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# Low-Grade B Cell Lymphomas of Mucosa-Associated Lymphoid Tissue (MALT-Type) Require CD40-Mediated Signaling and Th2-Type Cytokines for *in Vitro* Growth and Differentiation

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***To investigate the mechanisms of T cell dependence underlying the development of extranodal mucosa-associated lymphoid tissue (MALT)-type B cell lymphomas, the activation, proliferation, and differentiation of lymphoma B cells were studied using ligand binding to the CD40 membrane receptor. The activation and proliferative response of all investigated low-grade MALT-type lymphomas (n = 6) was strongly dependent on anti-CD40-mediated signals and was complemented by cytokines produced by T helper cells of the Th2 type (interleukin-4 (IL-4) and/or IL-10). Th1 cytokines (IL-2 and/or interferon-γ) had little effect. Low-grade, but less so high-grade, MALT-type lymphoma B cells were induced to secrete large amounts of tumor immunoglobulin in response to IL-10. In contrast, high-grade MALT-type lymphomas (n = 5) proliferated in response to both Th2- and Th1-type cytokines and CD40 stimulation, whereas Burkitt lymphomas (n = 3) could not be rescued from apoptosis by CD40 stimulation with or without cytokines. These results suggest that CD40 signaling in combination with Th2 cytokines are essential for the development and progression of low-grade MALT-type B cell lymphoma. We conclude that T cells, which activate B cells in a CD40-dependent fashion, may contribute to lymphoma pathogenesis. (Am J Pathol 1997, 150:1583–1593)***

The development of low-grade mucosa-associated lymphoid tissue (MALT)-type lymphoma is preceded by acquisition of inflammatory reactive lymphoid tissue; hence, Sjögren's syndrome, Hashimoto's thyroiditis, or *Helicobacter pylori* gastritis provide the background at these sites.[1] These conditions leading to secondary or acquired MALT are considered to be important preconditions of lymphomagenesis at extranodal sites.[2] During lymphoma progression, the tumor B cells show colonization of lymphoid follicle centers whereas subepithelial plasma cell differentiation suggests that the tumor is immunologically responsive *in vivo* to as yet unidentified signals.[3] Recent studies provide evidence that antigens may play a role in the pathogenesis of MALT-type lymphoma,[4,5] as the malignant B cells respond to antigen triggering *in vitro*.[6] They represent post-germinal center B lymphocytes[7] that have undergone somatic hypermutation, probably in response to antigen selection.[8] Although B cells of gastric low-grade MALT-type lymphomas recognize autoantigen,[4,7] they respond to *H. pylori*-activated autologous T cells.[9] Therefore, it is unclear at present whether receptor-ligand interaction between T and B cells and cytokines are involved in the pathogenesis of malignant B cells.[10–12]

It has been suggested that marginal zone B cells are the normal counterpart of MALT-type lymphoma tumor cells. The former are noncirculating memory B cells and are generated during T-cell-dependent antigen responses.[13] The CD40 molecule on B cells is the receptor for CD40 ligand (T-BAM, TRAP), an activation antigen on T helper cells,[14–16] and has been shown to play a central role in antigen-specific

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**Table 1.** *Characteristics of Tumors*

| Specimen | Histology* | Stage** | Immunoglobulin isotype |
|---|---|---|---|
| 1 Lung | Low-grade MALT | ND | IgM,λ |
| 2 Salivary gland | Low-grade MALT | ND | IgM,κ |
| 3 Thyroid | Low-grade MALT | ND | IgM,λ |
| 4 Stomach | Low-grade MALT | EI1 | IgM,λ |
| 5 Stomach | Low-grade MALT | EI2 | IgM,κ |
| 6 Stomach | Low-grade MALT | EI2 | IgM,κ |
| 7 Stomach | Large-cell MALT | EII1 | IgM,κ |
| 8 Stomach | Large-cell MALT | EII1 | IgM,λ |
| 9 Stomach | Large-cell MALT | EII2 | IgM,κ |
| 10 Stomach | Large-cell MALT | EII1 | IgM,κ |
| 11 Stomach | Large-cell MALT | EI2 | IgM,κ |
| 12 Stomach | Burkitt | EII1 | IgM,κ |
| 13 Stomach | Burkitt | EII2 | IgM,κ |
| 14 Stomach | Burkitt | EIII | IgM,κ |

*Classification according to REAL.[27,28]
†Stage according to modified classification of Musshoff,[29]; ND, not determined.

cognate B/T cell interactions.[17] CD40 activation appears to be critical for the generation of germinal center reaction,[18] for isotype switching, and for germinal center B cell maturation generating marginal zone B cells.[19,20] The maturation of B cells into efficient producers of high-affinity and high-specificity antibodies is regulated by various T cell subsets *via* cell surface molecules and soluble cytokines.[15] In particular, interleukin-2 (IL-2), IL-4, IL-6, and IL-10 have been demonstrated to be essential for B cell maturation.[21–23] Therefore, T cells may determine not only the type of B cell immune response but may also provide key molecules in B cell lymphoma initiation and progression.[24]

We therefore characterized the effect of CD40 activation *in vitro*, in combination with different cytokines, on proliferation and secretory function of MALT lymphomas.

This issue may be clinically relevant in view of the observations that eradication of the inflammatory stimuli in gastric MALT lymphomas is likely to induce tumor regression.[25,26]

## Materials and Methods

### Tissues and B Cell Purification

Different non-neoplastic tonsillar tissues with reactive tonsillitis (n = 8) and malignant lymphomas (n = 14; see Table 1) were obtained from biopsies immediately after surgical removal. In all cases, the diagnosis was confirmed by morphological and immunophenotypic analysis of fresh-frozen and paraffin-embedded sections.

Lymphocyte single-cell suspensions were isolated by density-gradient centrifugation and negatively depleted to remove macrophages, T cells, and germinal center lymphocytes using magnetic beads coupled with either anti-CD2, -CD14 or -CD10 (Dynal, Hamburg, Germany), except in Burkitt lymphomas where CD10 depletion was omitted.

Thereafter, lymphoma cells were further purified by negative depletion of non-neoplastic bystander B cells using magnetic beads coated with antibodies to heavy and light chains not expressed by the lymphoma, including IgD.

### Immunophenotyping of Isolated Cells

Three-color flow cytometric analysis was performed with a FACScan (Becton Dickinson, Mountain View, CA) using an argon ion laser tuned at 488 nm, with LYSIS II for data acquisition and analysis using directly conjugated MAb (CD19 (HD 37, Sigma Chemical Co., St. Louis, MO), CD3 (UCHT-1, Sigma), CD10 (ALB-2, Sigma), CD25 (ACT-1, Dako, Hamburg, Germany), CD69 (TP1/55.3.1, Dianova, Hamburg, Germany), IgD, (HJ9, Sigma), CD14, (Leu-M3, Becton Dickinson), κ (KP-53, Sigma), λ (HP6054, Sigma), CD40 (monoclonal antibody (MAb) 89)). Instrument set-up samples included an unstained sample and samples stained with fluorescein-isothiocyanate-, phycoerythrin-, and Quantum red-conjugated CD19 as well as directly conjugated isotype controls for each MAb, respectively. The instrument set-up was standardized using $CD19^+$ B lymphocytes from normal tonsils as reference. This was done by gating the fluorescence intensity of CD19 lymphocytes followed by adjustment of the light-scattering detectors to locate the B cells in a standard position in the correlative display of forward and sideward light scattering. The fluorescence detectors were adjusted using a tight light-scattering gate as obtained from the light scattering of the $CD19^+$ lymphocytes, followed by adjustment of the

three fluorescence detectors of an unstained sample. Each measurement contained 20,000 cells. Dead cell discrimination was performed with 7-amino-actinomycin D (7-AAD; Calbiochem, Frankfurt, Germany) in combination with dual-color immunofluorescence as described elsewhere.[30]

## *B Cell Cultures Using the CD40 System*

Purified normal and tumor B cells were cultured in RPMI 1640 supplemented with 1% gentamycin, 10% fetal calf serum, and 50 μg/ml transferrin (Sigma) in round-bottom 96-well plates at a final volume of 200 μl. For CD40 antigen activation, B cells were cultured in the presence of 0.1 μg/ml anti-CD40 MAb 89 (J. Banchereau) presented by a mouse $Ltk^-$ cell line stably expressing CDw32 according to the experimental procedure described previously.[31] Recombinant cytokines were added at the onset of culture at the following final concentrations: 100 U/ml interferon (IFN-γ) (Boehringer Mannheim, Mannheim, Germany), 50 U/ml IL-2, 100 U/ml IL-4 (W. Sebald), and 20 μg/ml IL-10 (PharMingen, San Diego, CA). DNA synthesis was determined by [$^3$H]thymidine incorporation as described.[7] Experiments were repeated three times, except for cases 4, 6, 10, and 11, which were tested once because of limited cell number after purification. The proliferation index was calculated by dividing [$^3$H]thymidine counts of a respective assay with different stimuli by the counts of a medium control without stimuli performed in parallel.

## *Immunoglobulin Production*

Supernatants were assayed in standard enzyme-linked immunosorbent assay (ELISA) as described recently[5] using microtiter plates (Falcon, Germany) coated with goat anti-human IgM heavy-chain isotype (Dako). After blocking with 0.5% bovine serum albumin, undiluted samples were added for 2 hours at 37°C. Finally, the plates were developed with rabbit anti-human μ-heavy-chain horseradish peroxidase conjugate isotype (Dako) and orthophenyl-diamine with hydrogen peroxide in citrate phosphate buffer and read at 490 nm in an automatic ELISA reader (Merlin, Berlin, Germany). The sensitivity of the assay was ≥50 ng of IgM/ml.

## *RNA Preparation and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)*

### *RNA Extraction*

Total RNA was prepared (TRIzol reagent, Life Technology, Paisley, UK) either from 20 approximately 10-μm frozen sections or from freshly isolated tonsil or tumor B cells. The integrity of RNA was controlled by electrophoresis of a 2% formaldehyde-agarose gel, and the yield of RNA was quantitated by measuring the optical density. To check for carry-over of material during the isolation step, extraction buffer without tissue was used as a negative control.

### *cDNA Synthesis*

First-strand synthesis was performed with 1 μg of total cellular RNA. RNA, 2 μg of dT-15 primer, and diethylpyrocarbonate water at a final volume of 8 μl were incubated for 10 minutes at 65°C. After chilling on ice, a master mix consisting of dNTPs and dithiothreitol (final concentrations, 1 mmol/l each and 10 mmol/l, respectively), 25 U of recombinant RNAse inhibitor (Promega, Heidelberg, Germany), reverse transcriptase buffer, and 200 U of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, MD) were added to a final volume of 25 μl. After 70 minutes of incubation at 37°C, the samples were heated to 98°C for 4 minutes. The efficiency of cDNA synthesis was estimated by PCR using glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific primers.

### *VH Gene Amplification*

The amplification of VH gene rearrangements was carried out in a GeneAmp PCR system 2400 (Perkin-Elmer Cetus, Emeryville, CA) in a final volume of 25 μl containing 1 μl of cDNA, 2.5 μl of 10X PCR buffer (USB, Braunschweig, Germany), 1.5 μl of 25 mmol/L $MgCl_2$, 0.5 μl of 10 mmol/L dNTPs, 0.5 μl of 20 pmol/ml each primer, and 0.5 U of *Taq* polymerase (USB). Amplifications consisted of a denaturation at 94°C for 1 minute followed by 35 cycles of 30 seconds at 94°C, 30 seconds of primer annealing at 61°C (VH1, VH2, VH5, and VH6 primers) or 65°C (VH3 and VH4 primers), and 30 seconds of extension at 72°C. After the last cycle, the reaction mixtures were incubated for 4 minutes at 72°C to insure complete extension of all products.

### *Oligonucleotides for PCR*

The VH-family-specific primers hybridize with framework region I of the immunoglobulin heavy chain variable region genes. The JH primer is a 5:1 mixture of the two oligonucleotides JH1–5 and JH6 and hybridize with the 5′ end of the JH fragments as described recently.[32]

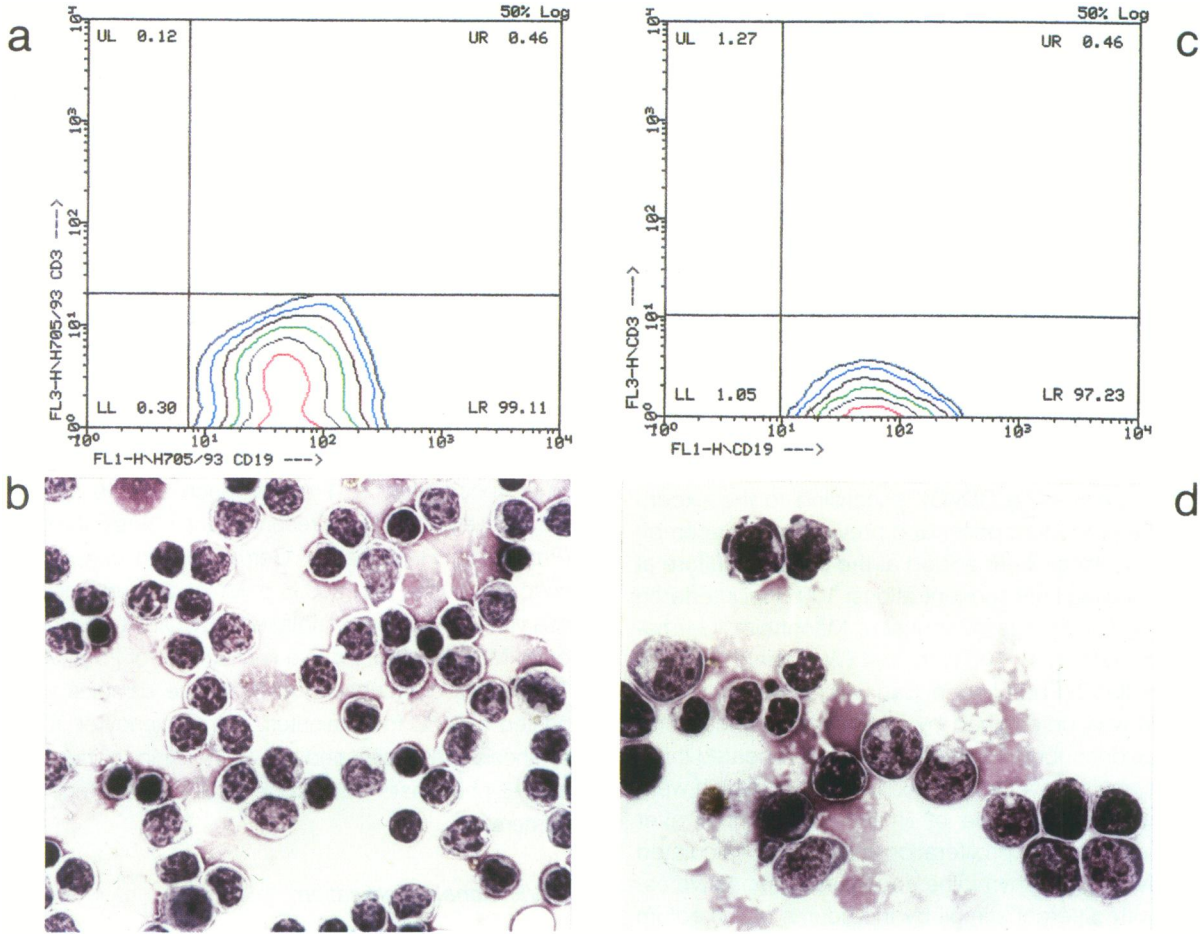


Figure 1. *Representative three-color FACS analysis of cell-sorting experiment showing 99% purity of lymphoma B cells (CD19$^+$/CD3$^-$) of low-grade MALT-type lymphoma with corresponding Pappenheim-stained cytospin demonstrating small lymphocytes with rim of clear cytoplasm (case 4,* **a** *and* **b**) *and 97.5% pure high-grade centroblastic lymphoma B cells (case 8,* **c** *and* **d**) *showing typical transformed chromatin structure. Magnification, ×1000 (*b *and* d).

### *Cloning, Sequencing, and Tumor-Specific Primers*

The RT-PCR products were cloned in pBluescript Sk− (Stratagene, Heidelberg, Germany). Sequence reactions were performed using the *Taq* dye desoxy termination cycle sequencing kit (Applied Biosystems, Foster City, CA) and were analyzed using an automatic sequencer (Applied Biosystems). Tumor-specific primers for the CDRIII region of the tumor B cells were generated as described recently.[8]

## *Results*

### *Immunophenotype of Isolated B Lymphocytes*

The purity of lymphocyte cell suspensions obtained after magnetic immunobeads depletion was between 97.5 and 99.7% B cells as calculated by FACScan (Becton Dickinson) analysis (see Figure 1, a–d). MALT lymphoma B cells expressed CD19, CD21, and CD40 and were negative for CD23, CD10, CD5, and IgD. Monoclonality was determined in FACS analysis on the basis of monotypic light and heavy chain expression.

### *Lymphoma B Cells Expanded in the CD40 System*

To test the efficiency of the cell-sorting experiments at the molecular level and to establish the origin of the MALT lymphoma B cells (during the cell culture experiments), analysis of the VH repertoire was performed in cases 3, 4, and 7. As shown in Figure 2, after PCR amplification, the tumor B cells express only one VH family. Furthermore, we investigated whether the freshly isolated MALT lymphoma B cells showed identical CDRIII segments by using tumor-specific primers. To this end, the cDNA were cloned and sequenced, proving identity of the tumor B cells in the surgical specimen (2B), after B cell purification (2C), and after

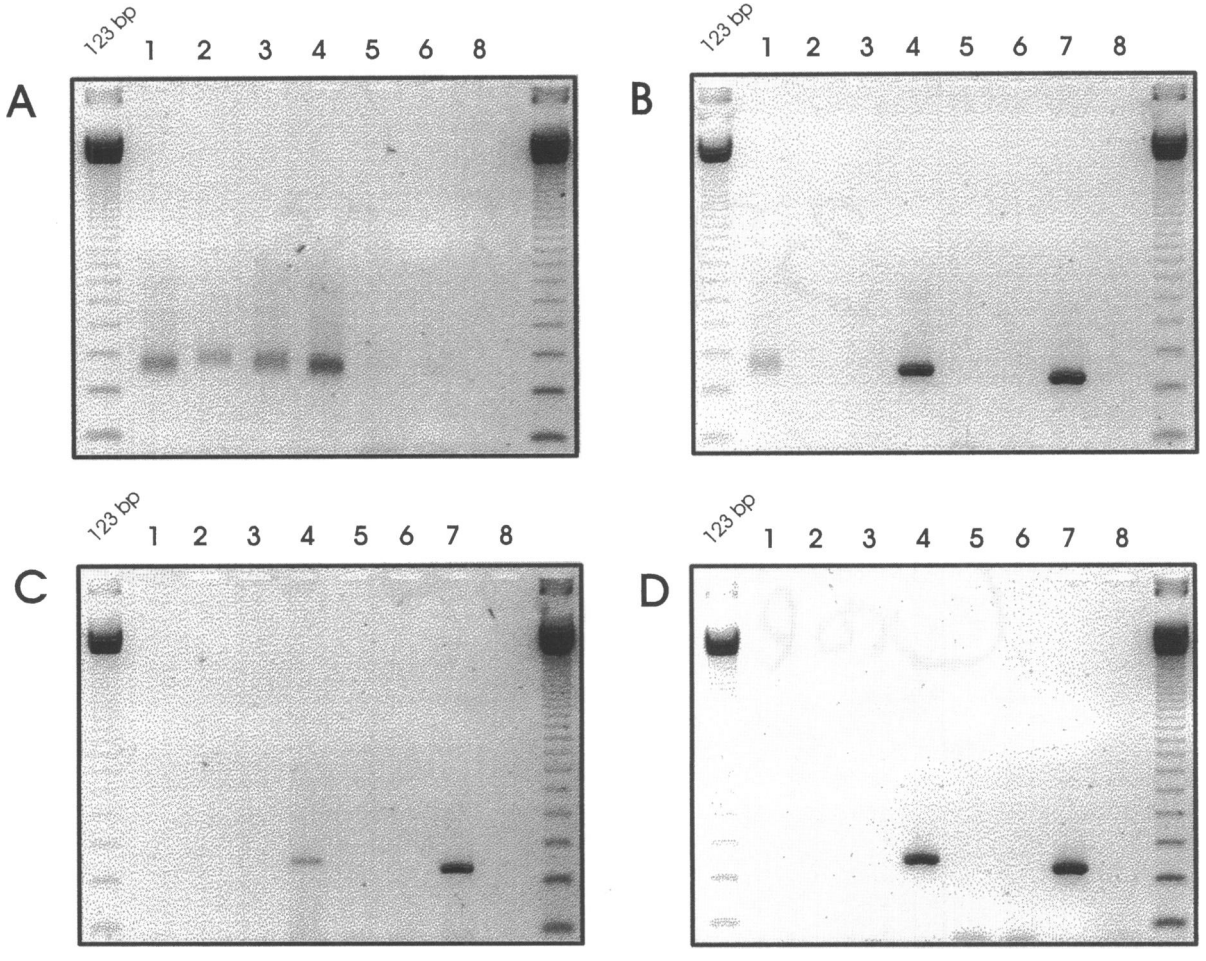


Figure 2. *VH-family-specific RT-PCR to distinguish tumor B cells (case 3) expand in the CD40 system from bystander B cells.* Lane 1, *VH1 family consensus forward primer × JH consensus reverse primer;* lane 2, *VH2 family consensus forward primer × JH consensus reverse primer;* lane 3, *VH3 family consensus forward primer × JH consensus reverse primer;* lane 4, *VH4 family consensus forward primer × JH consensus reverse primer;* lane 5, *VH5 family consensus forward primer × JH consensus reverse primer;* lane 6, *VH6 family consensus forward primer × JH consensus reverse primer;* lane 7, *to identify the tumor B cells, the VH4-family-specific forward primer was combined with a reverse CDRIII primer specific for the CDRIII region of the tumor B cells;* lane 8, *negative control.* A: *Positive control performed with purified normal polyclonal B cells of reactive tonsillitis.* B: *Freshly isolated tumor tissue before B cell purification identifying the monoclonal tumor B cells* (lanes 4 *and* 7) *and some oligoclonal bystander B cells* (lane 1). C: *Freshly isolated tumor B cells after purification and* (D) *after 7 days cultured in the CD40 system. In all three experiments* (B *to* D), *the tumor B cells are clearly identified by expressing the same VH family and CDRIII region.*

culture in the CD40 system (2D) without expanding contaminating non-neoplastic bystander B cells.

## *Functional Consequences of CD40 Stimulation of MALT Lymphoma B Cells*

Cultures of tonsil and MALT lymphoma B cells were maintained in the CD40 system with IL-4 for up to 4 weeks and grew in colonies on the fibroblast layer (Figure 3). Cells remained $CD19^+$, $CD10^-$, $CD23^-$, $IgD^-$ and continued to express CD40, whereas activation markers (CD69 and CD25) were rapidly expressed (Figure 4). No IL-2 production was detectable in tonsil or lymphoma cell culture supernatant by ELISA (Endogen; data not shown) throughout the culture period. Burkitt lymphoma B cells showed a rapid loss in viability as assessed by Trypan blue stain after 24 hours in the CD40 culture system (Figure 5a). Considerable apoptotic figures were detected in Pappenheim-stained cytospins (Figure 5b). Neither CD40 stimulation alone nor CD40 stimulation in combination with one of the cytokines tested (IL-2, IFN-γ, IL-4, or IL-10) could rescue the Burkitt lymphoma cells from rapid cell death.

## *MALT Lymphoma B Cells Require CD40 Signaling for Their Tumor Growth*

Cell counts were performed at different time points in an overall culture period and confirmed that the [$^3$H]thymidine incorporation reflected cell proliferation and resulted in a ~16-fold expansion of viable B

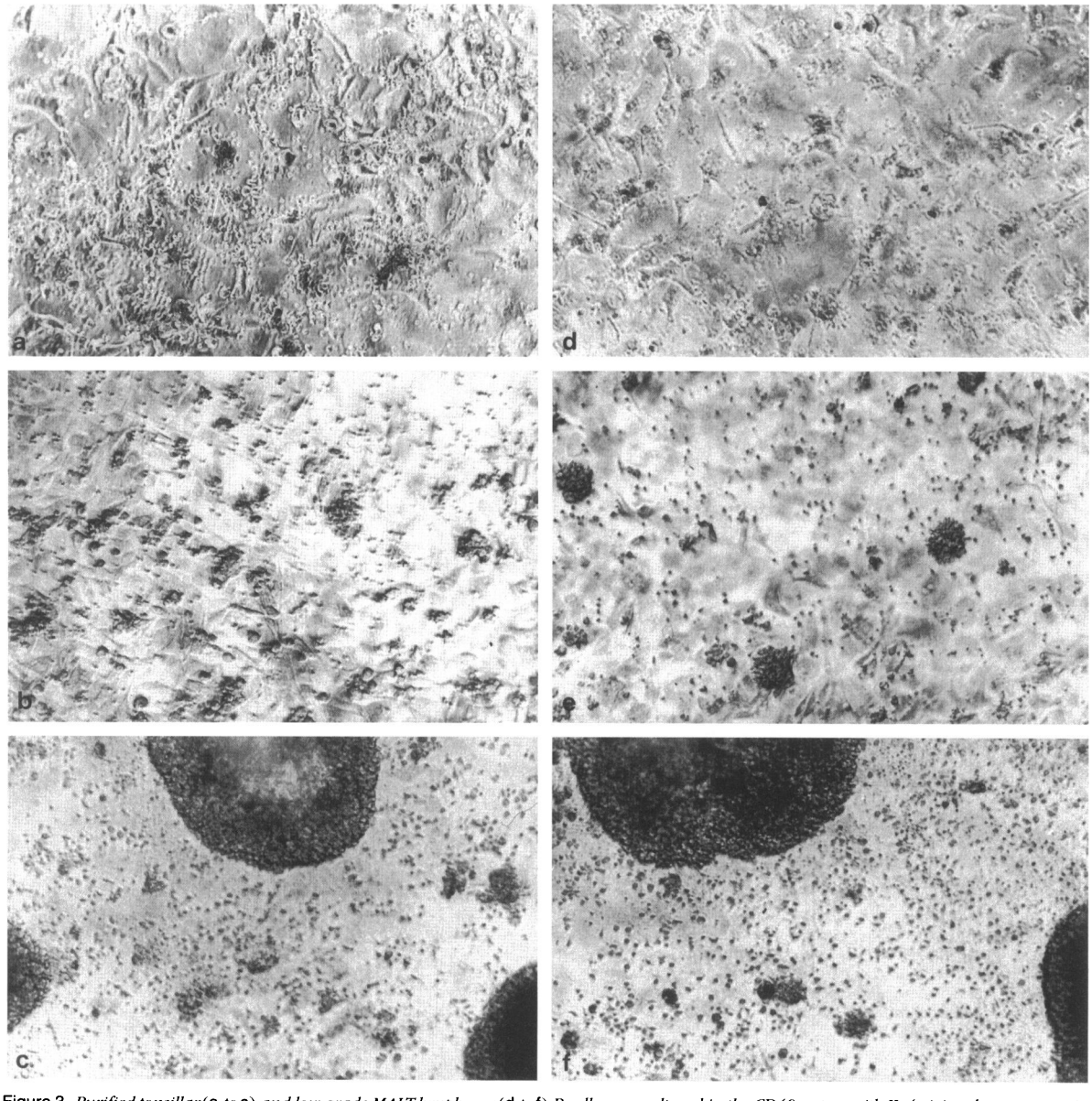


Figure 3. *Purified tonsillar* (a *to* c) *and low-grade MALT lymphoma* (d *to* f) *B cells were cultured in the CD40 system with IL-4 giving the same growing appearance at the microscope. After 1 day of cell culture* (a *and* d) *one can see adherence of single cells to the fibroblast layer. At day 3* (b *and* e), *small cell clumps arise that enlarge until day 7* (c *and* f).

cells within 16 days in the CD40 system (Figure 5a). Proliferation of MALT lymphoma B cells was dependent on CD40 signal and the added cytokine as shown in Figure 6. IL-4 as well as IL-10 in combination with CD40 signaling induced a marked proliferation in low-grade MALT lymphoma; combinations of both cytokines synergized to induce strong DNA synthesis. IL-2 and IFN-$\gamma$ had little effect on the proliferation of low-grade MALT lymphomas. In contrast, only high-grade MALT lymphomas responded significantly to CD40 signal alone and in combination with any cytokine offered.

## *Cytokines Induced MALT Lymphoma B Cells to Differentiate into Immunoglobulin-Secreting Cells*

Tonsillar and lymphoma B cells cultured in the CD40 system in the absence of cytokines produced only marginal quantities of immunoglobulin. IL-2 and IL-4 had no effect on the secretory activity in tonsil and low-grade lymphoma B cells and only a moderate effect in high-grade MALT lymphomas (Table 2). In contrast, addition of IL-10 triggered a pronounced immunoglobulin secretion that was more obvious in

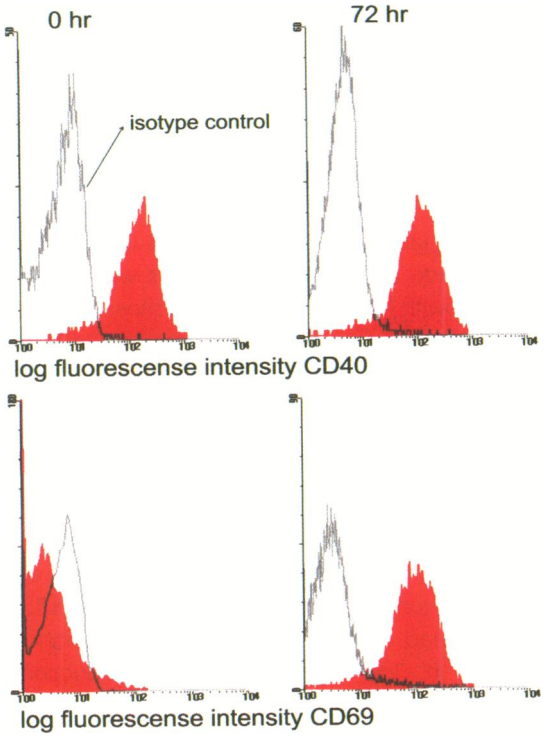


Figure 4. *Induction of activation markers by CD40 signaling. Histograms show fluorescence intensity of CD40 and CD69 staining* (solid graph) *superimposed with that of the isotype control antibody* (open graph) *using multicolor FACS analysis of low grade (case 5) MALT lymphoma B cell suspension freshly isolated after surgery (0 hr) and after 72 hours of CD40 culture. Cells were gated for viable lymphocytes, CD19$^+$, and 7-AAD using the same settings as for the freshly isolated B cells.*

low-grade than in high-grade MALT lymphomas and tonsil B cells. This effect could not be further enhanced by IL-2 or IL-4 in the low-grade lymphomas and only marginally in the high-grade tumors by IL-4. On cytospin preparations performed after the CD40/cytokine culture period, the secretory activity paralleled plasma cell morphology and cytoplasmic immunoglobulins.

## *Discussion*

To determine whether the tumor cell response in low-grade lymphoma might be due to autocrine effects or to effects solely mediated by tumor-infiltrating T cells, B cells were cultured in the so-called CD40 system. This allowed us to study the properties of B cells and T cells independently.[20,33–35] In particular, the present investigation shows that the CD40 system is an excellent tool for inducing proliferation and differentiation of low- and high-grade MALT-type B cell lymphoma. This, so far, has been very difficult to study because of limited cellular viability and low proliferative capacity *in vitro*. Extensive repeated cell selection using immunobeads subsequently verified by cyto- and immunophenotyping of the cultured cells and a VH-family-specific PCR using consensus VH-family and consensus JH oligonucleotides ascribed the effects to the malignant

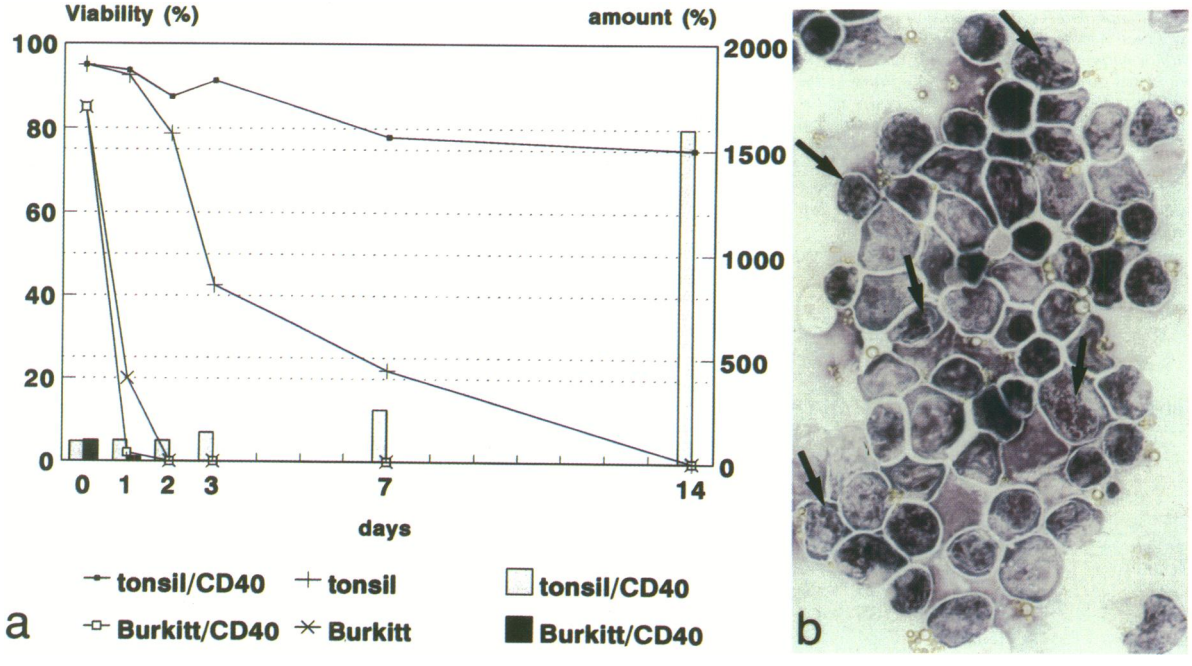


Figure 5. *Viability plot of purified tonsil and Burkitt lymphoma B cells in the CD40 system* (a) *with a marked decrease of viability of Burkitt lymphoma B cells within 1 day of culture that cannot be overcome by CD40 stimulation. Normal B cells proliferate and expand up to 16-fold after 14 days of culture only in the CD40 system. Pappenheim stain of purified Burkitt lymphoma B cells that had been exposed to anti-CD40 for 4 hours show morphological features of apoptosis* (b; arrow *to apoptotic cells*) *Magnification, ×1000.*

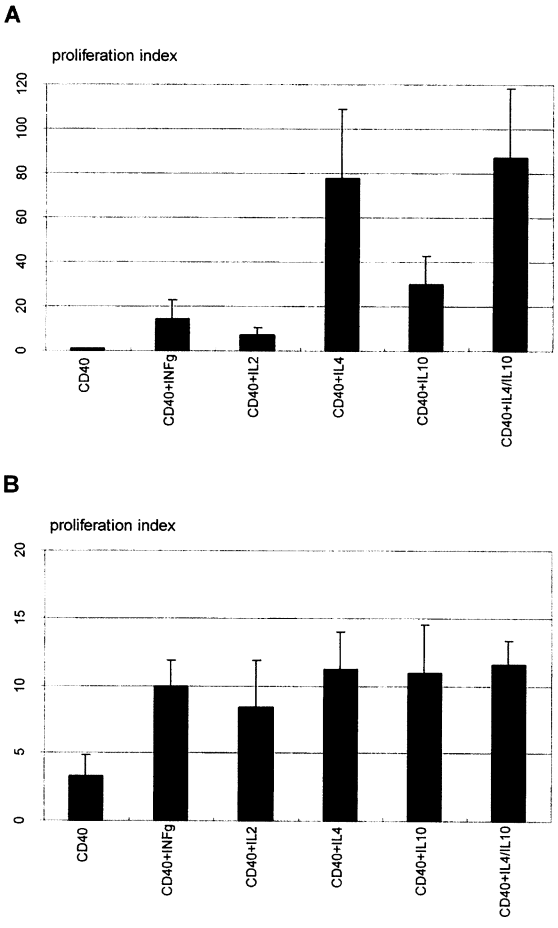


Figure 6. *Summary of low-grade* (A; $n = 6$) *and high-grade* (B; $n = 5$) *MALT lymphoma B cell proliferation that is induced by anti-CD40 and cytokines. A total of* $5 \times 10^4$ *purified B cells were cultured in triplicate with or without cytokines and anti-CD40 as indicated. B cell proliferation was determined at day 5 of culture by the addition of* [$^3$H]*thymidine present during the last 16 hours of the culture period. The proliferation index is expressed as cpm assay/cpm medium. The results give the cumulative mean value of all patients calculated from the means of three independent proliferation tests performed for each patient. Cases 4, 6, 10, and 11 were tested once because of a limited cell number after purification. Each error bar gives the standard deviation of the respective cumulative mean.*

and not residual bystander cells, as the tumor cells in the cell culture system showed clonal identity with the lymphoma cells they were derived from. In addition, using PCR with tumor-specific primers showed that low- and-high grade MALT lymphoma B cells were maintained during the whole CD40 culture period.

The long lasting effect of IL-4 in the CD40 system for low-grade MALT-type lymphomas was found to synergize with IL-10 for proliferation most efficiently. In particular, IL-4 allowed the generation of factor-dependent tumor B cell lines, whereas IL-10 induced a marked terminal differentiation into plasma cells, a characteristic feature found in low-grade MALT lymphoma *in vivo*.[36] Thus, IL-10 may play an important role in the amplification of immunoglobulin-mediated responses in MALT-type lymphoma. This contrasts with results from previous studies of normal B cells and control tonsillar B cells in this study, where IL-10 and IL-4 lead to increased cellular replication but resulted in low immunoglobulin production.[37] Whether IL-10 is produced inside MALT-type lymphomas and whether it originates from tumor-infiltrating T cells[38,39] or from other cell types *in vivo* remains to be established.

In contrast to anti-CD40-activated normal tonsillar B cells and B cell chronic lymphocytic leukemias (B-CLL),[23,40] low-grade MALT-type lymphomas did not proliferate or differentiate in response to IL-2 substantially. High-grade MALT-type lymphomas, like normal B cells, and low-grade MALT lymphomas responded both to IL-4 and IL-10, but only high-grade MALT lymphomas also grew after addition of IL-2 and IFN-γ alone.

These *in vitro* findings may be of decisive importance for the pathogenesis of low-grade MALT lymphomas *in vivo* considering that Th2 cells are essential for efficient mucosal antibody responses,[41,42] the preferential role of Th2 cells in forming germinal centers,[43] and the humoral response against common

Table 2. *IL-10 Elicits Secretory Activity of Anti-CD40-Activated MALT-Type Lymphoma B Cells to Secrete Immunoglobulin*

| | IgM production (μg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tonsil | Low-grade MALT | | | | | | High-grade MALT | | | | |
| | (average) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Medium | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.12 | <0.05 | <0.05 | <0.05 | 0.1 |
| IL-2 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.63 | 0.42 | <0.05 | <0.05 | 0.23 |
| IL-4 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | <0.05 | 0.85 | 0.61 | <0.05 | <0.05 | 0.14 |
| IL-10 | 0.18 | 7.34 | 6.68 | 10.23 | 4.22 | 7.64 | 3.62 | 1.22 | 1.11 | <0.05 | <0.05 | 0.89 |
| IL-10 + IL-2 | 0.42 | 7.22 | 6.99 | 9.58 | 4.45 | 9.50 | 4.82 | 1.43 | 1.19 | <0.05 | <0.05 | 1.13 |
| IL-10 + IL-4 | 0.22 | 7.28 | 7.29 | 10.63 | 4.87 | >10.0 | 4.82 | 1.99 | 1.54 | <0.05 | <0.05 | 0.97 |

A total of $5 \times 10^4$ purified B cells were cultured over 5 days in the CD40 system. Antibody production was measured by standard ELISA. Results are expressed as the mean of duplicate determinations. The SD was below 10% of the mean value.

environmental antigens.[44,45] As low- but not high-grade MALT lymphomas seem to have maintained a significant relationship to normal B cells, it is possible that IL-4 and IL-10 as well as the CD40 ligand, eg, expressed by activated T cells in reactive follicular gastritis, may trigger the antigen-dependent development of low-grade MALT-type lymphomas. This could happen either directly or by paracrine mechanisms and may explain gastric low-grade MALT lymphoma regression after eradication of *H. pylori*.[25,26]

It is unknown whether antigen-driven B cell proliferation is associated with secondary events that cause tumor transformation in MALT lymphoma. As in most nodal lymphomas, etiology and pathogenesis of lymphomas derived from MALT are not yet elucidated. In particular, unifying chromosomal aberration,[46,47] rearrangements of oncogenes,[48] or Epstein-Barr virus infections[49,50] which are thought to be first steps in the development of nodal lymphomas, have not been detected in lymphomas of MALT type. It is possible that antigen is necessary for survival of the initiating malignant tumor clone in combination with paracrine factors supported by reactive tumor-infiltrating T cells that express CD40 ligand.

In contrast, CD40 stimulation was found to inhibit cell proliferation in other lymphoma types, like Burkitt lymphoma in this study and nodal diffuse large-cell lymphoma or lymphoblastoid cell lines as published recently.[10] The responsible mechanisms are unclear, but several possibilities may be considered. First of all, although CD40 is present on all normal B cells and their malignant counterparts, receptor signaling may act different by means of cell cycle and stage of development, as demonstrated recently for different IL-2 signaling pathways regulating cellular proliferation mediated by c-*myc*, *bcl*-2, or *lck*.[51] Second, it may be possible that triggering of CD40 may deliver different signals if the CD40 receptor or CD40 signal transduction mediating proteins like CRAF1[52] or p23[53] are susceptible for mutations in lymphoma.

Our data provide evidence that the CD40 ligand expressed by activated non-neoplastic T cells *in vivo* may trigger the evolution of low-grade MALT-type lymphomas either directly or by paracrine mechanisms. Therefore, T cells may not only determine the type of B cell immune response but may also provide key molecules in B cell lymphoma initiation and progression. Both the histopathological and clinical features of MALT-type lymphomas suggest the possibility that the lymphomagenesis is, at least in part, T cell dependent.

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