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Expression of low-density lipoprotein receptors in peripheral blood and tonsil B lymphocytes

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SUMMARY

B lymphocytes, purified from peripheral leucocytes from young normolipaemic humans, expressed and internalized low-density lipoprotein receptors (LDLR). The expression was assessed by a monoclonal anti-LDLR. The internalization of LDL was assessed by LDL labelled with ^{125}I (^{125}I-LDL) and 1,1′-dioctadecyl-3,3,3′,3′ tetramethyl-indocarboxycyanine perchlorate (LDL-DiI). The expression of LDLR, assessed by anti-LDLR, was: $38 \pm 8\%$ ($n = 5$) for fresh purified cells, $60 \pm 10\%$ ($n = 12$) for non-stimulated cells, $79 \pm 5\%$ ($n = 10$) for IL-2 (100 U/ml)-stimulated cells and $95 \pm 5\%$ ($n = 8$) for pokeweed mitogen (PWM) (1:200 dilution)-stimulated cells. The optimal concentrations of agonist were 100 U/ml of IL-2, and 1:200 dilution of PWM. IL-2 and PWM increased the internalization of LDL-DiI by 1·5-fold. The internalization of LDL-DiI was maximal at 60 μg of protein/ml ($48 \pm 8\%$). Scatchard analysis revealed a Kd of $3{\cdot}2 \pm 0{\cdot}22 \times 10^{-8}$ M and 2180 ± 190 binding sites in non-stimulated cells, a Kd of $7{\cdot}73 \pm 0{\cdot}36 \times 10^{-9}$ M and $12\,500 \pm 430$ binding sites for IL-2 (100 U/ml)-stimulated cells, and a Kd of $7{\cdot}2 \pm 0{\cdot}43 \times 10^{-9}$ M and $13\,250 \pm 450$ binding sites for PWM (1:200 dilution)-stimulated cells. Lineweaver–Burk analysis of LDL binding (LDL-DiI) revealed that the apparent Kd for non-stimulated cells was $1{\cdot}3 \pm 0{\cdot}11 \times 10^{-8}$ M, and $9{\cdot}2 \pm 0{\cdot}2 \times 10^{-9}$ M and $7{\cdot}5 \pm 0{\cdot}25 \times 10^{-9}$ M for IL-2- and PWM-stimulated cells, respectively. B lymphocytes from tonsils also showed a high expression of LDLR assessed with anti-LDLR ($70 \pm 6\%$). The high expression of LDLR and the avid internalization of LDL suggest that LDL may be important for B cell physiological responses.

Keywords low-density lipoprotein low-density lipoprotein receptor B lymphocytes IL-2 pokeweed mitogen

INTRODUCTION

Low-density lipoprotein (LDL), the major carrier of cholesterol, has been implicated in the induction of cell cycle, protein glycosylation and mitochondria metabolism [1]. LDL is taken up by the cells through its specific receptor (LDLR) which is internalized along with LDL. Clinical and experimental data suggest that genetic defects in LDLR induce hypercholesterolaemia [1–5]. Several authors [1–6] have shown normal and defective expression of LDLR as well as normal and defective internalization of LDL by mononuclear lymphocytes. In T lymphocytes [7–10] and in natural killer (NK) cells [10–12], stimulation with IL-2 induced the expression of LDLR, suggesting a link between cytokines and lipoprotein metabolism. However, little is known about the expression and function of LDLR in B lymphocytes, despite the fact that these cells are present in the atheroma [3,13].

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B lymphocytes are differentiated from the other lymphocytes by their immunoglobulin expression and secretion as well as the phenotypic expression of other markers such as CD19, CD20, CD40 and CD72. Peripheral blood B cells are generally quiescent lymphocytes compared with B cells present in other lymphoid organs.

IL-2 and pokeweed mitogen (PWM) are able to activate B lymphocytes through different pathways [14–18]. IL-2 has been shown to be involved in B cell activation, growth and differentiation by the induction of multiple pathways (phosphorylation, oncogene transcription) [14,15]. On the other hand, PWM has been shown to be a polyclonal activator for B and T lymphocytes [17,18].

The aim of the present report was to study the expression of LDLR and the internalization of LDL in B lymphocytes purified

from peripheral blood leucocytes, the effect of IL-2 and lipopolysaccharide (LPS) on its expression, as well as the expression of this receptor in B cells purified from tonsils.

MATERIALS AND METHODS

Chemicals

Fetal calf serum (FCS), L-glutamine, PWM, penicillin–streptomycin and RPMI 1640 medium were purchased from GIBCO BRL (Gaithersburg, MD). 1,1′-dioctadecyl-3,3,3′,3′ tetramethyl-indocarbocyanine perchlorate (DiI) was purchased from Molecular Probes, Inc. (Eugene, OR). Percoll and Ficoll–Hypaque were purchased from Pharmacia LKB (Uppsala, Sweden). $Na^{125}I$ was purchased from New England Nuclear (Boston, MA). Recombinant human IL-2 (rhIL-2) was kindly donated by the Biological Response Modifiers Program (Frederick, MD) of the National Cancer Institute (Dr C. Reynolds). All other reagents were acquired from Sigma (St Louis, MO).

Antibodies

MoAbs anti-CD3–FITC, anti-CD56–FITC, anti-CD14–FITC, and anti-CD19-RD_1 (IgG1) were purchased from Coulter Immunology (Hialeah, FL). Anti-LDLR (IgG2b, clone 7), biotinylated goat anti-mouse IgG2b and streptavidin–FITC were obtained from Amersham (Aylesbury, UK). Anti-LDLR specificity has been assessed in different tissues of bovine and human origin [19,20].

Cell purification

B lymphocytes were obtained from: (i) Ficoll–Hypaque gradients of peripheral blood mononuclear cells (PBMC) of normolipaemic young (30 ± 5 years) donors, (ii) the tonsils of patients undergoing surgery for chronic tonsillitis (Central University Hospital). Written consent of the donor and approval of the Ethical Committee were obtained for both studies.

Peripheral blood B cells were purified after two cycles of E-rosette with sheep erythrocytes and non-rosetting B cells were separated using Ficoll–Hypaque as described previously [21]. These B cells were $>80\%$ $CD19^+$, $<8\%$ $MO2^+$, $<1\%$ $CD3^+$ and $<1\%$ $CD56^+$ assessed by flow cytometry. Tonsil B cells were purified as described previously [22]. The tonsils were finely minced with scissors, filtered through a wire mesh followed by a cycle of E-rosette and Ficoll–Hypaque centrifugation as described above. The purified cells were $>95\%$ $CD19^+$, $<1\%$ CD3, $<1\%$ $CD56^+$ and $<1\%$ MO2 as determined by flow cytometry.

B lymphocytes purified from peripheral blood were cultured overnight in RPMI 1640 in the presence of 0·5% bovine serum albumin fatty acid-free (RPMI–BSA) and stimulated with different concentrations of IL-2 or PWM (diluted according to manufacturer's instructions to 1:100, 1:200 and 1:300). The tonsil purified B cells were not cultured but for the assays of LDLR expression and internalization, they were incubated with RPMI–BSA.

In some experiments, B lymphocytes obtained from tonsils were fractionated by centrifugation on a seven-step Percoll gradient as described by Timonen *et al.* [23] instead of the four bands used by the standard method described by Mond & Brunswick [24]. The B cell fractions obtained from the different interfaces were assessed for LDLR expression.

Lipoprotein purification

LDL was separated from human plasma according to the method of Havel *et al.* [25]. Human plasma from healthy donors was centrifuged twice at 114 000 ***g*** for 20 h at 16°C, in the presence of inhibitors of lipid oxidation and peroxidation (1 mmol/*l* butylhydroxytoluene (BHT), 2 mmol/*l* reduced glutathione, 5 mmol/*l* ascorbic acid and 5 mmol/*l* EDTA). The purified plasma was adjusted to a density of 1·063 with the addition of KBr and centrifuged at 114 000 ***g*** for 20 h at 16°C for the separation of LDL. LDL was washed using a discontinuous gradient, 0·9% NaCl–KBr (density 1·063) at the top, and LDL–KBr (density $>1{\cdot}063$) at the bottom, and centrifuged as described above. The only protein content of this fraction was apolipoprotein B as determined by electrophoresis. No oxidative intermediates were detected in the purified LDL fraction using the thiobarbituric acid (TBARS) assay [26]. The purified lipoprotein was endotoxin-free as determined by the timed gel formation kit (Sigma).

LDL iodination

LDL iodination was performed as described previously by Shepherd *et al.* [27] with minor modifications. Briefly, 100 μl of freshly purified LDL (2 mg/ml of protein), dialysed against PBS, were mixed with 50 μl of $Na^{125}I$ (1 mCi/μmol) and 50 μl of chloramine T, 0·4% in PBS, vigorously for 45 s at room temperature. The reaction was stopped by adding 40 μl of 0·24% $Na_2S_2O_5$, 50 μl of 1% KI and 1 ml of 0·1 mol/*l* Tris–HCl/0·1 mol/*l* NaCl/1% BSA pH 8·0. Then, ^{125}I-LDL was separated from free iodine by passing it through Sephadex G-25. Eighty percent of the label was incorporated in the protein moiety of the lipoprotein.

^{125}I- LDL binding to purified B lymphocytes

Purified B lymphocytes (1×10^6) were mixed with different concentrations of ^{125}I-LDL and the assay was performed at 4°C for 1 h. After incubation, the cells were washed with PBS-gel in plastic RIA tubes and the cell pellet was counted in the gamma counter (LKB, Bromma, Sweden). Non-specific binding was assessed by incubating the cells with 100 μg/ml unlabelled LDL 1 h before addition of different concentrations of ^{125}I-LDL. The non-specific binding was $<30\%$ of the total bound ^{125}I-LDL.

The percentage specific binding was calculated according to the following formula:

$$\%\ \text{specific binding} = \frac{\text{total ct/min incorporated} - \text{non-specific bound } ^{125}\text{I-LDL}}{\text{total ct/min added}}$$

Scatchard analysis was performed using a computerized program developed by Munson & Robbard [28]. The value of Kd obtained in the Scatchard analysis was compared with the value obtained with the Lineweaver–Burk equation using LDL–DiI.

Labelling of lipoproteins with DiI

The labelling of LDL with DiI was performed as previously described [6]. LDL was adjusted to 2 mg/ml, labelled with 200 μl of 3 mg/ml DiI solution dissolved in dimethyl sulfoxide and then was added to 8 ml of lipoprotein-free plasma for 10 h at 37°C. LDL–DiI was centrifuged at 114 000 ***g*** for 18 h in order to eliminate the unbound fluorophore. The supernatant with the characteristic red colour was dialysed in PBS, adjusted to 2 mg/ml and filter-sterilized through a 0·45-μm Millipore filter. The labelling efficiency was determined by measuring the fluorophore at 480 nm. DiI is a hydrolysable and non-toxic fluorophore.

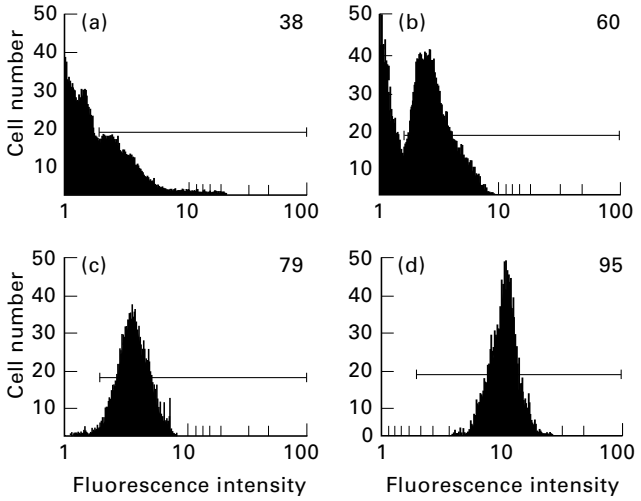

Fig. 1. Expression of low-density lipoprotein receptor (LDLR) using anti-LDLR. B cells purified from peripheral blood leucocytes were labelled with the anti-LDLR as described in Materials and Methods. A typical histogram of a normal donor is represented. The horizontal line represents the specific binding assessed. The number on the top right corner represents the positivity recorded. The different histograms represent: (a) fresh B cells, (b) non-stimulated cells incubated for 18 h with RPMI–bovine serum albumin (BSA), (c) IL-2-stimulated cells (100 U/ml), (d) pokeweed mitogen (PWM; 1:200 dilution)-stimulated cells. An increment in the mean channel fluorescence intensity is also observed depending on the stimulus.

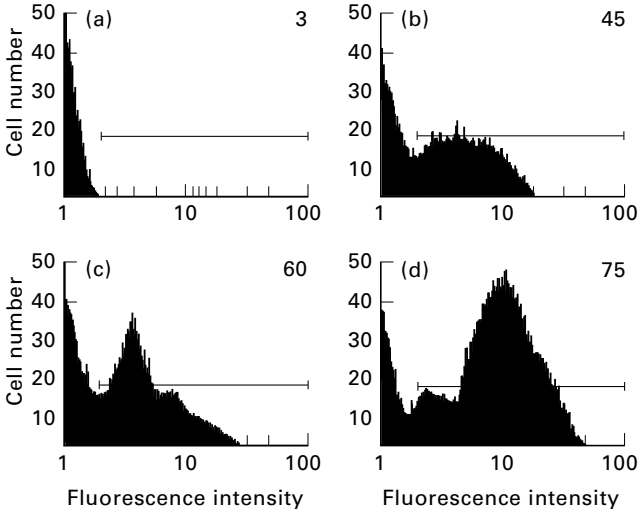

Fig. 2. Flow cytometry analysis of low-density lipoprotein (LDL)–1,1′-dioctadecyl-3,3,3′,3′ tetramethyl-indocarboxycyanine perchlorate (DiI) internalization by B lymphocytes. Purified B cells from peripheral blood leucocytes were incubated with LDL–DiI as described in Materials and Methods. A typical flow cytometry study of a normal donor is represented. The line represents the specific binding assessed. The number on the top right corner represents the positivity recorded. (a) The unspecific binding assessed by the incubation of unlabelled LDL (100 μg/ml) 1 h before the addition of 60 μg/ml of LDL–DiI. (b) Internalization of LDL–DiI in non-stimulated cells incubated for 18 h with RPMI–bovine serum albumin (BSA). (c,d) Internalization of LDL–DiI by cells stimulated with 100 U of IL-2/ml (c), and stimulated with pokeweed mitogen (PWM) 1:200 (d).

Flow cytometry studies

In order to quantify the uptake of LDL–DiI, the purified peripheral blood B cells were incubated for 18 h in RPMI–BSA in the presence or absence of different concentrations of IL-2 or PWM, washed with PBS, and resuspended at 1×10^6 cells/ml of RPMI–BSA that contained different concentrations of LDL–DiI. The B lymphocytes purified from tonsils were not incubated overnight, nor activated. Analysis of LDL–DiI binding to B cells allows determination of both surface binding and internalization of the ligand–receptor complex. The cells were incubated with different concentrations of LDL–DiI for a different period of time (0·5, 1, 2 and 4 h) at 37°C in the presence of 95% air and 5% CO_2 mixture. The maximum uptake of LDL–DiI was observed at 4 h following incubation (results not shown). After incubation, the cells were washed with PBS and analysed by flow cytometry (EPICS 753; Coulter). Forward angle (FALS) and 90° light scatter (90° LS) gates were established to exclude dead cells and cell debris. Fluorescence (> 570 nm) signal from the accumulated LDL–DiI in the cells was collected by the red photomultiplier (using a 600 nm dichroic short pass filter and a 645 nm band pass filter), processed and stored in one parameter as log scale histograms following the method of Suzuki *et al.* [29]. The data recorded was analysed in an Elite ESP software. The specificity of LDL–DiI binding was assessed by analysis of the competition between unlabelled LDL and LDL–DiI and pretreatment of the cells with EDTA. In the first case, the receptor is internalized with the unlabelled LDL, leaving the cell without receptors that can bind with LDL–DiI. In all cases, the amount of positive cells was < 3%. Maximum uptake was achieved when a concentration of 50–60 μg/ml of LDL–DiI/ml was used.

Table 1. Effect of pokeweed mitogen (PWM) or IL-2 stimulation on low-density lipoprotein (LDL)–1,1′-dioctadecyl-3,3,3′,3′ tetramethyl-indocarboxycyanine perchlorate (DiI) internalization by human B cells

Stimulus	Percent positive cells
Non-stimulated	48 ± 8
PWM dilution	
1:300	52 ± 10
1:200	95 ± 5
1:100	85 ± 10
IL-2 (U/ml)	
10	61 ± 5
50	70 ± 9
100	75 ± 6

B cells from five different donors were stimulated with different doses of PWM or IL-2 for 4 h at 37°C and internalization was assessed with 100 μg/ml of LDL–DiI. Results are expressed as the mean ± s.d. of positive cells recorded by flow cytometry.

Similar analysis was performed using specific anti-LDLR antibodies. Briefly, after 18 h incubation the cells were washed in PBS–0·1% sodium azide, incubated with anti-LDLR for 30 min at 4°C and subsequently washed extensively with PBS–azide–BSA 0·1%. A monoclonal goat anti-mouse IgG–FITC was added to the cell labelled with anti-LDL. Finally, the cells were washed in PBS containing sodium azide and BSA and resuspended in PBS for final flow cytometry analysis.

In order to assess the expression of LDLR in $CD19^+$ cells,

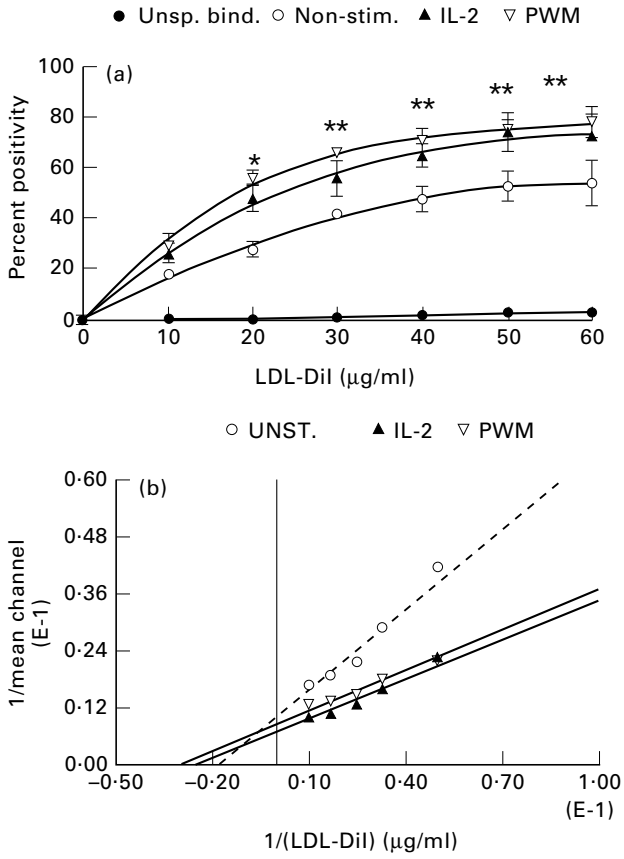

Fig. 3. Internalization of low-density lipoprotein (LDL)–1,1′-dioctadecyl-3,3,3′,3′ tetramethyl-indocarboxycyanine perchlorate (DiI) assessed by flow cytometry. B cells purified from peripheral blood leucocytes were either non-stimulated or stimulated with 100 U of IL-2 or with 1:200 dilution of pokeweed mitogen (PWM) for 18 h in the presence of RPMI–bovine serum albumin (BSA). Then, 1×10^6 cells/ml viable cells were incubated with different concentrations of LDL–DiI for 4 h as described in Materials and Methods. Percentage of positive cells was assessed by flow cytometry and the data presented in the figure represent the mean ± s.d. of 12 different donors. The figure represents unspecific binding (Unsp. bind.) assessed as the fluorescence intensity of the cells exposed to 100 μg/ml of LDL before adding the different concentrations of LDL–DiI (the cell internalized unlabelled LDL and did not express the receptor). Statistical significance (*$P<0{\cdot}05$; **$P<0{\cdot}01$) was observed when IL-2- or lipopolysaccharide (LPS)-stimulated cells were compared with the non-stimulated cells. (b) Lineweaver–Burk plot of LDL–DiI binding to the different leucocyte populations. Data represented in (a) were analysed with the following equation:

$$\frac{1}{\%\ \text{positivity}} = \frac{1}{(\text{LDL}-\text{DiI})}\times\frac{1}{(\text{Kd})}+\frac{1}{\text{Vmax}}$$

The calculated Kd for LDL was $1{\cdot}3\pm0{\cdot}11\times10^{-8}$ M for unstimulated, $9{\cdot}2\pm0{\cdot}2\times10^{-9}$ M for IL-2-stimulated and $7{\cdot}5\pm0{\cdot}25\times10^{-9}$ M for PWM-stimulated. This equation is generally used to calculate the apparent Km of enzyme kinetics and therefore represents only an approximation of the real Kd.

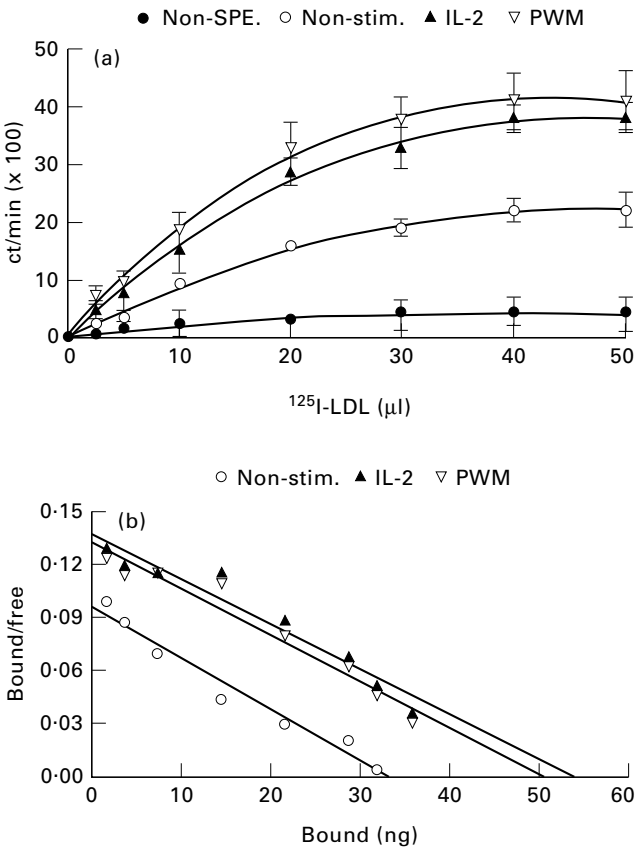

Fig. 4. Binding of ^{125}I-low-density lipoprotein (LDL) by purified B cells and Scatchard analysis. (a) The binding of ^{125}I-LDL by purified B cells unstimulated or stimulated with either 100 U of IL-2 or the 1:200 dilution of pokeweed mitogen (PWM). The cells were incubated with ^{125}I-LDL for 1 h at 4°C, and cell binding was determined using the gamma counter as described in Materials and Methods. Non-specific binding was assessed by incubating the cells with 100 μg/ml of unlabelled LDL 1 h before the addition of ^{125}I-LDL. The results represent the mean and s.d. of five different experiments. (b) Scatchard analysis of the data presented in (a). The number of binding sites was calculated to be 2180 ± 190 for unstimulated, 12 500 ± 430 for IL-2- and 13 250 ± 450 for PWM-stimulated cells. The different Kd are: $3{\cdot}2\pm0{\cdot}22\times10^{-8}$ M for unstimulated, $7{\cdot}73\pm0{\cdot}36\times10^{-9}$ M for IL-2- and $7{\cdot}2\pm0{\cdot}43\times10^{-9}$ M for PWM-stimulated cells.

double-labelling analysis was performed in an EPICS Elite flow cytometer (Coulter). Since anti-CD19 is an IgG1 antibody, and anti-LDLR is an IgG2b antibody, the expression of the receptor was assessed using a biotinylated MoAb anti-mouse IgG2b, which does not cross-react with IgG1, and streptavidin–FITC. Briefly, B cells were first labelled with anti-CD19-RD_1 for 30 min at 4°C, then the cells were washed with PBS–azide–BSA and subsequently incubated with 5 μg of anti-LDLR for 30 min at 4°C. The cells were then washed and incubated for 30 min at 4°C with 5 μl anti-mouse IgG2b and finally washed again and incubated with streptavidin–FITC in the same conditions as described above. Colour compensation was set up using the double-labelled isotype control (IgG1–RD_1, IgG2b–biotinylated streptavidin–FITC). The fluorescence intensity observed is represented in a log scale; the mean channel fluorescence intensity, then, represents the mean fluorescence recorded in log units.

Statistical analysis

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

RESULTS

The expression of LDLR, assessed by anti-LDLR, was: 38 ± 8%

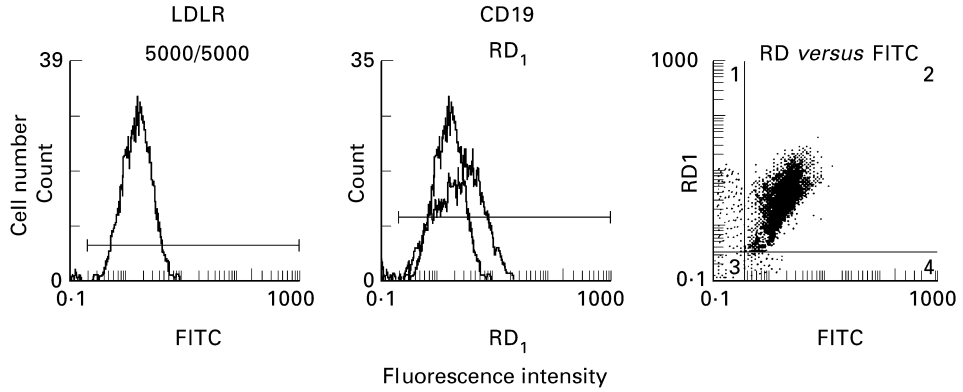

Fig. 5. Low-density lipoprotein receptor (LDLR) expression in $CD19^+$ cells from tonsils. B lymphocytes purified from tonsils were labelled with the two antibodies using first the anti-CD19 RD_1 and subsequently the complex anti-LDLR (IgG2b)–biotin anti-mouse IgG2b–streptavidin–FITC as described in Materials and Methods. The figure represents double-labelled cells and individual histograms analysed in an EPICS Elite flow cytometer (Coulter). The lines represent the specific binding. The cells co-expressing CD19 and LDLR represent 80% of the total cell population. The expression of $CD19^+$ was 95% while $LDLR^+$ expression was 85% assessed in the single histograms.

($n = 5$) for fresh purified cells, $60 \pm 10\%$ ($n = 12$) for non-stimulated cells, $79 \pm 5\%$ ($n = 10$) for IL-2 (100 U/ml)-stimulated cells and $95 \pm 5\%$ ($n = 8$) for PWM (1:200 dilution)-stimulated cells. Figure 1 represents a typical expression of the receptor. Stimulation with IL-2 and PWM (Fig. 1c,d) increased the mean channel fluorescence intensity by 1·5-fold (IL-2) and three-fold (PWM) compared with the non-stimulated cells (Fig. 1b).

The uptake of LDL–DiI by B cells purified from peripheral blood is illustrated in Figs 2 and 3. The optimal concentration of IL-2 and PWM, depicted in the figures, was determined by incubating the cells activated with different concentrations of IL-2 and PWM with 100 μg/ml LDL–DiI as illustrated in Table 1. A typical flow cytometry analysis of the internalization of LDL–DiI with the different stimuli is shown in Fig. 2. As specified in Fig. 2, IL-2 and PWM increased the positivity and mean channel fluorescence intensity (Fig 2c,d) compared with the non-stimulated control (Fig. 2b). There was an increment in positivity, and the mean channel fluorescence intensity was clearer in the PWM (Fig. 2d) stimulated cells compared with the IL-2-stimulated cells (Fig. 2c).

The uptake of LDL–DiI is depicted in Fig. 3. The internalization of LDL–DiI was specific (assayed in cells previously incubated with LDL or incubated with 2 mM EDTA) and maximal at 60 μg of protein/ml. A similar internalization was observed in tonsil cells (results not shown). Significant differences were observed among IL-2 or PWM stimuli compared with non-stimulated cells. The apparent Kd calculated using the Lineweaver–Burk equation is illustrated in Fig. 3b. The calculated Kd was: $1{\cdot}3 \pm 0{\cdot}11 \times 10^{-8}$ M for non-stimulated cells, $9{\cdot}2 \pm 0{\cdot}2 \times 10^{-9}$ M for IL-2 and $7{\cdot}5 \pm 0{\cdot}25 \times 10^{-9}$ M for PWM-stimulated cells. A Kd of $3{\cdot}2 \pm 0{\cdot}3 \times 10^{-7}$ M was observed in fresh T and B lymphocytes (reported previously [6]).

In Fig. 4, the binding of ^{125}I-LDL is depicted. In Fig. 4a, the binding is observed in non-stimulated and IL-2-stimulated cells. The Scatchard analysis of the two curves is represented in Fig. 4b. IL-2 and PWM induced a significant ($P < 0{\cdot}05$) increase in the binding of ^{125}I-LDL, and in addition there was an increase in the binding sites with a decrease in the Kd. The Kd of binding was $3{\cdot}2 \pm 0{\cdot}22 \times 10^{-8}$ M for non-stimulated cells, $7{\cdot}73 \pm 0{\cdot}36 \times 10^{-9}$ M for IL-2 and $7{\cdot}2 \pm 0{\cdot}43 \times 10^{-9}$ M for PWM-stimulated cells. In

Table 2. Expression of low-density lipoprotein receptor (LDLR) by tonsil B lymphocytes fractionated by a seven-step Percoll density gradient

Percoll fraction	Percent total cells	Percent positive cells	Relative expression of LDLR (%)	Mean channel
Unfractionated cells	100	70·0 ± 6·1	–	11·9 ± 0·3
1	35 ± 8	65·1 ± 15·4	22·8 (51·1)†	12·9 ± 0·6
2	29 ± 8	42·0 ± 14·0*	12·2 (27·4)	11·7 ± 0·8
3	15 ± 7	33·1 ± 10·0*	5 (11·2)	11·8 ± 1·8
4	11 ± 6	24·6 ± 7·4*	2·7 (6·1)	12·3 ± 0·8
5	8 ± 3	20·6 ± 3·4*	1·7 (3·8)	8·7 ± 2·6
6	2 ± 3	6·5 ± 1·0**	0·2 (0·4)	5·5 ± 2·8

†Percent of the total.

Tonsil B cells fractionated by Percoll density gradient of five different donors were assessed for LDLR expression with anti-LDLR MoAb. Data are expressed as the percentage of cells recovered in each band, % of the positive cells observed for LDLR using anti-LDLR, the relative expression of LDLR (calculated based on the expression of LDLR and the amount of cells in the fraction), and the mean channel fluorescence intensity of LDLR expression observed in these cells. The number in parentheses corresponds to the percentage of the total. Significant differences were observed in fractions 2–6 compared with fraction 1 (*$P < 0{\cdot}05$; **$P < 0{\cdot}01$).

addition, the number of binding sites increased from 2180 ± 190 to 12 500 ± 430 in IL-2-treated cells and to 13 250 ± 450 in PWM-stimulated cells. In fresh B lymphocytes, the number of binding sites was similar, 1900, but the Kd was lower: $1{\cdot}9 \pm 0{\cdot}21 \times 10^{-7}$ M (results not shown, $n = 3$).

The purified tonsil B cells, assessed by CD19 expression, were $LDLR^{+}$, as observed in Fig. 5. In concordance, the differential granularity of B lymphocytes, of five different donors, separated by Percoll gradients and LDLR expression, is illustrated in Table 2. The highest expression of LDLR is observed in the top two fractions of the Percoll. The cells recovered from these two fractions expressed around 78% of the total LDLR quantified with anti-LDLR. These fractions represent the most granular and activated cells, while the lower fractions were composed of non-activated or undifferentiated B cells. In addition, significant differences ($*P < 0{\cdot}05$ and $**P < 0{\cdot}01$) were observed in the positivity recorded between the first band and other Percoll fractions.

DISCUSSION

LDL uptake and cholesterol homeostasis by cells are important events within the context of cell survival, replication and metabolism. In fact, cholesterol and the mevalonic acid pathway have been involved in: (i) dolichols needed for protein glycosylation, (ii) ubiquinones needed for electron transport, (iii) isopentyl tRNA involved in DNA replication, and (iv) regulation of intracellular signals through GTP binding proteins. All these processes are regulated via cell cholesterol homeostasis through LDL uptake by LDLR and synthesis *de novo* of cholesterol [1,2].

Several receptors have been involved in the internalization of LDL: receptors for LDL native molecule and receptors for modified LDL [1–6]. Interestingly, blocking cholesterol synthesis with inhibitors of 3 hydroxy-methyl glutaryl CoA reductase (HMGCoA), similarly to antigen or anti-CD3 stimulation of these cells, induced LDLR expression in T lymphocytes [7–9] without apparent effect on scavenger receptors [5]. Likewise, in NK cells [10–12] the expression of LDLR is also related to cholesterol starvation and cell stimulation.

Cuthbert *et al.* did not find a direct immediate correlation between LDLR mRNA expression and LDLR protein expression in T cells [7,8]. The induction of LDLR transcription by mitogen was observed as soon as 1 h following the incubation of the cells, while LDLR protein expression was observed only 24 h later [7,8]. In NK cells, IL-2 stimulation did not affect the transcription of LDLR gene, but it induced an increase in cytosol to membrane sorting of the receptors [12]. Thus, post-transcriptional modifications and protein sorting may be essential in understanding LDLR expression and LDL internalization.

B lymphocytes purified from peripheral blood leucocytes express LDLR and are able to internalize LDL–DiI. This effect does not parallel the observations reported previously for T lymphocytes and NK cells [6–12]. Essentially, the expression of LDLR and the internalization of LDL–DiI in non-stimulated T lymphocytes and NK cells for non-stimulated cells was close to 12% [10]; in this study, LDLR expression and uptake was > 48%, which represents a four-fold increase in the expression of the receptor. As shown for the other lymphocytes [10,12], IL-2 stimulation increased the expression of LDLR, although this effect was lower than those reported for T and NK cells. The increment observed was 1·5-fold compared with three-fold reported previously [10].

The effect of PWM is not specific for B lymphocytes [16,18]. As expected, PWM stimulated the expression of LDLR in T lymphocytes. PWM resembled phytohaemagglutinin (PHA) stimulation, and the kinetics of receptor expression are similar to those described previously [1,7,8].

Peripheral blood B lymphocytes were also able to internalize and degrade ^{125}I-LDL (300 ng/6 h per mg protein, $n = 2$) after 18 h of incubation with RPMI–BSA. This value is similar to those reported for total lymphocytes after 72 h of incubation [30]. Upon stimulation, the amount of internalized ^{125}I-LDL doubled (620 and 650 ng/6 h per mg protein for IL-2 and PWM, respectively). The increase in LDL binding and internalization in peripheral blood B lymphocytes incubated in RPMI–BSA, compared with fresh cells, may be due not only to the lack of LDL, but also to the lack of fatty acids in the culture media which may modulate the membrane density of LDL receptors in these cultured cells [31].

In order to understand the importance of LDLR expression in these cells, we used B lymphocytes purified from tonsils. These cells are a heterogeneous B cell population which can be separated by its granularity using Percoll gradients. The high expression of LDLR in B cells obtained from tonsils and its correlation with granularity suggest that cell activation, in similar fashion as observed in T and NK cells, up-regulates LDLR expression. It may be proposed that LDL internalization may be important for the cell's metabolism and immunoglobulin production.

Recently, B lymphocytes have been shown to be present in the atheroma and to produce IgG [3,13]. The presence of B cells in the atheroma lesions may be important in the production of antibodies against native LDL or oxidized LDL (oxLDL), which in turn exacerbate the immune response in the lesion. Similarly, it has been suggested that B cell infiltration is dependent upon T lymphocyte presence and stimulation [3]. This hypothesis, in conjunction with the recognition of oxLDL by T lymphocytes purified from atheroma lesions [32] and the reports on the possible relationship of antibodies against oxLDL and cardiovascular diseases [33], has opened a new area of research in vascular pathology. It is concluded that further studies are required in order to understand the possible role of this lipoprotein and B lymphocytes in the pathology of cardiovascular diseases.

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