EFFECTS OF LOW DENSITY LIPOPROTEINS ON LYMPHOCYTE STIMULATION

Jörg HAGMANN*, Ingrid WEILER and Ernst WAELTI
Institute of Pathology, University of Bern, CH-3000 Bern, Switzerland

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1. Introduction

The regulatory functions of low density lipoprotein (LDL) have become a major focus of interest, following the proposal that suppression of endogenous cholesterol synthesis and stimulation of cholesterol re-esterification by LDL is mediated by a specific, high affinity LDL receptor on human fibroblasts and lymphocytes [1]. It has been reported that LDL also suppresses certain lymphocyte activities, namely DNA synthesis after stimulation with mitogens or allogenic cells in vitro and the primary immune response of mice to sheep red blood cells in vivo [2–4]. In these experiments the dose–response curve of the suppressive effect was the same with all mitogens tested.

We examined the effect of LDL on lymphocytes stimulated with different mitogens more closely. We show here that low doses of human LDL enhance the stimulatory effect of certain mitogens and that the pattern of the response differs, depending on whether B- or T-specific mitogens are used.

2. Materials and methods

Week 8–10 male CBA mice were obtained from Bomholtgard, Denmark. Mitogens were purchased from the following companies: phytohemagglutinin (PHA) and lipopolysaccharide (LPS) from Difco (PHA-P no. 3110-57 and LPS from *S. typhosa*, no. 0901), pokeweed mitogen (PWM) from Seromed and Concanavalin A (ConA) from Sigma (no. C-2010). The medium RPMI 1640 was from Gibco and the tritiated thymidine ([^{3}H]TdR) from Amersham (TRK 120).

LDL was isolated by sequential ultracentrifugation by the method in [5] and stored in 0.15 M NaCl containing 0.05 mM EDTA at 4°C for short periods of time. All preparations were pure by immunodiffusion criteria. LDL protein was determined by the method in [6].

Human peripheral blood mononuclear cells from healthy volunteers were isolated on a Ficoll/Hypaque gradient. The washed cells were incubated at 10^6 cells/ml in 0.2 ml amounts in Falcon microtest II tissue culture plates (no. 3040) for 56 h at 37°C in an incubator containing 5% CO_2 in air. Final mitogen concentrations were 25 μg/ml PWM, 2 μg/ml ConA and PHA diluted 1:2000. [^{3}H]TdR (10 Ci/mM) 0.5 μCi were added 8 h before harvesting the cultures on a Skatron harvester.

Mouse lymphocytes were isolated from spleens by teasing and contaminating erythrocytes were lyzed with 0.83% NH_4Cl for 10 min. The washed mononuclear cells were incubated at 5×10^6 cells/ml in medium containing 5% human serum delipidated by ultracentrifugation [7]. LPS was used at 20 μg/ml. Further conditions were identical to the ones used for the human cells.

3. Results and discussion

In a first series of experiments human peripheral blood lymphocytes isolated on a Ficoll/Hypaque gradient were stimulated with PHA, ConA or PWM

* Present address: Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20014, USA

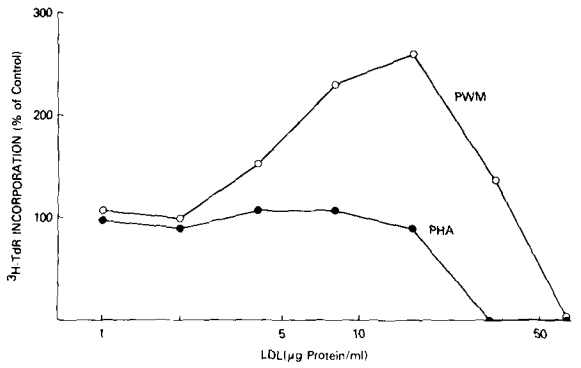

Fig.1. Effect of LDL on [^{3}H]TdR incorporation by human peripheral blood lymphocytes stimulated with PHA (•) or PWM (○).

at optimal concentrations in a serum-free microculture system. Various doses of LDL isolated from fresh human plasma were added at the beginning of the experiments, and the incorporation of [^{3}H]TdR was measured after an incubation period of 56 h by scintillation counting. Figure 1 shows that in the upper concentration range LDL was inhibitory, with doses leading to 50% inhibition (ID_{50}) ranging from 5–50 μg protein/ml in different experiments. However, at lower concentrations, LDL potentiated the effect of PWM. The extent of potentiation in different experiments varied from 1.5–3 times the values obtained in control cultures containing no LDL. With PHA (and ConA, data not shown), no potentiation or a very slight effect was observed. LDL alone, without added mitogens, inhibited the background incorporation of [^{3}H]TdR into lymphocytes at the higher concentrations. Parallel results were obtained when the labelling of lymphocytes with [^{3}H]TdR was determined autoradiographically or the formation of blast cells was determined morphologically. To exclude the possibility that inhibition was due to an interaction between mitogen and LDL, increasing mitogen concentrations were added to cultures containing a constant, inhibitory concentration of LDL. The inhibitory effect of LDL was not overcome by high concentrations of mitogen and, in the case of PHA, even enhanced (39% inhibition at a PHA dilution of 1:2000, 69% inhibition at a dilution of 1:400). It should be noted that viability, as determined by nigrosin exclusion, was not impaired at the LDL concentrations tested. Furthermore, identical results were obtained regardless of whether LDL was isolated by sequential ultracentrifugation or by precipitation with heparin, $MnCl_2$ and $MgCl_2$ [7].

It seemed that the pattern of the dose–response curve was due to two different effects, one inhibiting and one potentiating the stimulation of lymphocytes. Further experiments showed that storage of LDL at 4°C increased the inhibitory activity (ID_{50}<1 μg/ml) and abolished the potentiating effect (table 1). In addition, stored LDL caused the dose–response curves for PHA and PWM stimulation to become superimposable.

Since PHA and ConA are largely T cell-specific mitogens, and PWM is considered to stimulate both T and B lymphocytes, we examined the effect of LDL in cultures incubated with a B cell mitogen. Mouse splenic lymphocytes were stimulated with LPS or

Table 1
Effect of storage on the inhibitory activity of LDL

LDL	PHA			PWM		
(μg protein/ml)	cpm	SD[b]	% of control	cpm	SD	% of control
0 (control)	10 210	548	100	4903	381	100
1 μg fresh	11 351	932	111.2	4255	536	86.8
stored[a]	2522	400	24.7	1395	38	28.5
8 μg fresh	12 698	1057	124.4	11 270	1255	229.9
stored	406	136	4.4	196	43	4.0

[a] Stored for 2 months in 0.15 M NaCl containing 0.05 mM EDTA
[b] Standard deviation

The background incorporation of [^{3}H]TdR was 202 ± 81 cpm/well

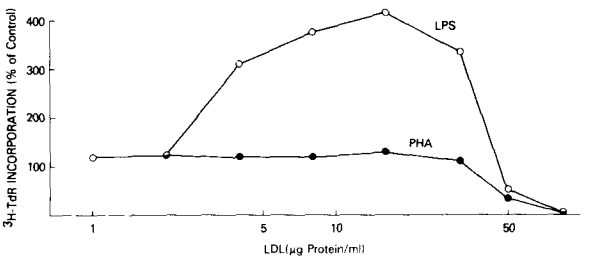

Fig.2. Effect of LDL on [^{3}H]TdR incorporation by mouse spleen lymphocytes stimulated with PHA (•) or LPS (○).

PHA in medium containing 5% lipoprotein-deficient human serum. Figure 2 shows the considerable potentiating effect of low doses of LDL on LPS-stimulated cells, whereas the inhibitory effect is more prominent in PHA-stimulated cultures. It should be noted that the proportion of B lymphocytes in mouse spleen is much higher (~40%) than in peripheral human blood (~10%), and that LPS, unlike PWM, is a mitogen specific for B cells. It thus seems that the potentiating effect of LDL regards the B cells. In this case B cells could be affected directly by LDL, or their stimulation could be enhanced by a preferential inhibition of T suppressor cells, as suggested [8]. This latter hypothesis could explain the results obtained with PWM-stimulated cultures. However, it cannot easily account for the findings in cultures stimulated with LPS.

Results of reports [2,3] on the effect of LDL on human lymphocyte stimulation differ from ours in the following respects:

(i) LDL concentrations needed for inhibition were higher;
(ii) No potentiation was observed;
(iii) Dose–response curves obtained with PHA- and PWM-stimulated lymphocytes were identical.

Some of these differences might be explained by differing culture conditions. For example, using 10% fetal calf serum in our cultures, we also observed a shift of the LDL dose–response curve to the right. Moreover, the potentiating effect of low doses of LDL was not detectable under these conditions (data not shown).

The question as to the active component(s) of LDL and the mechanisms of inhibition and potentiation remains open. Most studies of the inhibitory effect were directed to the apoproteins. However, our preliminary results have shown that inhibitory activity is also found in preparations of very low density lipoprotein and high density lipoprotein. A lipid component of liporoteins should therefore not be excluded as the responsible agent. Possibly oxygenated cholesterol derivatives are involved, some of which have been shown to suppress 3-hydroxy-3-methyl glutaryl CoA reductase, endogenous cholesterol synthesis and DNA synthesis in stimulated lymphocytes [9]. Such oxygenated cholesterol derivatives could also account for the increased inhibitory activity of stored LDL.

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