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Expression of transcription factors Pu.1, Spi-B, Blimp-1, BSAP and oct-2 in normal human plasma cells and in multiple myeloma cells

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Summary. Differentiation of B lymphocytes into plasma cells is regulated by the interaction of distinct transcription factors (TFs) which activate gene expression in a lineage- and stage-specific pattern. Using reverse transcription polymerase chain reaction, we studied the expression of five TFs (octamer binding factor oct-2, ets family members PU.1 and Spi-B, pax gene family member BSAP, and Blimp-1) in (1) human cell lines with a plasma cell phenotype, (2) primary malignant plasma cells [obtained from patients with plasma cell leukaemia (PCL) and multiple myeloma], and (3) normal human plasma cells generated *in vitro* or isolated from normal bone marrows. The expression pattern was compared with TFs expressed by normal $CD19^+$ B lymphocytes and by B cells from chronic lymphocytic leukaemia patients. Our results showed that plasma cells expressed a restricted set of TFs compared with $CD19^+$ B lymphocytes, with continued expression of Spi-B and oct-2, increased Blimp-1 expression, and downregulation of BSAP and PU.1. Cells from PCL lost Spi-B and PU.1 expression completely and expressed only oct-2 and Blimp-1, and thus resembled plasma cell lines. Human plasma cell differentiation therefore seems to be positively regulated by Blimp-1; whether this TF has any oncogenic potential will have to be analysed in future studies.

Keywords: plasma cell, multiple myeloma, plasma cell leukaemia, transcription factor, RT–PCR.

The process by which mature B cells differentiate into plasma cells is complex and associated with phenotypic and functional changes such as expression or loss of various cell surface molecules (e.g. gain of CD38 and CD138, loss of CD19 and CD20) and switch from the surface to the secreted form of immunoglobulin (Ig). The external signals that regulate this process in a B cell- and differentiation stage-specific manner include the antigen, extracellular growth factors [e.g. interleukin 6 (IL-6) (Jego *et al*, 2001] and cell–cell contact (e.g. CD40L) (Lagresle *et al*, 1995). Within a given B cell these stimuli are integrated by transcription factors (TFs) which, in response, execute the programme of differentiation by directly regulating gene expression (Liu & Banchereau, 1997; Reya & Grosschedl, 1998; Liberg & Sigvardsson, 1999).

Among TFs believed to influence B-cell differentiation are members of the 'ets' family of TFs. Binding sites for these TFs have been identified in several genes encoding B-cell receptor (BCR) signalling proteins, including Ig heavy and light chains, Ig J-chain, Igα, Igβ, and the tyrosine kinases Btk and blk (Garrett-Sinha *et al*, 1999). Although seven ets family members are expressed in B lymphocytes (Bassuk & Leiden, 1997), two of these proteins are particularly abundant: PU.1 and Spi-B. In PU.1 knock-out mice, myeloid and lymphoid cell development was completely blocked (Scott *et al*, 1994; McKercher *et al*, 1996); therefore, it was not possible to investigate the role of PU.1 in mature B lymphocytes and plasma cells in this mouse model. Only in one study was PU.1 expression analysed in a panel of human B-cell lines; PU.1 was found downregulated in a series of myeloma-, compared with pro-B, pre-B and mature B-cell lines (Pettersson *et al*, 1995).

Another TF considered to be a key regulator of B-lymphocyte development is the B cell-specific activator protein (BSAP), encoded by the PAX-5 gene. Its' critical role in early B-cell lymphopoiesis and for progression beyond the pro-B-cell stage has been established by targeted disruption of the mouse Pax5 gene (Urbanek *et al*, 1994). BSAP binding sites have been detected in the promoter regions of CD19 and CD20, of the tyrosine kinase blk, of RAG-2, of the J-chain, in the Ig heavy chain and kappa chain

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3′α-enhancer regions and in switch regions of Ig heavy chain genes (Neurath *et al*, 1995; Fong *et al*, 2000). The levels of BSAP were shown to decrease progressively in response to IL-2 and this change correlated with the differentiation of B cells into antibody-secreting plasma cells (Rinkenberger *et al*, 1996). On the other hand, overproduction of BSAP in a late B-cell line was shown to suppress differentiation into plasma cells (Usui *et al*, 1997). Conflicting results were reported by Mahmoud *et al* (1996), who found BSAP expression in normal plasma cells.

The oct-2 TF, a member of the octamer binding protein family, is expressed in B lymphocytes and binds to an octamer motif, present in all Ig promoters, in the mb-1 promoter, and in the 3′ Ig heavy chain enhancer (Latchman, 1996). Targeted mutation of the oct-2 gene in mice caused neonatal lethality and abrogated mitogen-induced proliferation and differentiation in Ig-secreting cells *in vitro*, arguing for a critical role of this TF in late B-cell maturation (Corcoran *et al*, 1993). Very recently, two groups reported the absence of oct-2 and its cofactor Bob-1/Obf.1 as the mechanism responsible for the lack of Ig gene expression in Hodgkin and Reed–Sternberg cells of classic Hodgkin's disease (Re *et al*, 2001; Stein *et al*, 2001).

Another TF with an essential role in terminal B-cell differentiation is the B lymphocyte-induced maturation protein (Blimp-1), which was originally isolated by subtractive cDNA cloning from a library enriched for genes specifically expressed in the IL-2- and IL-5-activated BCL1 murine B-cell lymphoma cell line (Turner *et al*, 1994). Transfection of Blimp-1 into BCL1 cells was accompanied by an increase in cell size and granularity, induction of J-chain mRNA, increased Ig protein secretion, and upregulation of syndecan-1 (Turner *et al*, 1994). Interestingly, another report showed that c-myc promoter activity was also repressed (Lin *et al*, 1997).

Taken together, these studies argue for a regulatory role of oct-2, PU.1 and Spi-B, BSAP and Blimp-1 in control of Ig production and in differentiation of B cell to plasma cell. Whether these TFs also play a role in the emergence of malignant plasma cells which accumulate excessively in the bone marrow of multiple myeloma patients is not known. We report here a comparative analysis of the expression of these TFs in normal and malignant human plasma cells.

PATIENTS AND METHODS

Monoclonal antibodies (mAb), cytokines, cell lines and other reagents. Anti CD-38-PC5 (Coulter Immunotech, Miami, FL, USA), anti-CD138-phycoerythrin (PE) (B-B4; Immuno-QualityProducts, Groningen, The Netherlands), anti-CD20-fluorescein isothiocyanate (FITC), and anti-CD45-FITC (BD Biosciences, Heidelberg, Germany), anti-CD138 (Immunotech, Marseille, France), IL-2, IL-6, IL-10 (Peprotech, Rocky Hill, NJ, USA), recombinant, soluble human CD40 Ligand (rhsCD40L, Alexis, Lausen, Switzerland), anti-FLAG M2 mAb (Sigma, Saint Louis, MO, USA), TO-PRO-3 iodide (Molecular Probes, Leiden, The Netherlands).

A series of different B-cell lines at various stages of differentiation was used: BJAB and Raji, two human cell lines derived from Burkitt lymphomas, BC-3, derived from a lymphoplasmocytoid primary effusion lymphoma (a kind gift of R. Nador, Department of Pathology, University Medical Centre, Geneva, Switzerland), OPM-2, established from a patient with advanced multiple myeloma, and L363, derived from a patient with plasma cell leukaemia (PCL) (both cell lines were obtained from the German collection of microorganisms and cell cultures, DSMZ, Braunschweig, Germany). The human T-cell leukaemia-derived cell line Jurkat was used as a control.

Patients and cell preparations. Bone marrow aspirations were obtained from five patients without haematological disease and from 10 patients with multiple myeloma (MM), according to institutional guidelines. According to the Durie/Salmon classification, three patients were stage IIA, one patient stage IIB, five patients stage IIIA and one patient stage IIIB. Peripheral blood was obtained from three patients with PCL. After enrichment of mononuclear cells with a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), cells were labelled with anti CD-45, anti-CD138, anti-CD38 and with TO-PRO, and analysed by flow cytometry (FACSCalibur, BD Biosciences). $CD138^+$ cells were purified using anti-CD138 and magnetic beads coated with sheep anti-mouse IgG (CELLection Pan Mouse IgG Kit, Dynal, Oslo, Norway), as described previously (Sun *et al*, 1997), and then resuspended in culture medium. Alternatively, plasma cells were purified using the cell sorter (FACStar+, BD) by gating on the $CD138^+CD38^{++}$ cell population. Purity and cell morphology were assessed by microscopic analysis on a cytospin and by re-analysis with flow cytometry.

Mononuclear cells from the peripheral blood of six healthy donors and of five patients with chronic lymphocytic leukaemia (CLL) (at diagnosis: two patients stage A, three patients stage B according to Binet) were obtained by centrifugation on a Ficoll-Paque gradient. $CD19^+$ cells were selected using magnetic beads (Pan-B Dynabeads M-450, Dynal, Oslo, Norway), according to the manufacturer's instructions, and then detached from the beads by overnight incubation at 37°C. Purity was verified by labelling with anti-CD20 and was in all cases > 90% (data not shown).

Purified cells were snap-frozen in liquid nitrogen and stored as dry pellets at −70°C for RNA analysis.

This protocol was approved by the Ethics committee of the Department of Internal Medicine, Geneva University Hospital.

Cell cultures. Cultures were performed at 37°C, in 5% CO_2, in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Basel, Switzerland), supplemented with 10% heat-inactivated fetal calf serum (FCS, Seromed, Berlin, Germany), 10 mmol/l Hepes buffer, 100 U/ml penicillin and 100 U/ml streptomycin (Gibco).

Two different culture systems were used for *in vitro* plasma cell generation:

(1) 1000 $CD19^+$ cells/well were cultured for 10 d together with 5×10^4 irradiated (50 Gy), mutagenized, murine EL-4 thymoma cells (clone 6·1.5·5, a kind gift of R. H. Zubler and C. Werner-Favre) and 5% T-cell supernatant in 200 μl cultures, as described (Matthes *et al*, 1993).

(2) $CD19^+$ cells (1×10^6 ml/well) were first stimulated with CD40L (100 ng/ml), anti-FLAG mAb (2 μg/ml), IL-2 (50 U/ml) and IL-10 (100 ng/ml) for 4 d. After harvesting, cells were counted, washed extensively and recultured at 10^5 cells/200 μl/well with IL-6 (10 ng/ml) and IL-10 (10 ng/ml) for 6 d (Jego *et al*, 2001).

At the end of the cultures the cells were harvested, analysed by flow cytometry, and stored for RNA analysis as dry cell pellets at −70°C.

Analysis of transcription factor mRNA expression. RNA was extracted from cell lines, and from purified plasma cells and CLL B cells using the method of Chomczynski & Sacchi (1987) and retrotranscribed into cDNA with AMV reverse transcriptase (Promega, Madison, WI, USA). Aliquots of the reverse transcription (RT) mixture were then amplified in a thermocycler (Biometra, Göttingen, Germany) using TAQ polymerase (Promega), as described previously (Matthes *et al*, 1993). The cycling conditions were 30 s at 94°C for denaturation, 30 s at temperatures between 56 and 64°C depending on the T_m of the pair of primers used, and 30 s at 72°C for elongation.

The primers had the following sequences, 5′- and 3′- respectively: β-2 microglobulin, GTCTG GGTTT CATCC ATCCG and TCATC CAATC CAAAT GCGGC; PU-1, CGACC ATTAC TGGGA CTTCC (P1) and TTCTT CTTCA CCTTC TTGAC C (P2); Spi-B, TCAGA GGAGG AAGAC TTACC (P1) and AGCTT CTGGT AGGTG ATGC (P2); Blimp-1, ACAGT GCCTT CTCCT TTACC (P1) and TGATG TCATC CTCCA CGTCC (P2); BSAP, CAACA AGCGC AAGAG AGACG (P1) and ACCTC CAGGA GTCGT TGTAC G (P2); oct-2, GAGGA GCTGG AGCAA TTCG (P1) and CTCTT CTCTA AGGCG AAGCG (P2).

For 'nested polymerase chain reaction (PCR)' or 'semi-nested PCR', 1/10 of the reaction volume of the first PCR was added to a fresh PCR mix with a second pair of primers having the following sequences, 5′- and 3′- respectively: PU-1, GCCGA GAACA ACTTC ACGG (P3) and CAGAT GCTGT CCTTC ATGTC G (P4); Spi-B, GGAGG AAGAC TTACC GTTGG (P3) and P2; Blimp-1, CACCT GAGAG TGCAC AGTGG (P3) and CACAA ACTGG GTGAA CTTGG C (P4); BSAP, P1 and AGTCA CGGCC TGTCA CAATG G (P3); oct-2, GACTT CAGCC AGACG ACCAT TT (P3) and CTCTA AGGCG AAGCG GACG (P4).

The primer pairs were designed to span over introns to allow the detection of potentially contaminating genomic DNA. Specificity was confirmed by digestion of amplified cDNA fragments with specific restriction enzymes or by sequencing (data not shown). An aliquot of the PCR product was then visualized after electrophoresis through 2% agarose gels by staining with ethidium bromide. To ensure the absence of sample contamination, an RNA extraction control was run in parallel to each PCR.

RESULTS

Expression of transcription factors in human cell lines

RT–PCR was performed for the five different TFs, oct-2, PU-1, Spi.B, BSAP and Blimp-1, and for the house-keeping gene β-2 microglobulin. Quantities of cDNA used for the TF-PCRs were adjusted to band intensities obtained with the PCR for β-2 microglobulin (Fig 1). Two different TF expression profiles were present: the cell lines BJAB and Raji strongly expressed BSAP, oct-2, PU.1 and Spi-B, and did not (BJAB) or only very weakly (RAJI) express Blimp-1, whereas the cell lines BC-3, L363 and OPM-2 with a lymphoplasmacytoid or plasma cell phenotype expressed Blimp-1, oct-2 weakly, but did not express BSAP, PU.1 or Spi-B.

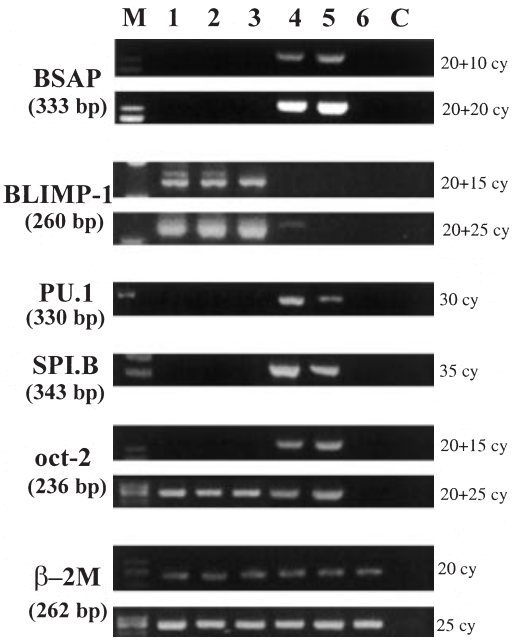

Fig 1. Transcription factor (TF) mRNA expression in five human B-cell lines. RNA was extracted from 10^6 cells of five human B-cell lines (BC3 (lane 1), OPM-2 (lane 2), L363 (lane 3), Raji (lane 4), BJAB (lane 5)) and from the T-cell line Jurkat (lane 6), retrotranscribed into cDNA and analysed by polymerase chain reaction (PCR) for expression of TFs and for β-2 microglobulin. cDNA quantities used for PCR of TFs were equilibrated according to band intensities obtained with β-2 microglobulin. To obtain optimal equilibrated samples, aliquots were analysed at two different cycle numbers. M, molecular weight marker; C, negative RNA–extraction control.

Expression of transcription factors in plasma cells from multiple myeloma patients and patients with CLL

For the analysis of primary multiple myeloma plasma cells, bone marrow aspirates were obtained at diagnosis from 10 patients with multiple myeloma as well as peripheral blood from three PCL patients. Flow cytometric analysis of the mononuclear cell suspension showed in each case a typical CD38 ($^+$ or $^{++}$), CD138 ($^+$ or $^{++}$) and CD45 (negative or weakly $^+$) plasma cell population (Fig 2), ranging from 5% to 62% (mean = 32%) of the cell suspension. After sorting with anti-CD138 coupled to magnetic beads, the purity of the obtained plasma cell population was assessed by labelling with anti-CD38; in each case >90% purity was obtained (Fig 2). In four cases, in which only a few cells were available, the cell sorter was used and a gate set on the

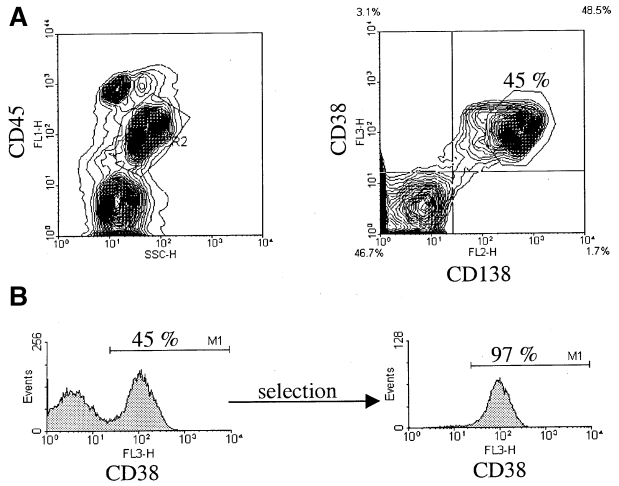

Fig 2. Flow cytometric analysis and isolation of bone marrow plasma cells from multiple myeloma patients. (A) After bone marrow aspiration and Ficoll-Paque separation, bone marrow mononuclear cells were labelled with anti-CD45, anti-CD138, anti-CD38 and TO-PRO, and analysed using flow cytometry. A typical result from a patient with $CD45^+$ (weak), $CD38^+$ and $CD138^+$ malignant plasma cells, constituting about 45% of the living (TOPRO negative) mononuclear cells, is shown. (B) $CD138^+$ cells were sorted with anti-CD138 coupled to magnetic beads and a 97% pure population of $CD38^+$ plasma cells was obtained. Histograms show CD38 labelling before (left) and after (right) sorting.

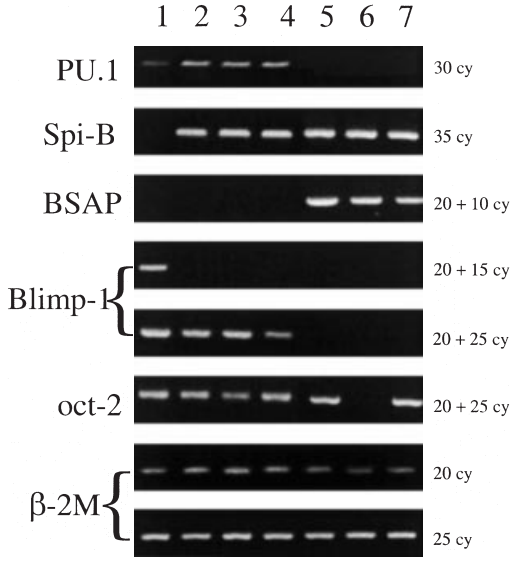

Fig 3. Transcription factor mRNA expression in primary multiple myeloma cells, in cells from PCL patients and from CLL B lymphocytes. A representative reverse transcription polymerase chain reaction (RT–PCR) analysis is shown, with purified plasma cells from one patient with plasma cell leukaemia (PCL) (lane 1) and from three patients with multiple myeloma (lanes 2–4), and with purified mature $CD19^+$ B lymphocytes from three patients with chronic lymphocytic leukaemia (CLL) (lanes 5–7). PCR was performed with the primers and cycle numbers as indicated, using cDNA quantities from the different samples previously equilibrated according to band intensities obtained from the β-2 microglobulin PCR.

$CD38^{++}CD138^+$ cells. Staining with anti-CD3 and anti-CD14 showed less than 5% contaminating T lymphocytes or monocytes, respectively, in each case and microscopic analysis on a cytospin showed cell populations with typical plasma cell morphology (data not shown). Between 10^5 and 10^6 plasma cells/sample were obtained.

cDNA quantities used for PCRs of TFs were again adjusted according to β-2 microglobulin band intensities (Fig 3). The primary malignant plasma cells exhibited a TF-expression pattern very similar to the one obtained from OPM-2 and L-363 cell lines; i.e. expression of Blimp-1, oct-2, and absence of BSAP (10/10). In contrast to the two cell lines, in which PU.1 and Spi-B were absent, all primary MM cells expressed both TFs. Interestingly, the three PCL samples were negative for Spi-B (3/3), negative (2/3) or weakly positive (1/3) for PU.1, and showed a stronger message for Blimp-1 than MM cells (3/3).

On the other hand, mature, malignant $CD19^+$ B lymphocytes from the peripheral blood of CLL patients showed a TF expression similar to that of RAJI and BJAB cell lines with BSAP (5/5) and oct-2 (4/5) expression and Blimp-1 absence (5/5). However, unlike in these two cell lines, PU.1 was not expressed (5/5) (Fig 3).

Generation of plasma cells in vitro

In order to compare TF expression patterns of malignant and normal cells, two strategies were used to obtain normal plasma cells. First, isolation from the bone marrow of patients without any clinically manifest haematological disease, by sorting $CD38^{++}CD138^+$ cells using flow cytometry. Second, generation *in vitro* using two different culture systems: the EL-4 culture system originally described by Zubler *et al* (1985), in which B cells are stimulated by murine thymoma cells, and a two-step culture system reported recently by Jego *et al* (2001), in which B cells are first stimulated by CD40 ligand, IL-2 and IL-10, and then, subsequently, by IL-6 and IL-10. Starting with purified $CD19^+$ mature B cells, both culture systems allowed us to generate $CD38^{++}CD138^+$ plasma cells after a 10-d culture (Fig 4).

Expression of transcription factors in in vitro-generated plasma cells

RT–PCR analysis was performed for normal plasma cells as already described. The TF expression profile for fresh bone marrow plasma cells (three experiments) and *in vitro* generated plasma cells (three experiments for each culture condition) was practically identical: expression of Spi-B (9/9) and oct-2 (9/9), at an intensity similar to that in $CD19^+$ cells, expression of Blimp-1 stronger than in $CD19^+$ lymphocytes (9/9), expression of PU.1 but in 4/9 cases weaker than in $CD19^+$ cells, and weak (5/9) or absent (4/9) expression of BSAP (Fig 5).

$CD19^+$ lymphocytes expressed all five TFs, with weak expression of Blimp-1 compared with plasma cells (3/3).

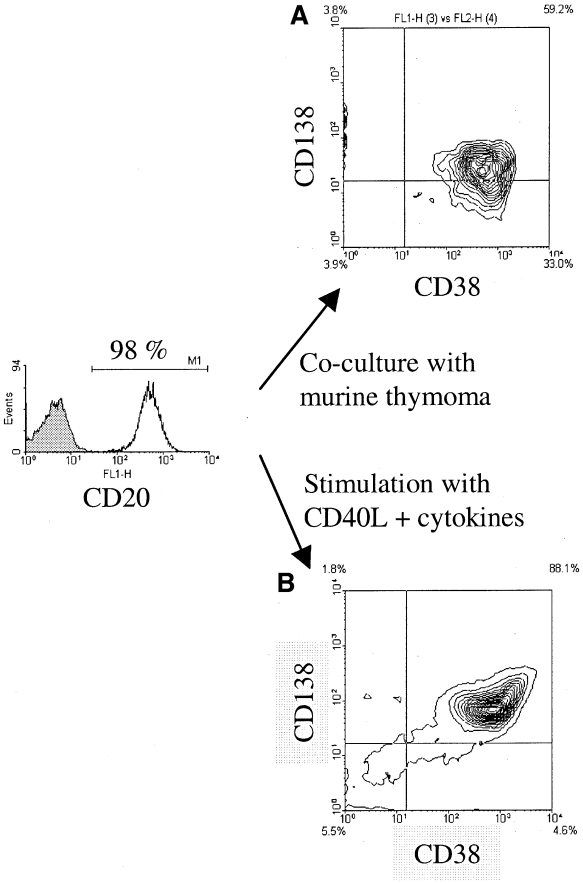

Fig 4. *In vitro* plasma cell generation. CD19$^+$ B lymphocytes were isolated from the peripheral blood of normal blood donors, analysed using flow cytometry to verify the purity of the obtained B cells (CD20$^+$ cells), and cultured *in vitro* for 10 d in (A) a co-culture system with a murine thymoma or (B) in a two-step culture system with stimulation by CD40L and interleukin 2 (IL-2)/IL-10, followed by IL-6/IL-10. Cells were harvested after 10 d and analysed using flow cytometry. Results of one representative experiment out of three are depicted.

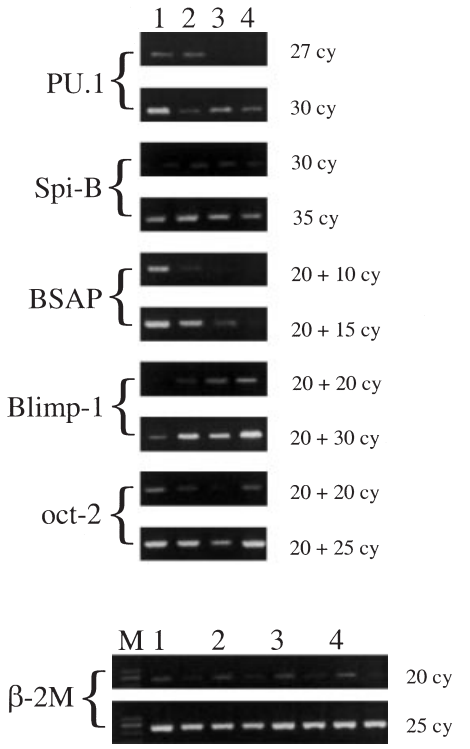

Fig 5. Transcription factor mRNA expression in normal CD19$^+$ B lymphocytes and in normal plasma cells. A representative reverse transcription polymerase chain reaction (RT–PCR) analysis (one experiment out of three) is shown, with purified CD19$^+$ B lymphocytes from the peripheral blood of a normal blood donor (lane 1), purified CD38$^+$CD138$^+$ plasma cells from the bone marrow (lane 2), *in vitro*-generated plasma cells using the EL-4 co-culture (lane 3) and the CD40L$^+$ cytokines stimulation (lane 4). PCR was performed with the primers and the cycle numbers as indicated, using cDNA quantities from the different samples previously equilibrated according to band intensities obtained from the β-2 microglobulin PCR. For equilibration, cDNA quantities were used at two different concentrations (x and 1/10 x) and analysed at two different cycle numbers.

DISCUSSION

In the present report we analysed the expression of five TFs with regulatory roles in B-cell differentiation in normal and malignant human plasma cells and cell lines (Table I). Several interesting conclusions can be drawn from this study.

Normal plasma cells, isolated from human bone marrow or generated *in vitro*, expressed a specific set of TFs compared with mature B lymphocytes: similar expression of Spi-B and oct-2, downregulation of PU.1 and BSAP, and upregulation of Blimp-1. The weak positive BSAP band in some samples was probably owing to contaminating CD19$^+$ B cells as, after *in vitro* culture, no purification of CD38$^+$CD138$^+$ was performed and not all cultured cells differentiated into plasma cells, as evident in the flow cytometric analyses (Fig 4). A similar reasoning could hold true for the weak PU.1 signal: < 1% of contaminating monocytes could account for the weak positivity, as PU.1 is not restricted to the B-cell lineage. These results are in agreement with most of the previously published murine studies. The discrepancy with the findings reported by Mahmoud *et al* (1996) of high BSAP expression in plasma cells is probably explained by their analysis of mRNA expression in a subpopulation of plasmablasts and not in terminally differentiated plasma cells (they used the CD38 but not the CD138 antibody for selection). Whether downregulation of BSAP constitutes a prerequisite for plasma cell differentiation, as suggested by Usui *et al* (1997), remains unproven.

MM plasma cells expressed the same set of TFs as normal plasma cells and at the same intensity (within the limits of our RT–PCR technique). No abnormalities in migration of PCR fragments on the agarose gels were detected in any of the patient samples (data not shown). It seems therefore

Table I. Expression of transcription factor cDNAs in normal and malignant plasma cells and CD19$^+$ B lymphocytes.

	CD19$^+$ mature B lymphocyte	Normal plasma cell	Plasma cell from multiple myeloma	Plasma cell leukaemia	Cell lines (OPM-2, L363)	CD19$^+$ B cells from CLL	Cell lines (RAJI, BJAB)
BSAP	+	–/weak	–	–	–	+	+
PU.1	+	weak/+	weak	–/weak	–	–	+
Spi-B	+	+	+	–	–	+	+
oct-2	+	+	+	+	+	–/+	++
Blimp-1	weak	+	+	++	++	–	–

Results from reverse transcription polymerase chain reaction (RT–PCR) experiments were denoted as negative (–), weakly positive (weak), positive (+) or strongly positive (++), according to band intensities and cycle numbers at which PCR amplification products became visible on ethidium-stained agarose gels. Dierences between ++ and +, and between + and weak signals were at least 5 cycle numbers.

improbable that major deletions of one of these TFs were present. However, point mutations or insertions cannot be excluded and, as a recent report showed in the case of the PU.1 gene in AML patients (Müller *et al*, 2001), might occur rather frequently in malignant haemopathies. It would therefore be interesting to analyse, for example, BLIMP-1 sequences, as in MM c-myc is overexpressed in up to 24% of cases, but mutations in the c-myc gene and its' promoter region have only been found in some of them (Selvanayagam *et al*, 1988). Mutations in the TF Blimp-1 could constitute a new mechanism by which c-myc escapes the normal downregulatory activity of Blimp-1. Point mutations in oct-2 or PU.1 with subsequent loss of function of these TFs could also explain the diminished Ig production in MM cells compared with normal plasma cells. Alternatively, some as yet not analysed TFs could be implicated in this regulation.

A major difference exists between cells from PCL and MM: PCL cells showed increased expression of Blimp-1 and loss of Spi-B. They therefore resemble established plasma cell lines which express only Blimp-1 and oct-2. Increased aggressiveness and proliferative capacity of malignant plasma cells seems to parallel loss of TFs and increased expression of Blimp-1. Whether Blimp-1 has in itself any oncogenic potential is not known. Although c-myc expression has not yet been analysed in PCL, it is interesting to note that, in a study performed on cell lines with a plasma cell phenotype, all lines studied expressed increased amounts of c-myc protein and had mutations in the 5′-UTR, thus arguing for an aberrant translational control of the c-myc gene in these cell lines (Willis *et al*, 1997). In contrast to blasts in murine erythroleukaemia, in which PU.1 is highly expressed and inhibits terminal differentiation (Rao *et al*, 1997), PU.1 is downregulated in malignant plasma cells, especially in cells from PCL, and totally absent in cell lines. This finding points to different roles and regulations of PU.1 depending on the haematopoietic lineage in which it is expressed, and could explain the loss of adhesion of PCL cells (and cell lines) to bone marrow stromal cells. PU.1 has in fact been shown to regulate homing, engraftment and adhesion of progenitor and stem cells to stroma by regulating expression of various integrins (Fischer *et al*, 1999).

In contrast to MM cells, malignant lymphocytes from CLL, fixed in their differentiation in a transition stage between immature and mature resting naive B cells, continued to express BSAP and did not upregulate Blimp-1, which remained undetectable (Fig 3). As normal B cells have been shown to react to IL-2 stimulation by BSAP downregulation (Rinkenberger *et al*, 1996), continued BSAP expression in CLL could explain their blocked differentiation stage, i.e. there could be a defect in the IL-2/BSAP signalling pathway. Alternatively, an incapacity to upregulate Blimp-1 could also explain this blockage.

In summary, we showed that normal and malignant human plasma cells typically express the TFs Blimp-1, oct-2 and Spi-B, and that differentiation of B cells into plasma cells is accompanied by upregulation of Blimp-1 and downregulation of PU.1 and BSAP. These studies agree to a great extent with previous work performed on murine cells and on cell lines. Anti-sense and transfection studies in primary cells are now warranted in order to elucidate more precisely the role of each TF in terminal B-cell differentiation.

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