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High Membrane Cholesterol in CLL B-Cells and Differential Expression of Cholesterol Synthesis Genes in IG GENE-Unmutated vs Mutated Cells

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Research Article

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ABSTRACT

Objectives: Chronic lymphocytic leukaemia (CLL) is associated with abnormalities of the B-Cell Receptor (BCR) signalling, including low responsiveness to antigenic stimulation and constitutive phosphorylation of several components of the signalling pathway. In B-cells, BCR-mediated signalling is regulated in part by the amount of membrane cholesterol. It was observed that Statins, pharmacological inhibitors of cholesterol synthesis, induce apoptosis of CLL cells *in vitro* and *in vivo*. Having previously reported that ectopic expression of CD5 in a B-cell line stimulated the transcription of genes involved in the synthesis of cholesterol, we investigated the expression and synthesis of cholesterol in CLL B-cells.
Study Design & Methodology: Plasma membrane cholesterol in CLL cells was evaluated by staining with Filipin and Flow cytometry in 26 patients. CLL cells were cultured with Lovastatin and subG1 cells and Gumprecht's shadows counted thereafter; surface expression of IgM, CD19 and CD5 was analysed. The expression of cholesterol synthesis genes was investigated in transcriptomic data from the MILE project (150 CLL and 110 controls).

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Results: We confirmed that leukemic B-cells contained more cholesterol in their plasma membranes than their normal counterparts. An enhanced expression of genes involved in the synthesis of cholesterol in CLL as compared to healthy controls was observed. Interestingly, among the 150 CLL patients analyzed, four cholesterol synthesis genes were activated in 65 "Ig-mutated" (M) in comparison to 69 "Ig-unmutated" (UM) CLLs. Leukemic cells cultured with Lovastatin exhibited a dose-response apoptosis, however surface IgM expression was unaffected and CD19 and CD5 were downregulated at highest concentrations only.
Conclusions: High membrane cholesterol in CLL cells may explain their sensitivity to Statins, with a potential difference between UM- and M-CLL.

Keywords: Chronic lymphocytic leukaemia; cholesterol; apoptosis; statins.

1. INTRODUCTION

Chronic lymphocytic leukemia (CLL) expands from B-cells endowed with resistance to apoptosis and frequently stereotyped antigen receptors, as inferred from restricted IGVH and IGVL gene usage and a frequently autoreactive BCR repertoire (Chiorazzi et al., 2005; Schroeder et al., 1994; Stevenson et al., 2004). Patients segregate in two major groups, one in which the immunoglobulin heavy (IgH) chain variable fragment (VH) is in germline configuration (unmutated, UM), and the other in whom the VH gene presents somatic mutations (mutated, M) (3). UM and M patients undergo severe and mild clinical evolution respectively (Hamblin et al., 1999). The BCRs of UM and M clones use distinct variable VH genes which suggest that they can recognize different antigens and that M CLL cells have encountered this antigen which could be an infectious agent or a self-antigen (Hamblin, 1999; Klein et al., 2001; Rosenwald et al., 2001; Hervé et al., 2005; Lanemo Myhrinder et al., 2008). Yet both M and UM CLL clones originate from autoreactive cells (Hervé et al., 2005) and the malignant B-cell clone of CLL patients often produce auto-antibodies (Ruzickova et al., 2008). Most likely, CLL B-cells display properties that allow them to escape from several physiological checkpoints in charge of deleting autoreactive B-cells in the bone marrow (Wardeman et al., 2003; Lagneaux et al., 1998) and in the periphery (Meffre et al., 2008).

CLL B-cells display surface phenotype and mRNA signatures characteristic of antigen-experienced memory B-cells (Stevenson et al., 2004; Hamblin et al., 1999; Klein et al., 2001), suggesting repeated stimulation of the BCR, a condition that may lead to BCR desensitization also called anergy (Villen et al., 1999). Accordingly, in contrast with normal B-cells, the response of leukemic cells to BCR stimulation is poor as evidenced by low protein phosphorylation and intracellular Ca2+ mobilization (Michel et al., 1993; Lankester et al., 1995). However and possibly reflecting their higher proliferation capacity, UM CLL-cells are less anergic than M CLL-cells as they respond better to BCR stimulation (Stevenson et al., 2004; Lanham et al., 2003).

Following antigen encounter, the BCR moves to cholesterol/sphingolipid- enriched lipid rafts. This first step occurs before phosphorylation of the BCR complex on CD79 and activation of BCR-associated molecules (Pierce et al., 2002; Gupta et al., 2007). Cholesterol is therefore essential in regulating BCR-mediated signals especially in anergic cells (Blery et al., 2006). CD5 expression is a feature of CLL B-cells and a prominent diagnostic marker of this disease (Matutes et al., 1994). We previously generated a permanent B-cell line with ectopic

expression of CD5 at the cell surface (Gary Gouy et al., 2002). Comparing vector- and CD5-transduced cells by DNA chip technology, we were surprised to find that most cholesterol synthesis genes were activated by CD5 (Gary Gouy et al., 2007). We therefore examined whether CLL B-cells might be enriched in membrane cholesterol (possibly as a consequence of their CD5 expression) in comparison to normal B-cells. We also analyzed transcriptomic data (Haferlach et al., 2010) in search for alterations in cholesterol synthesis genes in CLL. Finally, as Statins, drugs known for their impact on cholesterol metabolism, were reported to induce the apoptosis of CLL cells (Vitols et al., 1997; Chapman Shimshoni et al., 2003), we analyzed the impact of these drugs on the expression of IgM and other cell surface markers of CLL B-cells.

2. PATIENTS, MATERIALS AND METHODS

2.1 Patients

Peripheral blood samples from a total of 26 patients, 13 males and 13 females, with a mean age of 64 year old (median: 69 y.o, range: 43-80 y.o) was used for this study. The majority of them (N= 21), presented at diagnosis with a Binet-A stage, 2 with a Binet-B stage, and 3 with Binet-C stage. Three patients had been treated with Statins at the time of diagnosis. A first series of 16 donors without CLL and any treatment with Statins (8 males and 8 females, median age 61 y.o, range 22-82 y.o) from the local blood bank and 16 CLL patients was enrolled for the appreciation of membrane cholesterol. These patients were 8 men and 8 women with a mean age of 63 years old. All presented with untreated CLL, and Binet's stages were A for 7, B for 2 and C for 1.

A second series of patients was used to test the effect of in vitro treatment with Statins. These were 14 CLL patients (7 men, 7 women), with a mean age of 59 years old. Binet's stages were A for all at the time of sampling, two initial stages C having been induced to remission after treatment.

Informed consent was obtained from all patients according to the Declaration of Helsinki and the ethical committee of the “Université de Lorraine”.

2.2 CLL Cells

Lymphocytes were obtained by Ficoll gradient centrifugation from peripheral blood collected on EDTA.

2.3 Flow Cytometry

Separated lymphocytes were resuspended at 10^6/mL and 50 µL aliquots were incubated with PC5-conjugated CD5, PC7-conjugated CD19 monoclonal antibodies (Beckman Coulter, Miami, FL). For each sample, a second tube was prepared with the addition of Filipin (Robinson et al, 1980). Briefly, this antibiotic that selectively binds to cholesterol is also fluorescent and emits above 520 nm, i.e. in the FL1 channel of most flow cytometers. The best excitation is provided by UV light, but excitation is also possible with a 488nm argon laser (Muller et al, 1984). We therefore tested labeled cells for these three markers using an FC500 two lasers flow cytometer (Beckman Coulter). Filipin (Sigma), stored in DMSO at 25 mg/mL in the dark was used as working solution at 0.05 mg/mL in PBS. Normal B-cells (CD19+/CD5-), normal T-cells (CD19-/CD5+) and normal B-1 cells or CLL cells

(CD19+/CD5+) were first gated using a CD5/CD19 biparametric scattergram. The autofluorescence signal in FL1 was recorded for each subset, then the Filipin fluorescence signal of each subset was recorded in the second tube.

2.4 Transcripts Levels of Genes Involved in Cholesterol Synthesis in CLL and Controls

As published elsewhere, the MILE project analyzed on whole genome H60 chips (Affymetrix) samples from over 3000 hematological malignancies and healthy controls (Haferlach et al., 2010). Within this data base, information was extracted for 110 healthy controls and 150 CLL patients. Mutational status was known for 135, with respectively 69 UM CLL and 66 M CLL. The list of genes of interest, selected from results of the previous transcriptome study on CD5 transfected Daudi cells, that was used for this query is shown on table 1.

Table 1. Genes involved in cholesterol synthesis that were investigated in the MILE database

Acronym	Id#	Molecule
HMGCS1	205822	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)
HMGCS1	221750	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)
HMGCR	202539	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
HMGCR	202540	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
MVK	36907	mevalonate kinase
FDFT1	208647	farnesyl-diphosphate farnesyltransferase 1
FDFT1	210950	farnesyl-diphosphate farnesyltransferase 1
FDFT1	241954	Farnesyl-diphosphate farnesyltransferase 1
LSS	202245	lanosterol synthase (2;3-oxidosqualene-lanosterol cyclase)
CYP51A1	202314	cytochrome P450; family 51; subfamily A; polypeptide 1
CYP51A1	216607	cytochrome P450; family 51; subfamily A; polypeptide 1
SC4MOL	209146	sterol-C4-methyl oxidase-like
SC5DL	211423	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog; S. cerevisiae)-like
DHCR7	201790	7-dehydrocholesterol reductase
DHCR7	201791	7-dehydrocholesterol reductase

Nine genes encoding for enzymes involved in the synthesis of cholesterol from fatty acids were investigated. Their order from the top (Acetyl coA) to the bottom (cholesterol) of the cascade is: HMGCS1, HMGCR, MVK, FDFT1, LSS, Cyp51A1, SC4MOL, SC5DL, DHCR7.

2.5 Inhibition of Cholesterol by Statins

Isolated lymphocytes from 14 patients were cultured at 10^6 cells/ml for 24 hours to 6 days without (control) or with three different doses of Lovastatin (10^{-4}, 10^{-5}, 10^{-6}), at 37°C in RPMI-1640 (Gibco) with 10% Fetal Calf serum (Hyclone), in a water-saturated incubator. Apoptosis was measured in flow cytometry by DNA staining with propidium iodide using the DNA Prep kit (Beckman Coulter) by recording the percentage of sub-G1 apoptotic cells. The expression level of surface IgM, CD5 and CD19 was also measured in flow cytometry after staining as above and with an FITC-conjugated rabbit anti-human IgM antiserum. In parallel, cells were

spun down and stained with May Grünwald Giemsa in order to count Gumprecht's shadows and evaluate the live/dead cells ratio.

2.6 Statistics

Filipin expression in flow cytometry was expressed as mean fluorescence intensity (MFI) ratios comparing the MFI of the filipin signal and that of the autofluorescence in FL1. Transcriptome data were the expression levels of probesets as described elsewhere. All series were tested for normality with the Kolmogorov Smirnov test and consequently expressed as means or medians. Between groups comparisons were performed with Student's T test, paired Student's T test or Mann Whitney. Statistical significance was retained for P values lower than 0.05.

3. RESULTS AND DISCUSSION

3.1 Filipin Binding, as an Indicator of Cell Surface Cholesterol, is Higher on CLL B-Cells than in Control B-Cells

Having previously shown that CD5-transfected Daudi B-cells exhibited a higher content in membrane cholesterol than vector-transfected cells (Gary Gouy et al., 2007) we undertook to compare the amount of membrane cholesterol in (almost totally CD5+)-B cells from CLL with that of B-cells from age and sex-matched controls. As shown in Figure 1A, the fluorescence of Filipin-stained cells gated on CD19+ cells, was significantly enhanced in all but 3 CLL patients as compared to total normal B-cells (figure 1B). The mean MFI ratio was 5.7 ± 3.4 in CLL and 4.1 ± 1.6 in the group of healthy controls *(P* = 0.048). Of interest, retrospective analysis showed that the 3 samples that were slightly less fluorescent than controls turned out to belong to patients treated with Statins (patients 13, 14 and 16). Accordingly, when the analysis was limited to« Statin-free » samples, the MFI of CLL cells (5.9 ± 3.5) was increased as compared to controls : 3.9 ± 1.4 (*P* = 0.045) (Figure 1B). The difference in membrane cholesterol was also significant if CLL B-cells were compared to CD5+ B- cells from healthy controls, which account for some 10% of total B-cells (Gary Gouy et al., 2002), with respective MFI ratios of 5.7 ± 3.4 vs 4.2 ± 1.4 (*P* = 0.05). As the normal- CD5 negative- B-cell population is almost undetectable in CLL samples we could not compare it to the leukemic clone.

Altogether, we observed that CLL B-cells were enriched in membrane cholesterol in comparison to normal CD5+ or CD5- B-cells. We next analyzed the mRNA profiles of the transcripts coding for the enzymes involved in cholesterol synthesis.

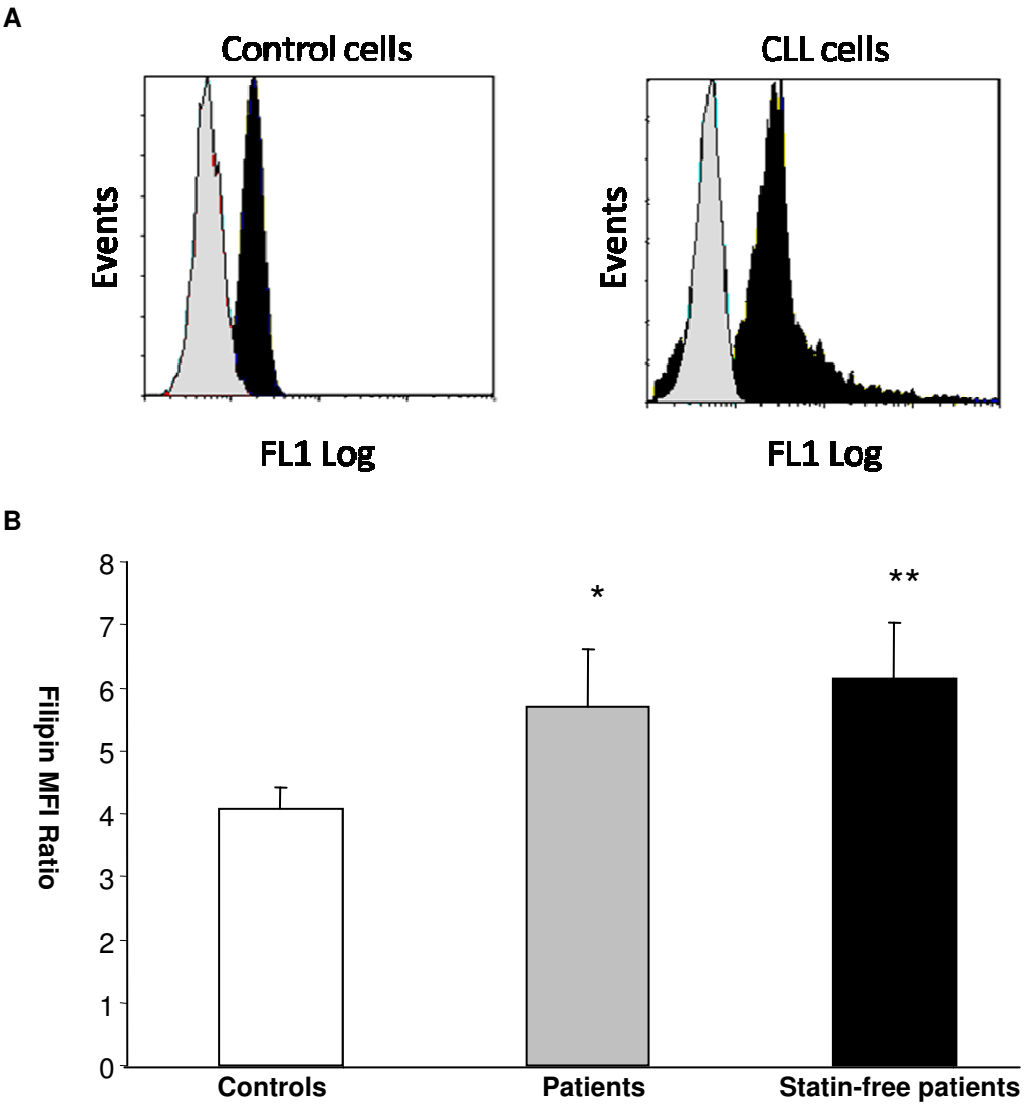

Figure 1. (A) Comparative FL1 fluorescence intensity of unstained (light gray) and Filipin stained (black) on CD19+/CD5- B-cells from a healthy control (left) and CD19+/CD5+ B-cells from a CLL patient (right). (B) Mean±SD of fluorescence ratios of all patients and controls (white histogram, n=16) or of Statin-untreated patients (black histogram, n=13). * p = 0.048, ** p = 0.044.

3.2 The Transcription of Genes Involved in Cholesterol Synthesis Vary in CLL Cells

Data from the MILE study were first plotted to compare the expression levels of 15 genes, shown to be modified by CD5 transfection of Daudi cells (Gary Gouy et al., 2007), between CLL patients and controls (table 2). Affymetrix chip analysis showed that the expression of 7 transcripts from the cholesterol synthesis pathway was significantly modified in CLL patients. This significant shift is an upregulation in 4 instances and a downregulation in 3. Interestingly the four upregulated genes, LSS, Cyp51A1, NSDHL, and SC5DL, act sequentially in the final step of the pathway to synthesize lanosterol, lathosterol and finally 7 dehydrocholesterol. However transcripts from the last gene, DHCR7, leading to the transformation of 7 dehydrocholesterol into cholesterol appear to be lower in CLL vs normal B-cells.

The second analysis performed by comparing UM and M CLL cells provided further information. It showed that all of the four significant differences implied genes of the cholesterol synthesis pathway in M-CLLs, all of which were upregulated (table 3). Among the 5 genes involved in the final pathway, LSS, SC5DL and DHCR7 were upregulated. In addition, FDFT1 acting upstream of LSS was also stimulated. In conclusion, the cholesterol synthesis pathway appears to be enhanced in M- as compared to UM-CLL.

Table 2. Transcription levels of Cholesterol Biosynthesis genes in CLL vs Controls*

Gene #	CLL N=150			P value Mann Whitney	Controls N=110		
	Median	Minimum	Maximum		Median	Minimum	Maximum
205822	36,75	8,2	103,2	NS	34,8	11,3	126,9
221750	157,1	40	396,4	<0.0001	109,15	47,3	227,7
202539	173,4	46,9	400,6	NS	184,45	78,4	400,6
202540	58,1	13,3	134	NS	53,9	4,4	132,7
36907	170,8	83,3	277,1	<0.0001	207,4	113,4	537,9
208647	771,15	326,2	2338,6	<0.0001	1265,55	585,7	3983
210950	630,85	148,9	2099,2	NS	447,2	156,2	3587
241954	83,8	20,2	309,4	NS	86,6	28,5	323,8
202245	260,05	105,8	684	<0.0001	184,2	67,6	542
202314	367	62,2	1122,6	<0.0001	260,5	65,7	668,2
216607	127,8	49,1	384,2	NS	150,55	40	302,7
209146	234,1	76,7	820,2	<0.0001	147,9	65,2	729,1
211423	280,35	75,1	1132,1	<0.0001	216	105	1148,5
201790	40	2,6	635,6	<0.0001	149,05	33,9	803,8
201791	37,75	0,7	644,4	<0.0001	125,4	5,6	683,2

** Light gray lines indicate significant downregulation in CLL cells compared to controls. Dark grey lines indicate significant upregulation in CLL cells compared to controls.*

As CD5 stimulated the cholesterol pathway genes in transfected Daudi B-cells, we wondered if the same applied to CLLs and compared the CD5 transcripts in UM-CLL vs M-CLL samples. However, CD5 transcripts levels were the same between UM (median 84.2, range 3.5-325) and M (median 84.9, range 3.7-302) CLL cells.

Table 3.Transcription levels of cholesterol synthesis genes in Unmutated vs Mutated-CLL

Gene #	Unmutated N=69			P value Mann Whitney	Mutated n=66		
	Median	Minimum	Maximum		Median	Minimum	Maximum
205822	42	8,2	103,2	NS	33,55	10,9	81,2
221750	171,2	43,1	299	NS	153,25	40	396,4
202539	180,6	54	310,7	NS	168,6	46,9	400,6
202540	55,2	13,3	128	NS	66,85	16	134
36907	164,9	88,2	277,1	NS	179,4	83,3	275,7
208647	727,5	326,2	1239,9	0.02	807,4	454,6	2338,6
210950	553,5	148,9	1523,7	NS	639,4	174,4	2099,2
241954	74,6	20,2	306,6	NS	85,65	35,3	309,4
202245	238,2	105,8	684	0.02	284,6	145,9	603,1
202314	357,3	62,2	1088,4	NS	415	91,9	1122,6
216607	128,1	49,2	301,3	NS	141	49,1	384,2
209146	235,7	88,2	529,7	NS	240,75	76,7	820,2
211423	248,6	75,1	808,3	0.003	357,85	112,2	1132,1
201790	33,2	3,2	635,6	0.03	44,55	2,6	109,3
201791	34,6	0,7	644,4	NS	42,4	5	113

** Light gray lines indicate significant downregulation in UM CLL cells compared to M CLL cells. The four genes are DHCR7, FDFT1, LSS and SC4MOL (see table 1).*

3.3 Lovastatin-Induced Cell Death and Modifications in Surface Markers of CLL Cells

For 14 patients with CLL, isolated lymphocytes were cultured for 6 days without or with three graded doses of Lovastatin from 10^{-6} to 10^{-4} M. Cells were then permeabilized and stained with propidium iodide and the percentage of hypodiploid (sub G1) cells evaluated as described previously (Gary Gouy 2007).

As shown in Figure 2, a significant increase in cell death was seen with increasing doses of lovastatin, whether the percentage of hypodiploid cells or Gumprecht's shadows were considered. In both cases Lovastatin was inefficient at 10^{-6}M but showed a significant effect at 10^{-5}M if Gumprecht's shadows were compared (p=0.002) and to a lesser extent if subG1 cells were considered (p=0.005).

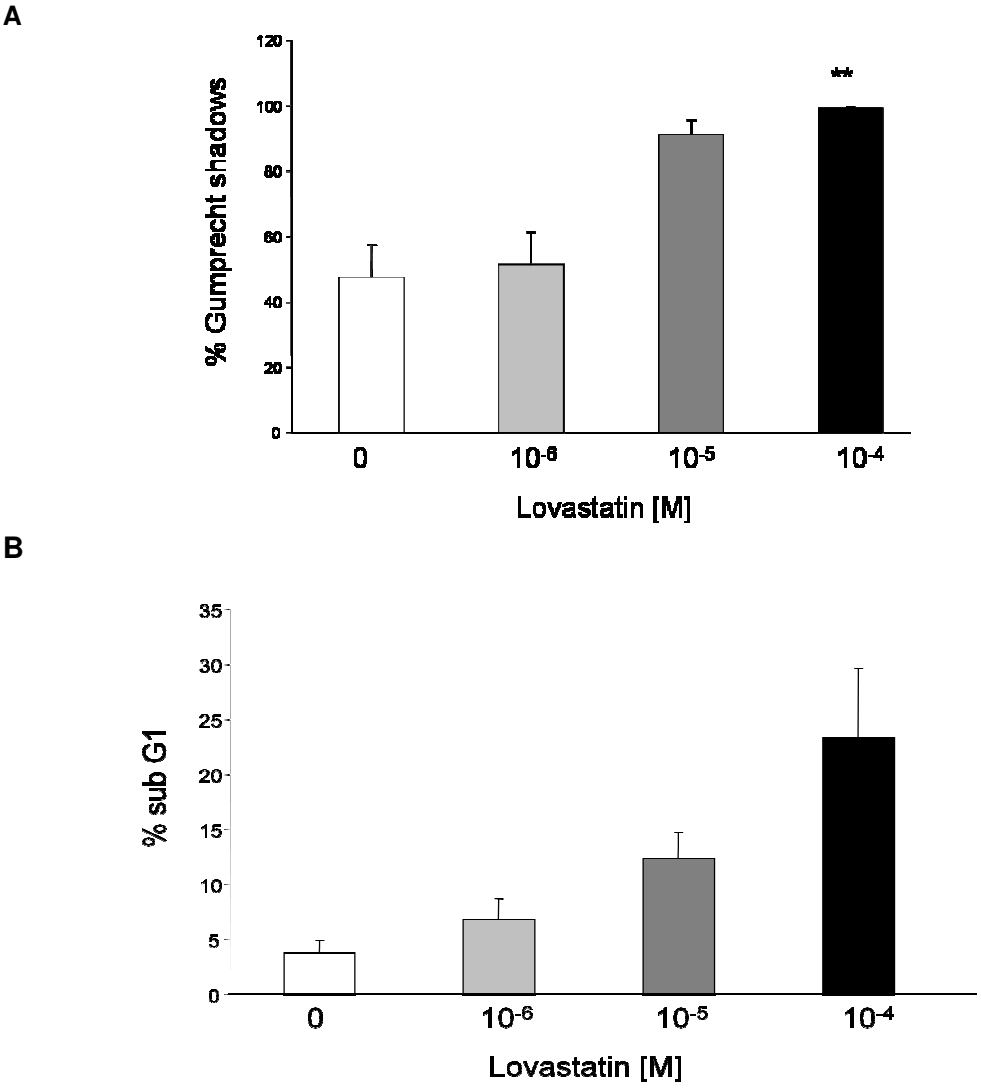

Figure 2. Apoptosis of CLL B-cells cultured for 6 days in medium without or with 3 graded doses of Lovastatin. (A): Gumprecht's shadows, (B): Hypodiploid (sub G1) cells. (n=14). ** p =0.002.

Concomitantly, the expression of IgM, CD19 and CD5 was assessed on the tumoral cells, and of CD5 on remaining T-cells. These data are shown in Table 4. Although no significant change was observed for the low expression of IgM, a significant decrease was noted on CLL cells for CD19 and CD5 expression at the highest Lovastatin concentration (p=0.01 and 0.03 respectively), and, interestingly, also for CD5 on resident T-cells upon incubation with Lovastatin (data not shown). A borderline dose-effect was also noticed, a concentration of 10^{-6} being less efficient than the two others.

Table 4. Variations in the expression of surface antigens upon 6day culture of CLL cells with Lovastatin (MFI ratios compared to day 0)

Antigen	Day 0	10^{-6} Lovastatin	P*	10^{-5} Lovastatin	P*	10^{-4} Lovastatin	P*
Surface IgM	1.1±0.6	1.2±0.8	NS	1.1±0.6	NS	1.0±0.4	NS
CD19	7.4±4.7	7.3±4.9	NS	5.4±2.7	0.01	5.3±2.6	0.01
CD5	23.1±15.1	22.9±14.1	NS	18.7±10.6	0.04	18.9±10.7	0.03
CD5 on T-cells	35.6±11.2	34.6±9.9	0.07	31±7.4	0.01	31±7.1	0.02

** Paired Student's T test's P value compared to D1*

3.4 DISCUSSION

Previous results from our group led us to hypothesize that CLL B-cells may be enriched in membrane cholesterol, possibly in relation with the expression of CD5; Data from the literature suggested that a high content in cholesterol at the plasma membrane may affect BCR trafficking and signalling (Bléry et al., 2006). Of interest, retention of the BCR in the cytoplasm of CLL cells has long been observed (Yasuda et al., 1982; Newell et al., 1983) suggesting trafficking abnormalities in this pathology. Membrane rigidity in CLL is a prominent cytological feature easily evidenced by the disruption of the cells and numerous Gumprecht's shadows on cytocentrifuged cells stained with May Grünwald Giemsa, and it is noteworthy that an increase in membrane cholesterol increases rigidity (Müller et al., 1970; An et al., 2010).

Our results demonstrate that there is more cholesterol in the plasma membranes of CLL B-cells than in the membranes of normal B-cells including the normal B-1 like CD5+ B-cell subpopulation. This finding was indirectly validated by the retrospective observation of lesser membrane cholesterol in the cells of patients receiving oral Statins than in the cells of the other patients, which is in keeping with the well known inhibitory activity of Statins on HMG-CoA reductase. Although it is tempting to attribute this difference to the effect of CD5, this is unlikely because CD5 expression was not diminished in the cells of the patients receiving Statins (data not shown) although it was inhibited *in vitro* by Lovastatin at the highest concentration (Table 4). Moreover, in our CD5-transfected cell line, 9 genes were stimulated, (as an example, a 4.5 fold stimulation was observed for MVK), and none were inhibited (Gary Gouy et al., 2007). This is at variance with the data gathered from the database of the MILE project (Haferlach et al., 2010). Although the expression of 4 gene transcripts involved in the synthesis of cholesterol is stimulated in CLL, the expression of 3 other genes is inhibited. How this could have modified cholesterol content at the membrane is difficult to interpret inasmuch as other parameters such as cholesterol trafficking and degradation should affect the whole picture and could not be analysed here. Interestingly however, we observed a difference between UM and M-CLL, which deserves a specific analysis in order to measure membrane cholesterol in both groups. Unfortunately only 2 out of 26 patients from our study were documented for IgVH mutational status (data not shown). Thus a dedicated study is needed to compare the amount of membrane cholesterol in both M and UM- CLL B-cells. How does membrane cholesterol affect BCR signaling? It has been shown that cholesterol is needed for BCR internalization in anergic but not in normal B-cells (Bléry et al., 2006). In this study on an HEL/anti-HEL double transgenic mouse model, the B-cells are anergic in vivo due to a permanent stimulation of surface immunoglobulins (sIg). Upon stimulation with antigen, sIg is internalized which desensitizes the cell to the antigen. This

process depends on cholesterol, indeed, when surface cholesterol was disrupted, sIg was restored and anergy was broken. This is reminiscent of what happened to the same mice bred in a CD5-null background (Hippen et al., 2000) which resulted in the loss of anergy characterized by autoimmune haemolytic anemia. Together with our data, the abovementioned results suggest a direct link between CD5 expression, membrane cholesterol and anergy in CLL B-cells. Here, the lower amounts of transcripts for relevant membrane cholesterol synthesis pathway molecules observed in UM-CLL compared to M-CLL is meaningful and may correlate with the "lesser anergic state" of the aggressive-subset in comparison to the indolent one.

As patients treated with Statins displayed lesser membrane cholesterol on their B-cells than untreated patients, we investigated *in vitro* the effect of Statins on the expression of IgM with the possibility in mind that it would be augmented. Although Lovastatin failed to change the expression of sIgM, it induced a significant decrease of that of CD19 and CD5. Moreover, we were able to confirm the results of others on the apoptotic effect of Statins (Vitols et al., 1997; Chapman Shimshoni et al., 2003). The action of these molecules is far from being limited to cholesterol. Statins may inhibit the farnesylation of key molecules such as ras (Cox et al., 1992) and lamins (Cox; Karp et al., 2001) involved respectively in cell proliferation and gene expression (Dechat et al., 2007). Finally, whether UM-CLL are more sensitive to Statins than M-CLL because of lower cholesterol content, should be explored as Statins could be useful adjuvants for chemotherapy (Fuchs et al., 2008; De Jong Peeters et al., 2009).

4. CONCLUSION

CLL B-cells are sensitive to inhibitors of cholesterol synthesis, and display an enhanced expression of cholesterol synthesis genes. Although it is tempting to attribute the increased sensitivity of CLL B-cells to Statins to their high cholesterol expression and possibly requirements in cholesterol, our study does not support this hypothesis. The relative amounts of membrane cholesterol and sensitivity to Statins between U- and UM-CLL should be evaluated in a specific study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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