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Advances in Brief

Cytokine Gene Expression in Neoplastic B Cells from Human Mantle Cell, Follicular, and Marginal Zone Lymphomas and in Their Postulated Normal Counterparts[1]

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Abstract

Cytokines may promote tumor growth by paracrine and/or autocrine pathways. Little information is available because malignant cells differ from their normal counterparts for the cytokine repertoire they express. Here we have investigated by reverse transcription-PCR the expression of 22 cytokine genes in neoplastic B lymphocytes from six patients with mantle cell lymphoma, 10 with follicular lymphoma, and 5 with marginal zone lymphoma and in their normal counterparts, *i.e.*, naive, germinal center, and memory B cells, purified from tonsils. The overall profiles of cytokine gene expression in neoplastic B cells and in the corresponding normal B-cell subsets were similar, but some "holes" in the repertoire of malignant *versus* normal B lymphocytes were detected. Different "hole" combinations were identified consistently in mantle cell lymphoma, follicular lymphoma, and marginal zone lymphoma, thus representing molecular fingerprints of each individual lymphoma entity.

Introduction

Multistep accumulation of genetic lesions results in cell transformation. According to the "seed and soil" hypothesis, however, the emergence of a fully malignant cell clone requires favorable microenvironmental conditions. This hypothesis is supported by the finding that cells bearing chromosomal translocations associated with certain hematological tumors are detected in blood or lymphoid tissues from healthy individuals (1). Cytokines are a major component of cellular microenvironments, where they play a pivotal role in the control of cell survival, proliferation, and differentiation. It has been shown that cytokines produced by malignant B lymphocytes can facilitate the growth of the neoplastic clone and be involved in the pathogenesis of some clinical manifestations associated with B-cell lymphoproliferative disorders, such as osteolysis, coagulation abnormalities, cachexia, and immune suppression (reviewed in Ref. 2). According to the REAL[3] classification, three types of human B-cell lymphoma, originating from normal naive, GC, and memory B cells, have been identified and designated as MCL, FL, and MZL, respectively (3). Thus far, little information in available on: (*a*) the cytokine repertoire expressed by neoplastic B lymphocytes from these lymphoproliferative disorders; and (*b*) the similarities or differences of such repertoire in comparison with that of the postulated normal counterparts. Here we have investigated by RT-PCR the expression of 22 cytokines in neoplastic B cells from MCL, FL, and MZL, as well as in their normal counterparts. The following cytokines have been studied: IL-1α and IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p35 and p40, IL-13, IL-15; IFN-γ, G-CSF, GM-CSF, M-CSF; TNF, LT-α, TGF-β1, and LIF. Most of these cytokines have a well-characterized functional activity on human B cells (2).

Materials and Methods

This investigation was performed after approval by a local institutional review board. Lymph nodes from 10 patients with FL (4 patients with grade I, 4 patients with grade II, and 2 patients with grade III FL), 6 patients with MCL (defined as a $CD5^+$, $CD23^-$ monoclonal B-cell proliferation), and 5 patients with MZL (monocytoid B-cell lymphoma) according to the REAL classification were studied (3).

Normal B-cell subsets representing the normal counterparts of FL, MCL, and MZL were isolated from tonsils. Twenty-five tonsils were obtained from patients undergoing tonsillectomy for inflammatory disorders, following informed consent.

MNCs were isolated by a Ficoll-Hypaque density gradient and were T-cell depleted by E rosetting. Macrophages and natural killer cells were subsequently removed by incubation with CD68 and CD56 mAbs, respectively, followed by immune rosetting. Neoplastic B lymphocytes were further purified by positive selection of malignant B cells according to the expression of monotypic immunoglobulin light chains (4). Naive B lymphocytes were isolated as IgD^+ cells from tonsil B lymphocyte suspensions by immune rosetting. The IgD^- B-cell fractions were further separated into $CD38^+$ (GC) cells and $CD38^-$ (memory) cells (5). In some experiments, GC B cells were isolated by a Percoll density gradient, followed by removal of IgD^+, $CD39^+$ B cells (4). All of the steps for tonsil B-cell subset isolation were performed at 4°C to prevent spontaneous apoptosis of GC B cells.

CD3, CD19, CD38, CD56, and CD68 mAbs were from Becton Dickinson (San Jose, CA). CD39 mAb was from PharMingen (San Diego, CA), whereas the anti-IgD mAb was from Dako (Glostrup, Denmark). Flow cytometric analysis was carried out as reported (4).

Normal or malignant B lymphocytes were cultured for 4–24 h in RPMI 1640 (Seromed-BiochromKG, Berlin, Germany) supplemented with 10% FCS (Seromed), in the presence or absence of CD40 mAb (1 μg/ml; Immunotech, Marseille, France) in combination with recombinant IL-4 (10 ng/ml; Genzyme, Cambridge, MA). Normal B-cell fractions were also incubated as above with: (*a*) anti-κ (1 $\mu g/10^6$ cells; Southern Biotechnology Associates, Birmingham, AL) and anti-λ (1 $\mu g/10^6$ cells) immunoglobulin light chain mAbs (Southern Biotechnology Associates); or (*b*) PMA (10 ng/ml; Sigma Chemical Co., St. Louis, MO) in combination with calcium ionophore (250 ng/ml; Sigma).

Supernatants from B cells cultured in the presence or absence of CD40 mAb and IL-4 were tested in triplicate for IL-6, IL-8, IL-10, TNF, and IFN-γ production by ELISA (Biosource, Camarillo, CA) or for G-CSF by the same technique (R&D Systems, Minneapolis, MN). The presence of IL-2, IL-7, and IL-15, which share the common γ chain of the IL-2 receptor for signal transduction (6), was investigated using the CTLL-2 bioassay (7). In this assay, recombinant IL-2, used as positive control, induces cell proliferation in a

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[3] The abbreviations used are: REAL, Revised European American Lymphoma; GC, germinal center; MCL, mantle cell lymphoma; FL, follicular lymphoma; MZL, marginal zone lymphoma; RT-PCR, reverse-transcription PCR; IL, interleukin; G-CSF, granulocyte-colony stimulating factor; M-CSF, macrophage-CSF; GM-CSF, granulocyte/macrophage-CSF; TNF, tumor necrosis factor; LT, lymphotoxin; TGF, transforming growth factor; LIF, leukemia inhibitory factor; MNC, mononuclear cell; PMA, phorbol myristate acetate; Th, T helper; mAb, monoclonal antibody; CD, cluster designation; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; mAb, monoclonal antibody.

dose-dependent manner (7). The identification of biologically active cytokine(s) in supernatants, which were tested in this study at the final concentration of 10% v/v, rests upon neutralization with anti-cytokine antibodies.

RNA was extracted from freshly isolated or cultured cells using Ultraspec (Biotecx Laboratory, Inc., Houston, TX) and subjected to RT-PCR as reported (8, 9). Primer sequences and profiles of amplification were described in previous studies (8, 9), with the exception of the following: IL-1α, 5′-TGACTCAGAGGAAGAAATCATC and 3′-TGGGCAACTGATGTGAAATAG; IL-1β, 5′-CTACGAATCTCCGACCACCAC and 3′-CGCTTTTCCATCTTCTTCTTTG; IL-7, 5′-ATGTTCCATGTTTCTTTTAGG and 3′-TCAGTGTTCTTTAGTGCCCATC; IL-13, 5′-GACCACGGTCATTGCTCTCAC and 3′-GTGTCTCGGACATGCAAGCTG; G-CSF, 5′-TGTGCCACCTACAAGCTGTGC and 3′-CCCAGTTCTTCCATCTGCTGC; LIF, 5′-TCTTGGCGGCAGGAGTTGTG and 3′-GTCGGCGGTGGCGTTGAGC; IL-15, 5′-CTGTTTCAGTGCAGGGCTTCC and 3′-GAATCAATTGCAATCAAGAAGTG; M-CSF, 5′-CTGCGTCCGAACTTTCTATGAG and 3′-CAGCAAGACCAGGATGACACT; LT-α, 5′-TGACACCACCTGAACGTCTC and 3′-GAGAAACCATCCTGGAGGAAG; CD3γ, 5′-GGTTCGGTACTTCTGACT and 3′-TGGTTTTGACTTGTTCTG; CD19, 5′-ACCTCCTCGCCTCCTCTTC and 3′-TCCCCTTCCTCTTCTTCTG; and CD68, 5′-CATCCAACAAGCAATAGCA and 3′-CTGAGCCGAGAATGTCCACT. The amplification profile was 94°C for 1 min, annealing 60°C (IL-1α and IL-1β), 65°C (LT-α and M-CSF), 48°C (CD3γ), 57°C (CD19), and 54°C (CD68) for 1 min, 55°C (IL-7) or 70°C (IL-13 and G-CSF and LIF) for 2 min, and extension at 72°C for 1 min. Each cycle of amplification was repeated 32 times. Ten μl of each sample were electrophoresed through a 1.5% agarose gel containing ethidium bromide. The specificity of amplification products was verified by confirming the known bp sequence length and by Southern blot.

Results

Purity of Neoplastic and Normal B-Cell Suspensions and Study Outline. The purity of B-lymphoma cells was >99%, as assessed by flow cytometric analysis of CD19 and monotypic immunoglobulin light chain expression (4). Likewise, >99% tonsil B cells expressed CD19. $CD3^+$ T cells, $CD56^+$ natural killer cells, and $CD68^+$ macrophages were absent (<1%) from the above cell suspensions.

The expression of the *CD3*γ, *CD68,* and *CD19* genes in normal or neoplastic B-cell fractions was next investigated by RT-PCR. CD3γ is selectively expressed in T cells, CD68 is a specific macrophage marker, and CD19 expression is restricted to the B-cell lineage. CD3γ and CD68 mRNA were never found in normal B-cell suspensions or in B-lymphoma cells, whereas they were detected in tonsil MNCs (Fig. 1). In contrast, CD19 transcripts were found in purified normal and neoplastic B-cell suspensions, as well as in tonsil MNCs (Fig. 1). The results shown in Fig. 1 refer to one representative case of MCL, FL, and MZL each and to a normal B-lymphocyte suspension. Normal and malignant B-cell fractions that contained even minute amounts of contaminant cell types, as assessed by flow cytometry and RT-PCR, were excluded from the study.

Purified normal or neoplastic B cells were tested for cytokine gene expression either immediately after isolation or after incubation with different stimuli. In particular, tonsil B-cell subsets were cultured for 4 h with CD40 mAb and IL-4, cross-linking anti-immunoglobulin mAbs or PMA and calcium ionophore. The former stimuli were selected to mimic *in vitro* T cell-dependent or -independent B-cell activation, respectively, whereas the combination of PMA and calcium ionophore represents a potent, nonphysiological stimulus. When feasible, because of the low numbers of purified cells available, B-lymphoma cells were incubated with CD40 mAb and IL-4 before RNA extraction.

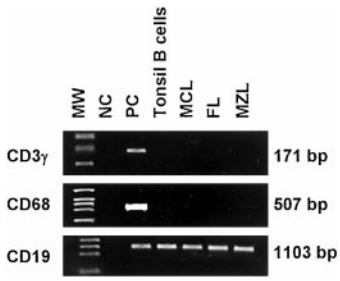

Fig. 1. *CD3*γ, *CD68,* and *CD19* gene expression in tonsil, MCL, FL, and MZL B cells, as assessed by RT-PCR. One representative experiment each is shown. *MW,* molecular weight markers; *NC,* negative control, represented by water in the place of cDNA; *PC,* positive control, represented by tonsil MNC. On the *right,* the expected molecular weights of the amplified bands are shown.

To check the correspondence between cytokine mRNA accumulation and protein secretion, normal B-cell suspensions were cultured for 24 h with or without CD40 mAb and IL-4. Supernatants were tested for IL-6, IL-8, IL-10, G-CSF, TNF, and IFN-γ by ELISA and for IL-2, IL-7, and IL-15 by the CTLL-2 bioassay. Although activated B-cell supernatants contained recombinant IL-4, RPMI 1640 supplemented with the same IL-4 concentration present in the CTLL assay (1 ng/ml) failed to induce cell proliferation, which was instead clearly detectable in the presence of recombinant IL-2 (data not shown). These experiments were not carried out with lymphoma cells because of the paucity of purified B lymphocytes obtained.

Cytokine Gene Expression in Neoplastic B Cells from MCL and in Naive Tonsil B Lymphocytes. Fig. 2 shows the patterns of cytokine gene expression in neoplastic B cells from two representative MCL cases and in two naive (IgD^+) tonsil B-cell fractions, tested immediately after isolation (see also Table 1). Expression of IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, GM-CSF, and M-CSF was observed consistently in both malignant and normal B cells (Fig. 2). IL-3, IL-4, IL-5, and IL-13 transcripts were never detected, either in MCL or naive B cells. IL-12 p40 and G-CSF mRNA were not expressed in any MCL sample but were always found in naive B cells (Fig. 2). These results were confirmed in 3 additional MCL cases and in 13 naive B-cell suspensions. The IL-2 transcript was found consistently in naive B cells but only in 2 of 5 MCL samples (Fig. 2 and data not shown).

The overall patterns of cytokine gene expression detected in both neoplastic and normal B cells after culture with CD40 mAb and IL-4 were similar to those observed in freshly isolated cells. However, IL-3, IL-5, and IL-13 mRNA were induced in activated naive B cells, whereas IL-3 and IL-13, but not IL-5, transcripts were expressed *de novo* in stimulated MCL B cells (data not shown). Notably, naive B-cell activation with anti-immunoglobulin mAbs or PMA and calcium ionophore had no effect on cytokine gene expression, as compared with the profiles detected in freshly isolated cells (not shown).

IL-6 and IL-8 were detected in the supernatants from both unstimulated and CD40 mAb plus IL-4-activated naive B cells at the following concentrations: IL-6, unstimulated cells, 533–1000 pg/ml, stimulated cells, 1000–2100 pg/ml, ranges from three different experiments; IL-8, unstimulated cells, 430–750 pg/ml, stimulated cells 980-1420 pg/ml, ranges from three different experiments. In contrast, IL-2, IL-7, IL-10, IL-15, G-CSF, TNF, and IFN-γ were below the threshold of detection.

Cytokine Gene Expression in Neoplastic B Cells from FL and in GC Tonsil B Lymphocytes. Fig. 3 shows the patterns of cytokine gene expression in neoplastic B cells from two representative FL cases and in two GC ($CD38^+$, IgD^-) tonsil B-cell fractions, tested after isolation (see also Table 1). Expression of IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL12 p40, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, and GM-CSF was found consistently in both malignant and normal B cells (Fig. 3). IL-3, IL-4, IL-5, IL-13, and M-CSF transcripts were never observed, either in FL or GC B cells. G-CSF mRNA was present in GC B cells but absent from FL B cells. These

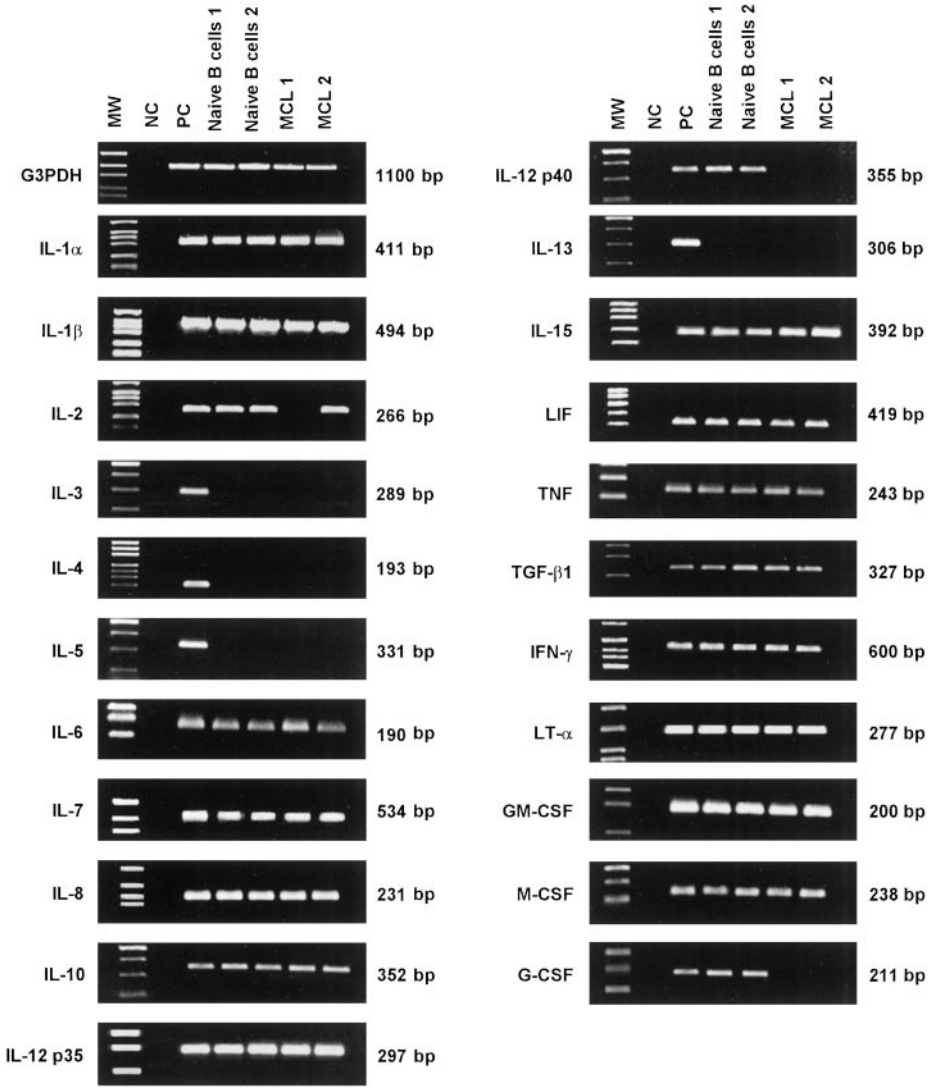

Fig. 2. Expression of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL-12 p40, IL-13, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, GM-CSF, M-CSF, and G-CSF in naive tonsil B lymphocytes and in their neoplastic counterparts, *i.e.*, MCL B cells. *MW*, molecular weight markers; *NC*, negative control, represented by water in the place of cDNA; *PC*, positive control, represented by normal peripheral blood MNCs stimulated with phytohemagglutinin for 6 h; naive B cell fractions, samples 1 (*Naive B cells 1*) and 2 (*Naive B cells 2*); MCL B cells, cases 1 (*MCL 1*) and 2 (*MCL 2*). The *first upper panel* of the *left column* shows the amplification product of the *G3PDH* housekeeping gene tested as control. On the *right side* of each *column*, the expected molecular weights of the amplified bands are shown.

results were confirmed in 8 FL cases and in 13 GC B-cell suspensions. The IL-2 transcript was detected always in GC B cells but only in 2 of 10 FL samples (Fig. 3 and data not shown).

Ten additional GC B-cell fractions were prepared by a Percoll density gradient and depletion of IgD^+, $CD39^+$ cells to exclude that CD38 triggering during the positive selection procedure could influence the patterns of cytokine gene expression. The latter hypothesis was ruled out by the identical results obtained using the two different cell purification techniques (not shown). The cytokine profile detected in freshly isolated FL cells was superimposable in all cases, independently of the grade according to the REAL classification.

Next, FL or GC B cells were stimulated *in vitro* with CD40 mAb and IL-4 before being tested for cytokine gene expression. In GC B cells, the only change observed was the induction *de novo* of IL-13 mRNA expression, whereas in FL cells IL-5, IL-13, and G-CSF transcripts were detected (data not shown). Again, incubation of GC B cells with cross-linking anti-immunoglobulin mAbs or with PMA and calcium ionophore was ineffective at inducing *de novo* expression of any cytokine gene (data not shown).

IL-6, IL-8, and G-CSF were found in supernatants from unstimulated and CD40 mAb plus IL-4-activated GC B cells. The ranges detected in three different experiments are the following: unstimulated cells, 238–533 pg/ml IL-6, 750-1090 pg/ml IL-8, and 100–170 pg/ml G-CSF; stimulated cells, 207–309 pg/ml IL-6, 1200–1340 pg/ml IL-8,

Table 1. Cytokine gene expression in freshly isolated neoplastic B cells from human MCLs, FLs, and MZLs and from their postulated normal counterparts

Cytokine mRNA[a]	Normal and malignant B-cell fractions[b]					
	Naive B cells	MCL	GC B cells	FL	Memory B cells	MZL
IL-1α	+	+	+	+	+	+
IL-1β	+	+	+	+	+	+
IL-2	+	−/+	+	−/+	+	−
IL-3	−	−	−	−	−	−
IL-4	−	−	−	−	−	−
IL-5	−	−	−	−	−	−
IL-6	+	+	+	+	+	+
IL-7	+	+	+	+	+	+
IL-8	+	+	+	+	+	+
IL-10	+	+	+	+	+	+
IL-12 p35	+	+	+	+	+	+
IL-12 p40	+	−	+	+	+	+
IL-13	−	−	−	−	−	−
IL-15	+	+	+	+	+	+
LIF	+	+	+	+	+	+
TNF	+	+	+	+	+	+
TGF-β1	+	+	+	+	+	+
IFN-γ	+	+	+	+	+	+
LT-α	+	+	+	+	+	+
GM-CSF	+	+	+	+	+	−
M-CSF	+	+	−	−	+	−
G-CSF	+	−	+	−	+	−

[a] As detected by RT-PCR.
[b] +, detected in all cases; −, not detected in any case; −/+, detected in some but not in other cases.

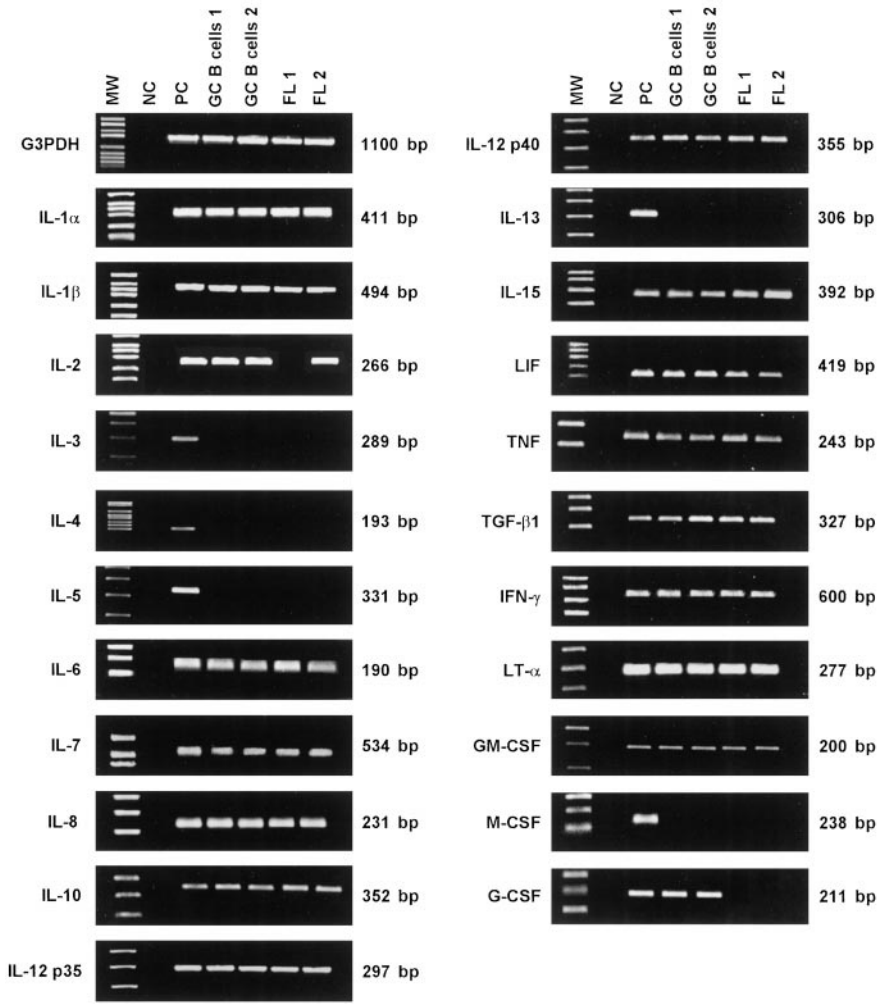

Fig. 3. Expression of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL-12 p40, IL-13, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, GM-CSF, M-CSF, and G-CSF in GC tonsil B lymphocytes and in their neoplastic counterparts, *i.e.*, FL B cells. *MW*, molecular weight markers; *NC*, negative control, represented by water in the place of cDNA; *PC*, positive control, represented by normal peripheral blood MNCs stimulated with phytohemagglutinin for 6 h; GC B cell fractions, samples 1 (*GC B cells 1*) and 2 (*GC B cells 2*); FL B cells, cases 1 (*FL 1*) and 2 (*FL 2*). The *first upper panel* of the *left column* shows the amplification product of the *G3PDH* housekeeping gene tested as control. On the *right side* of each *column*, the expected molecular weights of the amplified bands are shown.

and 200–300 pg/ml G-CSF. IL-2, IL-7, IL-10, IL-15, TNF, and IFN-γ were below the threshold of detection.

Cytokine Gene Expression in Neoplastic B Cells from MZL and in Memory Tonsil B Lymphocytes. Fig. 4 shows the patterns of cytokine gene expression in neoplastic B cells from two representative MZL cases and in two memory ($CD38^-$, IgD^-) tonsil B-cell fractions, tested after isolation (see also Table 1). Expression of IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL12 p40, IL-15, LIF, TNF, TGF-β1, IFN-γ, and LT-α was observed consistently in both malignant and normal B cells (Fig. 4). IL-3, IL-4, IL-5, and IL-13 transcripts were never detected, either in MZL or memory B cells. IL-2, G-CSF, M-CSF, and GM-CSF mRNA were always present in memory B cells but absent from MZL B cells. These results were confirmed in 3 additional MZL cases and in 13 memory B-cell suspensions. A summary of cytokine gene expression studies in freshly isolated B-cell subsets and in their malignant counterparts is shown in Table 1. *In vitro* stimulation of memory B cells or MZL B cells with CD40 mAb and IL-4 induced *de novo* IL-13 mRNA expression, whereas no effect was observed after culture with anti-immunoglobulin mAbs or PMA and calcium ionophore (data not shown).

Finally, IL-6 and IL-8 were detected in supernatants from unstimulated and CD40 plus IL-4-activated memory B cells. The ranges from three different experiments are the following: unstimulated cells, 99–486 pg/ml IL-6, 380–560 pg/ml IL-8; and stimulated cells, 156–453 pg/ml IL-6, 700-1090 pg/ml IL-8. IL-2, IL-7, IL-10, IL-15, G-CSF, TNF, and IFN-γ were below the threshold of detection.

Discussion

This study provides the first characterization of the patterns of expression of 22 cytokine genes in neoplastic B cells from human MCL, FL, and MZL and in their postulated normal counterparts, *i.e.*, naive, GC, and memory B cells, isolated from tonsils. Most of these cytokines were selected in view of their well-documented activity on human B lymphocytes (2). The aim of the study was to investigate whether there were differences in the repertoire of cytokines expressed by malignant B lymphocytes *versus* their normal counterparts.

The results obtained from the analysis of freshly isolated normal B-cell subsets indicate that naive, GC, and memory B lymphocytes display similar profiles of cytokine gene expression. Thus, IL-1α, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL-12 p40, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, G-CSF, and GM-CSF were expressed in all samples, whereas IL-3, IL-4, IL-5, and IL-13 transcripts were consistently absent from the three major B-cell subsets. M-CSF mRNA was detected in naive and memory B cells but not in GC B cells.

IL-13 mRNA induction was observed in all B-cell subsets upon culture with CD40 mAb and IL-4 but not with anti-immunoglobulin mAbs, suggesting that, in our experimental conditions, costimulatory interactions between CD40 on B cells and CD40 ligand on T cells, rather than B-cell receptor triggering, are of primary importance in the induction of cytokine gene transcription. This conclusion is reinforced by the finding that, in naive B cells, CD40 and IL-4 stimulation, but

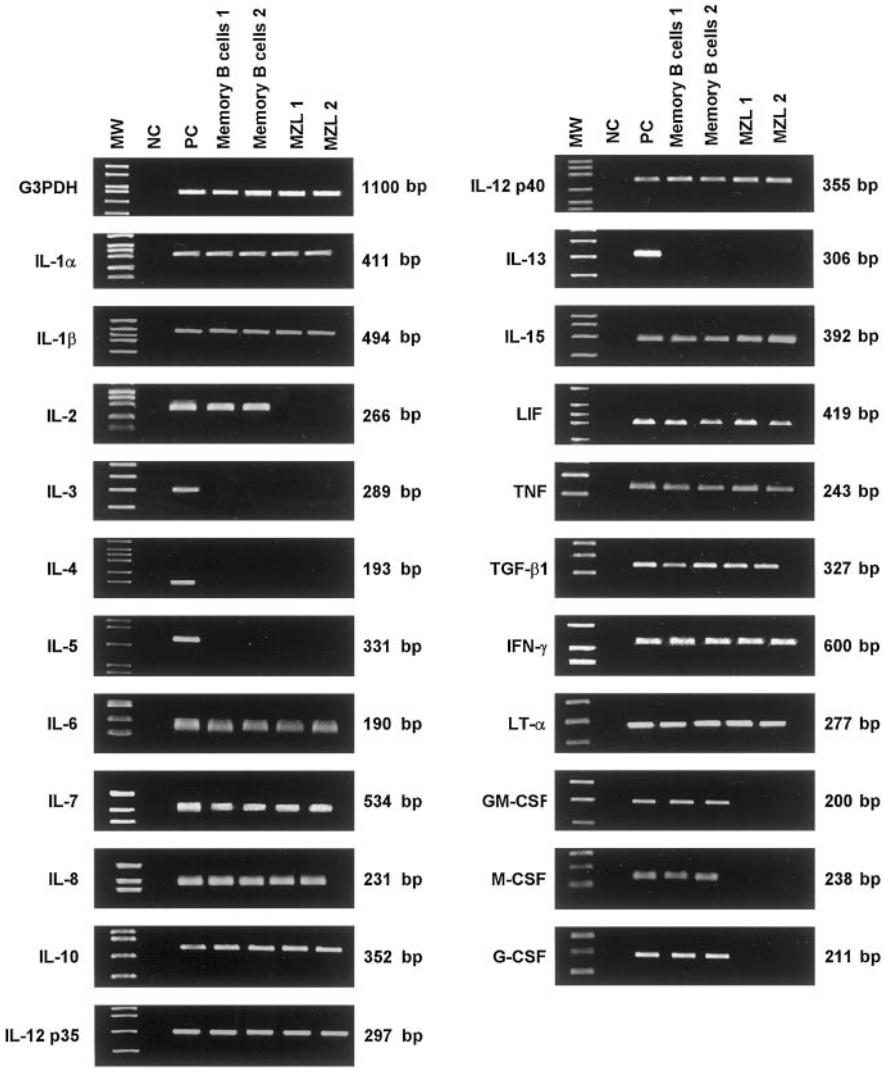

Fig. 4. Expression of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p35, IL-12 p40, IL-13, IL-15, LIF, TNF, TGF-β1, IFN-γ, LT-α, GM-CSF, M-CSF, and G-CSF in memory tonsil B lymphocytes and in their neoplastic counterparts, *i.e.*, MZL B cells. *MW*, molecular weight markers; *NC*, negative control, represented by water in the place of cDNA; *PC*, positive control, represented by normal peripheral blood MNCs stimulated with phytohemagglutinin for 6 h; memory B cell fractions, samples 1 (*Memory B cells 1*) and 2 (*Memory B cells 2*); MZL B cells, cases 1 (*MZL 1*) and 2 (*MZL 2*). The *first upper panel* of the *left column* shows the amplification product of the *G3PDH* housekeeping gene tested as control. On the *right side* of each *column*, the expected molecular weights of the amplified bands are shown.

not surface immunoglobulin cross-linking, also induced the transcripts of IL-3 and IL-5.

Malignant B cells from MCL, FL, and MZL displayed overall profiles of cytokine gene expression similar to that observed in their normal counterparts. However, some holes in the repertoire of neoplastic B cells were detected and found to be reproducibly associated with a given lymphoma category.

In comparison with naive B cells, freshly purified MCL cells consistently lacked G-CSF and IL-12 p40 mRNA. The latter finding indicates that MCL cells cannot assemble the IL-12 heterodimer (10).

In freshly isolated FL cells, at variance with GC B cells, G-CSF mRNA was absent, although it became detectable after CD40 and IL-4 stimulation. Notably, G-CSF was identified previously as an autocrine and/or paracrine antiapoptotic factor for GC B cells (11).

Freshly isolated MZL lacked IL-2, G-CSF, GM-CSF, and M-CSF mRNA in comparison with memory B cells. The functional relevance of these findings is thus far unknown and deserves further investigation. Minor differences between malignant B cells and their normal counterparts were observed as for *de novo* cytokine mRNA induction after CD40 and IL-4 stimulation. Activated B lymphocytes can present antigen to T cells (12). Murine studies support the hypothesis that this B cell-T cell interaction drives Th2 cell differentiation (13).

In this study, human CD40- and IL-4-stimulated B cells of all subsets were found to express the mRNA of cytokines that are instrumental for the induction of Th1 differentiation, *i.e.*, IFN-γ, IL-12 p35, and IL-12 p40 (10), whereas they lacked the transcript of IL-4, an essential regulator of Th2 commitment (14). IFN-γ was not detected in culture supernatants from unstimulated or activated B cells, but it is conceivable that additional signals, such as those delivered by IL-12, are required for induction of IFN-γ synthesis and release (10). Taken together, these findings would support the hypothesis that, in secondary lymphoid organs where most antigen-specific immune responses occur, activated B cells can promote Th1 differentiation. However, current models postulate that antigen-presenting B cells are committed to maintain the Th1 or Th2 phenotype of already differentiated $CD4^+$ cells through IL-12 or IL-6 release, respectively, rather than to promote such differentiation (15).

Production of IL-6 and IL-8 from the three major B-cell subsets and of G-CSF from GC B cells was detected both in the absence of stimuli and after CD40 mAb and IL-4 stimulation, whereas IL-2, IL-7, IL-10, IL-15, or TNF were not found in any supernatant. These results may be related to cytokine mRNA instability or to the requirement of additional signals for the induction of synthesis and release of the corresponding proteins.

Cytokines, such as IL-6, TNF, and IFN-γ, have been shown to be hyper-produced by malignant B lymphocytes from lymphoproliferative disorders other than those investigated here and to prolong survival or promote proliferation of tumor cells (2, 16–18). The issue

of cytokine production by lymphoma cells could not be here addressed because of limitations in the number of cells available.

In conclusion, this study demonstrates that the holes in the repertoire of cytokines expressed by malignant B cells *versus* their normal counterparts differ consistently in MCL, FL, and MZL and appear to represent molecular fingerprints of each type of lymphoma. Functional studies addressing the potential relevance of these findings for tumor growth are warranted.

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