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Secretion of cytokines by natural killer cells primed with interleukin-2 and stimulated with different lipoproteins

J. B. DE SANCTIS, I. BLANCA & N. E. BIANCO *Institute of Immunology, Central University of Venezuela, Caracas, Venezuela*

SUMMARY

Natural killer (NK) cells were shown to secrete differentially interleukins (IL), IL-1α, IL-1β, IL-2, IL-8, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), granulocyte–macrophage colony-stimulating factor (GM-CSF), and leukaemia inhibitory factor (LIF) upon stimulation with optimal concentrations of chylomicrons (CM), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL) or acetyl-modified low-density lipoprotein (AcLDL). CM, VLDL, LDL and AcLDL induced LIF secretion which was absent in non-stimulated cells. CM, VLDL, and LDL did not affect IL-1α secretion. CM stimulated IL-8 > TNF-α > IL-1α > IL-2 = IFN-γ, and decreased seventeenfold GM-CSF secretion. VLDL stimulated IL-8 secretion > IL-1α = IL-2 > IFN-γ > TNF-α and decreased fivefold GM-CSF secretion. LDL stimulated IL-8 secretion > IL-1α > IL-2 = IFN-γ, it did not modify TNF-α and inhibited five hundredfold GM-CSF secretion. HDL stimulated IL-2 secretion = IFN-γ > IL-8, it decreased GM-CSF secretion > IL-1α > IL-1β > TNF-α without affecting LIF. AcLDL stimulated IL-8 secretion > TNF-α > IL-1α > IL-2 = IFN-γ = IL-1β, and decreased GM-CSF secretion eightfold. When NK cells were primed with 10, 100 or 500 IU/ml of IL-2 before the addition of lipoproteins, a decrease in the secretion of cytokines was observed as compared with cells primed with IL-2 only. Differences in cytokine secretion were observed among the diverse type of lipoproteins used for cell stimulus. Thus, lipoproteins may condition NK cytokine secretion and cell activation.

INTRODUCTION

Lipoproteins, chylomicrons (CM), very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) have always been considered important for cell and tissue physiology although the deregulation of their metabolic control may be the cause of vascular pathologies.

Macrophages and T lymphocytes have been involved in the formation of the atheroma. It has been suggested that cell involvement depends upon the expression of different lipoprotein receptors.[1] Numerous receptors for the different native and modified lipoproteins have been shown to be present in macrophages, scavenger receptors,[1] LDL receptors,[2] VLDL receptors,[3] the α_2 macroglobulin/LDL receptor (α_2MR/LRP),[3] CD36,[4] HDL receptors,[5] and Fc receptors.[6] These various types of receptors seem to be regulated by cell metabolism and in consequence regulate different cellular mechanisms. Despite abundant reports on these receptors, little is known about the influence of lipoproteins on cytokine production and secretion.

Interleukin 2 (IL-2) is a cytokine secreted by T and natural killer (NK) cells which generates a broad range of effects in different leukocytes.[7] Both tumoricidal activity and proliferation of NK cells are enhanced following stimulation with IL-2.[8,9] This cytokine has been shown to affect lipoprotein metabolism and lipoprotein receptor expression in different cells.[10,11]

Recently, De Sanctis *et al.*,[10–12] have shown that NK cells express lipoprotein receptors and that these receptors modulate proliferative and spontaneous cytotoxicity of NK cells. In addition, IL-2 induced an increase in the expression of these receptors,[10–11] and in the case of LDLR, this increment was not due to an increase in mRNA, but of protein sorting.[11] Based upon these experiments, the aim of the study was to assess the effect of the different lipoproteins on the secretion of several cytokines in non-stimulated and IL-2 stimulated NK cells.

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Abbreviations: CM, chylomicrons; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-2, interleukin-2; IFN-γ, interferon-γ; TNF α, tumour necrosis factor-α; IL-8, interleukin-8; GM-CSF, granulocyte–macrophage colony-stimulating factor; LIF, leukaemia inhibiting factor; NK, natural killer cells.

Correspondence: Dr Juan B. De Sanctis, Instituto de Inmunología, Aerocav Care off. #1216, PO Box #02–5304. Miami, Fl 33102–5304, USA.

MATERIALS AND METHODS

Chemicals and antibodies

Recombinant human interleukin 2 (rhIL-2) was kindly donated by Dr Craig Reynolds from the Biological Response Modifiers

Program, National Cancer Institute (Frederick, MD), fetal calf serum (FCS), L-glutamine, penicillin–streptomycin and RPMI-1640 medium were purchased from Gibco BRL (Gaithersburg, Md). Percoll and Ficoll-paque® was purchased from Pharmacia LKB (Uppsala, Sweden). Anti-Leu11c-phycoerythrin (PE) (CD16) was purchased from Becton Dickinson (Mountain View, Ca); NKH-1 RD1 (CD56), unlabelled CD3, CD3–fluoroscein isothiocyanate (FITC) and CD14–FITC antibodies were purchased from Coulter Immunology (Hialeah, Fl). All other reagents were acquired from Sigma Chemical Co. (St. Louis, Mo).

Lipoprotein purification

All lipoproteins, chylomicrons (CM) VLDL, LDL and HDL, were purified according to the method of Havel.[13] Human plasma from healthy donors was centrifuged at 114 000 ***g*** for 20 min at 16° in the presence of inhibitors of lipoprotein oxidation (1 mM butylhydroxytoluene, BHT, 2 mM reduced gluthatione, 5 mM ascorbic acid and 5 mM ethylene diamine tetra-acetic acid (EDTA)) in order to separate chylomicrons from the plasma. CM were subsequently washed using a discontinuous gradient (0·9% NaCl on the top and CM–KBr at the bottom) and centrifuged as described above. The remaining plasma was used to separate VLDL by centrifuging for 20 hr 114 000 ***g*** at 16° and the purified fraction was washed with discontinuous gradients as described previously. LDL was purified adjusting the plasma density to 1·063 g/ml, centrifuged and cleaned as described for CM. The plasma that was left was adjusted to 1·2 g/ml to purify HDL and centrifuged and cleaned as described for CM. All lipoproteins were dialysed extensively against phosphate-buffered saline (PBS)–BHT–EDTA and, before the assays, against PBS alone. No oxidative intermediates were detected in the purified fractions using the thiobarbituric acid (TBARS) assay.[14]

Lipoprotein acetylation

LDL was acetylated using acetic anhydride as described previously by Basu *et al.*[15] Briefly, the purified LDL fractions (16 mg/ml) were dialysed and then an equivalent volume of saturated sodium acetate was added with continuous stirring in an iced water bath. Acetic anhydride was added in small quantities up to an amount equal to 1·5 times of the mass of protein. After 30 min of mixing, the modified lipoprotein was dialysed against PBS, filter sterilized and subsequently incubated with the cells. The electrophoretic mobility of this fraction was different from that of unmodified LDL (results not shown).

Cell purification

Blood samples were taken from normal healthy donors (blood bank of the Central University Hospital, Caracus, Venezuela). Human large granular lymphocytes (LGL) were separated by passage of the non-adherent mixed population of cells through nylon wool, subsequent centrifugation on Percoll gradients and anti-CD3 monoclonal antibody plus complement treatment to deplete $CD3^+$ cells, as described previously.[16]

The purified fraction, assessed for CD16 and CD56 positivity in an EPICS 753 flow cytometer (Coulter Corporation, Hialeah, FL), as described previously,[11] was >85% CD16–CD56, <2% CD3 and <1% CD14. The purified NK cells were then cultured for 18 hr in RPMI-1640 medium supplemented with 0·5% bovine serum albumin (BSA; fatty acid free (RPMI–BSA)) in the presence of different concentrations of IL-2. After the incubation, cell viability was >90%, as determined by trypan blue exclusion.

Lipoprotein stimulation of NK cells

After the 18 hr incubation described above, the cells were washed twice with PBS, adjusted to 1×10^6 cells/ml in RPMI–BSA and then incubated in the presence of 20 μg/ml of lipoprotein for another 18 hr. This concentration of lipoprotein (represented in amount of protein/ml) was previously shown to be the optimal concentration for proliferative and cytotoxic responses observed for most lipoproteins.[12]

The same supernatant was assayed for the different cytokines.

Cytokine quantification by enzyme-linked immunosorbent assay (ELISA)

The cytokines were quantified with the commercial ELISA kits following the manufacturer's instructions. IL-2 and interferon-γ (IFN-γ) were quantified with Intertest-2x® and Intertest γ® from Genzyme Corporation (Cambridge, MA). Tumour necrosis factor-α (TNF-α), IL-1α, IL-1β, IL-8, granulocyte–macrophage colony-stimulating factor (GM-CSF) and leukemia inhibitory factor (LIF) were quantified using Quantikine® from R&D systems (Minneapolis, MN).

No differences were observed when standard curves were performed with RPMI–BSA alone or RPMI–BSA with the different lipoproteins. Lipoproteins, at this concentration of 20 μg/ml did not affect cytokine determination using this assay.

Statistical analysis

ANOVA, and paired Student's *t*-test were employed in order to analyse the different set of experiments.

RESULTS

Figure 1 illustrates an example of the cell population used in this study. The purified cell population was assessed for both markers CD16–FITC and CD56–RD (86% positiveness for both markers). The cytotoxic activity of these cells was published previously.[12]

The effect of the different lipoproteins on the secretion of IL-1α and β is depicted in Fig. 2. In Fig. 2a, acetyl modified LDL (AcLDL) significantly increased the secretion of IL-1α in 100 IU IL-2 ($P<0·05$) and 500 IU IL-2 ($P<0·005$) primed cells. In contrast, HDL in non-stimulated cells and CM in 500 IU of IL-2 primed cells significantly decreased the secretion of the cytokine ($P<0·05$). In Fig. 2b, there is a significant decrease only in non-stimulated cells treated with HDL ($P<0·05$) in IL-1β secretion. No major differences were observed for IL-2 primed cells except for cells stimulated with LDL primed with 100 IU of IL-2 ($P<0·05$).

The secretion of IL-2 by NK cells is illustrated in Fig. 3a. For non-primed cells, the secretion of IL-2 was significantly increased upon stimulation with VLDL ($P < 0·05$), LDL or AcLDL ($P<0·01$), and HDL ($P<0·005$). Conversely, IL-2 primed cells treated with CM, VLDL, LDL, HDL and AcLDL secreted significantly lower amounts of this cytokine at all

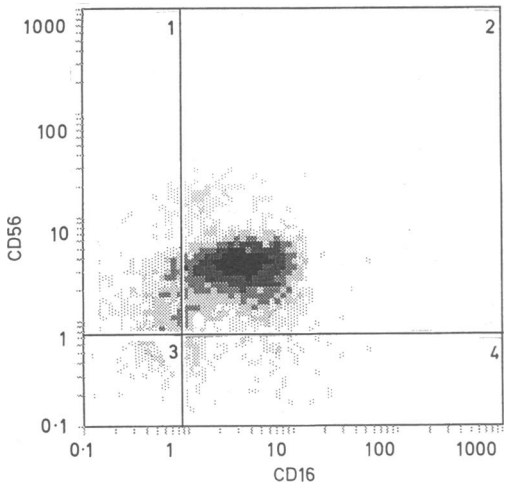

Figure 1. Expression of CD56 and CD16 on the purified NK cell population. The figure represents a typical expression of CD56–RD1 (Y axis) and CD16–FITC (X axis) in the cell population used for this study. The horizontal and perpendicular lines represent non-specific binding. The purified cell population was 86% positive for both markers (rectangle #2).

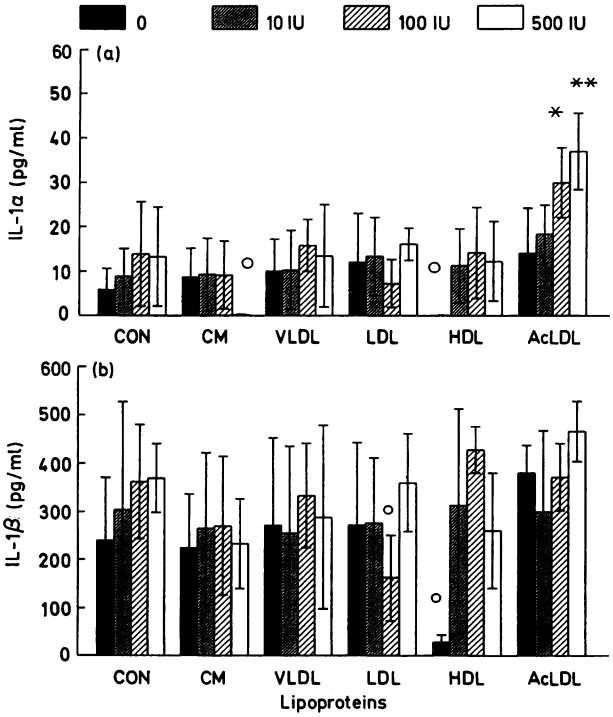

Figure 2. Effect of lipoproteins and IL-2 on the secretion of IL-1α and IL-1β. The figure depicts the amount of cytokine detected by ELISA in the supernatants of NK treated cells as described in Materials and Methods versus the type of assay performed. The controls (CON) correspond to cells that were not treated with lipoproteins. The bars represent the mean $\pm$ SD of five different donors. (a) IL-1α secretion and (b) IL-1β secretion in pg/ml of cultured media. Each lipoprotein treated cells was compared to cells non-treated with lipoproteins. The statistical significance observed are as follows: $*P<0{\cdot}05$ and $**P<0{\cdot}01$ represent an increase and $°P<0{\cdot}05$ a decrease with respect to the controls.

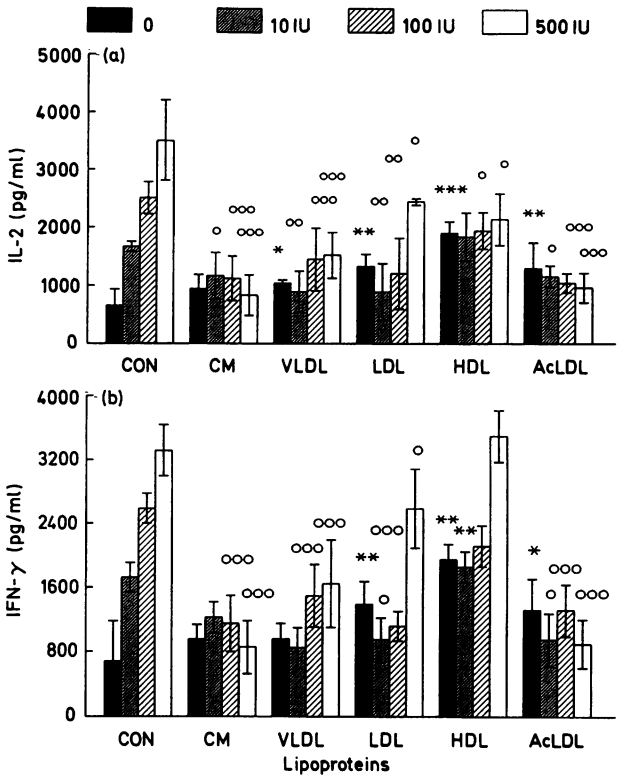

Figure 3. Effect of lipoproteins and IL-2 on the secretion of IL-2 and IFN-γ. The figure depicts the amount of cytokine detected by ELISA in the supernatants of NK treated cells as described in Materials and Methods versus the type of assay performed. The controls (CON) correspond to cells that were not treated with lipoproteins. The bars represent the mean $\pm$ SD of five different donors. (a) IL-2 secretion and (b) IFN-γ secretion in pg/ml of cultured media. Each lipoprotein treated cells was compared to cells non-treated with lipoproteins. The statistical significances observed are as follows: $*P<0{\cdot}05$ and $**P<0{\cdot}01$ represent an increase and $°P<0{\cdot}05$,$°°$ $P<0{\cdot}01$$°°$ $°P<0{\cdot}005$ a decrease with respect to controls.

concentrations tested ($P<0{\cdot}05$, $P<0{\cdot}01$ and $P<0{\cdot}005$ depending on the condition). In Fig. 3b of the figure, the secretion of IFN-γ was represented. LDL, HDL ($P<0{\cdot}01$) and AcLDL ($P<0{\cdot}05$) increased the secretion of this cytokine in non-primed cells. Conversely, the secretion of IFN-γ decreased significantly in CM, VLDL, LDL, HDL and AcLDL stimulated IL-2 primed cells ($P<0{\cdot}05$, $P<0{\cdot}01$, and $P<0{\cdot}005$ depending on the condition tested), and unmodified in 10 and 500 IU IL-2 primed cells stimulated with HDL.

TNF-α secretion (shown in Fig. 4a) increased significantly when non-primed cells were stimulated with CM and AcLDL ($P<0{\cdot}01$) while no differences were observed with the other lipoproteins. In IL-2 primed cells, CM, VLDL, LDL, HDL and AcLDL significantly decreased ($P<0{\cdot}005$, $P<0{\cdot}0005$, $P<0{\cdot}0001$ depending on the condition tested) TNF-α secretion. The amount of secreted IL-8 is depicted in Fig. 4b. All lipoproteins, except HDL, significantly increased (CM, VLDL, LDL $P<0{\cdot}05$, and AcLDL $P<0{\cdot}005$) the secretion of this cytokine in non-primed cells. In addition, cells stimulated with LDL (100 IU) and AcLDL at any concentration of IL-2 secreted significantly ($P<0{\cdot}005$ and $P<0{\cdot}0001$ depending on

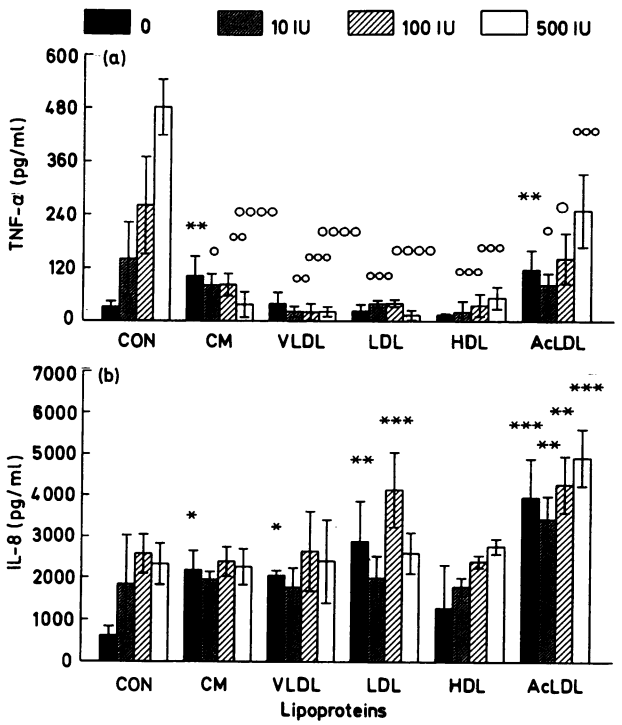

Figure 4. Effect of lipoproteins and IL-2 on the secretion of TNF-α and IL-8. The figure depicts the amount of cytokine detected by ELISA in the supernatants of NK treated cells as described in Materials and Methods versus the type of assay performed. The controls (CON) correspond to cells that were not treated with lipoproteins. The bars represent the mean ± SD of five different donors. (a) TNF-α secretion and (b) IL-8 secretion in pg/ml of cultured media. Each lipoprotein treated cells was compared to cells non-treated with lipoproteins. The statistical significance observed are as follows: $*P<0.05$, $**P<0.01$ and $***P<0.005$ represent an increase and $°P<0.05$,$°°$ $P<0.01°°°$ $P<0.005$ a decrease with respect to controls.

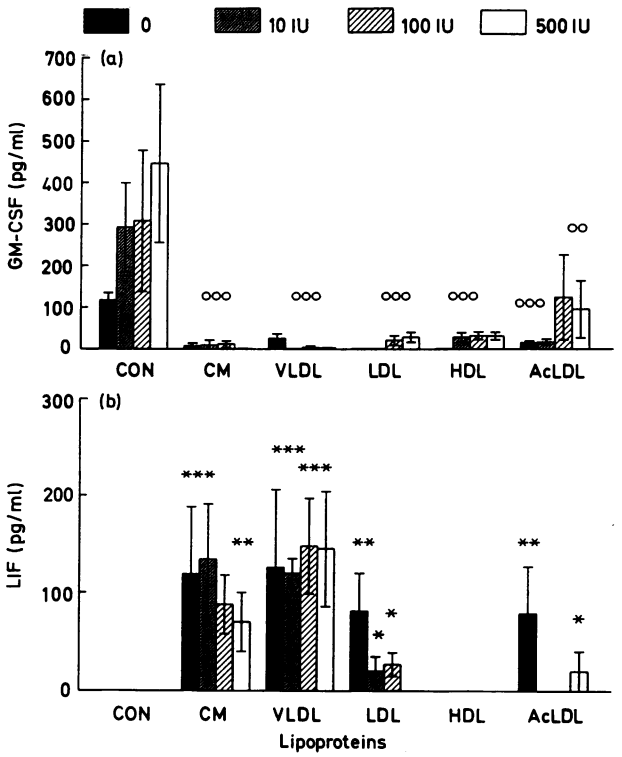

Figure 5. Effect of lipoproteins and IL-2 on the secretion of GM-CSF and LIF. The figure depicts the amount of cytokine detected by ELISA in the supernatants of NK treated cells as described in Materials and Methods versus the type of assay performed. The controls (CON) correspond to cells that were not treated with lipoproteins. The bars represent the mean ± SD of five different donors. (a) GM-CSF secretion in pg/ml of cultured media. Each lipoprotein treated cells was compared to cells non-treated with lipoproteins and statistical differences were observed ($°°P<0.005$,$°°$ $°P<0.00001$). (b) LIF secretion in pg/ml of cultured media. The statistical significances observed are $*P<0.05$, $**P<0.005$, $***P<0.0001$.

the condition tested) higher amounts of IL-8 when compared to the controls.

Figure 5a illustrates the effect of lipoproteins in GM-CSF secretion. For all types of lipoprotein in non-primed cells there is a significant decrease ($P<0.000\,01$) in the secretion of GM-CSF. Only in the case cells primed with IL-2 and treated with AcLDL this effect is lower ($P<0.005$). The secretion of LIF is pictured in Fig. 5b. There is no production of LIF in the cells used as controls; however, for non-primed cells, CM, VLDL, LDL and AcLDL significantly increased the secretion of LIF ($P<0.005$ and $P<0.0001$ depending on the condition tested). Treatment with CM, and VLDL induced significant changes in the secretion of LIF in IL-2 primed cells ($P<0.005$ and $P<0.0001$ depending on the condition tested). The effect of LDL is lower than the effect observed with CM and VLDL. A modest increment was also observed with AcLDL. HDL, independently of the treatment used, did not induce LIF secretion.

In summary, marked changes have been observed in non primed NK cells treated with lipoproteins. CM, VLDL, LDL and AcLDL induced LIF secretion which was absent in controls. CM preferentially simulated IL-8 (four-fold) > TNF-α (three-fold) > IL-1α (two-fold) > IL-2 (one and a half-fold) = IFN-γ (one and a half-fold); it did not affect IL-1β secretion and it decreased 17-fold GM-CSF secretion. VLDL increased IL-8 secretion (three-fold) > IL-1α (two-fold) = IL-2 (two-fold) > IFN-γ (one and a half-fold) > TNF-α (1·3-fold) without altering IL-1β secretion and decreasing five-fold GM-CSF secretion. LDL increased the secretion of IL-8 (five-fold) > IL-1α (two and a half-fold) > IL-2 (two-fold) = IFN-γ (two-fold); it did not affect IL-1β nor TNF-α secretion and it inhibited GM-CSF secretion five hundred-fold. HDL increased the secretion of IL-2 (three-fold) = IFN-γ (three-fold) > IL-8 (two-fold); it decreased the secretion of GM-CSF (five hundred-fold) > IL-1α (two hundred and fifty-fold) > IL-1β (nine-fold) > TNF-α (two-fold) without altering LIF secretion. AcLDL increased the secretion of IL-8 (six-fold) > TNF-α (four-fold) > IL-1α (three-fold) > IL-2 (two-fold) = IFN-γ (two-fold) = IL-1β (two-fold), and decreased GM-CSF secretion eight-fold.

ANOVA analysis of the effects of the different lipoproteins on the secretion of the different cytokines is illustrated in Table 1. The significance observed was due to the fact that lipoproteins produced modifications in the amount of cytokine secreted by the cells. In order to compare the extent of the effect of each lipoprotein on NK cytokine secretion, the effect

Table 1. *P* values of the ANOVA analysis representing the effect of lipoprotein stimulation on cytokine secretion of IL-2 primed cells

IL-2 (IU/ml)	IL-1α	IL-1β	IL-2	IFN-γ	TNF-α	IL-8	GM-CSF	LIF
0	0·07	0·007	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0007
10	0·42	>0·8	0·0006	<0·0001	0·0003	0·002	<0·0001	<0·0001
100	0·005	0·0006	<0·0001	<0·0001	<0.0001	<0·0001	<0·0001	<0·0001
500	<0·0001	0·04	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001	<0·0001

Table 2. *P* values of the ANOVA analysis comparing the effect of each lipoprotein on cytokine secretion of non-primed cells

	CM versus	VLDL versus	LDL versus	HDL versus	AcLDL versus
IL-1α	—	HDL $P=0·07$	HDL $P=0·03$	AcLDL $P=0·02$	
IL-1β	HDL $P=0·03$	HDL $P=0·02$	HDL $P=0·02$	AcLDL $P=0·002$	CM $P=0·09$
IL-2	LDL $P=0·05$	HDL $P=0·0009$	HDL $P=0·009$	CM $P=0·0004$	CM $P=0·07$
IFN-γ	LDL $P=0·06$	LDL $P=0·06$	HDL $P=0·01$	CM $P=0·001$ VLDL $P=0·01$	HDL $P=0·01$
TNF-α	VLDL $P=0·009$	AcLDL $P=0·0009$	CM $P=0·003$	CM $P=0·001$	LDL $P=0·0009$ HDL $P=0·0005$
IL-8	HDL $P=0·08$	AcLDL $P=0·003$	HDL $P=0·008$ AcLDL $P=0·05$	AcLDL $P=0·0004$	CM $P=0·005$
GM-CSF	VLDL $P=0·009$	LDL $P=0·002$ AcLDL $P=0·09$	AcLDL $P=0·03$	VLDL $P=0·002$	HDL $P=0·03$
LIF	HDL $P=0·005$	HDL $P=0·004$	HDL $P=0·03$	—	AcLDL $P=0·04$

Table 3. *P* values of the ANOVA analysis comparing the effect of each lipoprotein on cytokine secretion of cells primed with 10 IU/ml IL-2

	CM versus	VLDL versus	LDL versus	HDL versus	AcLDL versus
IL-1α	NS	NS	NS	NS	NS
IL-1β	NS	NS	NS	NS	NS
IL-2	HDL $P=0·02$	HDL $P=0·003$	HDL $P=0·003$		HDL $P=0·02$
IFN-γ	VLDL $P=0·006$	HDL $P=0·0004$	HDL $P=0·0008$	CM $P=0·0064$	HDL $P=0·0008$
TNF-α	VLDL $P=0·04$	AcLDL $P=0·04$		CM $P=0·04$	HDL $P=0·03$
IL-8	AcLDL $P=0·006$	AcLDL $P=0·003$	AcLDL $P=0·007$	AcLDL $P=0·003$	
GM-CSF	NS	NS	NS	NS	NS
LIF	LDL $P<0·0001$	HDL $P<0·0001$	VLDL $P<0·0002$	CM $P<0·0001$	CM $P<0·0001$ VLDL $P<0·0001$

NS, not significant.

Table 4. *P* values of the ANOVA analysis comparing the effect of each lipoprotein on cytokine secretion of cells primed with 100 IU/ml IL-2

	CM versus	VLDL versus	LDL versus	HDL versus	AcLDL versus
IL-1α	AcLDL $P=0.005$	AcLDL $P=0.03$	AcLDL $P=0.003$	AcLDL $P=0.02$	
IL-1β	HDL $P=0.04$	LDL $P=0.03$	HDL $P=0.003$		LDL $P=0.01$
IL-2	HDL $P=0.01$		HDL $P=0.02$		HDL $P=0.009$
IFN-γ	VLDL $P=0.09$	LDL $P=0.07$	HDL $P=0.0003$	CM $P=0.0003$ VLDL $P\leqslant 0.0001$	VLDL $P=0.01$ HDL $P=0.001$
TNF-α		AcLDL $P=0.02$	AcLDL $P=0.04$	AcLDL $P=0.03$	
IL-8	LDL $P=0.003$	LDL $P=0.007$ AcLDL $P=0.004$	HDL $P=0.003$	AcLDL $P=0.002$	CM $P=0.002$
GM-CSF	AcLDL $P=0.06$	AcLDL $P=0.05$	AcLDL $P=0.08$		
LIF	VLDL $P=0.004$	LDL $P=0.0001$ HDL $P<0.0001$	CM $P=0.04$	CM $P=0.0004$	CM $P=0.0004$ VLDL $P<0.0001$

Table 5. *P* values of the ANOVA analysis comparing the effect of each lipoprotein on cytokine secretion of cells primed with 500 IU/ml IL-2

	CM versus	VLDL versus	LDL versus	HDL versus	AcLDL versus
IL-1α	VLDL $P=0.04$	AcLDL $P=0.002$	CM $P=0.02$ AcLDL $P=0.004$	CM $P=0.05$ AcLDL $P=0.002$	CM $P=0.0001$
IL-1β	AcLDL $P=0.01$	AcLDL $P=0.04$		AcLDL $P=0.02$	
IL-2	VLDL $P=0.03$	LDL $P=0.007$	CM $P=0.0003$ AcLDL $P=0.0004$	CM $P=0.0009$ VLDL $P=0.04$	VLDL $P=0.06$ HDL $P=0.002$
IFN-γ	VLDL $P=0.01$	LDL $P=0.006$ AcLDL $P=0.02$	CM $P=0.0001$	CM $P=0.002$ VLDL $P=0.01$	LDL $P=0.0001$ HDL $P=0.0002$
TNF-α	AcLDL $P<0.0001$	AcLDL $P<0.0001$	AcLDL $P<0.0001$		
IL-8	AcLDL $P=0.0001$	AcLDL $P=0.0002$	AcLDL $P=0.0003$	AcLDL $P=0.0005$	
GM-CSF					
LIF	VLDL $P=0.003$	LDL $P=0.0001$	CM $P=0.004$ VLDL $P=0.0001$	CM $P=0.02$	CM $P=0.004$ VLDL $P<0.0001$

of each treatment was compared. In Tables 2–5, the effect of lipoprotein stimulation was compared for all cytokines secreted by non-stimulated and IL-2 primed NK cells. Only non-significant changes were observed in IL-1α, IL-1β and GM-CSF when the cells were primed with 10 IU of IL-2 and in GM-CSF for cells primed with 500 IU of IL-2. The decrease in GM-CSF secretion was independent of the lipoprotein used for stimulus.

DISCUSSION

Lipoprotein receptors are expressed in mononuclear cells when they are cultured in the absence of lipoproteins and fatty acids[1] and depend on complex post-transcriptional and post-translational mechanisms.[1,2] Macrophages, NK cells, and T lymphocytes internalize lipoproteins through different pathways.[1] Macrophages express scavenger receptors, Fc receptors, and the CD36 antigen that bind preferentially modified LDL, the VLDL receptor, and the α_2 macroglobulin receptor/LDL receptor related protein (internalized CM and VLDL).[1–6] NK cells internalized all types of lipoproteins suggesting the presence of different receptors in these cells.[12] The lipoproteins tested induced NK cell proliferation and some induced an increase in spontaneous cytotoxicity (CM, VLDL, and LDL) while the others decreased it (HDL and AcLDL).[12] The extent of the effect depended upon lipoprotein concentration;[12] in most cases, the optimal concentration was 20 μg of protein/ml. In T lymphocytes a similar proliferative response was observed.[10] Both types of lymphocytes have been shown to express LDL receptors following their activation.[10] In addition, IL-2 was able to increase lipoprotein uptake in NK cells and T lymphocytes and the extent of the proliferative response in IL-2 primed cells was higher when it was compared to the non-stimulated ones.[10] Some of the effects of lipoproteins may be due to a direct cell activation through its receptor or to an autocrine effect induced probably by cytokines secreted in this system.

In this report we studied the effect of each lipoprotein on the secretion of cytokines in conditions in which optimal concentrations of lipoproteins that were added to culture media, RPMI–0·5% BSA fatty acid free.[10–12] In addition, the quantity of lipoproteins required for optimal responses were less than the amount observed if 10% fetal calf serum was used for cell culture.[10–12] The media with lipoproteins was used to perform the standard curve of the assay observing that there was no net effect of lipoprotein detection by ELISA (results not shown). Furthermore, enzyme linked assays were used instead of biological assays since lipoproteins may alter the extent of the bioassay and as consequence invalidate the interpretation of the result. This particular effect has been observed in the bioassay for interleukin-2 using CTLL cells, LDL potentiated their proliferative response driven by IL-2.[17] It is concluded then, that the assays used were sensible enough to determine cytokines in cultured media and that lipoproteins did not interfere with these assays.

In all conditions tested in this report, there were significant changes in the secretion of cytokines IL-2, IFN-γ, TNF-α, IL-8, GM-CSF and LIF. These effects were lipoprotein type and IL-2 dose dependent. A change in IL-1α secretion was only observed when the cells were primed with either 100 or 500 IU of IL-2. On the contrary, the secretion of IL-1α and IL-1β was not affected when NK cells were primed with 10 IU of IL-2. Likewise, except for cells treated with HDL there were no major differences in IL-1β secretion. HDL also induced a decrease in IL-1α secretion, as compared to the other lipoproteins. All lipoproteins increased the secretion of IL-2, IL-8 and IFN-γ and decreased the secretion of GM-CSF in non-primed cells. TNF-α secretion increased with CM and AcLDL treatments while decreasing with LDL and HDL treatments. CM, VLDL, LDL and AcLDL increased LIF secretion, while HDL did not modify it. LDL stimulated cells secreted less TNF-α and IL-8 as compared to AcLDL stimulated cells. Furthermore, LDL treated cells produce more IL-1α, IL-1β, LIF and IL-8 than HDL stimulated cells, and HDL stimulated cells more IL-2 and IFN-γ than LDL treated cells.

In comparison to other reports[18] an increase in IFN-γ and a decrease in GM-CSF secretion has been reported for IL-12 stimulated cells, probably potentiating cellular immunity. The contrary, an increase in GM-CSF and a decrease in IFN-γ secretion has been observed in IL-7 stimulated cells[18] The increase in GM-CSF secretion seems to be related to bone marrow stimulation or cellular differentiation. Therefore, it may be suggested that the lipoprotein stimulus on NK cells may drive the cells towards cellular immunity contrary to potentiating cellular differentiation.

The differential secretion of cytokine secretion depending upon the lipoprotein used to stimulate the cells suggest a different pathway of activation. For example, HDL, a lipoprotein that was shown to induce NK proliferative response and inhibit spontaneous cytotoxicity,[12] induced the secretion of IL-2, IFN-γ and IL-8 and decreased the secretion of IL-1α, IL-1β and GM-CSF as compared with non-treated controls. It could be suggested that the deregulation of the secretory pattern of inflammatory cytokines like IL-1 and TNF-α may be responsible for the decrease in spontaneous cytotoxicity. However, this seems not to be the case since AcLDL, a lipoprotein that mediated similar effects on NK cell proliferative and cytotoxic activity,[12] induced the secretion of most of the cytokines studied in this report except for GM-CSF. It is possible that the effect of HDL and AcLDL on cytokine secretion is different but the net outcome may converge at one point which is NK cytotoxicity inhibition. A similar episode is observed for CM and VLDL. CM and VLDL stimulated similarly proliferative and cytotoxic responses;[10] however, CM induced the secretion of TNF-α, which was not observed upon VLDL stimulation. TNF-α secretion is probably not the limiting cytokine that affects NK proliferative and cytotoxic responses.

Contrary to previous expectations, the T lymphocytes observed in atheroma are partially activated cells (high DR and CD45RO, but low CD25 expression) that belong to a heterogeneous subpopulation (50% CD4, and 50% CD8) suggesting complex pathways of cell activation.[19] Furthermore, *in vivo* elimination of T lymphocytes increased the size of intimal thickening after balloon injury in mice.[19] It was suggested then that the lack of T lymphocytes in the lesion may be related to a decrease in IFN-γ production in the lesion that would decrease smooth muscle cell proliferation.[19] Even though a simple decrease in one cytokine may not be, at first glance, enough to produce this effect, the cells that surround the lesion may be controlled by this cytokine either by inhibiting cell metabolism of by inhibiting the secretion of cytokines that activate other cells in paracrine fashion. A support for this hypothesis is that IFN-γ down-regulated scavenger receptors in macrophages and endothelial cells.[20] Thus, IFN-γ secretion, induced by HDL in parallel to a decrease in the secretion of the other inflammatory cytokines IL-1α, IL-1β and TNF-α, may be responsible for the protective effect of this lipoprotein. Eventually, AcLDL may promote cellular aggression since it induced the secretion of high quantities of inflammatory cytokines as compared to IFN-γ.

In addition, IL-8 secretion was higher in AcLDL treated cells as compared to HDL treated cells suggesting that peripheral cells are attracted by IL-8 and eventually activated by the inflammatory cytokines produced upon AcLDL activation.

The induction of LIF secretion by lipoproteins may be a negative feedback for lipoprotein metabolism. LIF inhibited lipoprotein lipase (LPL) catalytic activity and, similarly to TNF-α it decreased the enzyme transcription in 3T3-L1.[21] An overproduction of LIF may reduce LPL secretion and activity generating an accumulation of triglyceride rich lipoproteins and its remnants. Lipoprotein remnants have been involved in atheroma formation.[1]

Clinical data suggest the importance of lipoproteins in lipoprotein metabolism. In transplant atherosclerosis, cardiac allografts that were treated with pravastatin (an inhibitor of cholesterol synthesis) experienced a decrease in NK cytotoxic activity which was correlated with reduced rejection.[22] These effects envision the role of NK cells, lipoproteins and cytokines in the allograft response besides T lymphocytes and IL-2.[23]

The biological spectrum of cell response upon lipoprotein activation is bimodal. Certainly there are many cytokines and other cells products which have not been studied, but there is an interesting relationship between five specific cytokines IFN-γ, IL-2, IL-8, GM-CSF and LIF. One may picture that, contrary to a general hypothesis, lipoproteins do not overdrive NK cells to secrete cytokines involved in a typical inflammatory reaction, but to a complex cellular response. It is possible then that in stimulated or attracted cells the sum of secreted cytokines upon NK activation with lipoproteins may be responsible for part of the cellular response observed in the atheroma.

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