saline (pH 7.4) for 48 h at 4°C. After dialysis, the lipoprotein preparations were analyzed for protein concentration by either the method of Lowry et al. (6), or that of Schaffner and Weissman (7); the latter method was used if the lipid content of the preparations (VLDL and IDL) interfered with the method of Lowry et al. The various preparations were also assayed for their concentrations of cholesterol and triglyceride with a Technicon AutoAnalyzer I (Technicon Instruments Corp., Tarrytown, N. Y.) using the method N-24a for cholesterol (8) and a modification (9) of the Kessler and Lederer technique (10) for triglycerides. The relative proportions of protein and of each lipid were found to be in the expected range (5, 11, 12) for each different lipoprotein fraction.

After the protein and lipid assays, the preparations were dialyzed against the RPMI 1640 culture medium (see below) for 24 h with several changes of dialysis solution. The volumes were then adjusted with culture medium to the desired protein concentrations for addition to the cell culture mixtures.

Lymphocyte Isolation. Peripheral blood lymphocytes were isolated from heparinized venous blood by a modification of the method of Böyum (13). 40–50-ml samples of blood were obtained from normal donors, and the erythrocytes were allowed to sediment by gravity at 37°C for 1–2 h. The resulting plasma-leukocyte mixture was collected and gently mixed with an equal volume of sterile isotonic saline solution. Two volumes of the saline-plasma-leukocyte mixture were layered over one volume of Ficoll-Conray solution (the latter consisting of 10 ml of 33.4% Conray (meglumine iothalamate, from Mallinckrodt Inc., St. Louis, Mo.), prepared from a 60% aqueous solution, plus 24 ml of 9% Ficoll (Pharmacia Fine Chemicals, Piscataway, N. J.). The tubes were centrifuged at 1,500 rpm for 30 min at 4°C. The cells, collected from the interface, were washed three times with RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) and supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM L-glutamine. The cells, usually comprising ≅85% lymphocytes and 15% monocytes, were subsequently cultured in this medium with the addition of 10% (final volume) autologous plasma, 10% type AB plasma heated to 56°C for 30 min, or 20–25% LPDP. This amount of LPDP provided plasma protein equivalent to that contained in 10% whole plasma because of the dilution occurring during the ultracentrifugal isolation of LPDP.

Mitogen Stimulation. The effects of the addition of a mitogen alone, or together with lipoproteins, were assessed by determining the extent to which there was stimulation of the incorporation of [^{3}H]thymidine into DNA of cultured cells. 0.25-ml triplicate cultures containing 1 or 2×10^5 lymphocytes per well were placed in microtiter plates (IS FB 96, Linbro Chemical Co., New Haven, Conn.) and incubated at 37°C in an atmosphere of 5% CO_2:95% air for periods of 3–5 days. At the start of the incubation, an optimal amount of mitogen (see below), with or without lipoproteins, was added to each well. At 15–16 h before the cells were harvested, 1 μCi [*methyl*-^{3}H]thymidine (6.7 Ci/mmol, from New England Nuclear, Boston, Mass.) was added per well. The microtiter cultures were harvested and washed extensively with isotonic saline on glass fiber filters (grade 934 AH, H. Reeve Angel & Co., Inc., Clifton, N. J.), using a Mash II Harvester (Microbiological Associates, Bethesda, Md.) (14). The filters were dried and placed in 12 ml of Instabray (Yorktown Research Inc., Hackensack, N. J.). Radioassay for ^{3}H was carried out in a model 3320 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The concentrations of the various lipoprotein preparations and the other conditions employed in the different experiments are indicated in Results. The results were expressed, for each set of triplicate samples, as the mean ± SEM of the counts per minute of [^{3}H]thymidine incorporated into DNA per culture. Cell viability as determined by trypan blue dye exclusion was 82–88% at the end of culture.

The mitogens used included phytohemagglutinin-P (PHA-P, from Difco Laboratories, Detroit, Mich.) and Con A (from Calbiochem, San Diego, Calif.). Dose-response curves with PHA-P and Con A were performed in a previous study, and lymphocytes from all normal individuals tested responded strongly to the doses of each mitogen employed in the present experiments (15). When used, Con A was added to a final concentration of 5 μg/ml in the culture mixture. PHA-P was added from a pretested and standardized stock solution and when used, the cultures contained either 1 or 5 μl of the stock PHA-P solution per ml of culture mixture.

Allogeneic Cell Stimulation. Unidirectional mixed lymphocyte cultures were prepared by first incubating the stimulating lymphocytes at 2×10^6 cells/ml for 1 h at 37°C with 25 μg/ml

mitomycin-C (Sigma Chemical Co., St. Louis, Mo.), and then washing them three times with large volumes of RPMI 1640. Preliminary cultures were performed with unrelated donors, and the most reactive ratios of stimulator to responder cells (1:1 or 2:1; see Results, e.g. 2×10^5 of each kind of cells per 0.25 ml culture mixture) were used in the experiments. Bidirectional mixed lymphocyte cultures were performed with an equal number of lymphocytes from each donor, usually 2×10^5 cells from each donor per 0.25 ml of culture mixture. Cultures were carried out under the conditions described above; lipoproteins were added at the start of the culture in the amounts indicated in Results. After 3-5 days, the cultures were assayed for the extent of stimulation of the incorporation of [^{3}H]thymidine into DNA by the procedures described above.

Results

Effects of Lipoproteins on Allogeneic Cell Stimulation. A series of experiments was first conducted to compare the effects of three classes of normal plasma lipoproteins (VLDL, LDL, and HDL, isolated by sequential flotation at densities of 1.006, 1.063, and 1.21) on the stimulation of lymphocyte proliferation produced by allogeneic cells. Fig. 1 presents the results of an experiment comparing the effects of increasing concentrations of each lipoprotein on the unidirectional mixed lymphocyte reaction (MLR) in 10% type AB plasma. Addition of either LDL or VLDL markedly inhibited allogeneic cell stimulation. The LDL fraction produced the greatest inhibition, with 50% inhibition occurring at 50 μg LDL protein/ml, and complete inhibition of the MLR at 100 μg/ml. The VLDL fraction also markedly inhibited the MLR, with 60% inhibition occurring at 100 μg VLDL protein/ml and almost complete inhibition at 150 μg/ml. HDL also strongly inhibited the MLR, but only at much higher concentrations. In this experiment, inhibition of the MLR was not observed at 250 μg HDL protein/ml, but almost 90% inhibition was achieved at 500 μg/ml. Similar results were obtained in two other experiments using the same classes of lipoproteins from two other individuals added to lymphocytes cultured in type AB plasma, and in another three experiments using a completely autologous system with the lipoproteins, plasma, and lymphocytes originating from the same individual.

Since the type AB and autologous plasmas utilized in the above experiments contained lipoproteins, it was decided to perform all of the remaining experiments in LPDP, to eliminate the possibility of endogenous lipoproteins in the culture medium contributing to the effects of the exogenously added lipoproteins. Furthermore, since LDL (d = 1.006–1.063) produced the strongest inhibition of the MLR (see above), it was decided to fractionate the d = 1.006–1.063 lipoproteins into IDL (d = 1.006–1.019) and LDL (d = 1.019–1.063) fractions, and to study these separately in subsequent experiments. In addition, the lipoprotein fractions utilized in the subsequent experiments were generally prepared from a pooled plasma sample comprising plasma collected from three normal individuals, to minimize the possibility of minor unrecognized individual variations in lipoproteins with regard to their effects on cell proliferation.

Fig. 2 shows the effects of increasing concentrations of each of the four lipoprotein fractions on the bidirectional MLR, performed in LPDP. The IDL fraction produced the most marked inhibition of lymphocyte proliferation stimulated by allogeneic cells, with complete inhibition being observed at 10 μg IDL protein/ml. In fact, the incorporation of [^{3}H]thymidine into DNA in the presence of IDL at concentrations of 10–250 μg protein/ml was suppressed

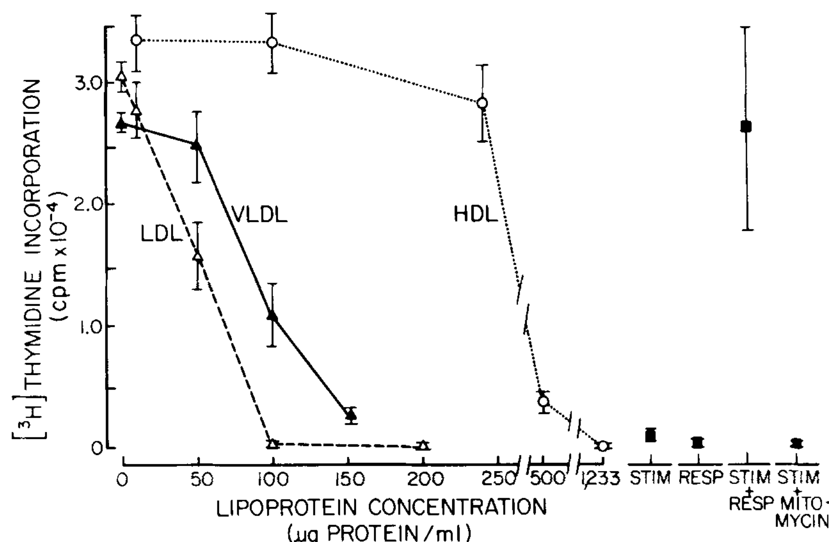

FIG. 1. The effects of lipoproteins on the unidirectional MLR. The VLDL, LDL (d = 1.006–1.063), and HDL fractions were all isolated from blood from one normal donor. Each 0.25-ml culture mixture contained 2×10^5 stimulator and 2×10^5 responder lymphocytes in 10% heat-treated type AB plasma (Materials and Methods). Lipoprotein concentrations are expressed as micrograms of lipoprotein protein per milliliter of culture, and [^{3}H]thymidine incorporation into DNA as the mean ± 1 SEM of triplicate cultures for each data point. The four control data points (■) represent the values seen without added lipoproteins, for the responder cells alone (RESP), the stimulator cells alone (before mitomycin treatment; STIM, and after; STIM + MITOMYCIN) and for the mixed stimulator (mitomycin treated) + responder cells (STIM + RESP).

below the values seen for the resting controls (unstimulated cells) of both donors. VLDL and LDL were both strongly inhibitory, although only at singificantly higher concentrations than IDL. VLDL did not inhibit allogeneic cell stimulation when added at 1–10 μg VLDL protein/ml. Approximately 80% inhibition occurred, however, at 50 μg VLDL protein/ml, and complete inhibition at 100 μg/ml. LDL produced nearly 90% inhibition at 150 μg/ml and suppression of [^{3}H]thymidine uptake into DNA below that of resting controls between 300 and 500 μg/ml. HDL was not inhibitory at concentrations up to 500 μg/ml, but at 3,000 μg HDL protein/ml, >90% inhibition was observed.

Similar findings were obtained in two experiments using a completely autologous unidirectional MLR, with lymphocytes, lipoproteins, and LPDP from the same individual, and also in another three experiments with bidirectional MLR using lipoproteins from pooled plasma and LPDP from pooled AB plasma. In all of these experiments, the relative inhibitory potency of the different lipoprotein fractions was always found to be IDL > VLDL > LDL ≫ HDL, and inhibition was observed at lipoprotein concentrations similar to those shown in Fig. 2.

Effects of Lipoproteins on Mitogen Stimulation. Experiments were also carried out to study the effects of lipoproteins on lectin-stimulated lymphocytes. Fig. 3 shows the effects of VLDL, IDL, LDL, and HDL, on PHA-P-stimulated lymphocytes cultured in LPDP. Each of the lipoprotein fractions was able to

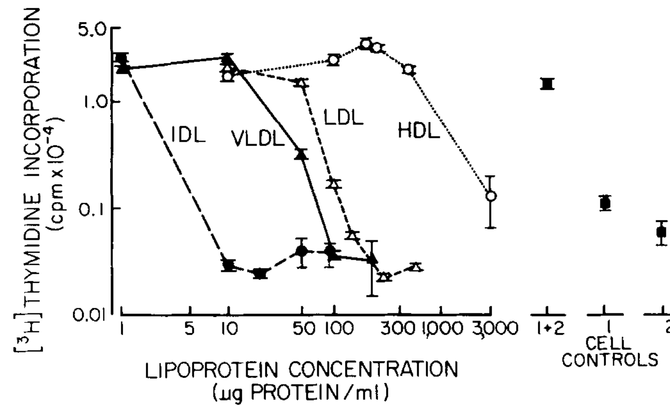

FIG. 2. The effects of VLDL, IDL, LDL, and HDL on the bidirectional MLR. Each 0.25-ml culture mixture contained 2×10^5 cells from each donor in 25% LPDP. The lipoprotein fractions were isolated from pooled plasma from three individuals. Lipoprotein concentrations are expressed as micrograms of lipoprotein protein per milliliter of culture and [^{3}H]thymidine incorporation into DNA as the mean ± SEM of triplicate cultures for each data point. The three control data points (■) represent the values seen without added lipoproteins for the cells from each donor cultured alone (CELL CONTROLS 1 and 2) and for the cells cultured together (1 + 2).

completely inhibit the mitogen-induced stimulation of lymphocyte proliferation. The rank order of inhibitory potency by the four lipoproteins was the same as that seen with the MLR. Thus, IDL was the strongest inhibitor, followed by VLDL, LDL, and finally HDL, which inhibited only at higher concentrations. IDL inhibited lectin stimulation >90% at 10 μg IDL protein/ml, with almost complete inhibition observed at 20 μg/ml. VLDL produced ≅75% inhibition at 10 μg VLDL protein/ml and complete inhibition at 50 μg/ml. LDL provided almost 90% inhibition at 50 μg LDL protein/ml, with complete inhibition at 100 μg/ml. HDL was not inhibitory at 100 μg HDL protein/ml; it did, however, produce >50% inhibition at 250 μg/ml, almost 90% inhibition at 500 μg/ml, and complete inhibition at 3,000 μg/ml. As with the MLR, the lipoproteins suppressed [^{3}H]thymidine incorporation into DNA below that of the resting control cultures (no PHA-P added) at the highest lipoprotein concentrations tested.

Similar results were obtained in three other experiments with PHA-P. One experiment used a completely autologous system of lymphocytes, lipoproteins, and autologous plasma, and the other two experiments used lipoproteins from pooled plasma and LPDP from pooled AB plasma.

Fig. 4 describes the effects of the same four lipoprotein fractions on lymphocytes stimulated by another lectin, Con A. With minor variations, the results were almost identical to those observed with PHA-P (see Fig. 3). The relative potency for inhibition of lectin-stimulated lymphocyte proliferation was, as before, IDL > VLDL > LDL ≫ HDL. Moreover, as in the above experiments, the highest concentrations of all of the lipoproteins suppressed [^{3}H]thymidine incorporation into DNA below the level seen with the resting control cultures.

Other Studies. HDL preparations isolated by a single ultracentrifugal flotation between densities 1.063 and 1.21 may be contaminated with small

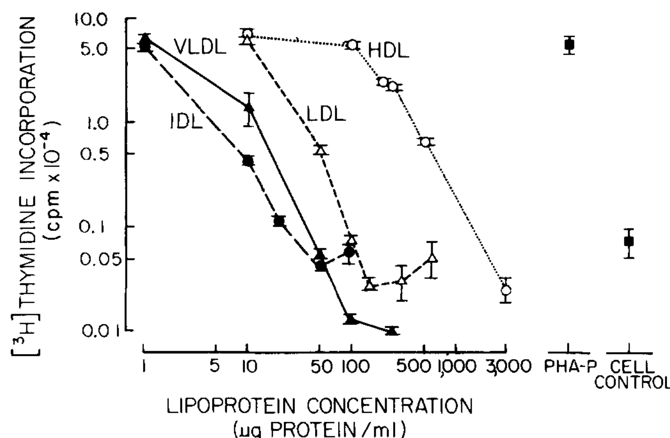

FIG. 3. The effects of VLDL, IDL, LDL, and HDL on PHA-P-stimulated lymphocytes. Each 0.25-ml culture mixture contained 2×10^5 lymphocytes in 25% LPDP. PHA-P was used at a concentration of 5 μl of the pretested stock solution/ml of culture. Isolated lipoprotein fractions, obtained from pooled plasma from three individuals, are expressed as micrograms of lipoprotein protein per milliliter of culture. The data represent mean ± SEM values for [^{3}H]thymidine incorporation into DNA for triplicate cultures for each point. The two control data points (■) represent the values seen without added lipoprotein for the cells alone (CELL CONTROL) or the cells + PHA-P.

amounts of LDL. The question was therefore raised whether or not the inhibitory effects of HDL, seen at relatively high concentrations in the above experiments (Figs. 1-4), might be largely the result of the presence of small amounts of contaminating LDL. To examine this question, lipoproteins were prepared from normal plasma by sequential ultracentrifugal flotation at densities of 1.006, 1.019, 1.063, 1.085, and 1.21. The HDL preparation isolated between densities of 1.085 and 1.21, and accordingly free of contaminating LDL, was then tested for its effects on stimulated lymphocytes. Two experiments were conducted in which the effects of VLDL ($d < 1.006$), LDL (d = 1.019-1.063), and HDL (d = 1.085-1.21) were examined on: (a) the bidirectional MLR, and (b) PHA-P-stimulated lymphocytes. The methods employed were similar to those described above for the experiments shown in Figs. 2 and 3. In both experiments, the HDL (d = 1.085-1.21) inhibited stimulated lymphocytes in a manner that was quantitatively similar to the inhibition previously seen with HDL (d = 1.063-1.21). In the lectin (PHA-P) experiment, the inhibitory effects seen at a given HDL concentration (d = 1.085-1.21) were quantitatively very similar to those shown for HDL in Fig. 3. In the bidirectional MLR experiment, inhibitory effects were seen at HDL concentrations (d = 1.085-1.21) somewhat lower than those which produced inhibition in the experiment shown in Fig. 2, and more similar to the HDL concentrations which produced inhibition in the experiment shown in Fig. 1. In these two experiments with HDL (d = 1.085-1.21), moreover, the effects of VLDL and LDL were quantitatively similar to those shown in Figs. 2 and 3. These experiments thus indicate that the inhibitory effects seen with HDL were not the result of the presence of small amounts of contaminating LDL.

Although it has not been extensively studied, the inhibition of lymphoprolif-

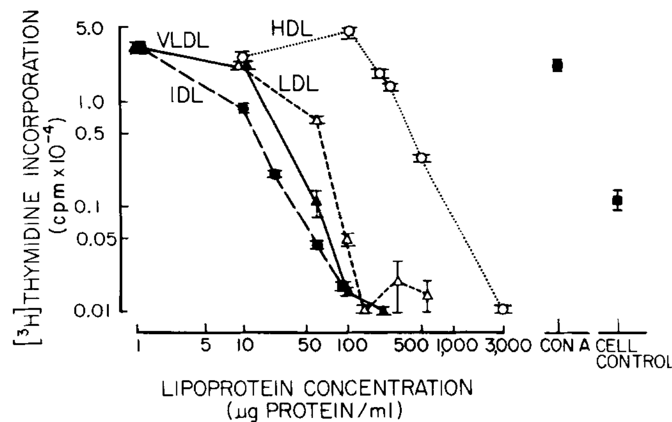

FIG. 4. The effects of VLDL, IDL, LDL, and HDL on Con A-stimulated lymphocytes. Each 0.25-ml culture mixture contained 2×10^5 lymphocytes in 25% LPDP. The lipoprotein fractions were obtained, and the data are expressed, as described in the legend to Fig. 3. The two control data points (■) represent the values seen without added lipoprotein for the cells alone (CELL CONTROL), or the cells + Con A.

eration by normal plasma lipoproteins could also be documented by examining the numbers of lymphoblasts present as well as by [^{3}H]thymidine incorporation. 50% inhibition of [^{3}H]thymidine incorporation by LDL of PHA-stimulated cultures also produced 50% inhibition of blast transformation when examined either directly by phase microscopy or by light microscope examination of Wright's stained smears.

Discussion

The results presented here demonstrate that all of the classes of normal plasma lipoproteins can strongly inhibit the proliferation of lymphocytes stimulated by allogeneic cells or lectins. Inhibition was observed when lipoproteins were added to lymphocytes cultured in autologous, heterologous, or LPDP. Generally similar degrees of inhibitory potency were observed with the different lipoproteins in all of the different experiments performed. In each of the experiments involving the four different lipoprotein classes, both for allogeneic cell and lectin stimulation, and for incubations containing whole plasma or LPDP, the rank order of inhibitory potency was always the same. Thus, in every instance, IDL was the strongest inhibitor, followed by VLDL, LDL, and HDL, which inhibited at higher concentrations. In addition, the concentration of a given lipoprotein required for inhibition was of a similar order of magnitude in the various experiments involving both allogeneic cell and phytohemagglutinin stimulation. Moreover, at relatively higher concentrations, the lipoproteins $d < 1.063$ (VLDL, IDL, and LDL) consistently (and in the case of HDL, often) suppressed the incorporation of [^{3}H]thymidine into DNA below the levels seen with resting, unstimulated control lymphocytes.

The potential physiological significance of these observations is illustrated in Table I, which summarizes the concentrations of each lipoprotein found to provide 50% and almost complete (>95%) inhibition of allogeneic cell stimulation: roughly similar concentrations of the lipoproteins also provided a similar

TABLE I
Lipoprotein Concentrations for Inhibition of Allogeneic Cell Stimulation, Compared to Normal Plasma Levels

LP class	LP density	Approximate LP levels to achieve inhibition of*		Normal LP levels in plasma*
		50%	>95%	
VLDL	<1.006	2–4	8–12	10–20
IDL	1.006–1.019	0.4–0.8	2–4	6–9
LDL	1.019–1.063	5–10	15–30	50–80
HDL	1.063–1.21	50–100	200–300	125–175

* Lipoprotein (LP) levels are expressed as milligrams of LP protein per deciliter. The representative normal plasma levels listed were estimated from the published values for normal levels of the plasma lipoproteins and lipoprotein lipids (11, 12).

order of magnitude of inhibition of lectin stimulation. The concentrations of VLDL, IDL, and LDL required for complete inhibition of stimulated lymphocyte proliferation were all considerably below the levels of each of these lipoproteins normally found in human plasma. In addition, the concentration of HDL required for 50–90% inhibition was in the range of HDL levels normally found in human plasma. These observations suggest that circulating lymphocytes may normally be highly suppressed by the combined effects of all of the different endogenous plasma lipoproteins, and that lipoproteins may, accordingly, play important roles in vivo in modulating lymphocyte functions and responses.

A puzzling but unexplained feature of these studies derives from the fact that effective lymphocyte stimulation, and lipoprotein inhibition of stimulated lymphocytes, was observed when lymphocytes were cultured in 10% whole plasma (Fig. 1). Significant inhibition of lymphocyte stimulation might have been anticipated in such experiments from the lipoproteins present in the 10% whole plasma. The basis for the apparent absence of such effects is not clear, and will require future study. It should be noted, however, that most of the experiments reported here were conducted with LPDP to avoid this problem.

The inhibition of stimulated human lymphocytes by VLDL and LDL has been observed by other investigators (2, 3), although the effects of these different lipoproteins were neither compared with each other, nor investigated in LPDP. Waddell et al. (3) reported that plasma from patients with type IV or V hyperlipoproteinemia inhibited both spontaneous and stimulated (by mitogens and antigens) incorporation of [^{3}H]thymidine by cultured mononuclear cells. The inhibitory effect was identified with the chylomicron and VLDL fractions isolated from plasma. Curtiss and Edgington (2) reported that LDL (d = 1.006–1.063), isolated from normal human serum by three different methods (ultracentrifugal flotation, affinity chromatography on Con A-Sepharose 4B, and polyanion precipitation) inhibited both mitogen and allogeneic cell-induced lymphocyte proliferation. A subfraction of LDL (LDL-In) was isolated and gave the greatest inhibition. LDL-In had a mean buoyant density of 1.055 g/ml, and was clearly distinguishable from the lipoprotein fraction

referred to as the T-lymphocyte, E-rosette inhibitory factor, previously studied by Chisari and Edgington (1). In more recent studies, intravenous injection of preparations of partially purified LDL-In strongly suppressed the in vivo mouse anti-sheep erythrocyte hemagglutination response (16). The activity of the partially purified preparations of LDL-In studied by these investigators was reported in terms of the amount of lipoprotein protein required for 50% inhibition of [^{3}H]thymidine uptake by PHA-stimulated human peripheral blood lymphocytes (2, 16); this degree of inhibition was usually achieved with 10-20 μg LDL-In protein/ml.

In the studies reported here, 50% inhibition of [^{3}H]thymidine uptake by PHA-stimulated lymphocytes was observed at IDL protein concentrations of 2-4 μg/ml. Thus, IDL appears to be a significantly more potent inhibitor of stimulated lymphoproliferation than the partially purified LDL-In preparations studied by Curtiss and colleagues (2, 16). Moreover, VLDL often produced 50% inhibition of PHA-stimulated lymphocytes at concentrations of 5-10 μg protein/ml (Fig. 3). These findings do not support the concept that there is a particular subspecies of LDL (i.e., LDL-In) that serves specifically as an immunoregulatory lipoprotein (2, 16). The results suggest instead, that all normal lipoproteins of $d < 1.063$, and particularly the IDL fraction, have potent immunosuppressive activity, and that all normal lipoproteins (including HDL) may play important immunoregulatory roles in vivo. There is also the broader issue of whether the effects of lipoproteins are restricted to lymphoid cells or whether lipoproteins might also influence the growth of other kinds of cells as well. For example, Leffert and Weinstein (17) have observed inhibition of DNA synthesis of regenerating liver cells by VLDL.

The studies reported here did not explore the mechanism of lipoprotein inhibition of lymphoproliferation, nor did they evaluate whether the lipoproteins interfered with lymphocytes, monocytes, or both. Mechanistically, it would be of interest to investigate whether or not the lipid or the apolipoprotein moiety of the lipoproteins are mainly responsible for the inhibition. Apolipoprotein B is common to all the lower density ($d < 1.063$) lipoproteins, representing the major apoprotein of LDL and also comprising ≅35% of VLDL protein (12, 18). The lower density lipoproteins ($d < 1.063$) also share the property of interacting strongly with polyanions and being readily precipitated by appropriate combinations of polyanion (e.g., heparin or dextran sulfate) and divalent cation (19, 20). Future studies will be required to explore whether these or other characteristics of the lipoproteins might be involved in the inhibitory phenomenon.

The experiments involving the MLR indicate that the lipoproteins must interact in some way with the cells in order to produce the observed inhibitory effects. Waddell et al. (3) have shown that [^{3}H]thymidine does not bind to VLDL and chylomicrons, and there is no reason to think that the labeled nucleotide might bind to any of the other lipoproteins. Lower density lipoproteins are, however, known to interact with certain (but not all) lectins, such as Con A (21, 22). The possibility of significant binding of PHA to VLDL and chylomicrons was evaluated by Waddell et al. (3) using ^{125}I-PHA. They found that the labeled lectin was not bound to the lipoproteins in appreciable

amounts. Curtiss and Edgington (2) also concluded that the possibility of LDL-In competing with cellular sites for lectin binding was unlikely. Most important, however, are the findings that the various lipoproteins strongly inhibited the MLR, since available data suggest that the cellular sites which bind Con A are not involved in the MLR (23).

The fact that lipoproteins can bind to lymphocytes and regulate cellular processes has been demonstrated by the elegant studies of Brown and Goldstein and their colleagues. These workers have shown that freshly isolated human lymphocytes (4) and long-term human lymphoid cell lines (24) have cell surface receptors for LDL that play a key role in the regulation of cholesterol and lipoprotein metabolism by the cells in a manner similar to that demonstrated in fibroblasts (25). Thus, it is possible that lipoprotein interaction with the lymphocyte surface might affect in some way the cell surface sites involved in the MLR or in lectin stimulation, and that this in turn might lead to the observed inhibition of lymphoproliferation.

One mechanism whereby lipoproteins might alter cell membrane characteristics is in influencing the composition of the membranes. Shinitzky and Inbar (26) have noted that lymphoma cells have decreased surface membrane cholesterol levels and microviscosity, compared to normal lymphocytes. Both the membrane microviscosity and cholesterol levels could be raised by incubation of lymphoma cells with lecithin-cholesterol (1:1) liposomes. More recently, it has been reported that the mitogenic response of human peripheral lymphocytes to lectins can be decreased by brief treatment of the cells with lecithin-cholesterol liposomes (27). Lipids have also been reported to affect immune functions in other systems. Specific inhibition of lectin and/or antigen-stimulated lymphocytes by polyunsaturated fatty acids and by prostaglandins has been reported (28, 29), and lipids are known to depress reticuloendothelial phagocytosis (30). As cited previously, Curtiss et al. (16) have shown suppression of the primary antibody response to a thymic-dependent antigen, sheep erythrocytes, by a species of LDL. However, patients with familial hyperlipidemia are not known to be clinically immunodeficient, and very little information is available pertaining to the regulation of other parameters of the immune response by lipoproteins other than the inhibition of in vitro-stimulated lymphocytes.

There is a growing list of naturally occurring circulating immunosuppressive factors which have recently been reviewed by Cooperband et al. (31). These substances are usually immunosuppressive at near physiologic concentrations, are not antigen-dependent, inhibit only lymphoid cells, and frequently lack species specificity. Most are assayed by inhibition of lymphoproliferation. The effects of the best characterized factors have, however, also been examined with regard to other important immune parameters, such as the ability to inhibit T-dependent and T-independent antibody formation in vivo and in vitro, as well as prolongation of homografts. Among the best characterized are an α-globulin (32), now called α-immunoregulatory globulin, and its peptide (33), C-reactive protein (34), the SAA serum factor associated with amyloidosis (35), and α-fetoprotein (36). Recently, plasmin cleavage of fibrinogen has been shown to produce low molecular weight degradation products capable of immunosuppression (37). The results reported here suggest that all of the

classes of normal plasma lipoproteins, but particularly those of $d < 1.063$, should also be considered as another group of naturally occurring immunosuppressive factors.

Summary

Lipoproteins, isolated by sequential flotation at densities 1.006, 1.019, 1.063, and 1.21, were examined for their ability to inhibit human lymphocytes stimulated by allogeneic cells and by lectins (phytohemagglutinin-P and concanavalin A). All the classes of normal plasma lipoproteins inhibited lymphoproliferation when peripheral blood lymphocytes were cultured in autologous, heterologous, or lipoprotein-deficient plasma ($d > 1.21$). The rank order of inhibitory potency was intermediate density lipoprotein (IDL) > very low density lipoproteins (VLDL) > low density lipoproteins (LDL) ≫ high density lipoproteins (HDL), regardless of the mode of stimulation. The concentrations of IDL, VLDL, and LDL required for complete inhibition of stimulated lymphoproliferation were considerably below the levels of each of these lipoproteins normally found in human plasma. In addition, the concentration of HDL required for 50–90% inhibition was in the range of HDL levels normally found in human plasma. Moreover, at relatively higher concentrations, lipoproteins suppressed the incorporation of [^{3}H]thymidine into DNA below the levels seen with resting, unstimulated lymphocytes. The results suggest that circulating lymphocytes may normally be highly suppressed by the combined effects of all the endogenous lipoproteins and that the lipoproteins may play important roles in vivo in modulating lymphocyte functions and responses.

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