# Regulatory effects of lipoprotein lipase on proliferative and cytotoxic activity of NK cells

**Juan B. De Sanctis,[1] Isaac Blanca, and Nicolas E. Bianco**

Instituto de Immunologia, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela

**Abstract** Lipoprotein lipase (LPL) induced, in a dose-dependent fashion, a 2-fold and 11-fold increase in the proliferative response of peripheral blood lymphocytes (PBL) at 48 and 72 h, respectively; a 4- and 12-fold increase in natural killer (NK) cells, respectively; and a maximal 3-fold induction in interleukin-2 (IL-2)-treated NK cells at 72 h. T lymphocytes did not proliferate independently of the concentration of LPL used. LPL decreased the proliferative response of K562 and U937 cell lines. The effect on NK cells could be blocked by anti-LPL if it was added before LPL binding to the cell membrane. Contrary to its effects on NK proliferative response, LPL inhibited spontaneous cytotoxicity and lymphokine-activated killer activity (LAK). The effect was dose-dependent, target-dependent (U937 was more sensitive than K562 in LAK assays), but not LPL-binding time-dependent. Treatment of NK cells with heparinase overcame the inhibitory effect of LPL in spontaneous cytotoxicity. LPL binding to cell membranes, as assessed by flow cytometry, was as follows: K562 cells > monocytes > NK cells > LAK cells > U937 cells, absent in T lymphocytes and partially sensible to heparinase and IL-2 treatments. Protein kinase C translocation was observed upon treatment of NK cells with LPL. Three proteins in NK cell membrane (76, 57.2, and 27.2 kD), two in the cytosol (57.2 and 27.2 kD), and only one in ANA-1 cell membrane (76 kD) were precipitated with LPL-Sepharose. LPL receptors seem to be responsible for the proliferative and cytotoxic response observed in LPL-stimulated NK cells.—**De Sanctis, J. B., I. Blanca, and N. E. Bianco.** Regulatory effects of lipoprotein lipase on proliferative and cytotoxic activity of NK cells. *J. Lipid Res.* 1996. **37:** 1987–2000.

**Supplementary key words** interleukin-2 • K562 and U937 cell lines • flow cytometry • lymphokine activated killer activity • cell membranes

Lipoprotein lipase (LPL, E.C. 3.1.1.34) is a key enzyme in the catabolism and anabolism of triglyceride-rich lipoproteins, chylomicrons, and very low density lipoprotein, as well as other lipoproteins (1). It has been established that LPL is secreted by adipose tissue (white/brown), heart, mammary gland (lactating), skeletal muscle, adrenal, ovary, thoracic aorta, spleen, small intestine, testis, lung, kidney, brain (hippocampus), neonatal liver (1), macrophages (2–4), and natural killer (NK) cells (5). The cell surface receptor for LPL has been proposed to be a proteoglycan containing heparan sulfate, or a glycosaminoglycan to which LPL is linked through phosphatidylinositol. This latter receptor, sensitive to phosphatidylinositol-phospholipase C has been discovered in cardiac myocytes, but it has not been observed in macrophages (1).

LPL is thought to be important in the development of atherosclerosis because it generates a high quantity of lipoprotein remnants that are avidly taken up by macrophages (6). This overproduction of remnants or modified lipoproteins may also alter the physiological response of other cells such as T lymphocytes and NK cells (7–9). In addition, when LPL was added to murine macrophages in culture, it induced the transcriptional activation of tumor necrosis factor (TNF) (10), a process that is protein kinase C (PKC)-dependent (11). This induction of TNF by LPL may be related to the increased level of this cytokine reported to be in the atheroma (7).

NK cells ($CD3^-CD16^+CD56^+$) represent a subset of lymphocytes distinguishable from T and B lymphocytes by their morphology, phenotype, and functional capacity to spontaneously kill tumor cells or virally infected cells (12, 13). Treatment of NK cells with interleukin 2 (IL-2) results in a significant augmentation of their tumoricidal activity (12, 13). On the other hand, IL-2 is required but not sufficient to produce a maximal proliferative response of NK cells (14).

NK cells have been involved in the metabolism of lipoproteins as these cells express and secrete LPL (5) and display different lipoprotein receptors (8, 9, 15). Furthermore, when NK cells were incubated with lipoproteins, all of them induced NK proliferative response and the extent of the response was lipoprotein- and

Abbreviations: LPL, lipoprotein lipase; NK, natural killer cells; LAK, lymphokine activated killer; IL-2, interleukin-2; FITC, fluorescein isothiocyanate; PKC, protein kinase C.

[1]To whom correspondence should be addressed.

concentration-dependent (8). Addition of lipoproteins to IL-2-stimulated T lymphocytes and NK cells also augmented their proliferative response (9). On the other hand, not all lipoproteins augmented NK cytotoxic response in the absence of other stimuli (8). The different lipoproteins originate various physiological responses in NK cells, depending on the specific receptor to which the lipoprotein is bound (8). Likewise, this recognition may be dependent on LPL expression and secretion, as it has been shown that LPL increased lipoprotein recognition by the α2 macroglobulin receptor/LDL receptor-related protein (16), and it induced lipoprotein internalization through cell proteoglycans that bind LPL (17).

The aim of the present study was to investigate the effect of LPL on the proliferative and cytotoxic response of NK cells in the absence of other stimuli and/or in the presence of IL-2. The expression of a possible receptor for LPL on NK cells, responsible for these effects, was also studied.

## MATERIALS AND METHODS

### Chemicals

Fetal calf serum (FCS), L-glutamine, penicillin–streptomycin, RPMI 1640, and RPMI-1640 Selectamine medium were purchased from Gibco BRL (Gaithersburg, MD). Human recombinant interleukin-2 (IL-2) was kindly donated by the Biological Response Modifiers Program, National Cancer Institute (Frederick, MD). Percoll and Ficoll-paque were purchased from Pharmacia LKB (Uppsala, Sweden). $Na^{51}Cr$, Expre$^{35}S^{35}S$ labeling kit, and En$^3$Hance were purchased from New England Nuclear (Boston, MA). Heparinase type III (1 unit generates 0.1 μmol saturated uronic acid/h at pH 7.5), and all other reagents were acquired from Sigma Chemical Co. (St. Louis, MO).

### Antibodies

Anti-Leu11c-PE (CD16, FcrIII receptor, expressed in NK cells) was purchased from Becton Dickinson (Mountain View, CA). Anti-NKH-1 $RD_1$ (CD56, asialo $GM_1$, expressed in NK cells), anti-T3 (CD3, expressed in T lymphocytes) unlabeled, anti-T3- FITC (CD3), anti-4B4-FITC (CD19, expressed in B lymphocytes) and anti-MO2- FITC (CD14, expressed in monocytes) antibodies were purchased from Coulter Immunology (Hialeah, FL). Anti-CD44 (hyaluronic acid receptor) was purchased from Sigma Chemical Co. (St. Louis, MO).

### LPL purification

LPL was purified from human postheparin plasma using Intralipid (Vitrus, Stockholm, Sweden) and two steps of heparin-Sepharose columns, as previously described (18). The enzyme was eluted from the affinity column with 2 M NaCl, and the purity of the protein and of anti-LPL was assessed by polyacrylamyde gel electrophoresis and Western blot analysis, respectively (19).

### Anti-LPL antibody

Anti-LPL antibody was described previously (5). It is capable of binding LPL monomer and dimer and inhibit LPL catalytic activity (5).

### Determination of endotoxin concentrations

The endotoxin content of all media and LPL preparations was determined by a quantitative limulus amebocyte lysate assay (Whittaker, Wakersville, MA). The endotoxin content in the LPL preparations and in the media were found to be lower than 0.007 ng/ml.

### Labeling of LPL with FITC

LPL was labeled using fluorescein isothiocyanate (FITC) as described in detail elsewhere (20) and adjusted to 1 mg/ml in PBS–0.02% Na azide. LPL-FITC conserved LPL catalytic activity and anti-LPL retained the same reactivity for LPL-FITC, as determined by ELISA (results not shown).

### Cell purification

Blood samples were taken from normal healthy donors (blood bank of the Central University Hospital). Human peripheral blood mononuclear cells were separated by a gradient of Ficoll-Hypaque. The cells were then washed, resuspended to $5 \times 10^6$ cells/ml in RPMI-10% FCS and separated from adherent cells by incubation on plastic Petri dishes (Becton-Dickinson, Mountain View, CA). The non-adherent peripheral blood lymphocytes (PBL) were used in proliferative assays.

Human large granular lymphocytes (LGL) were separated by passage of the non-adherent mixed population of cells through nylon wool columns, and subsequent centrifugation on Percoll gradients (21). The low density (mostly LGL) cells isolated from the Percoll gradients were treated with anti-CD3 monoclonal antibody plus

complement, to deplete $CD3^+$ cells. The resulting preparation was >80% $CD16^+$, <1% $CD14^+$, <1% $CD3^+$, and <1% $CD19^+$.

T lymphocytes were isolated from the high density bands of the Percoll gradients, and treated with anti-CD56 monoclonal antibody to deplete NK cells. The purified T cells were >95% $CD3^+$, <1% $CD14^+$, <1% $CD56^+$.

For flow cytometry assays, monocytes were purified from the top band of the Percoll gradient. The cells were >85% $CD14^+$, <1% $CD3^+$, <1% $CD56^+$.

### Cell proliferative assays

In order to determine the effects of LPL on the proliferative response of non-adherent peripheral blood lymphocytes (PBL), and T and NK cells, the cells were adjusted to a concentration of $1 \times 10^6$/ml of RPMI-1640–5% fetal calf serum, and 100 µl was added to the wells of a 96-well plate that contained 100 µl of LPL diluted at different concentrations with RPMI 1640-BSA (fatty acid-free) 0.5%. The cells were cultured for 48 or 72 h and were labeled 18 h prior to the end of the incubation period with 1 µCi/well [$^3$H]thymidine (prepared in RPMI-BSA). The incorporation of [$^3$H]thymidine was measured in a beta-plate counter (LKB, Sweden). The LPL used for this study was the same as the one used for flow cytometry studies and cytotoxic assays.

In some experiments, anti-LPL was added to LPL prior the incubation of the cells with the enzyme. Anti-LPL blocked only the effect of soluble LPL and not the effect of membrane-bound LPL.

K562 (human erythro leukemia) and U937 (human histiocytic) cells were also tested for proliferative response in the presence of LPL as they were the targets cells for spontaneous NK and lymphokine activated killer cytotoxicity, respectively. The cell cycle of both cells is 48 h. The assays were performed using $5 \times 10^4$ cells/well and LPL as described above. LPL did not affect cell viability as assessed by trypan blue exclusion.

### NK cytotoxic assay

A short term (4 h) radiolabeled release assay using $^{51}$Cr-labeled K562 cells as targets was performed as described previously (5). Briefly, $5 \times 10^6$ K562 cells were labeled with 150 µCi of $Na^{51}Cr$ (200–500 µCi/mmol), for 1 h at 37°C. Labeled cells were washed three times in RPMI medium plus 5% FCS and resuspended at $5 \times 10^4$ cells/ml in RPMI containing 10% FCS. A fixed number ($5 \times 10^3$ cells in 0.1 ml) of labeled K562 cells was mixed with 0.1 ml of effector cells at different effector-to-target cell ratios (40:1, 20:1, and 10:1). The combination of target and effector cells was seeded in triplicate into 96-well U-bottomed microtest plates (Falcon Plastics, Oxnard, CA).

Generally, the effector cells were previously incubated with different concentrations of LPL for a minimum of 30 min up to 24 h at 37°C (the effect of LPL is independent of the time of incubation). The cells were then washed and adjusted for each effector-to-target ratio. In other experiments, $1 \times 10^6$ cells were pre-incubated either with anti-LPL (0.5 or 1 µg/ml) or anti-CD44 (0.5 or 1 µg/ml) for 30 min at 4°C or 10 IU/ml of heparinase type III for 30 min at 37°C and then washed before the addition of LPL. In some assays, these latter treatments, with anti-LPL, anti-CD44, or heparinase, were performed after the incubation of the effector or the target cells with LPL.

$^{51}$Cr release was measured in 100-µl samples of supernatants using a gamma counter (Compugamma, Wallac, LKB, Sweden). Total release of radioactivity was determined by counting the radioactivity released from $5 \times 10^3$ labeled K562 cells treated with 1% Triton X-100. The percent of lysis was calculated by the following formula:

$$\% \text{ Specific lysis} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total release} - \text{Spontaneous release}}$$

### Determination of LAK activity

Purified NK cells were stimulated for 72 h with 1000 IU of IL-2/$10^6$ cells in order to induce lymphokine activated killer activity (LAK) as previously described (22). These cells were tested for cytotoxicity against a human histiocytic cell line U937 that is resistant to NK spontaneous cytotoxicity or short priming with IL-2. In parallel, the same cells were tested for cytotoxicity using K562 cells as target. The cytotoxicity assay was performed as described for NK cytotoxic assay. Spontaneous NK and LAK cytotoxicity were compared.

### Enzyme binding to cell surface

The expression of membrane-bound LPL was monitored by flow cytometry, using an EPICS 753 flow cytometer (Coulter), equipped with a 5-W argon laser and dye laser. The instrument was calibrated for fluorescence and light scatter using DNA check calibrating beads (Coulter Immunology). Excitation wavelength was set at 488 nm and all parameters except forward angle light scatter (FALS) were assessed using a logarithmic scale. LPL-FITC was added at different concentrations in PBS–0.02% sodium azide for 30 min at 37°C.

The cells were then washed twice with PBS and analyzed by flow cytometry. Non-specific binding was assessed by incubating the cells with 10 g LPL before adding LPL-FITC. A set of experiments was performed by adding LPL, for 30 min at 37°C, and subsequently anti-LPL-FITC obtaining similar results as with LPL-FITC.

In order to assess the possible receptor for LPL, the cells were treated, either before or after LPL-FITC incubation, with 10 units of heparinase (type III) per million cells, at 37°C for 30 min, and the bound LPL-FITC was quantified by flow cytometry. Likewise, some experiments were performed adding anti-CD44, 0.5 μg/ml for 30 min at 4°C, prior to the incubation with LPL-FITC. In another assay, the cells were incubated with LPL-FITC, washed, and then treated with 1000 IU of IL-2/ml for 30 min at 37°C.

Binding experiments with LPL-FITC were also performed with purified monocytes (>90% $CD14^+$), purified T lymphocytes (>90% $CD3^+$), either non-stimulated or stimulated with 100 IU of IL-2/$10^6$ cells, and U937 and K562 cell lines growing in log phase.

### Determination of PKC activity

The measurement of PKC activity was performed as described by the manufacturer (Gibco BRL) using the protocol described by Thomas, Gopalakrishna, and Anderson, (23) and modified by Yasuda et al. (24). The assay is specific as it is performed with a particular pseudosubstrate inhibitor, PKC(19–36). Briefly, 20 × $10^6$ purified NK cells were stimulated with 1 μg/ml of LPL for 1 h, then the cells were lysed by homogenization in a dounce homogenizer using the ice-cold lysing solution provided by the kit (20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 25 g/ml leupeptin, and 25 g/ml apoprotinin). The membrane fraction and cytosol fraction were separated by ultracentrifugation at 100,000 *g* for 60 min at 4°C. The membrane fraction was solubilized again with 1% (w/v) of Nonidet P-40 and then recentrifuged at 100,000 *g* for 60 min at 4°C. Then, the enzyme from both fractions was partially purified through DEAE52 columns following the kit's instructions and eluted with a solution containing 20 mM Tris, pH 7.5, 0.5 mM EDTA, 0.5 mM EGTA, 10 mM mercaptoethanol, and 0.2 M NaCl. The eluted fraction was analyzed for PKC activity following the optimum conditions of the assay (20 mM Tris, pH 7.5, 20 mM $MgCl_2$, 1 mM $CaCl_2$, 20 M ATP, 50 M acetyl myelin basic protein, 0.28 mg/ml phosphatidylserine, and 100 nM PMA) suggested in the kit's instructions. After the assay, the samples were spotted in DEAE-cellulose disks that were subsequently acid-washed and then counted in the beta counter (LKB-Bromma, Sweden). The specificity of the assay was determined by subtracting the radioactivity obtained in the presence of the pseudosubstrate inhibitor PKC(19–36) from the total radioactivity of the assay. The results are expressed as percentage of control values.

In order to assess the effects of activation of PKC by LPL, 20 μM of H7 (1-(5-isoquenoline sulfonyl)-2-methylpiperazine dihydrochloride) was added 1 h before the addition of LPL; a set of experiments without addition of LPL were used as control.

### Identification of LPL receptors

LPL receptor from NK cells was precipitated using the standard protocol for immunoprecipitation described elsewhere (25), with a minor modification, namely an LPL-Sepharose (10 mg of LPL/ml of gel) was used and samples were taken from the cytosol and membrane of NK cells. Briefly, purified NK cells were rinsed with methionine/cysteine-free medium (RPMI-1640 Selectamine) and metabolically labeled with 0.1 mCi/ml of Expre$^{35}$S$^{35}$S (labeling kit) overnight in the presence of dialyzed 10% FCS. The cells were then centrifuged and lysed in phosphate-buffered saline (PBS) solution containing 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, and 0.1 mM leupeptin. After centrifugation of the lysed cells to remove nuclei and unbroken cells, the supernatant was separated and the membrane and cytosol fractions were separated by ultracentrifugation and sucrose gradient, respectively (25). The membrane fraction was then washed by centrifuging at 100,000 *g* in the lysis buffer, but with 0.1% NP-40. The pellet was resuspended in 0.5 ml lysis buffer. Both membrane and cytosol fractions were tested for alkaline phosphatase activity using *p*-nitrophenyl phosphate as substrate and measuring the product, *p*-nitrophenol. Phosphatase activity was present only in the membrane fraction. An aliquot of each fraction, 50 μl, was pretreated with 20 μl of albumin-Sepharose (1 mg/ml). The mixture was incubated for 2 h at room temperature, centrifuged, and the supernatant was mixed with 20 μl of LPL-Sepharose, prepared as detailed elsewhere (18), for 1 h at room temperature. The mixture was centrifuged, and the pellet was washed twice with 0.05% NP-40 and 1 mM PMSF, and twice with 0.05 M Tris-HCl, pH 6.8. Finally, the pellet was resuspended in SDS buffer and heated at 95°C for 10 min. Samples were then subjected to SDS-polyacrylamide gel electrophoresis using 7% acrylamide as separating gel. After the electrophoresis, the gels were soaked in En$^3$Hance, dried, and autoradiographed with an amplified screen using Kodak Xomat AR film (Rochester, NY).

As controls, samples of membrane and cytosol were treated with LPL-Sepharose that was previously treated

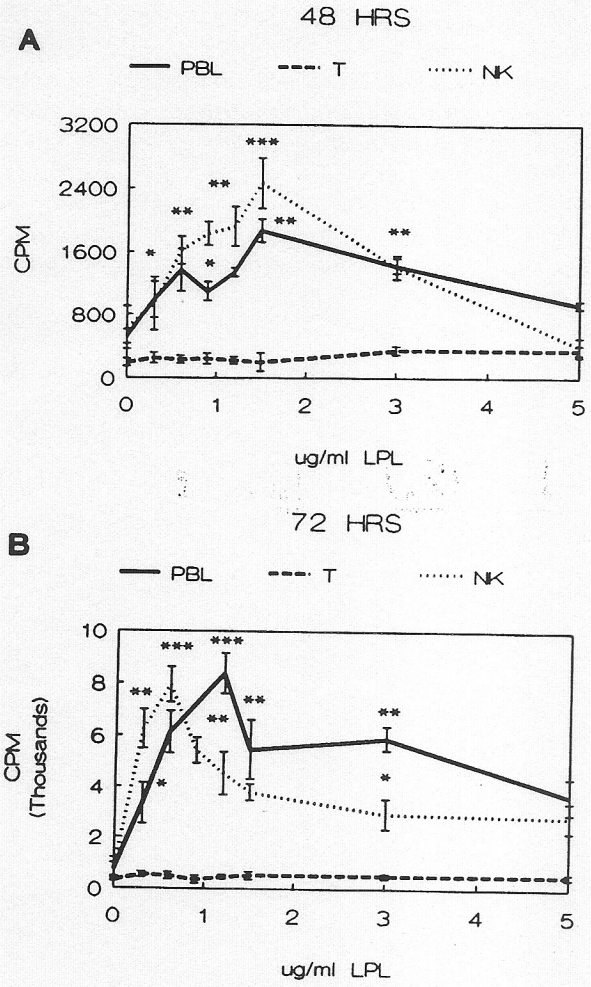


**Fig. 1.** Effect of LPL on the proliferative response of peripheral blood non-adherent lymphocytes (PBL), NK cells and T lymphocytes. The cells were separated and treated as described in Materials and Methods and each curve represents the mean ± standard deviation of ten different donors performed in triplicate. Part A of the figure illustrates the effect of different concentration of LPL on the proliferative response of PBL, NK, and T cells at 48 h. PBL and NK cells, but not T lymphocytes, proliferate in the presence of LPL. These increments in proliferative response were significant at different concentrations of LPL (*$P < 0.01$, **$P < 0.001$, ***$P < 0.0001$) as compared to non-treated cells. Part B of the figure depicts the effect of different concentration of LPL on the proliferative response of PBL, NK, and T cells at 72 h. These increments in proliferative response were significant at different concentrations of LPL (*$P < 0.01$, **$P < 0.001$, ***$P < 0.0001$) as compared to non-treated cells.

with 20 μl of anti-LPL. Thus, anti-LPL blocked LPL binding to its receptors.

### Statistical analysis

The paired and unpaired Student's *t* test was used for statistical analysis.

## RESULTS

### LPL effect in proliferative response

**Figure 1** illustrates the effect of LPL on the proliferative response of peripheral non-adherent blood mononuclear lymphocytes (PBL), T lymphocytes, and NK cells. At 48 and 72 h, LPL induced the proliferative response of PBL and NK cells, but it did not modify T lymphocyte response (Fig. 1, parts A and B). In part A of the figure, a 4-fold increment in NK proliferative response was observed at concentrations ranging from 0.9 to 1.5 μg/ml of LPL, while there was only a maximum 2-fold induction for PBL in this range of concentrations. NK proliferative response was significantly higher in NK cells as compared with PBL ($P < 0.05$, n = 10) at the same range of concentrations. At 72 h, part B of the figure, the induction of the proliferative response was similar for PBL and NK cells (11-fold and 12-fold, respectively), but there was a shift in the concentration of LPL needed to reach this maximum. While the optimal concentration of LPL required for the maximal induction of PBL proliferation was 1.2 μg/ml, similar to the value observed at 48 h, the quantity required for maximal proliferative response of NK cells was 0.5 (g/ml, 3-fold less as compared to 48 h.

The effect of LPL on NK cells is specific as the addition of anti-LPL prior to the addition of LPL to cell culture blocked the proliferation induced by LPL as observed in **Fig. 2,** part A. With increasing concentrations of the antibody (0, 0.5, and 1 μg/ml), the amount of LPL required to induce proliferative response was higher. This effect was significant ($P < 0.05$, n = 4) up to 1.2 μg/ml of LPL for both concentrations of antibody used. At high concentrations of LPL, a decrease in the proliferative response was observed as reported in Fig. 1, part A. The inhibition of proliferative response was not observed when anti-LPL was added after cell incubation with LPL; anti-LPL did not affect the proliferative response induced by LPL (results not shown).

Two cell lines were also used to study the effect of LPL on the proliferative response and compared to fresh cells. Instead of increasing the proliferative response, LPL decreased it in both cell lines in a dose-dependent fashion (Fig. 2, part B). This decrease was significant at concentrations higher than 0.9 μg/ml ($P < 0.01$), and it reached a plateau at 3 μg/ml. The concentration required for a 50% reduction in the proliferative response was 2.0 and 1.5 μg/ml of LPL for K562 and U937, respectively.

In order to monitor the effect of IL-2 and LPL on NK cells, NK cells were either stimulated for 18 h with 100 IU of IL-2/ml (primed) and then washed before the

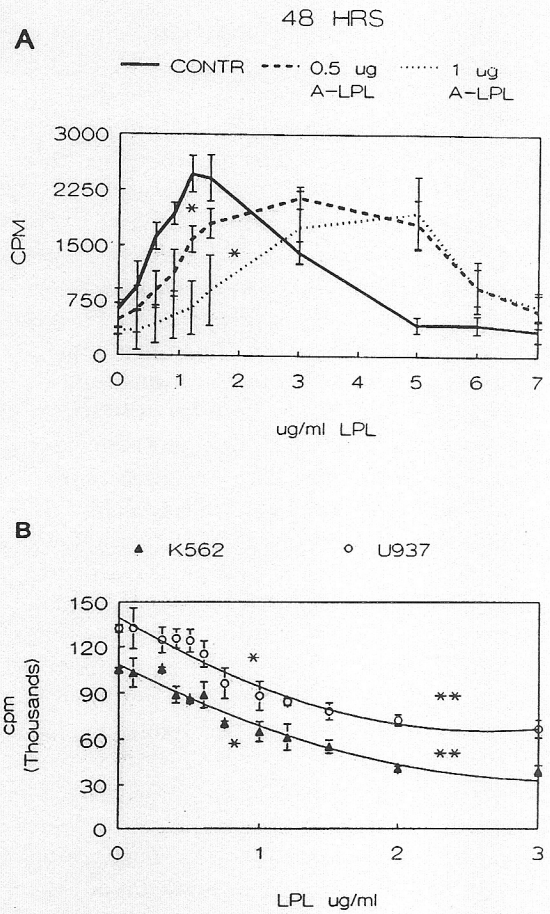


**Fig. 2.** Effect of LPL and anti-LPL on the proliferative response of NK cells, and K562 and U937 cell lines. Part A: The figure illustrates the effect of the addition of anti-LPL to LPL prior to the stimulation of the cells with anti-LPL. Anti-LPL binds LPL avidly in solution and blocks the effect of LPL on NK proliferative response. The assay was performed in ten donors in the absence of anti-LPL and four donors for each concentration of LPL. Significant differences ($*P < 0.05$) were observed at concentrations ranging from 0.5 up to 1.2 μg/ml for each concentration of anti-LPL used, 0.5 μg (0.5 μg A-LPL) or 1 μg (1 μg A-LPL) as compared to the proliferative response observed in the absence of anti-LPL (CONTR). Part B: Effect of LPL on the proliferative response of K562 and U937 cell lines. The cell lines were cultured and treated as described in Materials and Methods and the proliferative response was assessed at 48 h. Significant differences ($*P < 0.01$, $**P < 0.001$, n = 16) were observed when LPL treated cells were compared to non-treated cells.

addition of LPL, or the 100 IU of IL-2/ml was added together (costimulated, NK + IL-2) with LPL in culture. The effect at 48 and 72 h is depicted in **Fig. 3.** PBL incubated with IL-2 was used as a control. Contrary to the previous observation with non-stimulated cells, either group of NK cells, primed or costimulated, proliferated less than PBL at 48 h (2-fold less comparing the maximum). When PBL were stimulated with LPL, there was only a 1.6-fold induction, at 1.2 μg/ml, in the proliferative response when it was compared to non-treated cells. Even though the maximal proliferative response at 48 h was similar for primed or costimulated cells, the concentration of LPL required to reach a maximum response was 1.2 μg/ml for primed cells as compared to 0.3 μg/ml for costimulated cells (Fig. 3, part A). At 72 h, either in the presence or absence of LPL, the NK primed cells proliferated significantly less

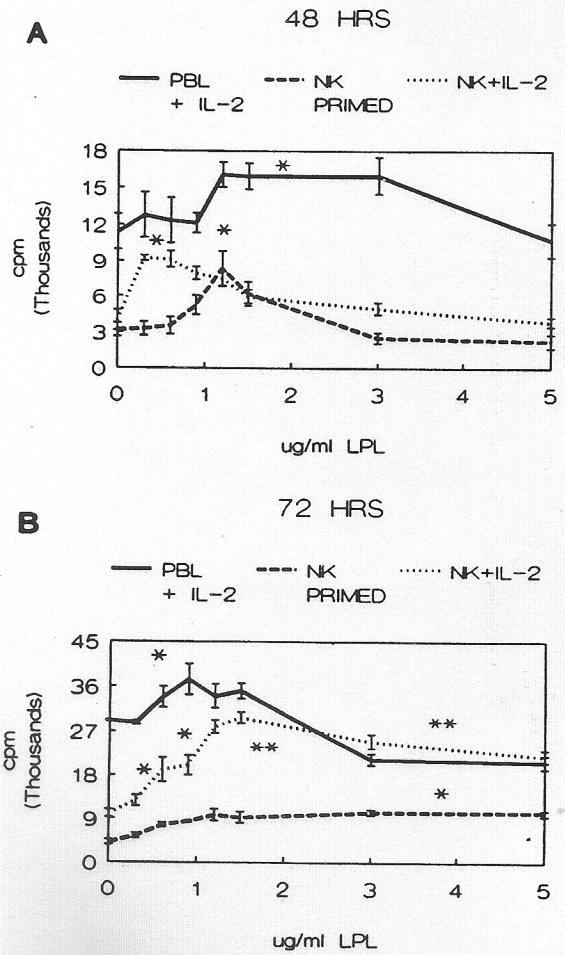


**Fig. 3.** Effect of LPL on the proliferative response of IL-2-treated PBL and NK cells. PBL and NK cells were separated and then either costimulated (+ IL-2) or primed (stimulated with 100 IU/ml of IL-2 for 18 h prior to the addition of LPL) as described in Materials and Methods. The assay was performed with ten different donors in triplicate. Part A of the figure represents the effect in the proliferative response at 48 h. Significant differences ($*P < 0.01$) were observed for each group at the maximum proliferative response when compared to cells not treated with LPL. NK either primed or costimulated proliferate significantly less ($P < 0.01$) than PBL at 1.2 μg/ml LPL. Part B of the figure illustrates the effect in the proliferative response at 72 h. Significant differences ($*P < 0.001$, $**P < 0.0001$) were observed when the treated cells were compared with cells not treated with LPL. NK primed cells proliferate significantly less ($P < 0.0001$) as compared to costimulated cells. NK costimulated and primed cells proliferate significantly less ($P < 0.001$ and $P < 0.0001$ respectively) at concentrations ranging from 0 up to 1 μg/ml as compared to PBL.

($P < 0.0001$, n = 10) as compared to costimulated cells. The increment in the proliferative response was only 2-fold for primed cells and 3-fold (2.8) for costimulated cells. Similarly, at 1.2 μg/ml of LPL, the proliferative response of costimulated cells was 3-fold higher than the observed value for primed cells. In the absence of LPL, the difference was 2-fold. Finally, LPL induced only a marginal, 1.2-fold, increment of the proliferative response in PBL cells. The reduction of PBL proliferative response at concentrations of LPL higher than 3 μg/ml was not significant. At 48 h, NK cells, either primed or costimulated, proliferated significantly less ($P < 0.01$) than PBL. At 72 h, NK primed cells proliferated significantly less ($P < 0.0001$) as compared to costimulated cells, and NK costimulated cells proliferated significantly less ($P < 0.001$) at concentrations ranging from 0 to 1 μg/ml when compared with PBL. In primed or costimulated T lymphocytes, LPL did not enhance proliferative response at 48 or 72 h (results not shown).

### Effect of LPL on NK and LAK cytotoxic response

LPL was able to inhibit NK-mediated cytotoxic activity in a concentration-dependent fashion as illustrated in **Fig. 4,** part A. The inhibition of NK cytotoxic activity was independent of the time of incubation of the cells with the enzyme (results not shown). A 50% reduction in the cytotoxic response was observed at 1 μg/ml of LPL for most effector-to-target (E:T) ratios. The cytotoxic response was blocked completely at concentrations higher than 3 μg/ml of LPL. The effect of LPL was independent of the time of incubation of the effector cells with the enzyme as the same inhibition was observed when the cells were incubated with LPL for 30 min up to 18 h (results not shown).

When anti-LPL was mixed with LPL before it was incubated with the cells, anti-LPL prevented LPL inhibition of the cytotoxic activity as shown in Fig. 4, part B (A-LPL LPL). The opposite effect was observed when anti-LPL was added after the incubation with LPL (A-LPL). Moreover, the addition of anti-CD44 (0.5 μg) did not prevent the inhibitory effect observed with LPL. The effect observed with anti-CD44 was independent of the concentration used (other concentrations not shown) or whether it was added before or after LPL.

As observed in **Fig. 5,** part A, LAK activity in the presence of LPL was less affected when K562 cells were used as targets as compared with spontaneous cytotoxicity. In order to achieve a 50% reduction of the cytotoxic response, the concentration of LPL was 3 μg/ml for each ratio, 3-fold higher than the concentration required to achieve the same response in spontaneous cytotoxicity. However, when U937 was used as target, LPL inhibited the cytotoxic response of LAK cells (Fig. 5, part B) at lower concentrations. A 50% reduction in cytotoxic response was observed at 0.8 μg/ml of LPL for most E:T ratios. This concentration is lower than the recorded concentration for NK spontaneous cytotoxic-

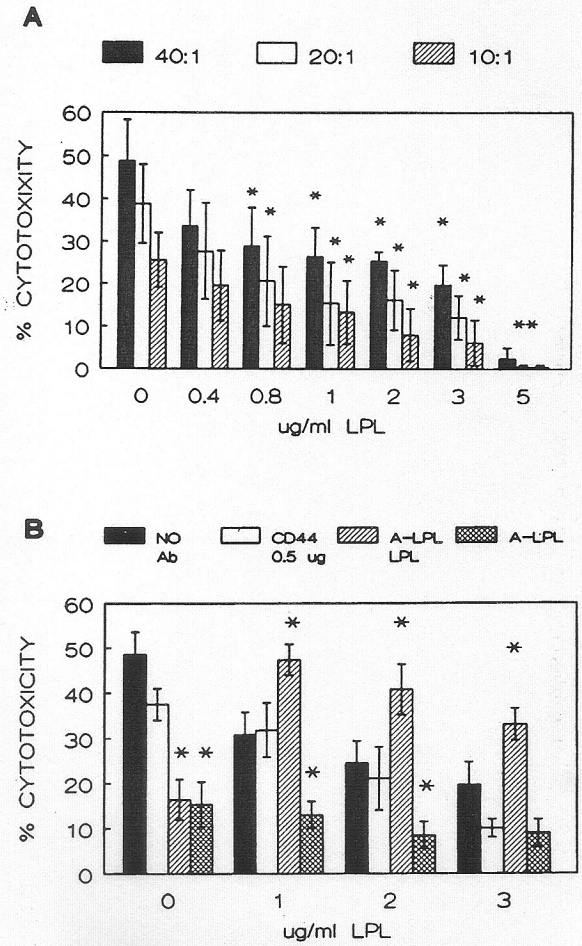


**Fig. 4.** Effect of LPL on NK spontaneous cytotoxic activity. The cells were separated and treated and the cytotoxic activity was performed in ten donors in triplicate as described in Materials and Methods. Part A of the figure represents the effect of LPL on the spontaneous cytotoxicity at different effector:target ratios in the presence of different concentrations of LPL. Significant differences were observed when comparing the cytotoxicity recorded with non-treated cells with respect with LPL incubated cells (*$P < 0.05$, **$P < 0.01$). No spontaneous cytotoxic activity was observed at concentrations higher than 3 μg/ml. Part B of the figure depicts the effect of different concentrations of LPL on the cytotoxic response observed for different treatments of the cells at an E:T ratio of 40:1. The graph represents the percentage of cytotoxicity versus the amount of added LPL; 0 corresponds to no LPL added to the cells. Four different treatments are illustrated: *1)* cells incubated in the absence of antibodies (No Ab); *2)* cells treated with 0.5 μg/ml of anti-CD44 prior to the addition of LPL; *3)* 1 μg/ml of anti-LPL was mixed along with LPL (A-LPL LPL); and *4)* 1 μg/ml of anti-LPL was added after the incubation of the cells with LPL (A-LPL). The concentrations of antibodies, in each case, was kept constant. Significant differences were observed (*$P < 0.05$) when the different treatments were compared to the control (No Ab).

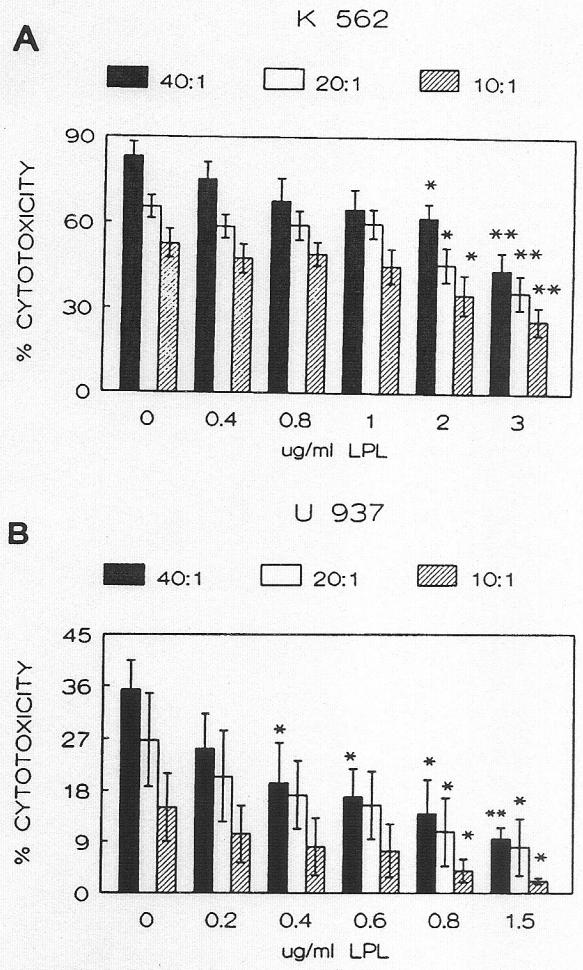


**Fig. 5.** Effect of LPL on LAK cytotoxicity. The purified NK cells were stimulated with IL-2 to generate LAK cells and then were treated with different concentrations of LPL and the cytotoxic assay was performed as described in Materials and Methods. The assays were performed in parallel for ten different donors. Part A of the figure illustrates the effect of LAK cytotoxicity against K562 cells at different concentrations of LPL. Significant differences were observed depending of the concentrations of LPL (*$P < 0.01$, **$P < 0.001$). Part B of the figure illustrates the effect of LPL on LAK cytotoxicity using U937 as targets. The cytotoxicity recorded is less than that observed for K562. However, significant differences (*$P < 0.05$, **$P < 0.01$) were obtained at different concentrations of LPL. Note that the values represented in the X and the Y axis are different from part A.

ity (1 μg/ml). The concentration of LPL required for 50% inhibition was significant when spontaneous and LAK cytotoxicity using K562 cells as targets were compared ($P < 0.001$) and when LAK cytotoxicity using K562 and U937 were compared ($P < 0.01$).

In order to assess the importance of LPL binding to a receptor in NK cytotoxicity, heparinase was added either to K562 cells or to purified NK cells previously treated with LPL. The treatments generated different responses as observed in **Table 1.** The addition of heparinase slightly increased the spontaneous cytotoxic response of NK cells. Moreover, heparinase treatment of NK cells previously incubated with LPL showed an increment in the cytotoxic response as compared to the non-treated cells and the heparinase-treated K562 cells. The increment for heparinase-treated NK cells was close to 2-fold compared to the non-treated NK cells and K562-treated cells and was significant ($P < 0.01$) at 0.5 and 1.0 μg/ml LPL at both effector-to-target ratios tested.

### Binding of LPL to the surface of purified cells

LPL-FITC binds to the surface of NK cells and other cell lines. **Figure 6,** part A, illustrates the total, specific, and nonspecific binding of LPL-FITC. LPL-FITC bound avidly to K562 cells and monocytes and to a lesser extent to U937 cells, NK cells, LAK cells, and T lymphocytes (Fig. 6, parts B, C, and D). In T lymphocytes, either non-stimulated or stimulated with IL-2 (results not shown), there was no incorporation of LPL-FITC o the surface of these cells as assessed by the lack of increment of the mean channel fluorescence intensity recorded. The effect observed in NK and LAK cells was different from the one observed in T cells: there was a significant increment in the mean channel fluorescence intensity with respect to the basal value. This increment, in NK

TABLE 1. Effect of heparinase on the cytotoxic activity of NK cells

| E:T Ratio | [LPL] | Heparinase Treatment | % Cytotoxicity* |
|---|---|---|---|
| | μg/ml | | |
| 40:1 | 0 | - | 38.0 ± 2.4 |
| | 0 | K562 | 33.8 ± 2.7 |
| | 0 | NK | 42.0 ± 2.0 |
| | 0.5 | - | 27.9 ± 3.1 |
| | 0.5 | K562 | 32.8 ± 2.3 |
| | 0.5 | NK | 45.0 ± 3.0[a] |
| | 1.0 | - | 21.0 ± 2.4 |
| | 1.0 | K562 | 16.0 ± 2.4 |
| | 1.0 | NK | 38.0 ± 2.5[a] |
| 20:1 | 0 | - | 29.4 ± 1.5 |
| | 0 | K562 | 24.1 ± 2.4 |
| | 0 | NK | 32.1 ± 3.2 |
| | 0.5 | - | 20.6 ± 2.8 |
| | 0.5 | K562 | 19.3 ± 2.3 |
| | 0.5 | NK | 35.5 ± 2.6[a] |
| | 1.0 | - | 16.5 ± 2.5 |
| | 1.0 | K562 | 14.2 ± 1.8 |
| | 1.0 | NK | 32.0 ± 3.1[a] |

The spontaneous cytotoxicity of NK cells was assayed after the incubation of either the effector or the target cells with different concentrations of LPL and then treatment with either 0 or 10 units of heparinase before the cytotoxic assay was carried out. The type of treated cells is illustrated in the column labeled Heparinase Treatment. The assay was performed in triplicate for three different donors as described in the Materials and Methods section and the percentage of cytotoxicity refers to the mean ± standard deviation. Statistical significance was observed ([a]$P < 0.01$) when the % cytotoxicity of the non-treated heparinase cells was compared with the % cytotoxicity of NK cells treated with heparinase.

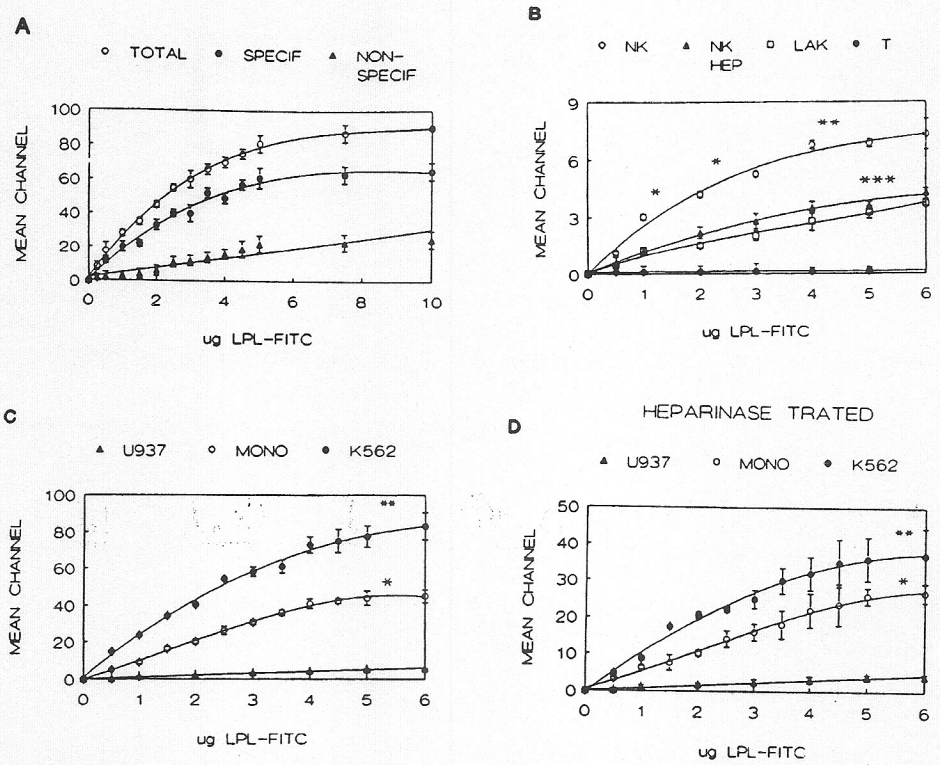


**Fig. 6.** Binding of LPL-FITC to different cell types. The cells were separated and incubated with different concentrations of LPL-FITC as described in Materials and Methods. The binding, assessed by flow cytometry, is reported as a mean channel fluorescence intensity (specified as Mean Channel in the figure). The figures represent the Mean Channel versus the amount of LPL-FITC added (each point represents the mean ± SD of eight different experiments). In some experiments, the cells were treated with 10 units of heparinase/$10^6$ cells prior to the addition of LPL-FITC. Part A of the figure represents a typical calculation of LPL specific binding by flow cytometry performed with K562 cells. The specific binding was calculated based on the subtraction of the total LPL-FITC binding minus the background fluorescence recorded when cells were previously treated with 10 μg/ml before adding LPL-FITC. Part B of the figure illustrates the binding of LPL-FITC to NK cells, heparinase treated NK cells LAK cells and T cells. Significant differences were observed when NK cells were compared to T cells (*$P < 0.05$, **$P < 0.01$) and LAK cells (***$P < 0.05$). Heparanase treatment also significantly reduced LPL-FITC binding ($P < 0.05$) as compared to non-treated cells. Part C of the figure depicts the binding of LPL-FITC to K562 cells, monocytes and U937 cells. The binding was significantly higher (*$P < 0.001$, **$P < 0.0001$) as compared to non-treated cells. In part D of the figure, the effect of heparanase for monocytes, K562 and U937 cells is recorded; the amount of LPL bound is still significantly higher in K562 cells and monocytes as compared to U937 cells (*$P < 0.001$ and **$P < 0.0005$, respectively).

and LAK cells, was, however, significantly lower when it was compared to the values obtained for monocytes ($P < 0.05$ and $P < 0.01$) and K562 cells ($P < 0.001$ and $P < 0.0001$) at concentrations higher than 2.5 μg and 0.5 μg of LPL, respectively. There was a significant reduction in the binding of LPL-FITC when the cells were treated with heparinase (Fig. 6, parts B and part D). The decrease in binding was higher in K562 and monocytes ($P < 0.001$) as compared to NK and U937 cells ($P < 0.05$). No major decrease in LPL-FITC binding was observed when LAK cells were treated with heparinase (results not shown). A similar effect was observed when the cells were treated with heparinase before or after LPL binding (results not shown). Anti-CD44 did not inhibit the binding of LPL-FITC to cell membrane (results not shown) in a similar fashion as observed in NK spontaneous cytotoxicity (Fig. 4, part B).

LPL bound differently to each cell population tested. As there was no binding of LPL-FITC in T cells, and the recorded fluorescence corresponded to the non-specific autofluorescence, the mean fluorescence intensity of T cells was used to calculate the difference in the maximum binding of LPL-FITC. Taking this last point into account, K562 cells bound 500-fold more LPL-FITC than T cells; similarly, monocytes bound 214-fold more, NK cells 49-fold more, LAK cells 20-fold more, and U937 27-fold more than the basal value. Fundamentally, monocytes bound 4.5-fold more LPL than NK cells, NK

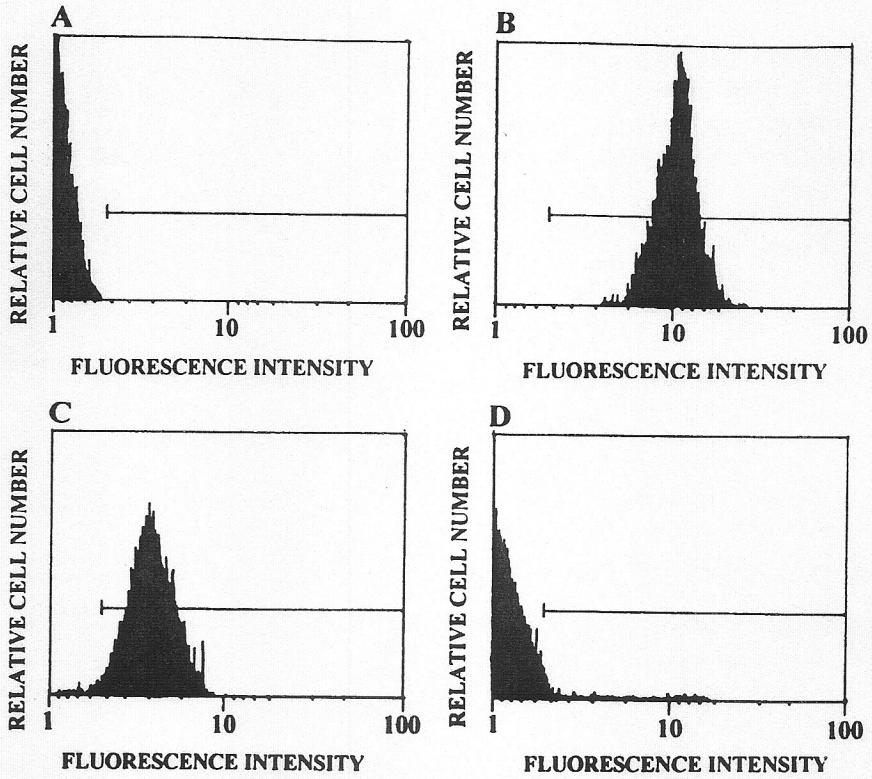


**Fig. 7.** Flow cytometry analysis of the binding of LPL-FITC to NK cells. A typical flow cytometry experiment of LPL-FITC binding to the surface of NK cells is depicted in this figure. In part A of the figure, the background fluorescence (cell autofluorescence) was recorded. Part B illustrates the histogram recorded when 6 μg of LPL-FITC was bound to NK cells. The histogram represents total binding. Part C illustrates the histogram recorded when the cells were treated with 10 IU/ml heparinase after the addition of LPL-FITC. The fluorescence intensity is less than that recorded in part B of the figure. In part D, the histogram represents the effect of stimulating the cells for 30 min at 37°C with 1000 IU/ml of IL-2 after LPL-FITC binding.

cells 2-fold more than LAK cells, K562 2-fold more than monocytes, and K562 14-fold more than U937 cells. The calculated $K_m$ of LPL (required amount of LPL for 50% binding) for each cell type was as follows K562 = $2.9 \times 10^{-6}$ M, monocytes = $3.2 \times 10^{-7}$ M, NK cells = $1 \times 10^{-7}$ M, LAK cells = $2.1 \times 10^{-7}$ M, and U937 = $5 \times 10^{-8}$ M.

A typical flow cytometry experiment of LPL-FITC binding to the surface of NK cells is shown in **Fig. 7.** In part A of the figure, the background fluorescence is recorded and in part B the maximum uptake of LPL-FITC in NK cells is assessed (6 μg/ml). When these cells were treated with heparinase the fluorescence intensity decreased proportionally to the membrane-bound LPL-FITC (part C of the figure). In part D, a histogram represents the effect of stimulating the cells for 30 min at 37°C with 1000 IU/ml of IL-2 after LPL-FITC incubation. The bound LPL-FITC diminished significantly as compared to the observed value in parts B and C ($P < 0.001$, n = 5).

### PKC activity of NK stimulated cells

LPL induced a greater than 60% translocation of PKC from the cytosol to the membrane, parallel to a decrease in the cytosolic form of the enzyme. This increment of PKC in the particulate fraction was blocked by H7 as is shown in **Fig. 8.** H7 did not affect PKC translocation by itself.

### LPL receptor

LPL receptor was determined by precipitation of cells metabolically labeled with [$^{35}$S]methionine using LPL-Sepharose. As shown in **Fig. 9,** the precipitation of LPL-binding proteins from non-stimulated NK cells led the resolution of bands of $Mw_{app}$ of 76,000, 57,200, and 27,200 in the membrane fraction and only bands of

57,200 and 27,200 in the cytosol fraction. ANA-1 macrophages, which produce high amounts of LPL and may be activated by the addition of LPL, showed a single band of $Mw_{app}$ 76,000 in the membrane fraction. The control experiments, performed by adding anti-LPL to LPL-Sepharose prior to incubation with the membrane or cytosolic fraction, illustrate the specificity of the assay.

## DISCUSSION

Lipoprotein lipase (LPL) is a key enzyme in the complex physiology of lipid metabolism. In vivo studies have shown the importance of LPL in different lipoprotein disorders, e.g., hyperchylomicronemia and atherosclerosis. In the hyperchylomicronemia syndrome, LPL is either absent or dysfunctional (26), or is catalytically inactive due to the lack of the essential activator, apoC-II (27, 28). On the other hand, overproduction of LPL by smooth muscle cells and macrophages or an increase in the amount of LPL in the vascular endothelium generates lipoprotein remnants that seem to play an important role in the development of atherosclerosis (1, 6, 29, 30). Renier and colleagues (19) have shown that an association exists between LPL production by macrophages and genetic predisposition to murine atherosclerosis. The LPL mRNA expression and secretion of LPL was found to be higher in strains of mice identified as being susceptible to atherosclerosis, as compared to their resistant counterparts. In addition, LPL was found to induce TNF-α transcription in macrophage cells (10), a process that is

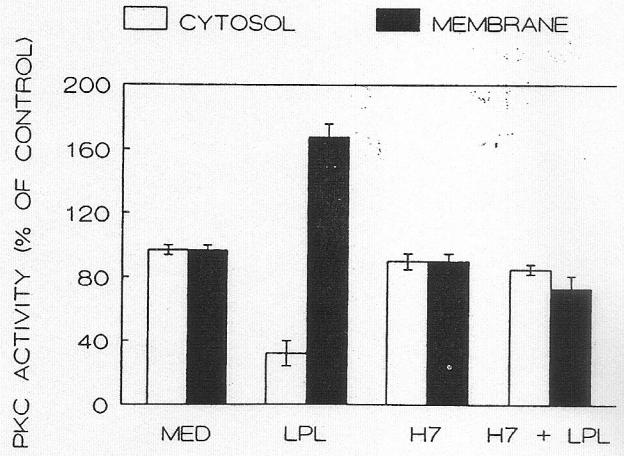


**Fig. 8.** PKC activity of NK stimulated cells. NK cells were separated and treated with LPL and the assay was performed as described in Materials and Methods. The figure represents the percentage of PKC activity, with respect to control, assessed in the cytosol and membrane fraction of the cells treated with 1 μg/ml LPL, 20 μM H-7 (1-(5-isoquenoline sulfonyl)-2-methylpiperazine hydrochloride) and 20 μM H-7 plus 1 μg/ml LPL.

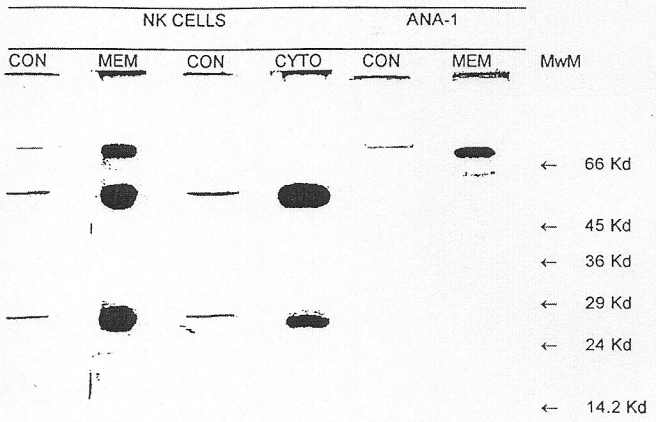


**Fig. 9.** LPL receptors were determined by precipitation of metabolic labeled cells, with [$^{35}$S]methionine, using LPL-Sepharose as described in Materials and Methods. LPL receptors were assessed in the membrane (MEM) and cytosol (CYTO) fraction of NK cells and in the membrane (MEM) fraction of ANA-1 cells. In order to test specificity, the fractions were incubated with LPL-Sepharose previously treated with anti-LPL (CON). The recorded bands can also be observed when the gel is colored with Coomassie brilliant blue. Molecular weights of the standards (MwM) are illustrated at the right side of the picture. The bands recorded in the membrane fractions of NK cells have $Mw_{app}$ of 76,000, 57,200, and 27,200, the bands recorded in the cytosol fraction, 57,200 and 27,200, and the band observed in the membrane fraction of ANA-1 cells is 76,000.

PKC-dependent (11). Thus, the modulation of LPL gene expression and protein secretion may dramatically affect the equilibrium of lipoprotein anabolism and catabolism, leading to various metabolic disorders including atherosclerosis.

In the present study, LPL induced an increase in the proliferative response that was selective for NK cells as no proliferative response was observed in T lymphocytes. In addition, the quantity of LPL that induced the maximum proliferative response in NK cells, decreased from 48 to 72 h and remained unaffected for PBL. This effect is specific as it can be blocked by anti-LPL. Anti-LPL may bind to an epitope required for LPL binding to its receptor. The activation through LPL is partially dependent on PKC activity, similar to the reported effect in ANA-1 cells (11). The translocation of PKC to the membrane may explain the inhibitory effect of LPL on the proliferative response of K562 and U937 cell lines. Hence, cell differentiation induced by LPL increased hemoglobin presence in K562 cell culture, assessed by 3,3′,5,5′ tetramethylbenzidine (results not shown) and an increased adherence to plastic in U937 cells.

The inhibitory effect of LPL on the spontaneous cytotoxic response of NK cells is dependent on the concentration of the enzyme, but it is independent of the time of incubation of the effector cells. It is probable that the binding of LPL to its receptor is of high affinity. Likewise, as LPL binding in effector cells is partially

sensitive to heparinase, an enzyme that hydrolyzes only heparan sulfate from cell proteoglycans, and this receptor is critical for cytotoxic activity, the effect on the response appears to be dependent on the available sites of LPL binding or on the saturability of this receptor in these cells. Even though LPL binding to target cells also hampered NK cytotoxicity, the mechanism by which this effect is achieved is different. Heparinase treatment of K562 cells did not restore NK cytotoxic activity. It is has been reported (31) that glycophorin protects K562 from NK attack, and a similar mechanism may be proposed for LPL.

The binding of LPL to the surface of the cells, which seems to be greater in monocytes and NK cells than in T lymphocytes, may be due to the presence of heparan sulfate or related proteoglycans in the cell surface area. Heparinase treatment decreased the amount of LPL bound to different cell membranes and increased NK cytotoxic response. Likewise, IL-2 was able to release most of the LPL-FITC that was bound to NK cell surface as well as that endogenously produced as reported previously (5). The mechanism by which IL-2 induces the release of LPL may be dependent on a phosphatidylinositol-phospholipase C sensitive anchorage. LAK cells express fewer receptors for LPL-FITC that are less sensitive to heparinase compared to resting NK cells.

The consequences of IL-2 stimulation and LPL treatment of NK cells provide evidence of the wide variety of physiological responses of these cells. Even though IL-2 stimulation increased the concentration of LPL required for 50% inhibition of the cytotoxic activity using K562 cells as targets, IL-2 was not able to completely overcome LPL inhibition that was observed using U937 cells as targets. Likewise, the co-stimulatory effect of LPL plus IL-2 induced a marked increase in the proliferative response of NK cells at 72 h, an effect not observed in NK-primed cells. These effects suggest that LPL stimulation through its receptor as well as IL-2 stimulation alone are necessary but not sufficient to induce the maximal NK proliferative response as reported by Robinson et al. (14) for IL-2, and that the LPL receptor may decrease the recognition of the target.

In order to understand the properties of the LPL receptor, and in accord with previous reports that postulated heparan sulfate as an LPL receptor (1), we investigated CD44 as a potential candidate. CD44 antigen is a chondroitin sulfate proteoglycan, with only E splice variants identified as heparan sulfate proteoglycans (32). This antigen has been considered the receptor for hyaluronic acid and other components of the extracellular matrix (32). The addition of anti-CD44 did not block the binding of LPL to the membrane of NK cells. This effect could be due to the binding of the antibody to an epitope that is independent of the site of the protein that binds LPL or simply that CD44 is not the receptor for LPL in these cells. A possible argument that has to be considered is that CD44 is expressed in all the cells of the immune system and endothelial cells. Therefore, LPL should bind to all these cells with the same avidity; this seems not to be the case as shown in this report.

The LPL receptor in NK cell membrane, however, seems to be a complex array of proteins. Using metabolic labeling studies, three proteins with molecular weights of 76,000, 57,200, and 27,200 were precipitated in the membrane fraction, and two, 57,200 and 27,200, in the cytosolic fraction. It is possible that 57,200 and 27,200 proteins are part of the 76,000 protein (the sum is 84,400) or that each protein binds LPL independently. In ANA-1 cells with functional LPL receptors (11), the 76,000 protein seems to be responsible for LPL binding. The specificity of the precipitated proteins was assessed by using anti-LPL. Further experiments should assess the specificity of these proteins to LPL.

The interaction of LPL with its receptors seems to condition the physiological response of NK cells in vitro. In vivo experiments performed in rats have shown that an intravenous bolus of heparin plus 10% Intralipid, a clinically used coconut fat emulsion for parenteral nutrition, increases NK cytotoxic activity (33). These results suggest a link between LPL, lipoprotein metabolism, and NK function. Specifically, heparin induces the release of LPL from the capillary endothelium, macrophages, and NK cells, providing more LPL to degrade the Intralipid emulsion and other triglyceride-rich lipoproteins. Augmented levels of fatty acids due to an increase in LPL activity may be responsible for the modulation of NK cell functions, especially NK cytotoxicity as described by Kurzer et al. (33).

In the complex physiological regulation of cytotoxicity and inflammation, it has been shown that disaccharides are able to inhibit TNFα cytolytic properties (34). Specifically, a disaccharide is liberated from the extracellular matrix by heparanase once there is an inflammatory reaction (34). When the inflammatory reaction is amplified and the pH of the inflammed site drops, the heparanase is activated releasing the disaccharide which then acts as a negative feedback of inflammation (34). Similarly, heparin has been shown to inhibit NK cytotoxic responses (35) although the mechanism is not fully understood. Recently, a 70 kD peptidoglycan has been shown to be the receptor of heparin and other sulfated heparinoids (36) and it is possible that this receptor is similar to the 76 kD protein observed in this report. If the cells are stimulated with heparin and then LPL is released, the excess heparin may interact with this receptor which, in turn, will produce an inhibitory effect on the cytotoxic response. It can be proposed then that

the regulation of these saccharides and glycoproteins condition the local immune response and spontaneous NK cytotoxicity that seems to be independent of the proliferative response of these cells.

Recently, receptors for polymorphic major histocompatibility complex type I have been described in NK cells (37). These receptors, designated killer-cell inhibitory receptors (37), belong to the immunoglobin superfamily and have a molecular mass of 58 kD and 70 kD. It can be proposed that LPL may bind to these receptors producing the inhibition of NK cytotoxic response. Furthermore, as postulated for T lymphocytes (37), the binding of LPL to these receptors may activate a tyrosine kinase that activates the inhibitory MHC receptor. However, during this activation, cytokine secretion (IL-2, IL-1 and TNF) may be responsible for the increase in NK proliferative response.

Overall, it has been shown that LPL induced an increase in the proliferative response, and an inhibition of cytotoxic response in both non-stimulated and stimulated NK cells. These effects may be due to the interaction of LPL with a specific receptor that is involved in the translocation of PKC from the cytosol to the membrane. This last effect on NK activation might be selective enough to increase NK proliferative response, but not cytotoxicity. LPL effects on NK cells suggest that these cells might respond to LPL as a stimulus. The presence of a negative regulator for NK cytotoxicity suggests a protective effect of LPL or its receptor in cell injury that could condition the genesis of atherosclerosis.

This research was supported by grants S1-2516 and S1-95-000568 from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) and the GENIC Program (Caracas, Venezuela). The authors wish to thank Drs. Danuta Radzioch for providing the ANA-1 cell line, Stephen Tillett, Lorea Baroja, Felix Toro, and José Corado for useful discussions, and Henry Rivera and Carlos Vivas for technical assistance.

*Manuscript received 21 March 1996 and in revised form 28 May 1996.*

## REFERENCES

1. Braun, J. E. A., and D. L. Severson. 1992. Regulation of the synthesis, processing and translocation of lipoprotein lipase. *Biochem. J.* **287:** 337–347.
2. Khoo, J. C., E. M. Mahoney, and J. L. Witzum. 1981. Secretion of lipoprotein lipase by macrophages in culture. *J. Biol. Chem.* **256:** 7105–7108.
3. Mahoney, E. M., J. C. Khoo, and D. Steinberg. 1982. Lipoprotein secretion by human monocytes and rabbit alveolar macrophages in culture. *Proc. Natl. Acad. Sci. USA.* **79:** 1639–1643.
4. Wang-Iverson, P., A. Ungar, J. Blumis, P. R. Buckberg, J. C. Gibson, and W. V. Brown. 1982. Human monocytes in culture synthetize and secrete lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **104:** 923–928.
5. De Sanctis, J. B., I. Blanca, D. Radzioch, and N. E. Bianco. 1994. Lipoprotein lipase expression in natural killer cells and its role in their cytotoxic activity. *Immunology.* **83:** 232–239.
6. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60:** 473–481.
7. Ross, R. 1993. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature.* **326:** 801–809.
8. De Sanctis, J. B., I. Blanca, and N. E. Bianco. 1995. Expression of different lipoprotein receptors in NK cells and their role on NK proliferative and cytotoxic activity. *Immunology.* **86:** 399–407.
9. De Sanctis, J. B., I. Blanca, and N. E. Bianco. 1995. Effects of lipoproteins on the proliferative response of interleukin 2 activated T lymphocytes and large granular lymphocytes. *Clin. Sci.* **89:** 511–520.
10. Renier, G., E. Skamene, J. B. De Sanctis, and D. Radzioch. 1994. Induction of tumor necrosis factor α gene expression by lipoprotein lipase. *J. Lipid Res.* **35:** 271–278.
11. Renier, G., M. Olivier, E. Skamene, and D. Radzioch. 1994. The induction of tumor necrosis factor α gene expression by lipoprotein lipase requires protein kinase C activation. *J. Lipid Res.* **35:** 1413–1421.
12. Ortaldo, J. R., and R. B. Herberman. 1984. Heterogeneity of natural killer cells. *Annu. Rev. Immunol.* **2:** 359–380.
13. Trinchieri, G. 1989. The biology of NK cells. *Adv. Immunol.* **47:** 187–487.
14. Robertson, M. J., T. J. Maney, C. Donahue, C. Levine, and J. Ritz. 1993. Costimulatory signals are required for optimal proliferation of human natural killer cells. *J. Immunol.* **150:** 1705–1714.
15. De Sanctis, J. B., I. Blanca, D. Radzioch, and N. E. Bianco. 1996. Expression and function of low density lipoprotein receptors in $CD3^-$ $CD16^+$ $CD56^+$ cells: effect of interleukin-2. *Cell. Immunol.* **167:** 18–29.
16. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P. H. Iverius, and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/α2-magroglobulin receptor in vitro. A process facilitated by cell surface proteoglycans. *J. Biol. Chem.* **268:** 14168–14175.
17. Mudler, M., P. Lombardi, H. Jansen, T. J. C. Van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9375.
18. Posner, I., and J. B. De Sanctis. 1987. Kinetics of product inhibition and mechanisms of lipoprotein lipase activation by apolipoprotein C-II. *Biochemistry.* **26:** 3711–3717.
19. Renier, G., E. Skamene, J. B. De Sanctis, and D. Radzioch. 1993. High macrophage lipoprotein lipase is correlated in inbred strains with susceptibility to atherosclerosis. *Arterioscler. Thromb.* **13:** 190–196.
20. Goding, J. W. F. 1976. Conjugation of antibodies with fluorochromes: modifications of the standard method. *Immunol. Methods.* **13:** 215–222.
21. Timonen, T., J. R. Ortaldo, and R. B. Herberman. 1981. Characteristics of human large granular lymphocytes and relationship to natural killer cells and K cells. *J. Exp. Med.* **153:** 569–571.

22. Caldera, L. H., M. León-Ponte, G. Aquatella, N. E. Bianco, and I. Blanca. 1992. Bone marrow and peripheral blood natural killer cell activity in lymphomas. Its response to IL-2. *Clin. Exp. Immunol.* **88:** 143–149.
23. Thomas, T. P., R. Gopalakrishna, and W. B. Anderson. 1987. Hormone- and tumor promoter-induced activation or membrane association of protein kinase C in intact cell. *Methods Enzymol.* **141:** 399–411.
24. Yasuda, I., A. Kishimoto, S. Tanaka, M. Tominaga, A. Sakurai, and Y. Nishizuka. 1990. A synthetic peptide substrate for selective assay of protein kinase C. *Biochem. Biophys. Res. Commun.* **166:** 1220–1227.
25. Springer, T. 1991. Immunoprecipitation. *In* Current Protocols in Immunology. Vol. 1. J. E. Coligan, A. M. Krugsberg, D. H. Margulies, E. M. Shevach, and W. Strober, editors. Wiley-Intercience, New York. 8.3.1–8.3.12.
26. Chait, A., and J. D. Brunzell. 1991. Chylomicronemia syndrome. *Adv. Intern. Med.* **37:** 249–265.
27. Posner, I., J. B. De Sanctis, and I. Golfetto. 1986. Two newborn siblings with congenital apolipoprotein C-II deficiency and inverted lipoprotein $CIII_1/CIII_2$ ratios. *Med. Sci. IRSC.* **14:** 1155–1156.
28. Posner, I., J. B. De Sanctis, and I. Golfetto. 1987. Plasma lipoproteins, apolipoproteins and post-heparin plasma lipolytic activities in an infant with apolipoprotein C-II deficiency. *Med. Sci. Res.* **15:** 1049–1050.
29. O'Brien, K. D., D. Gordon, S. Deeb, M. Ferguson, and A. Chait. 1992. Lipoprotein lipase is synthetized by macrophage-derived foam cells in human coronary atherosclerotic plaques. *J. Clin. Invest.* **89:** 1544–1549.
30. Ylä-Herttuala, S., B. A. Lipton, M. E. Rosenfeld, I. J. Goldberg, D. Steinberg, and J. L. Witzum. 1991. Macrophages and smooth muscles cells express lipoprotein lipase in human and rabbit atherosclerosic lesions. *Proc. Natl. Acad. Sci. USA.* **88:** 10143–10147.
31. El Ouagari, K., J. Tissié, and H. Benoist. 1995. Glycophorin A protects K562 cells from natural killer cell attack. Role of oligosaccharides. *J. Biol. Chem.* **270:** 26970–26975.
32. David, G. 1993. Integral membrane heparan sulfate proteoglycans. *FASEB J.* **7:** 1023–1030.
33. Kurzer, M., D. Tice, M. M. Meguid, and E. R. Reinitz. 1989. Natural killer cell activity in rats infused with Intralipid. *J. Clin. Lab. Immunol.* **29:** 33–35.
34. Lider, O., L. Cahalon, D. Gilat, R. Hershkoviz, D. Siegel, R. Margalit, O. Shosevoy, and I. R. Cohen. 1995. A disaccharide that inhibits tumor necrosis factor α is formed from the extracellular matrix by the enzyme heparanase. *Proc. Natl. Acad. Sci. USA.* **92:** 5037–5041.
35. Yamamoto, H., S. Fuyama, S. Arai, and F. Sendo. 1985. Inhibition of natural killer cytotoxicity by heparin. *Cell. Immunol.* **96:** 409–415.
36. Dziarski, R., and D. Gupta. 1994. Heparin, sulfated heparinoids, and lipoteichoic acids bind to the 70-kDa peptidoglycan/lipopolysaccharide receptor protein on lymphocytes. *J. Biol. Chem.* **269:** 2100–2110.
37. Lanier, L. L., and J. H. Phillips. 1996. Inhibitory MHC class I receptors on NK cells and T cells. *Immunol. Today.* **17:** 86–91.