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Expression of different lipoprotein receptors in natural killer cells and their effect on natural killer proliferative and cytotoxic activity

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SUMMARY

Natural killer (NK) cells take up chylomicrons (CM), very low density (VLDL), low density (LDL), high density (HDL) and acetyl-modified low density (AcLDL) lipoproteins through different receptors, VLDL being the lipoprotein with the highest uptake and HDL the lowest. The uptake of LDL can be selectively blocked by the anti-LDL receptor, which does not affect the uptake of CM, VLDL, HDL and AcLDL. Although the uptake of lipoproteins assessed by flow cytometry using DiI is not very high, the lipoproteins are able to induce an increase in proliferative responses, VLDL, AcLDL and HDL being the most important ones with 12- and 17-fold increments, respectively. CM, VLDL and LDL at low concentrations increase NK cytotoxic activity, while HDL and AcLDL inhibit, in a dose-dependent fashion, the killing of NK cells against K562. These results suggest the presence of four different receptors that are responsible for the cytotoxic and proliferative responses observed.

INTRODUCTION

Lipoproteins, chylomicrons (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL), have always been considered important for cell and tissue physiology.[1] However, the deregulation of its metabolic control may be the cause of vascular pathologies such as atherosclerosis.[2,3]

Macrophages and T lymphocytes are involved in the formation of atheroma. The effects of oxLDL and other remnants in the formation of foam cells have been documented,[1–3] and different subpopulations of T cells are present in lesions.[1,3] Moreover, anti-CD3-activated T cells show an enhanced expression of LDL receptor (LDLR).[4] Recently, LDL and modified LDL, produced by activated macrophages, were shown to affect T-lymphocyte function.[1,5] Several receptors for different lipoproteins have been shown to be present in macrophages, the scavenger receptors,[6] the α_2-macroglobulin/LDL receptor (α_2MR/LRP),[6] CD36,[7] HDL receptors,[8] VLDL receptor[9] and Fc receptors.[10] All of these seem to be regulated and induce different cell physiological responses. Despite these reports, little is known about the role of other lymphocytes, which may be essential in the early stages of the atheroma formation.

Natural killer (NK) cells ($CD3^-$ $CD16^+$ $CD56^+$) represent a subset of lymphocytes distinguishable from T and B lymphocytes by their morphology, phenotype and functional capacity to kill tumour cells or virally infected cells, spontaneously[11,12] and are one of the most important secretory cells of the immune system. In peripheral blood, NK cells account for about 5–15% of circulating lymphocytes, but in some organs, e.g. the liver, they represent up to 45% of tissue-infiltrating lymphocytes,[11] suggesting a role of NK cells in the normal function of this organ.

Even though NK cells have not been directly involved in the development of atherosclerosis, recently it has been shown that diet,[13] lipid emulsions,[14,15] and oxidized LDL by polymorphonuclear leucocytes[16] alter NK lytic efficiency against K562 and P815 cells. Furthermore, experiments with lovastatin *in vitro* have shown it to inhibit the lymphocyte proliferative response and NK cytotoxicity[17,18] that can be overcome by the addition of interleukin-2 (IL-2).[18] Similarly, we have previously shown that lipoprotein lipase,[19] a key enzyme in lipoprotein metabolism, is expressed in NK cells and that its expression affects NK cytotoxic activity. However, to our knowledge, there are no reports on lipoprotein receptor expression and function in NK cells.

In this study, our aim was to investigate the presence of the different lipoprotein receptors in NK cells and their effect on the proliferative and cytotoxic responses of these cells.

MATERIALS AND METHODS

Chemicals

Fetal calf serum (FCS), L-glutamine, penicillin, streptomycin and RPMI-1640 medium were purchased from Gibco BRL (Gaithersburg, MD). 1,1′-dioctadecyl-3,3,3′,3′ tetramethyl

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indocarbocyanine perchlorate (DiI) was purchased from Molecular Probes Inc. (Eugone, OR). Percoll- and Ficoll-Paque were purchased from Pharmacia LKB (Uppsala, Sweden). All other reagents were acquired from Sigma Chemical Co. (St Louis, MO).

Antibodies

Anti-Leu-11c-phycoerythrin (PE) (CD16) was purchased from Becton Dickinson (Mountain View, CA); NKH-1 RD_1 (CD56), unlabelled anti-CD3, anti-CD3-fluorescein isothiocyanate (FITC) and anti-CD14-FITC antibodies were purchased from Coulter Immunology (Hialeah, FL). Anti-LDLR was purchased from Amersham Int. (Amersham, UK).

Lipoprotein purification

All lipoproteins, CM, VLDL, LDL and HDL, were purified according to the method of Havel.[20] 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 glutathione, 5 mM ascorbic acid and 5 mM EDTA] in order to separate CM 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 were 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. Protein, triglyceride, phospholipid and cholesterol content was determined for each fraction using standard kits (Sigma Chemical Co.). No oxidative intermediates were detected in the purified fractions using the TBARS assay.[21]

LDL acetylation

LDL was acetylated using acetic anhydride as described previously by Basu *et al.*[22] Briefly, the purified LDL fractions (16 mg/ml) were dialysed and then an equivalent volume of a saturated sodium acetate was added with continuous stirring in an iced water bath. Acetic anhydride was added in small amounts up to an amount equal to 1·5 times of the mass of protein. After 30 min of mixing, the modified lipoproteins were dialysed against PBS, filter sterilized and subsequently used for flow cytometry analysis, proliferative and cytotoxic studies. The electrophoretic mobility of acetyl-modified modified LDL (AcLDL) was different from that of unmodified LDL (results not shown).

Labelling of lipoproteins with DiI

The labelling of each lipoprotein with DiI was performed as described previously.[23] Briefly, lipoproteins were adjusted at 2 mg/ml and then labelled with 200 μl of a 3 mg/ml solution of DiI dissolved in dimethyl sulphoxide, and the mixture was added to 8 ml of lipoprotein-free plasma for 10 hr at 37°. Lipoprotein-DiI was centrifuged at 114 000 *g* for 20 min (CM) or for 18 hr (VLDL, LDL, AcLDL and HDL) in order to eliminate the unbound chromophore. The supernatant, with the characteristic red colour, was dialysed against PBS, adjusted at 2 mg/ml and filter-sterilized through a 0·45 μm Millipore filter (Bedford, MA). The labelling efficiency was determined by the emission of the chromophore in solution at 480 nm in a fluorometer (Perkin-Elmer-Cetus, Norwalk, CT). The ratio lipoprotein-DiI/total DiI was always close to 50%. The fresh sterile lipoprotein-DiI was used for flow cytometry, proliferative and cytotoxic studies.

Cell purification

Blood samples were taken from normal healthy donors (blood bank of the Central University Hospital, Caracas, Venezuela). Human large granular lymphocytes (LGL) were separated by passage of the non-adherent mixed population of cells through nylon wool, and subsequent centrifugation on Percoll gradients.[24] The cells isolated from the Percoll gradients were treated with anti-CD3 monoclonal antibody plus complement, to deplete $CD3^+$ cells. The purified fraction was assessed for CD16 and CD56 positively in an EPICS 753 flow cytometer (Coulter Corporation, Hialeah, FL). The purified cell fraction contained > 80% CD16 and CD56, < 2% CD3 and < 1% MO2 positively. Purified NK ($CD3^-$ $CD16^+$) cells were then cultured for 18 hr in RPMI-1640 medium supplemented with 0·5% bovine serum albumin (BSA; fatty acid free) in order to induce the expression of lipoprotein receptors. After the incubation, NK cell viability was > 90%.

Flow cytometric studies

After the 18-hr incubation in RPMI—BSA, the cells were washed in PBS adjusted to 1×10^6/ml, resuspended with different concentrations of lipoproteins-DiI in RPMI-BSA, and incubated for 4 hr at 37°. The unspecific binding was assessed by (1) the positivity determined by adding lipoproteins-DiI to cells previously treated with 1 mM EDTA; and (2) the positivity determined from cells treated with lipoproteins-DiI and incubated for 4 hr at 4°. After incubation the cells were washed extensively with PBS and its positivity determined by flow cytometry. Fluorescence (< 570 nm) signals emitted by the accumulated lipoproteins-DiI in the cells were collected by the red photomultiplier using 488 nm excitation with an argon laser. In some experiments, NK cells were incubated with 5 μg of anti-LDL receptor for 30 min at 4° before the addition of lipoproteins-DiI.

Cell proliferative studies

After 18 hr incubation in RPMI-BSA, the cells were washed extensively with PBS, adjusted to $1 \times 10^5/100\ \mu$l and added to the well of a Primaria 96-well plate (Beckton Dickinson) which contained the different lipoproteins diluted at different concentrations with RPMI-BSA. The cells were incubated for 72 hr (best proliferative response observed) and labelled 18 hr prior to the end of the incubation with 1 μCi/ml [^{3}H]thymidine (prepared in RPMI-BSA). The incorporation of [^{3}H]thymidine was measured in a β-plate counter (LKB, Stockholm, Sweden).

In some experiments, the cells (1×10^6 cells/100 μl) were incubated for 30 min at 4° with 5 μg anti-LDLR or an irrelevant mouse IgG2b as a control. Then the cells were adjusted to 1×10^6/ml and added to the plates that contained the different concentrations of LDL, and the proliferative experiments were performed as described above.

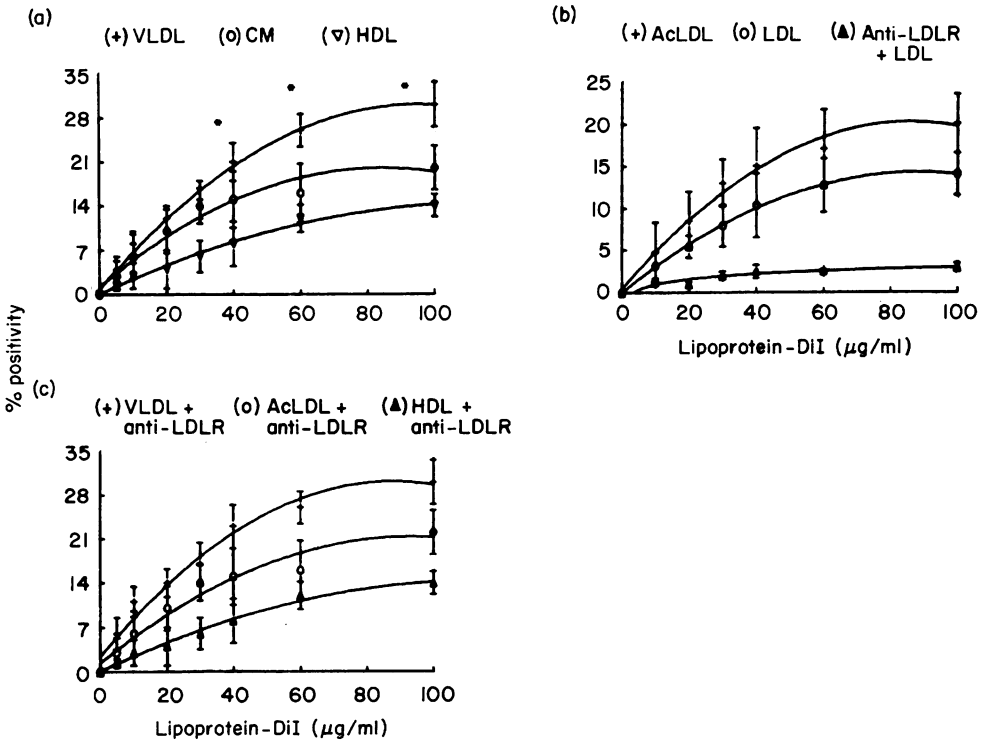

Figure 1. Uptake of lipoproteins labelled with DiI by NK cells. 1×10^6 cells/ml purified NK cells were labelled with different concentrations of CM–DiI, VLDL–DiI, LDL–DiI, AcLDL–DiI or HDL–DiI, as described in the Materials and Methods. Positivity was assessed by flow cytometry with the red photomultiplier for the lipoprotein–DiI. The positivity reported corresponds to the specific (subtracting unspecific binding) mean plus SD of five different experiments. (a) Positivity of cells incubated with VLDL–DiI, CM–DiI and HDL–DiI. The highest uptake was observed with VLDL–DiI and the lowest with HDL–DiI. The difference between VLDL–DiI and HDL–DiI was significant (*$P < 0{\cdot}05$). (b) Uptake of AcLDL–DiI, LDL–DiI and LDL–DiI to cells incubated previously with 5 μg anti-LDLR. (c) Effects of 5 μg anti-LDL receptor incubation with NK cells for 30 min at 4° before the addition of the different lipoproteins labelled with DiI. VLDL–DiI, AcLDL–DiI and HDL–DiI were uptaken similarly, as reported in (a) and (b).

NK cytotoxic assay

NK cells were incubated with different concentrations of lipoproteins for 4 hr in RPMI–BSA, with the different concentrations needed for the cytotoxic assay (2×10^6, 1×10^6, $0{\cdot}5 \times 10^6$ and $0{\cdot}25 \times 10^6$ cells/ml) in separate tubes. After the incubation, the cells were washed, viability assessed and the cells then resuspended in RPMI–BSA for the assay.

A short-term (4-hr) radiolabelled release assay using ^{51}Cr-labelled K562 cells as targets was performed as described previously.[19] Briefly, 5×10^6 K562 cells were labelled with 150 μCi of Na^{51}Cr (200–500 μCi/mmol; Amersham Int.) for 1 hr at 37°. Labelled cells were washed three times in RPMI medium plus 5% FCS and resuspended at 5×10^4 cells/ml in RPMI containing 10% FCS. A fixed number (5×10^3 cells in 0·1 ml) of labelled K562 cells was mixed with 0·1 ml of effector cells at four different effector : target (E : T) cell ratios (40 : 1, 20 : 1, 10 : 1 and 5 : 1). The combination of target effector cells was seeded in triplicate into 96-well U-bottomed microtest plates (Falcon Plastics, Oxnard, CA). ^{51}Cr release was measured in 100-μl samples of supernatants using a γ-counter (Compugamma; Wallac, LKB). Total release of radioactivity was determined by counting the radioactivity released from 5000 ^{51}Cr-labelled K562 cells. The percentage lysis was calculated by the formula:

$$\% \text{ specific lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}}.$$

In some experiments, the cells (1×10^6 cells/100 μl) were incubated for 30 min at 4° with 5 μg anti-LDLR or an irrelevant mouse IgG2b as a control. Then the cells were added to the plates that contained the different concentrations of LDL and incubated for 4 hr, as described above. The cells were then washed and the cytotoxicity assay performed as described before.

Statistical analysis

The paired Student's *t*-test was employed for analysing the different sets of experiments.

RESULTS

Figure 1 illustrates the uptake of the different lipoproteins labelled with DiI. All lipoproteins were taken up by NK cells. The uptake in each case reached a maximum between 60 and 80 μg/ml of lipoprotein–DiI. CM, VLDL and HDL were compared (Fig. 1a); VLDL–DiI was taken up more avidly than CM–DiI and HDL–DiI ($P < 0{\cdot}05$ compared to HDL). As

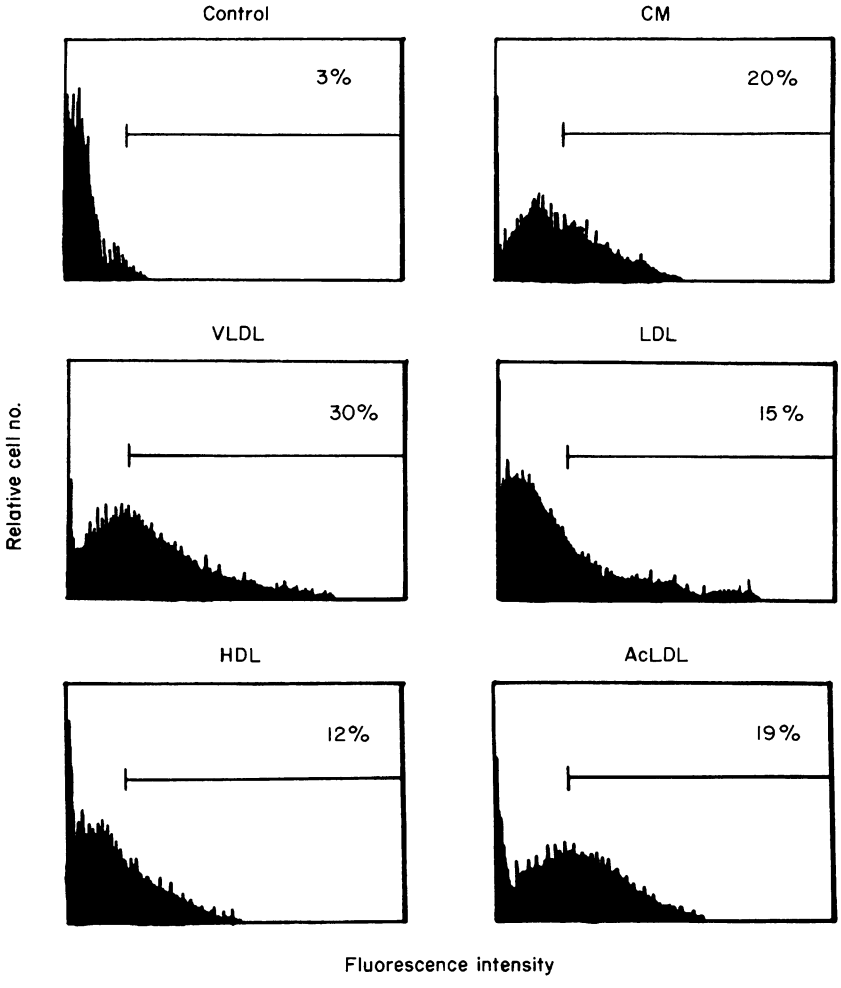

Figure 2. Expression of lipoprotein receptors quantified with lipoprotein–DiI. A typical expression of the maximum uptake by NK cells of the different lipoproteins is shown. The cells were incubated as described in Fig. 1, but with 100 μg/ml of one of the following lipoproteins; CM–DiI, VLDL–DiI, LDL–DiI, AcLDL–DiI or HDL–DiI. The line represents the cursor used for quantifying fluorescence intensity and positivity. In the right hand corner of each figure the specific positivity is reported. The control value corresponded to the positivity recorded by incubating the cells with any labelled lipoprotein at 4°.

shown in Fig. 1b, the acetylation of LDL was taken up more avidly than LDL even at low concentrations; this difference, however, was not significant. The blockage of LDL receptor resulted in a specific impediment of LDL uptake (78% inhibition with 5 μg of antibody). Fig. 1c illustrates the effect of anti-LDL receptor uptake of different lipoproteins, VLDL, AcLDL and HDL, which was unaffected.

A typical maximal uptake of the different lipoproteins labelled with DiI is shown in Fig. 2. The control illustrates that the unspecific binding of lipoproteins labelled with DiI was always less than 3% for logarithmic units >2; the test of the figure illustrates the positivity assessed for CM–DiI, VLDL–DiI, LDL–DiI, HDL–DiI and AcLDL–DiI. The uptake of VLDL–DiI, CM–DiI and AcLDL–DiI was higher than that observed for LDL–DiI and HDL–DiI (Fig. 1). In addition, the fluorescence intensity was not the same for each lipoprotein.

In order to assess the effects of the different lipoproteins on the physiological responses of NK cells, the proliferative and cytotoxic responses were evaluated in parallel with the flow cytometry experiments. In Figs 3, 4, 5 and 6, (a) illustrates the NK proliferative response and (b) illustrates the cytotoxic response of NK cells in the presence of increasing concentrations of CM (Fig. 3), VLDL (Fig. 4), LDL and AcLDL (Fig. 5) and HDL (Fig. 6). Each lipoprotein had different effects in both functions.

The effect of CM (Fig. 3) on the proliferative response of NK cells was maximal at concentrations between 20 and 40 μg/ml, in which the proliferative response increased eightfold with respect to the untreated control. Concentrations higher than 60 μg/ml impeded proliferative responses. In Fig. 3b, the cytotoxic response was increased at low E:T ratios (20:1, 10:1 and 5:1), the maximal increase being at 8 and 20 μg/ml. However, at concentrations higher than 30 μg/ml, there was a marked decrease in the NK cytotoxicity, being completely absent at 60 μg/ml.

Figure 4 illustrates the effects of VLDL on NK proliferative and cytotoxic responses. In the proliferative response, the

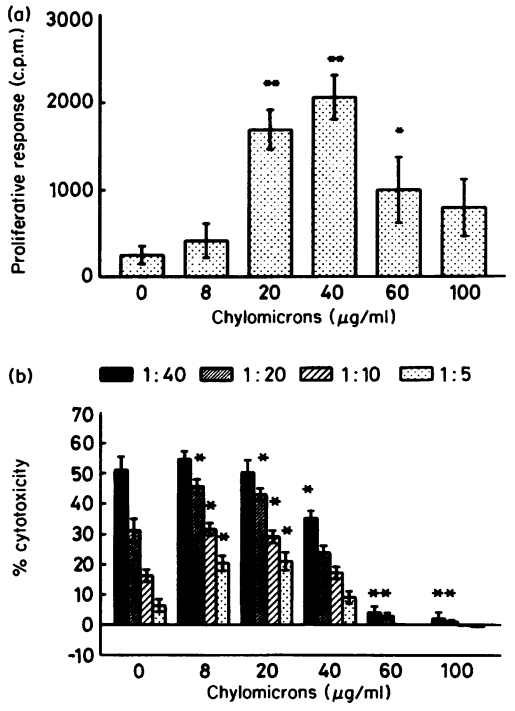

Figure 3. Proliferative and cytotoxic responses of NK cells with CM–DiI. NK cells were washed after 18 hr and incubated with different concentrations of CM for proliferative and cytotoxic responses, as described in the Materials and Methods. (a) Effect of chylomicrons on the proliferative activity of these cells; (b) the cytotoxic response. The differences observed were significant ($*P < 0.05$, $**P < 0.01$) and corresponded to the statistical analysis of the increment in proliferative response and the increase and decrease in the cytotoxic response observed compared to the untreated control.

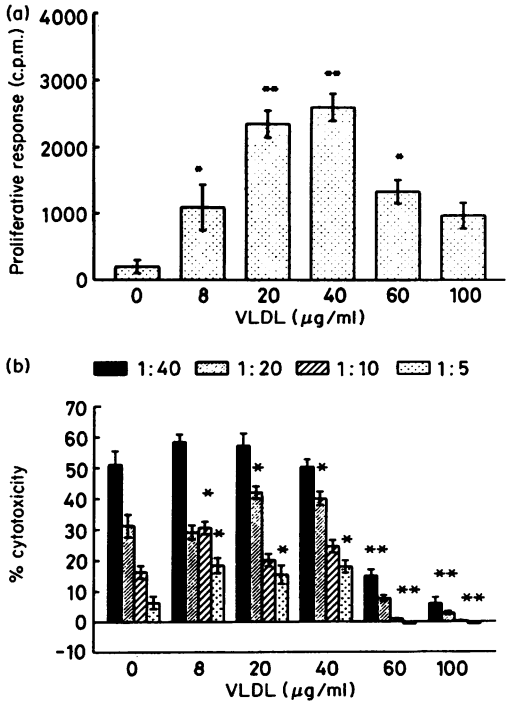

Figure 4. Proliferative and cytotoxic response of NK cells with VLDL–DiI. NK cells were washed after 18 hr and incubated with different concentrations of VLDL, as described in the Materials and Methods. (a) Effect of VLDL on the proliferative activity of these cells; (b) the cytotoxic response. The differences observed were significant ($*P < 0.05$, $**P < 0.01$) and corresponded to the statistical analysis of the increment in proliferative response and the increase and decrease in the cytotoxic responses observed compared to the untreated control.

increase was maximal between 20 and 40 μg/ml (12-fold), similar to CM. NK cytotoxicity increased at E:T ratios of 20:1, 10:1 and 5:1 for concentrations between 10 μg/ml and 40 μg/ml. The concentrations of VLDL required for the induction were higher than that observed for CM. Concentrations higher than 40 μg/ml of VLDL reduced NK cytotoxicity.

In order to study in detail the effects of LDL and AcLDL and their uptake by different receptors, the biological effects of both lipoproteins was investigated (Fig. 5). AcLDL induced a maximum 12-fold increase in the proliferative response, while the maximum LDL induction was eightfold. At 60 μg/ml of AcLDL the effect of proliferative response became significantly different with respect to 60 μg/ml of LDL ($P < 0.05$). The NK cytotoxic response increased in the presence of LDL, reaching a maximum at 20 μg/ml, and then, at concentrations higher than 40 μg/ml, there was a decrease in the cytotoxic response. In contrast, AcLDL diminished the NK cytotoxic response in a concentration-dependent fashion. This effect was the opposite of that observed on the proliferative response.

Table 1 illustrates the effect of anti-LDLR on the proliferative and cytotoxic response of NK cells treated with LDL. The antibody abrogated the proliferative and cytotoxic responses induced by LDL.

At increasing concentrations of HDL (Fig. 6), there was gradual increment of the proliferative response that was maximal (17-fold induction) at 100 μg/ml. In contrast, the cytotoxic response diminished as HDL concentrations increased. These effects are in contrast to the results shown in previous figures (Figs 3–5), in which the cytotoxic and proliferative responses increased at low concentrations of lipoprotein and decreased at high concentrations.

A detailed study on the proliferative and cytotoxic responses (E:T ratio of 20:1) of the different lipoproteins is shown in Fig. 7. The proliferative response was induced in the presence of any lipoprotein, as shown in Fig. 7a, b. Figure 7a illustrates the effects of increasing concentrations of triglyceride-rich lipoproteins (CM and VLDL), and Fig. 7b illustrates the effect of cholesterol-rich lipoproteins (LDL, AcLDL and HDL). The effects observed could be divided into three: (1) the effect of triglyceride-rich lipoproteins (CM and VLDL), which were maximal at 40 μg/ml; (2) the effect of apolipoprotein B (apo B) cholesterol-rich lipoproteins (LDL and AcLDL), which reached a maximum at 50 and 60 μg/ml, respectively; and (3) the effect of HDL, which did not reach a maximum.

In Fig. 7c, d, the effect of lipoproteins on NK cytotoxicity (E:T ratio of 20:1) was evaluated. The observed effects could be divided into two, (1) an augmentation of the cytotoxicity and (2) a marked inhibition. AcLDL and HDL showed a similar pattern of inhibition depending on the concentration of the lipoprotein (Fig. 7d), whereas CM, VLDL and LDL at low concentrations increased the cytotoxicity against K562 cells. The maximum peaks on the cytotoxic response for each lipoprotein was 8 μg/ml for CM, 30 μg/ml for VLDL (Fig. 7c)

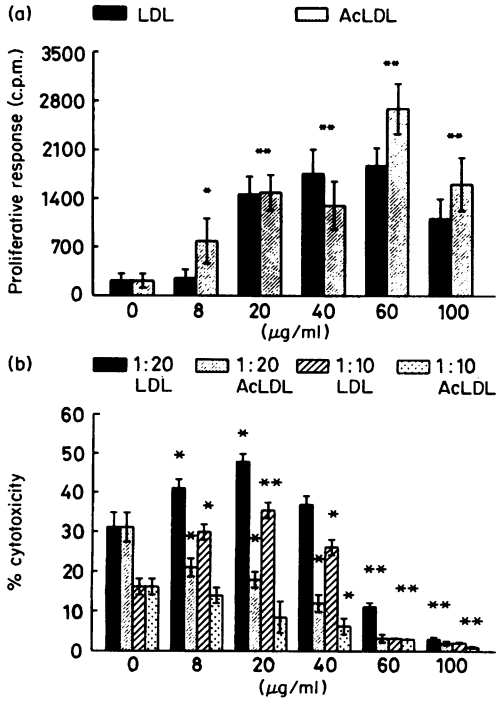

Figure 5. Proliferative and cytotoxic response of NK cells with LDL and AcLDL–DiI. NK cells were washed after 18 hr and incubated with different concentrations of LDL or AcLDL, as described in the Materials and Methods. (a) Effect of LDL and AcLDL on the proliferative activity of these cells; (b) the cytotoxic response in E : T ratios of 20 : 1 and 10 : 1 for both lipoproteins. The differences observed were significant ($*P < 0{\cdot}05$, $**P < 0{\cdot}01$) and corresponded to the statistical analysis of the increment in proliferative response and the increase and decrease in the cytotoxic response observed compared to the untreated control.

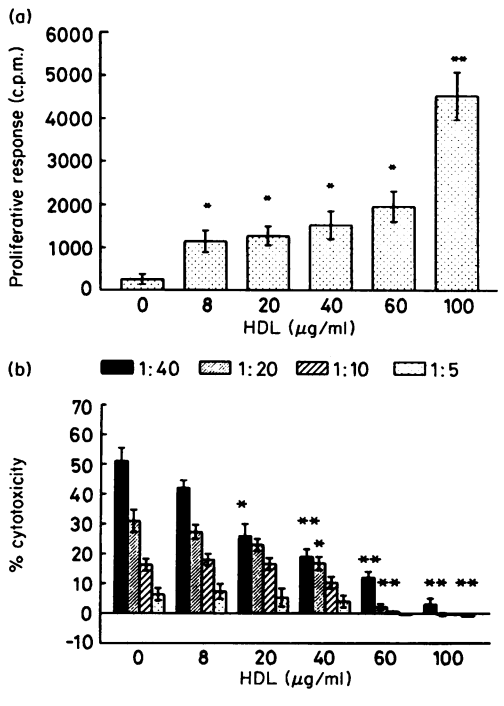

Figure 6. Proliferative and cytotoxic response of NK cells with HDL–DiI. NK cells washed after 18 hr and incubated in the presence of different concentrations of HDL, as described in the Materials and Methods. (a) Effect of HDL on the proliferative activity of these cells; (b) the cytotoxic response. The differences observed were significant ($*P < 0{\cdot}05$, $**P < 0{\cdot}01$) and corresponded to the statistical analysis of the increment in proliferative response and the increase and decrease in the cytotoxic response observed compared to the untreated control.

and 20 μg/ml for LDL (Fig. 7d). However, this effect was inverted at concentrations higher than 30 μg/ml for CM and higher than 40 μg/ml for VLDL and LDL.

DISCUSSION

The expression of the different lipoprotein receptors is enhanced in mononuclear cells when they are cultured in the absence of lipoproteins and fatty acids,[1] and depends on complex post-transcriptional and post-translational mechanisms.[1] T lymphocytes and macrophages take up lipoproteins through different mechanisms.[1–10] T lymphocytes have been shown to express LDL receptors following their activation with mitogen or with anti-CD3,[6,25] and modified LDL receptor induces their proliferation.[6,25] Macrophages have been shown to express (1) receptors for modified LDL (scavenger receptors, Fc receptors and CD36);[6,7,10] (2) receptors for VLDL;[9] (3) receptors for HDL;[8] and (4) the α_2MR/LRP, which is involved in the uptake of CM and VLDL.[6,26–28]

In NK cells, dietary lipids,[13] liquid emulsions[14,15] and oxidized LDL[16] alter cell cytotoxicity and antibody-dependent cell-mediated cellular cytotoxicity (ADCC), suggesting that lipoprotein receptors may modulate NK functions. Furthermore, lovastatin, an inhibitor of the key enzyme of the cholesterol pathway (hydroxyl methyl glutaryl coenzyme A reductase), *in vitro*, was able to decrease the proliferative response due to mitogenic stimulus, and suppressed 50% of the cytotoxic response of NK cells.[17,18] Despite its importance, these reports did not assess the expression and function of lipoprotein receptors in NK cells.

In this report, we have shown that all lipoproteins are taken up by NK cells. NK cell uptake of lipoproteins was higher when the cells were incubated in the absence of lipoproteins and were free from fatty acids. The uptake of lipoproteins, even though it was not very high, induced an increase in the proliferative response and had a bimodal effect on NK cytotoxicity, suggesting that cholesterol homeostasis and lipid supplementation may be essential for NK physiological responses. The uptake of triglyceride-rich lipoproteins and LDL resulted in a bimodal induction of both responses at low concentrations. In contrast, HDL and AcLDL diminished the NK cytotoxic response at all concentrations, despite their effect on the NK proliferative response. The receptor for LDL was shown to be blocked by a specific monoclonal antibody and the lack of expression of this receptor did not modify the uptake of other lipoproteins. Furthermore, this antibody blocked the effect of LDL on NK proliferative and cytotoxic responses. These results suggest the presence of at least three different lipoprotein receptors, one for CM and VLDL, one for LDL and one for HDL.

We have recently shown that NK cells express lipoprotein lipase (LPL) at the surface, which is a key enzyme responsible

Table 1. Effect of anti-LDLR on the proliferative and cytotoxic responses of NK cells treated with LDL

Proliferative response (c.p.m.)

Conc. LDL (μg/ml)	Pretreated with IgG2b antibody	Pretreated with anti-LDLR
0	269 ± 119	580 ± 259
8	1470 ± 229*	650 ± 453
20	2070 ± 258**	390 ± 355
40	2550 ± 328**	530 ± 452

Cytotoxic response (% of ^{51}Cr release)

E:T ratio	Control	LDL (20 μg/ml)	Anti-LDLR + LDL (20 μg/ml)
1:20	31·2 ± 3·7	48·0 ± 2·2*	28·0 ± 4·3
1:10	16·2 ± 2·11	35·5 ± 2·6*	18·6 ± 2·6

NK cells were cultured as described in the Materials and Methods. Before the proliferative and cytotoxic experiments were performed, NK cells were incubated for 30 min at 4° with either 5 μg of anti-LDLR or an irrelevant mouse IgG2b. Then the cells were washed and cultured in the presence of different concentrations of LDL, as described in the Materials and Methods. The results represent the mean ± SD of three different experiments performed in triplicate.

Significant differences were observed (*$P < 0.05$, **$P < 0.01$) in both responses when the cells treated with LDL were compared with the anti-LDLR pretreated cells.

for the hydrolysis of triglyceride-rich lipoproteins.[19] LPL was shown to bind CM and VLDL and this selective binding induced the uptake of the complex through α_2MR/LRP in macrophages.[26–28] A tentative explanation for the uptake of CM and VLDL by NK cells is that LPL is released from the NK surface due to its affinity for triglycerides, and the complex LPL–CM or LPL–VLDL is internalized through α_2MR/LRP. The interaction of the complex LPL–CM or VLDL–CM with α_2MR/LRP could induce, as shown in macrophages,[26–28] an increase in inositol, a crucial molecule for NK cell degranulation and cytotoxicity.[12] Indirect support for this hypothesis comes from experiments with Intralipid in rats[14] and humans.[15] Kurzer *et al.*[14] demonstrated that an infusion of Intralipid into rats induced an increase in the cytotoxic response of NK cells, a mechanism dependent only on triglyceride content, and Sedman *et al.*[15] demonstrated that parenteral nutrition regimens that contained medium-chain and long-chain triglycerides induced an increment in NK and lymphokine-activated killer (LAK) activity.

Another possibility is that VLDL receptors are expressed in NK, as shown in macrophages,[9] but its expression does not explain CM uptake by NK cells.

The process of activation through LDL receptors is more specific than the observed expression in α_2MR/LRP, as the interaction of LDL with its receptor, and the biological effects induced by this binding, could be blocked with anti-LDL receptor antibody, which does not affect other lipoprotein interactions. The different effects observed with LDL and AcLDL on the cytotoxic response of NK cells, and the lack of effects of anti-LDL receptor on AcLDL stimulation, suggest

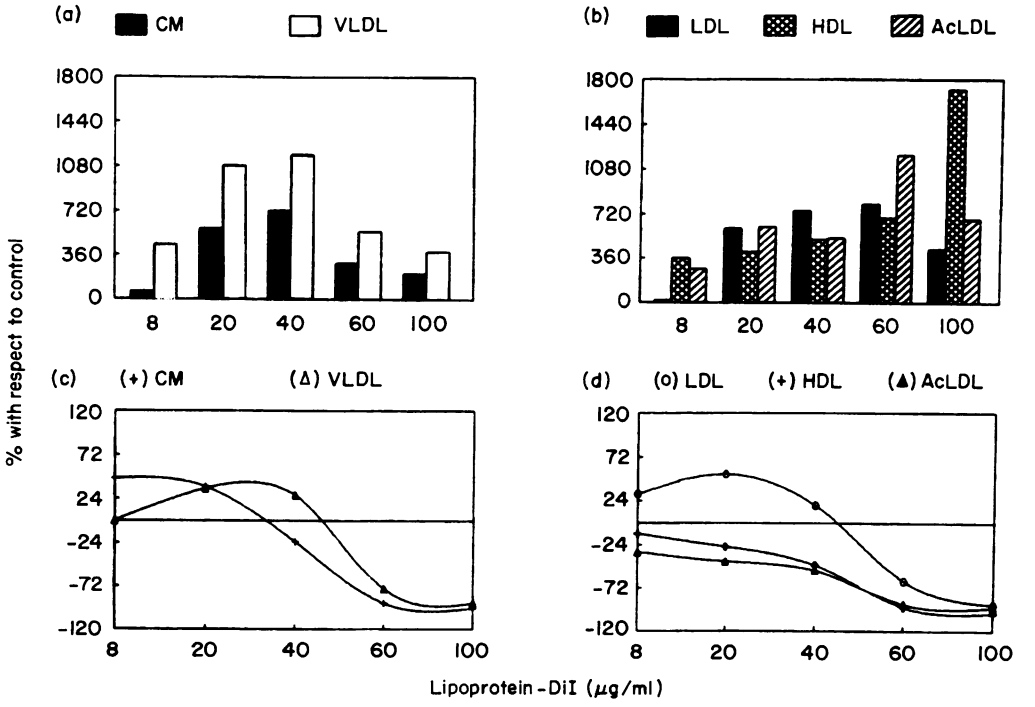

Figure 7. Summary of the effects of the different lipoproteins on the proliferative and cytotoxic responses of NK cells. (a) The induction of triglyceride-rich lipoproteins on the proliferative response of NK cells. (b) The induction of cholesterol-rich lipoproteins on the proliferative response of NK cells. (c) The effect of triglyceride-rich lipoproteins on the cytotoxic activity of NK cells at a ratio of 20:1. (d) The effect of cholesterol-rich lipoproteins on the cytotoxic activity of NK cells at a ratio of 20:1.

the presence of two independent receptors. Tanabe *et al.*,[16] in support of this hypothesis, reported that oxLDL and not native LDL inhibited ADCC activity of NK cells. It is probable that the uptake of oxLDL is dependent on Fc receptors, as the binding of NK cells to antibody-coated P815 was affected by oxLDL.

In macrophages it was shown that HDL receptors were present,[8] and its internalization did not involve lysosomal degradation.[29] In the present report, we have shown that HDL uptake is the lowest of all lipoproteins and probably this effect is the same as the one observed in macrophages. Despite this effect, the interaction of HDL with its receptor augments the NK proliferative response, as reported by Jürgens *et al.*[30] with a whole lymphocyte population. In addition, Chen & Bonavida[31] have shown that HDL inhibits the NK cytotoxic response in a similar fashion as apolipoprotein AI (apo AI) suggesting that apo AI is involved in the recognition of the receptor.

The results presented here suggest that NK biological functions may be modified by lipoproteins *in vivo*. Kurzer *et al.*[14] and Sedman *et al.*[25] have shown that a triglyceride infusion increases peripheral blood NK cytotoxic activity. It could be proposed that the high cytotoxic response observed in human hepatic NK cells, compared to peripheral blood,[11,12] might be due to the influence of lipoproteins which are anabolized and catabolized in this organ. Recently, Katznelson *et al.*[32] have shown that heart transplant patients that were treated with Primvastatin^R (another inhibitor of the hydroxymethyl glutaryl coenzyme A reductase) showed a lower peripheral NK cytotoxic activity but a higher life expectancy in comparison to the controls. This study suggests a connection between NK cell activity, cholesterol homeostasis and life expectancy, which should be examined further.

Overall, we have shown that NK cells uptake lipoproteins. This uptake may be dependent on at least three different receptors: (1) LDL receptor; (2) α_2MR/LRP receptor; (3) scavenger receptor, HDL receptor or Fc receptor. These receptors seem to activate the cells through different mechanisms, as LDL receptor and α_2MR/LRP induced an increase in the proliferative and cytotoxic responses, while HDL receptors, scavenger or Fc receptors induced proliferative responses markedly diminishing the NK cytotoxic response. The biochemical mechanisms for these effects are unknown but should give more insights on the importance of triglyceride, phospholipid and cholesterol homeostasis on NK physiological responses and their probable role in atherosclerosis.

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