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Lipoprotein metabolism and NK cell function

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ABSTRACT

Several evidences suggest the importance of the immune system in the genesis and progression of atheromas; however, few reports have dealt with the effect of lipoprotein metabolism on leukocyte function. Here, we discuss the interactions between lipoprotein metabolism and NK (CD3-CD16+CD56+) cells and provide new hypothesis to ascertain the role of these cells in the genesis of coronary disease.

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INTRODUCTION

Lipoprotein anabolism and catabolism are involved in a complex array of different pathways of intermediary metabolism. The quantification of the different normal lipoproteins: chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) has been widely used to study lipoprotein metabolism. The absence or the abnormal accumulation of a specific lipoprotein is defined as a dyslipemia. Generally, the dyslipemias, classified according to Frederickson, are related either to an abnormal triglyceride metabolism, mainly regulated by

lipoprotein lipase (LPL), or to an anomalous cholesterol homeostasis regulated by the 3-hydroxy-methyl-glutaryl CoA reductase (HMGCoAR), the lecithin cholesterol acyl-transferase (LCAT) and lipoprotein receptors. Thus, a broad picture of lipoprotein metabolism may be assessed studying the expression, activity and function of key enzymes and receptors. Brown and Goldstein as well as other authors [1-4] have shown that the hypercholesterolemia, due to an impaired uptake of LDL, produced the accumulation of this lipoprotein that subsequently undergoes oxidative and chemical modifications. This modified LDL, not recognisable by normal LDL receptors, may accumulate in the arterial intima and subsequently induce the formation of an atheroma [1-4].

Different hypotheses have been proposed to explain the genesis of atheromas [1-4]. Several researchers have proposed that leukocytes are responsible for the formation of the lesion. Leukocytes, directly or indirectly, are involved in the chemical modification lipoproteins and in the removal of remnants [1-4]. In animals and in human transplant recipients, various evidences have shown that immunosuppressive therapy may prevent atheroma formation [3]. There is no clear connection, however, between the formation and the removal of a fatty streak and the generation of an inflammatory reaction that may evolve into an atheroma. Then, what is the relationship between lipoprotein metabolism and immune response?

Lipoprotein lipase (LPL) and atherosclerosis

The importance of LPL in the genesis of atherosclerosis has been widely suggested. Zilversmith [5] was the first to propose a relationship among enzyme activity, lipoprotein remnants and arteriosclerosis. Different groups have found high levels of LPL and its mRNA in atherosclerotic lesions [6-7]; however, a clear definition of the cell type responsible of this increased amount of LPL in the lesion has not been encountered. A general consensus is that LPL is secreted by adipose cells and

incorporated on the extracellular matrix (rich in heparan sulphate) of arterial endothelial cells [8]. LPL is then released by its affinity to triglyceride rich lipoproteins [8]. Nevertheless, LPL is transcribed and secreted by different cell types and tissues suggesting that this enzyme is ubiquitous and its presence in the lesion may be independent of the enzyme produced by adipose tissue.

Renier *et al.* [9] showed that peritoneal macrophages from mice susceptible to develop atherosclerosis transcribe and secrete higher quantities of LPL in comparison to their resistant counterparts, suggesting that LPL may be responsible for strain's susceptibility. Likewise, the addition of LPL to a murine macrophage cell line induced the transcription and secretion of TNF α without altering IL-1 β gene expression [10]. This effect, mediated by protein kinase C (PKC) activation [11], suggests that LPL, by interacting with its receptor, induces a proinflammatory event which may be crucial in the genesis of an atheroma. A marked decrease in LPL production, and consequently in the number of arteriosclerotic lesions, was observed in mice of the susceptible strain fed with a polyunsaturated fatty acid diet [12]. These results lead us to postulate that lipoproteins may play a role in the regulation of LPL transcription and secretion.

Macrophages are not the only leukocytes that produce LPL. We found that LPL is transcribed, expressed and secreted by human NK cells [13]. Anti-LPL was shown to inhibit NK cytotoxicity against K562 cells [13] and IL-2 activation produced the release of LPL overcoming the effect of anti-LPL [13-14]. However, addition of LPL to NK cultured cells promoted proliferative, hampered NK cytotoxic responses [14], induced CD25 expression and the secretion of IFNγ, IL-2, IL-8, TNFα while decreasing GM-CSF and not affecting IL-1 and LIF secretion (table 1).

In addition, we found that LPL was also able to induce the translocation of PKC from cytosol to the membrane particulate fraction. LPL binds to three

EFFECT OF LPL ON NK PROLIFERATIVE, CYTOTOXIC, ACTIVATION AND SECRETION OF CYTOKINES.

[LPL]	PRO.	CYT.	CD69	CD25	CYTOKINE SECRETION	
					INCREASE	DECREASE OR NO CHANGE
0.1 μg/ml	↑	↓	-	-	IFNγ, IL-2, TNFα	GM-CSF, IL-8, IL-1α, IL-1β, LIF,
1 μg/ml	↑↑	↓↓	-	↑	IFNγ, IL-2, IL-8, TNFα	GM-CSF, IL-1α, IL-1β, LIF

TABLE 1.

The effect of LPL on NK cell is depicted in the table. The effects are represented by: increase (↑), decrease (↓) or no change (-) in the different physiological responses and markers assessed. The cytokines that appear in the table follow a sequence, the most prevalent is the first and the least prevalent is the last. The words PRO and CYTO refer to proliferative response and cytotoxicity respectively.

different proteins on NK membrane. One of them, 58 kDa protein, belongs to the family of killing inhibitory receptors (KIR) [14-15]. In a physiological condition, it may be suggested that LPL expression on the endothelial cell surface (along with glycosaminoglycans and heparan sulphate proteoglycans) may protect this cell from NK cytotoxicity. An inflammatory reaction, on the contrary, may activate heparanase and other enzymes, which are able to degrade glycosaminoglycans and proteoglycans from the cell surface, as postulated by Lider *et al.* [16], releasing LPL and exposing endothelial cells to NK damage.

EXPRESSION AND BINDING OF LPL ON NON-STIMULATED AND IL-2 STIMULATED NK CELLS.

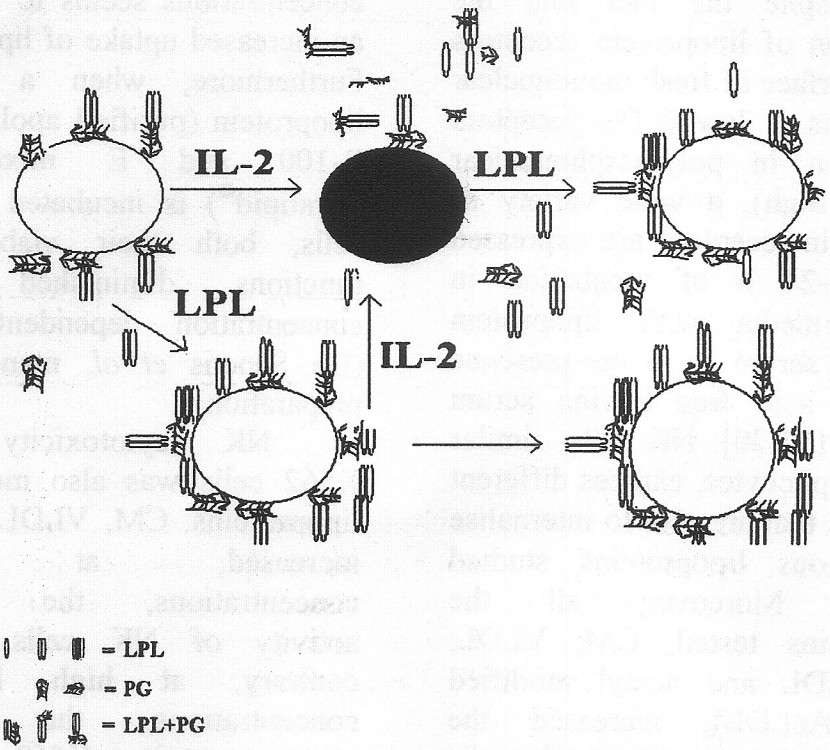

Fig. 1

Expression and binding of LPL to non-stimulated and IL-2 stimulated cells.
Non-stimulated NK cells express LPL in their surface along with proteoglycans (PG). When the cells are stimulated with IL-2, LPL is released from the cell surface, it is highly cytotoxic and its proliferative response is enhanced (cell drawn in black). If this activated cell is incubated with LPL, the proliferative response is potentiated and the cytotoxic response is hampered. Similarly, if the non-stimulated cells are incubated with LPL (bottom of the figure), the cells proliferate more avidly and their cytotoxic response diminishes as the numbers of LPL receptors are occupied. IL-2 can release these newly bound LPL in a similar fashion as above (not shown in the figure).

Lipoprotein receptors and NK function

Despite the fact that the expression of lipoprotein receptors on the surface of fresh mononuclear leukocytes is low (LDL receptors expression in polymorphonuclear cells is high), a wide variety of lipoprotein receptors are expressed after 18-24 h of incubation in culture media with lipoprotein deficient serum or in the presence of fatty acid free bovine serum albumin [17-20]. NK cells, similar to T lymphocytes, express different receptors that are able to internalise the various lipoproteins studied [17-19]. Moreover, all the lipoproteins tested, CM, VLDL, LDL, HDL and acetyl modified LDL (AcLDL), increased the proliferative response of NK cells [17-18] at optimal concentrations (table 2). At high concentrations of protein (80 to 150 μg/ml depending on the lipoprotein tested), most of the lipoproteins inhibited cell growth and induced cell apoptosis. Experiments performed with a lipid emulsion (Intralipid®) revealed that cells are viable and functional in culture with concentrations up to 3 % of emulsified lipid. Therefore, the dramatic decrease in cell viability at high lipoprotein concentrations seems to be due to an increased uptake of lipoproteins. Furthermore, when a synthetic lipoprotein (purified apolipoprotein B-100 and E mixed with Intralipid®) is incubated with NK cells, both their viability and functions diminished in a concentration dependent manner (De Sanctis *et al.* manuscript in preparation).

NK cytotoxicity against K562 cells was also modified by lipoproteins. CM, VLDL and LDL increased, at optimal concentrations, the cytotoxic activity of NK cells. On the contrary, at high lipoprotein concentrations, the cytotoxic response against K562 cells was impaired [17]. HDL and AcLDL inhibited, at every concentration tested, the cytotoxic activity of NK cells [17]. This effect could not be diminished by the addition of other lipoproteins. Interestingly, treatment with IL-2 was able to partially suppress the inhibitory effect of HDL and AcLDL.

Since several transcriptional, post-transcriptional and post-

EFFECT OF LIPOPROTEINS ON NK PROLIFERATIVE, CYTOTOXIC, ACTIVATION AND SECRETION OF CYTOKINES.

	PRO.	CYT.	CD69	CD25	CYTOKINE SECRETION	
					INCREASE	DECREASE OR NO CHANGE
CM (10 μg/ml)	↑	↑	↑	↑	LIF, IL-8, TNFα, IL-1α, IFNγ, IL-2	GM-CSF, IL-1β
VLDL (20 μg/ml)	↑	↑	↑	↑	LIF, IL-8, IL-2, IL-1α, TNFα, IFNγ	GM-CSF, IL-1β
LDL (20 μg/ml)	↑	↑	↑↑	↑↑	LIF, IL-8, IL-1α, IFNγ, IL-2	GM-CSF, IL-1β, TNFα
HDL (20 μg/ml)	↑↑	↓	-	↑↑	IL-2, IL-8, IFNγ,	GM-CSF, IL-1α, IL-1β, TNFα, LIF
AcLDL (20 μg/ml)	↑↑	↓	-	↑	LIF, IL-8, TNFα, IL-1α, IL-2, IFNγ, IL-1β	GM-CSF

TABLE 2.

The effect of lipoproteins on NK cell is depicted in the table. The effects are represented by: increase (↑), decrease (↓) or no change (-) in the different physiological responses and markers assessed. The cytokines that appear in the table follow a sequence, the most prevalent is the first and the least prevalent is the last (for complete information see references 23, 31). The words PRO and CYTO refer to proliferative response and cytotoxicity respectively.

translational mechanisms are involved in the control of the different lipoprotein receptors [21-22], it has been speculated that their expression is enhanced upon cell activation. IL-2, an activator of NK physiological responses, promoted in a dose dependent fashion, an increase in the internalisation of various lipoproteins and enhanced the proliferative response of T lymphocytes and NK cells [18-19]. This cytokine potentiated the proliferative response induced by lipoproteins in a dose dependent fashion. It has been suggested that IL-2 activation promotes cell metabolism, which in turn enhances the uptake and degradation of lipoproteins.

In order to understand the effect of IL-2 on lipoprotein receptors, NK cells were incubated, with different concentrations of IL-2 in the absence of lipoproteins. The stimulation with IL-2 did not affect LDL receptor transcription [19]. Instead, IL-2 promoted the membrane expression of the cytoplasmic pool of LDL receptors [19], suggesting that post-translational controls are crucial for the expression of these receptors in this particular cell type. The effect of LDL on NK cytotoxicity was not observed upon IL-2 stimulation [19]. It is possible that either both types of receptors activate a similar pathway or that an autocrine secretion of IL-2 is responsible for the effect observed.

Analysis of the expression cell activation markers, CD69 (early) and CD25 (late), provided the first evidence that different pathways are responsible for lipoprotein induced NK cell activation. CD69 (dependent of PKC activation) and CD25 (a non-specific marker for cell activation and division expression) were found to be enhanced depending on the lipoprotein used (table 2). CD69 expression was enhanced most efficiently by an optimal concentration of LDL and to a lesser extent by VLDL and CM. HDL and AcLDL did not enhance CD69 expression. On the contrary, CD25 expression was efficiently enhanced by optimal concentrations of HDL and LDL and to a lesser extent by CM, AcLDL and VLDL. At optimal concentration, the expression of cell surface markers correlated

EXPRESSION OF LIPOPROTEIN RECEPTORS IN NON-STIMULATED AND IL-2 STIMULATED NK CELLS.

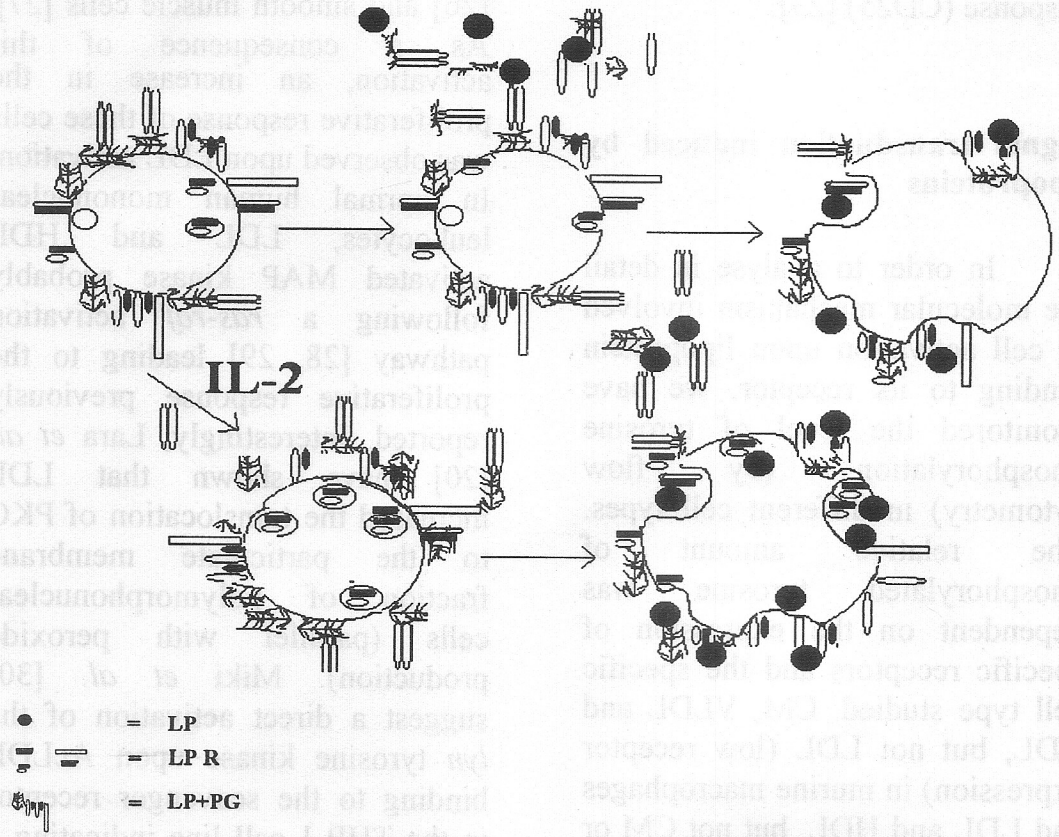

Fig. 2

Expression of lipoprotein receptors on non-stimulated and IL-2 stimulated NK cells.
Non-stimulated NK cells express a wide variety of lipoprotein (LP) receptors (LP R). These receptors internalize lipoproteins either by directly interacting with their ligand or by complex formed with proteoglycans (LP+PG) and complex of proteoglycans and LPL (LPL+PG) as illustrated in the previous figure. When the cells are stimulated with IL-2, NK cells express more lipoprotein receptors which in turn internalize more lipoprotein (bottom part of the figure).

with NK cell cytotoxicity (CD69) and T and NK proliferative response (CD25) [23].

Signal transduction induced by lipoproteins

In order to analyse in detail the molecular mechanism involved in cell activation upon lipoprotein binding to its receptor, we have monitored the level of tyrosine phosphorylation (by flow cytometry) in different cell types. The relative amount of phosphorylated tyrosine was dependent on the expression of specific receptors and the specific cell type studied. CM, VLDL and HDL, but not LDL (low receptor expression) in murine macrophages and LDL and HDL, but not CM or VLDL in K562 cells increased the amount of phosphorylated tyrosine by twofold [24]. On the contrary, an increase in phosphorylated tyrosine is observed in NK cells stimulated with most lipoproteins except AcLDL [25].

LDL activation was shown to induce the phosphorylation of the mitogen activated protein kinase (MAP) in the U937 cell line [26] and smooth muscle cells [27]. As a consequence of this activation, an increase in the proliferative response of these cells was observed upon LDL activation. In normal human mononuclear leukocytes, LDL and HDL activated MAP kinase probably following a *ras-raf* activation pathway [28, 29] leading to the proliferative response previously reported. Interestingly, Lara *et al.* [20] have shown that LDL increased the translocation of PKC to the particulate membrane fraction of polymorphonuclear cells (parallel with peroxide production). Miki *et al.* [30] suggest a direct activation of the *lyn* tyrosine kinase upon AcLDL binding to the scavenger receptor in the THP-1 cell line indicating a direct involvement of the kinase with the receptor. These reports, along with other preliminary results of our laboratory, leads us to postulate that Janus kinases (JAK) and inositol kinases, besides PKC, MAP and *lyn* kinases, are involved in lipoprotein induced signal transduction.

Lipoproteins and cytokine secretion

Most recently, we have evaluated the effect of lipoproteins on NK cytokine secretion (table 2) [31]. We have found that NK cells produced high amounts of IL-2 and IFN γ upon lipoprotein stimulation. Secretion of high amounts of IL-8 and LIF was also observed without major effects on TNF α, IL-1α and IL-1 β. GM-CSF secretion was markedly decreased. The increase in IL-8 secretion by NK cells parallels the effect reported in human macrophages loaded with cholesterol and in foam cells [32]. The sustained increase in IL-8 secretion by macrophages and NK cells activated with lipoproteins suppose that this inflammatory cytokine might be important in the attraction of cells in the inflammatory lesion. In general, lipoprotein stimulus may selectively activate NK cells and this activation may promote important autocrine and paracrine networks crucial for immune response [31]. It is suggested that the lipoprotein stimulus may drive NK cells towards cellular immunity instead of potentiating bone marrow cell differentiation [31].

Physiological and clinical implications of lipoprotein metabolism on NK function.

Do the effects of lipoproteins and LPL on NK cells *in vitro* resemble an *in vivo* response? Kurzer *et al.* [33] and Sedman *et al.* [34] have shown that triglyceride infusion increased peripheral blood NK cytotoxic activity. These increases seem to be dependent on cell's metabolic regulation. An impaired cell metabolism may explain the diminished NK cytotoxicity reported in cancer and virus infected patient's [35]. Likewise, the high cytotoxic response observed in human hepatic NK cells as compared to peripheral blood; [35] could be influenced by lipoproteins, which are anabolized and catabolized, by this organ. In hepatitis C virus infection, Corado *et al.* [36] have shown that NK cytotoxicity is impaired in these patients despite a normal T cell response; this impairment may be due to the uptake of virus complexed to lipoproteins [37]. Thus, lipoproteins may influence NK cell responses *in vivo*.

In cardiac transplant patients treated with Pravastatin® (an inhibitor of the HMGCoAR, a key enzyme in cholesterol synthesis), Kobashigawa *et al.* [38] have shown an increased life expectancy in comparison to controls. NK cytotoxic activity was inversely correlated with patient's survival. It can be proposed that a diminished NK cytotoxic activity may protect these patients from transplant associated atherosclerosis. Thus, the use of cyclosporine along with Pravastatin® may prolong transplant patient's survival.

All the evidence presented here lead us to suggest that LPL and lipoproteins modulate NK physiological responses. Furthermore, one may envision that, through several mechanisms, NK cells may be involved in the genesis of atherosclerosis: 1) viral or other factors that produce morphological modifications along with a decreased LPL expression may predispose endothelial cells to NK lysis, 2) lipoproteins may prime NK cells making them more responsive to a normal physiological stimulus and 3) the secretion of cytokines upon lipoprotein activation may condition the progression of the atheroma. These hypotheses opened a new area of research in coronary diseases and confirmed the importance of lipoprotein metabolism on leukocyte's function.

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