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Biology of Human Tumors

Clinical Cancer Research

T-Cell Levels Are Prognostic in Mantle Cell Lymphoma

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Abstract

Purpose: The purpose of this study was to investigate the impact of T-cell subsets on pathologic and clinical features including disease outcome in mantle cell lymphoma (MCL).

Experimental Design: Cell populations were investigated using flow cytometry in diagnostic MCL ($n = 153$) and reactive ($n = 26$) lymph node biopsies. Levels of tumor cells, T cells, T-cell subsets, and the CD4:CD8 ratio were assessed and related to pathologic and clinical parameters.

Results: MCL cases with diffuse and nodular histologic subtypes showed lower levels of T cells, especially $CD4^+$ T cells, than those with mantle zone growth pattern. Both CD3 and CD4 levels were lower in the nodular subtype than in mantle zone ($P = 0.007$; $P = 0.003$) and in the diffuse compared with the nodular subtype ($P = 0.022$; $P = 0.015$). The CD4:CD8 ratios were inversely correlated to tumor cell proliferation ($P = 0.003$). Higher levels of $CD3^+$ and $CD4^+$ T cells and higher CD4:CD8 ratios were associated with indolent disease ($P = 0.043$, 0.021, and 0.003 respectively). In univariate analysis, a high CD4:CD8 ratio, but not the histologic subtype, was correlated to longer overall survival (OS). In multivariate analysis, the CD4:CD8 ratio correlated with OS independently of Mantle Cell Lymphoma International Prognostic Index (MIPI) and high p53 expression ($P = 0.023$).

Conclusion: $CD3^+$, $CD8^+$, and particularly $CD4^+$ T-cell levels are higher in indolent MCL and decrease with more aggressive histology as reflected by a diffuse growth pattern. High CD4:CD8 ratio correlated independently of other high-risk prognostic factors with longer OS, suggesting a prognostic role for T cells in MCL. *Clin Cancer Res; 20(23); 6096–104. ©2014 AACR.*

Introduction

Mantle cell lymphoma (MCL) is a mature B-cell neoplasm that constitutes 5% to 10% of non-Hodgkin lymphomas. Patients with MCL have a poor prognosis with a median overall survival (OS) of 3 to 5 years (1–3). A subset of MCL with clinically indolent disease course was recognized already 15 years ago (4), but reliable markers for predicting clinically indolent disease are still lacking (5–7) Certain indolent MCL are associated with non-nodal, leukemic presentation and splenomegaly, less genomic complexity, mutated immunoglobulin genes, and lack of expression of SOX11 (5, 8, 9). However, also a subset of nodal, nonleukemic MCL may demonstrate an indolent clinical behavior, not requiring immediate treatment (6). These cases may be associated with a partial mantle zone growth pattern and are frequently $SOX11^+$ (7). Most MCL carry the chromosomal translocation t(11;14)(q13;q32) causing overexpression of the cyclin D1 protein and deregulation of the cell cycle (10). Although the t(11;14) is considered to be the first step in oncogenesis, other aberrations are necessary for the development of an overt lymphoma (11, 12; reviewed in ref. 5). Tumor cell proliferation (13, 14) and p53 aberrations (15, 16), as well as the clinical Mantle Cell Lymphoma International Prognostic Index (MIPI; ref. 17), are strongly associated with outcome.

MCL cells are dependent on signals from stromal cells and cytokines in the microenvironment for their survival (18–22). However, compared with follicular lymphoma (FL) and chronic lymphocytic lymphoma/small cell lymphoma (CLL/SLL), much less is known about how the nonmalignant cells affect disease development and prognosis in MCL. The t(11;14) translocation occurs at the pre-B-cell stage (23) and the cells carrying the translocation migrate to the lymph nodes and often reside in the mantle zone of the B-cell follicle. It has been hypothesized that the normal counterpart of at least some MCL variants could be

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Mantle cell lymphoma (MCL) is a B-cell neoplasm with poor prognosis compared with other B-cell lymphomas. MCL tumor cells are dependent on microenvironmental signals for their survival but little is known about how the nonmalignant cells affect disease development and prognosis. The levels of T cells and their subsets were measured by flow cytometry in diagnostic MCL lymph nodes and associations to clinicopathologic features were analyzed. High T-cell levels were positively correlated to mantle zone histology and clinically indolent disease. High T-cell CD4:CD8 ratio correlated with low proliferation and indolent disease. In multivariate analysis, a high CD4:CD8 ratio predicted longer overall survival (OS) independent of Mantle Cell Lymphoma International Prognostic Index (MIPI). These data are of interest in view of novel treatments that target the lymphoma microenvironment.

the mantle/marginal zone B lymphocytes (24–26) and that the lymphoma cells are dependent on the specific microenvironment of these tissue compartments (27). Clonal MCL lesions with only a few cyclin $D1^+$ cells surrounding the germinal center or forming small extrafollicular noduli (*in situ* MCL) have been described previously (28–30). On the basis of architectural growth pattern, three different histologic subtypes of MCL have been described: mantle zone, nodular, and diffuse. These growth patterns possibly reflect different ways of interactions between tumor cells and the nonmalignant cells. In some clinical studies, the type of growth pattern has been associated with differences in patient outcome but is less predictive than other pathologic parameters such as tumor cell proliferation (31–35).

We used flow cytometry to analyze the levels of T cells and T-cell subsets in MCL lymph nodes at diagnosis and correlated the quantitative differences in T-cell subset composition to clinicopathologic features including tumor cell proliferation, histologic subtype, and patient outcome. Our findings provide novel information on the role of T cells in MCL and are of additional relevance in view of recent treatment options targeting the lymphoma microenvironment (36–39).

Materials and Methods

Patients and clinical data

All MCL cases ($n = 243$) diagnosed in the Stockholm region between January 1, 1998 and December 31, 2012 were retrieved from the Regional Cancer Center registry and the database of the Department of Pathology, Karolinska University Hospital (Stockholm, Sweden) and S:t Göran Hospital (Stockholm, Sweden). This cohort partly overlaps with our previously published Stockholm cohort ($n = 186$) that included all MCL cases diagnosed between January 1, 1998 and June 30, 2010 (7). However, in this study, 34 cases diagnosed later than June 30, 2010 are added and only MCL cases where the diagnostic lymph nodes had been examined by flow cytometry were included ($n = 153$). Thirty-eight of 153 patients underwent high-dose treatment and autologous stem cell transplantation (ASCT) in accordance with the Nordic MCL2 (35) or MCL3 (40) study protocols. Seventeen patients did not require treatment and two received only local irradiation therapy. The 96 remaining patients mostly received chemotherapy with rituximab according to the Swedish National guidelines; however, 29 patients did not get rituximab as first-line therapy, the majority of those were diagnosed before rituximab was introduced as part of the therapy for MCL in Sweden.

The pathology review of the cases was done before the review of the flow cytometry data, and thus without knowledge of the quantification of T cells by flow cytometry. The histologic subtype could be evaluated in 107 out of 153 cases. In the remaining 46 cases, the diagnosis was based on core biopsies or fine-needle aspirations of lymph nodes and verified in bone marrow biopsies, and thus the histologic subtype in the lymph nodes could not be investigated. Clinical parameters were retrieved from hospital charts. OS was calculated from time of diagnosis to the time of death or last follow-up (May 15, 2013). Median follow-up time for the surviving patients was 4.13 (range, 0.36–15.13) years.

Diagnosis, IHC analysis, and flow cytometry

The MCL diagnosis followed the criteria of the World Health Organization (WHO) classification (10). All cases included were cyclin D1–positive by immunohistochemistry (IHC) and/or positive for t(11;14)(q13;q32) translocation by interphase FISH. All available tissue sections from excised diagnostic lymph nodes ($n = 107$) were reviewed and the predominant histological subtype (mantle zone, nodular, or diffuse; Supplementary Fig. S1) was determined as well as the presence of partial mantle zone pattern in any part of the tumor tissue. IHC for cyclin D1, Ki67, SOX11, and p53 was performed on whole sections of paraffin-embedded lymph node biopsies as previously described (7). IHC staining was semi-automated and performed on a Bond Max robot using Vision Biosystems TM bond Polymer Refine and Bond DAB Enhance, as recommended by the manufacturer (Leica Microsystems). Four- to eight-color flow cytometry was performed on lymph node cell suspensions (57 fine-needle biopsies and 96 whole lymph nodes or core biopsies) on a FACSCanto (BD Sciences) and analyzed with FACSDiva Software (BD Sciences). The fluorochrome-conjugated monoclonal antibodies used are shown in Supplementary Table S1.

Statistical methods

Statistical analysis was performed with the programs Stata 9.2 and OriginPro 8. We calculated associations with OS using the Kaplan–Meier method, log-rank test, and Cox multivariate analysis. For other correlations, the Student *t*, Spearman, Fisher exact, and Mann–Whitney–Wilcoxon tests were used, according to the nature of the variables. All *P* values are two-tailed and a $P < 0.05$ was considered statistically significant.

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Table 1. Clinical and pathologic features of included cases

Clinical and pathologic features	All ($n = 153$)
Median age (range)	69.5 (32.1–97.7)
Male sex, %	113/153 (73.9)
Age > 65 y, %	95/153 (62.1)
B symptoms, %	54/150 (36)
ECOG $\geq$ 2, %	5/146 (3.4)
>Four nodal sites, %	109/151 (72.2)
Splenomegaly, %	72/143 (50.3)
Ann Arbor IV, %	127/150 (84.7)
WBC > 10×10^9/L, %	38/150 (25.3)
Lymphocytes > 5×10^9/L, %	30/147 (20.4)
High serum LDH[a], %	58/142 (40.8)
MIPI high risk, %	50/132 (37.9)
Ki67 high $\geq$ 30%, %	61/127 (48)
SOX11 positivity, %	125/130 (96.2)
p53 positivity, %	19/128 (14.8)
ASCT first-line treatment, %	38/143 (26.6)
Blastoid variant, %	20/136 (14.7)
Indolent disease, %	15/128 (11.7)
Median OS, y	3.94

[a]Defined by Karolinska University Hospital Laboratory.

Ethical permission

The Regional Central Ethical Review Board at Karolinska Institutet has approved the research and given all necessary ethical permissions.

Results

Clinical and pathologic features of the included cases

The clinical and pathologic characteristics of the 153 patients (113 males and 40 females) are presented in Table 1. The median age at diagnosis was 69 years and 62% of the patients were older than 65 years at the time of diagnosis. High-risk MIPI score was present in 38% of the patients and 85% were in stage IV. The vast majority of the cases, 96%, were SOX11$^+$ and in 48% of the cases, high proliferation ($\geq$30%) was noted. Fifteen percent of the cases showed blastoid morphology.

A subset of asymptomatic MCL patients does not require immediate treatment and has been suggested to have a more indolent disease course and a better outcome (6). For the definition of "indolent disease," we here required that the patient did not need treatment within 2 years after diagnosis. Using this definition, 15 patients (12%) had indolent disease. Median OS for the 153 patients was 3.9 years (Fig. 1A). As expected, high and intermediate MIPI score predicted for shorter OS ($P < 0.00005$; Fig. 1B). High p53 protein expression (> 20% of tumor cells strongly positive) was associated with very poor OS ($P < 0.00005$; Fig. 1C). High tumor cell proliferation was also associated to impaired OS, both when analyzed with the cutoff $\geq$ 30% ($P = 0.002$) and in tertiles (Supplementary Fig. S2). The median OS was significantly longer in patients <70 years of age (8.1 years compared with 2.3 years for older patients; $P < 0.00005$; Supplementary Fig. S2), mainly due to treatment differences because the young patients more often got high-dose therapy with ASCT.

Flow cytometry

Cell frequencies as analyzed by flow cytometry (Table 2) are presented as percentage of cells in mononuclear gate. In most cases, MCL tumor cells expressed CD19, CD20, and CD5, but 10 cases (6.5%) were CD5$^-$ (<10% of CD5$^+$ tumor cells). These 10 cases were all SOX11$^+$ and the clinical and pathologic features of these cases did not differ from CD5$^+$ MCL. The analysis of the levels of tumor cells by flow cytometry was based on a combination of markers (CD19, CD20, and CD5) and light chain restriction. Very few nonmalignant B cells (median, 0.20%; range, 0%–14.8%) were detected in the MCL lymph nodes (Table 2). In the majority of cases, MCL tumor cells constituted the most frequent population with a median value of 82%. However, there was a wide distribution with a range from 10% to 99%. CD3$^+$ T cells varied from 1% to 84% with a median value of 14%. The frequencies of T and B cells added together in the flow cytometry analysis of the lymphocyte gate should be equal to 100%, and the CD3 levels always decrease when the local lymph node tumor burden, as reflected by CD19$^+$ MCL cells, increases. The CD4:CD8 ratio is, however, independent of the T-cell levels in the sample. Therefore, the CD4:CD8 ratio was calculated in all samples. The median ratio was 1.84 (range, 0.3–10.4).

Correlation of T-cell levels and CD4:CD8 ratio to clinicopathologic features

No correlation was found between the patient age and the levels of T-cell, T-cell subsets, the CD4:CD8 ratio, or the histologic subtype. Blastoid and classical MCL variants did not differ significantly in proportions of total CD3$^+$ cells, T-cell subsets, B cells, or CD4:CD8 ratio (all $P > 0.05$).

Total CD3$^+$ and the CD3$^+$CD4$^+$ subset T-cell levels as well as CD4;CD8 ratios were significantly lower in males than in females ($P = 0.009$, 0.004, and 0.027, respectively). The T-cell and T-cell subset levels and CD4:CD8 ratio did not show any significant correlation to expression of p53 by IHC ($P > 0.05$), but higher CD4:CD8 ratios negatively correlated with tumor cell proliferation ($P = 0.003$; Fig. 2). Higher levels of CD3$^+$ cells, CD3$^+$CD4$^+$ cells, and CD4:CD8 ratio were associated with indolent disease ($P = 0.043$, 0.021, and 0.003, respectively), and higher CD4:CD8 ratios also correlated with longer OS in univariate analysis ($P = 0.018$; Fig. 1D).

Comparison of T-cell levels and CD4:CD8 ratios in histologic MCL subtypes and reactive lymph nodes

We hypothesized that T-cell levels were higher in lymph nodes with partial MCL involvement as reflected by the

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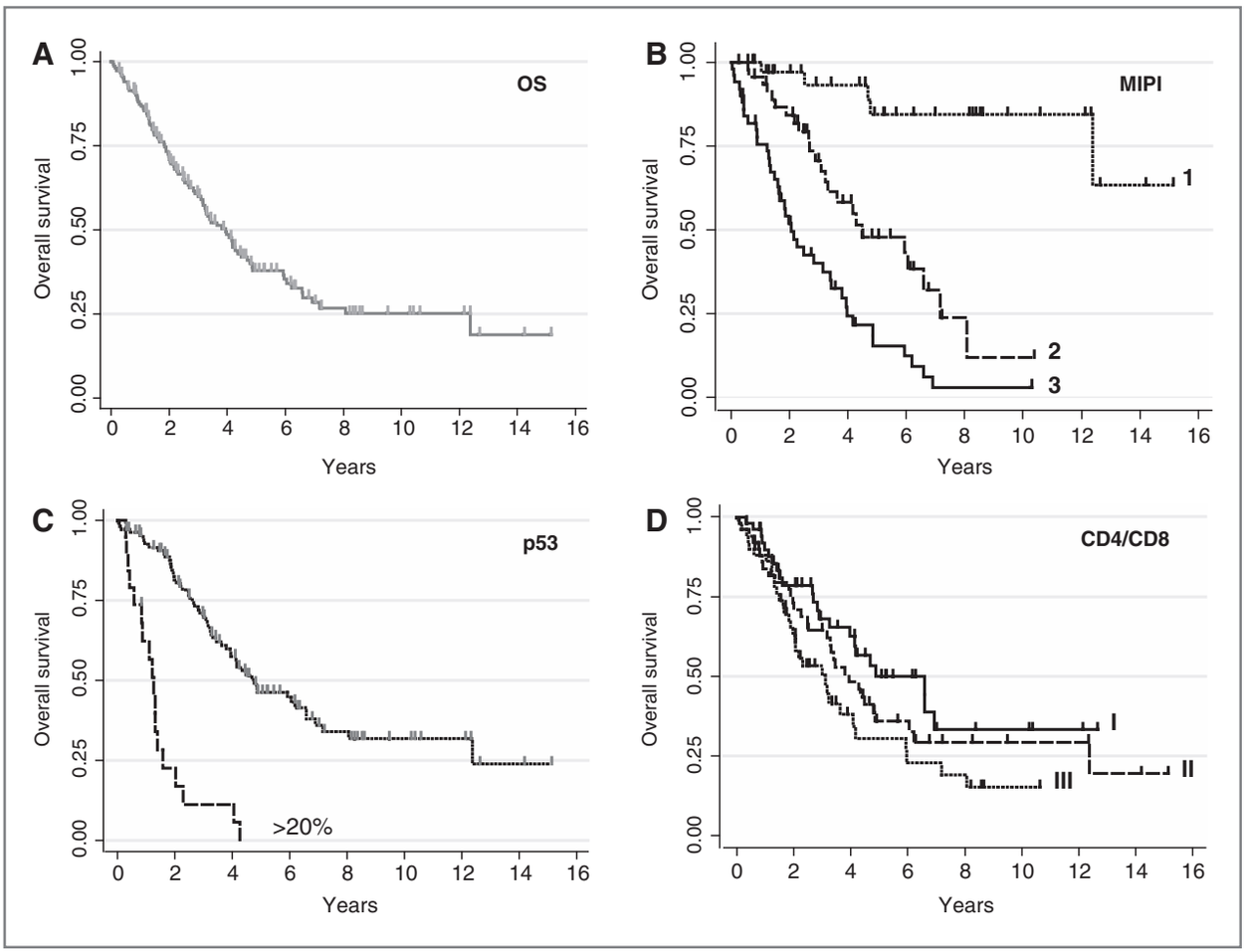

Figure 1. OS in relation to MIPI, p53 protein expression, and CD4:CD8 ratio. A, median OS of the entire cohort ($n = 153$) was 3.9 years. B, median OS according to MIPI. In MIPI 1 patients ($n = 36$), the median OS was not reached; in the MIPI 2 group ($n = 46$), the median OS was 4.5 years; and in the MIPI 3 group ($n = 50$), OS was 2.1 years; P (log-rank test for trend) <0.00005. C, median OS according to p53 protein expression was 1.3 years for cases with >20% $p53^+$ cells and 4.8 years for $p53^-$ cases; $P < 0.00005$. D, median OS according to CD4:CD8 ratio in diagnostic lymph nodes. Cases were subgrouped in tertiles based on CD4:CD8 ratio. Median OS was 4.9 years for the first tertile (I) with the highest CD4:CD8 ratio, 3.9 years for the middle tertile (II), and 3.1 years for the third tertile (III) with the lowest CD4:CD8 ratio P (log-rank test for trend) = 0.018.

Table 2. Results of flow cytometry on diagnostic MCL lymph nodes

Cell population%[a]	Median	Range
MCL tumor cells[b]	81.6	10.4–98.9
Nonclonal B cells	0.20	0.00–14.8
$CD3^+$	14.20	1.00–84.0
$CD3^+CD4^+$	8.80	0.60–63.2
$CD3^+CD8^+$	4.50	0.20–21.1
CD4:CD8 ratio	1.84	0.35–10.4

[a]Percentage of cells in mononuclear gate (median, 88.2%; range, 21.5%–99%).
[b]On the basis of CD19, CD20, CD5, and monotypic light chain.

tumor growth pattern (mantle zone compared with nodular and diffuse subtypes; Supplementary Fig. S1). The histologic subtype evaluated in 107 cases, showed a predominant mantle zone pattern subtype in 5, whereas 20 cases, including three blastoid variants, had a partial mantle zone pattern with a few remaining residual germinal centers surrounded by an expanded mantle zone of cyclin D1–positive cells. Thus, predominant or partial mantle zone histology was seen in 25 of 107 (23%) cases and these cases were grouped together in the further analyses ("mantle zone growth pattern"). The nodular and diffuse subtype was seen in 29 (27%) and 53 (50%) cases, respectively. The T-cell levels and CD4:CD8 ratios in these MCL histologic subtypes and in reactive lymph nodes ($n = 26$) are presented in Fig. 3 and Supplementary Table S2. The total T-cell levels in reactive lymph nodes were higher than in MCL irrespective of histologic subtypes (Fig. 3A–C). The CD3 levels were significantly higher in cases with mantle zone growth pattern

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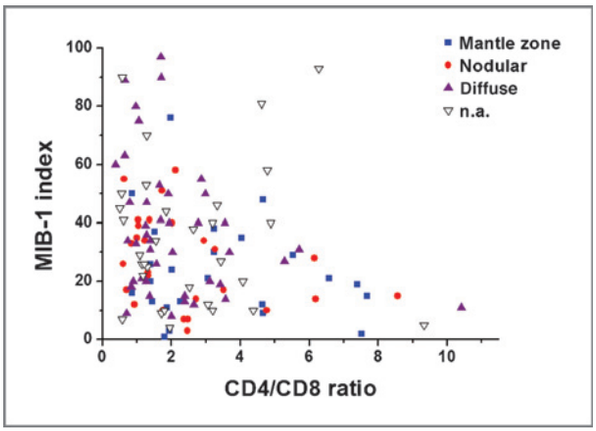

Figure 2. MIB-1 index in relation to CD4:CD8 ratio in diagnostic MCL lymph nodes with different growth patterns. MIB-1 index, as measured by the proportion of Ki67 staining cells by IHC, was evaluated in tumor cell areas of 127 of 153 MCL lymph nodes. CD4:CD8 ratio was calculated from flow cytometric T-cell subset analysis ($n = 153$). In 107 of 153 cases, the MCL histologic subtypes could be evaluated as indicated by color-coding in the figure. High CD4:CD8 ratio was significantly correlated with low tumor cell proliferation ($P = 0.003$). When proliferation was analyzed in the different histologic subtypes, a significant difference between mantle zone and diffuse subtypes was found using the cutoff of 30% Ki67$^+$ cells ($P = 0.021$).

(median, 30.4%) than in those with nodular (median, 16.7%) or diffuse subtype (median, 10.4%; $P = 0.007$ and $P < 0.001$, respectively). Furthermore, cases with nodular histologic subtype had higher CD3 levels than those with the diffuse subtype ($P = 0.022$). The lower levels of total T cells in nodular and diffuse subtypes of MCL were mainly due to a reduction in CD4$^+$ T cells (Fig. 3B) while the CD8 levels were less affected (Fig. 3C). Thus, the CD4:CD8 ratio changed with tumor growth pattern (Fig. 3E) with a higher CD4:CD8 ratio in cases with mantle zone growth pattern (median, 3.1) than in those with the diffuse subtype (median, 1.7; $P = 0.002$; Fig. 3E). The CD19$^+$ B-cell levels were higher in nodular and diffuse subtypes compared with mantle zone (Fig. 3D).

Features associated with histologic subtypes

Because a decreased CD4:CD8 ratio was detected in the more aggressive diffuse histologic subtype, we further analyzed clinical and pathologic features potentially associated with different histologic subtypes. Patients with nodular or diffuse subtype of MCL more frequently showed an elevated serum level of lactate dehydrogenase (LDH) compared with cases with predominant or partial mantle zone growth pattern ($P = 0.0024$ and 0.0027, respectively). Significantly higher tumor cell proliferation rate (Ki67 $\geq$30%) was seen in the diffuse subtype compared with cases with mantle zone growth pattern ($P = 0.0214$). Clinically indolent disease was more common in MCL with mantle zone growth pattern compared with those with a diffuse subtype ($P = 0.011$). None of the other parameters investigated (listed in Table 1) or OS were significantly associated with the histologic subtypes.

Risk stratification by the CD4:CD8 ratio

In univariate analysis, a high CD4:CD8 ratio was the only immune cell variable significantly correlating to a longer OS ($P = 0.018$). When grouping the patients according to the CD4:CD8 ratio in tertiles, three groups, each containing 51 patients, were generated. All prognostic factors significant in univariate analysis were then competed in multivariate analysis ($n = 110$), demonstrating that a high CD4:CD8 ratio was an independent predictor of longer OS ($P = 0.023$; Table 3). Also when excluding patients who underwent ASCT, the CD4:CD8 ratio retained its prognostic impact in competition with MIPI and p53 (CD4:CD8 ratio, $P = 0.027$; MIPI and p53 both, $P < 0.0001$; $n = 88$).

Discussion

The immune microenvironment in the lymphoma tissue has raised considerable interest in recent years due to the assumption that lymphoma cells are dependent on signals from nonmalignant cells for their survival. A disruption of this necessary interaction of tumor cells with their environment has been proposed to form the basis for novel therapeutic strategies (41). Gene expression studies in FL, performed in the pre-rituximab era, demonstrated that patient outcome was highly influenced by the composition and activation status of nonmalignant cells in the tumor microenvironment (42, 43). These pivotal and challenging findings stimulated a number of further studies in FL mainly using IHC and/or flow cytometry. In these investigations, a role for T cells and macrophages in tumor progression and patient outcome has been demonstrated; however, the relative impact of various nonmalignant cells is not yet fully clarified (reviewed in ref. 44). Also in CLL, T cells are of importance for survival of tumor cells and disease progression (reviewed in ref. 45).

MCL is a clinically challenging lymphoma because it responds well to conventional immunochemotherapy but mostly relapses quickly. The role of the microenvironment in the pathogenesis and progression of MCL is unclear.

The presence of stereotyped immunoglobulin receptors and selective VH-gene usage in MCL suggests a role for antigen stimulation during disease development (24, 25) and cell adhesion, cytokine profiles as well as signaling from mesenchymal cells is of importance for proliferation and tumor cell survival (22, 46–48). Less is known on the potential role of T cells in MCL. To investigate this issue, we retrieved data from diagnostic MCL lymph node samples. We included all cases diagnosed at a single institution over a limited time period and chose to use flow cytometry for cell enumeration because this technique provides quantitative information on cell levels and subsets in lymphoma (49, 50). The major cell populations assessed were clonal B lymphocytes and the two main T-lymphocyte populations characterized by expression of CD4 and CD8. Other cell types, such as monocytes and natural killer (NK) cells, were in most instances infrequent and therefore not included in the final analysis. T-cell levels were correlated to clinical and

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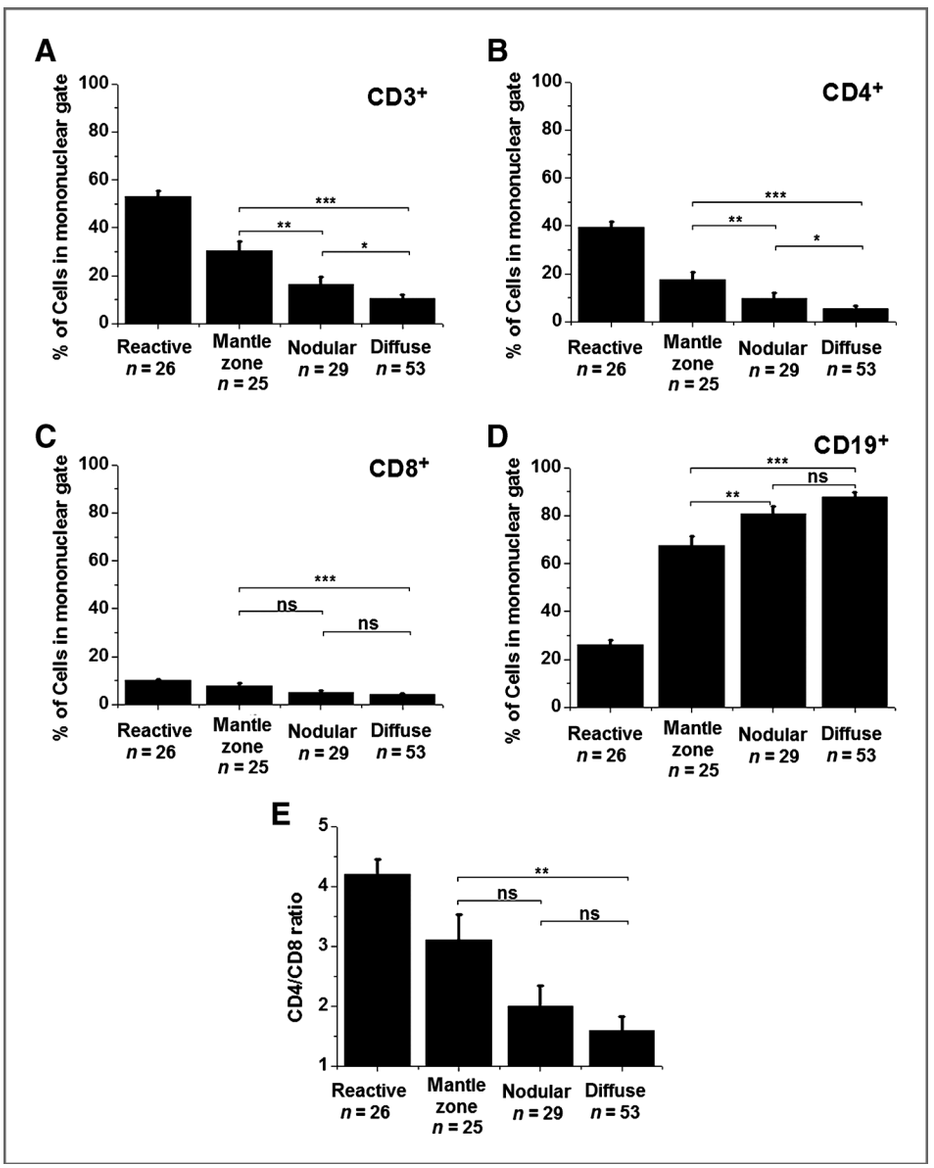

Figure 3. Levels of total T cells, $CD4^+$, and $CD8^+$ T-cell subsets and B cells and CD4:CD8 ratio in reactive lymph nodes and MCL with different growth patterns. A–D, the levels of T cells, T-cell subsets, and B cells in diagnostic samples from MCL with different histologic growth patterns ($n = 107$) and from reactive lymph nodes ($n = 26$) were evaluated by flow cytometry. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. A, $CD3^+$ T-cell levels were lower in MCL compared with reactive lymph nodes irrespective of subtype ($P < 0.001$, all comparisons) and within MCL a further reduction occurred in more aggressive subtypes; mantle zone to diffuse, $P < 0.001$; mantle zone to nodular, $P = 0.007$; nodular to diffuse, $P = 0.022$. B, $CD4^+$ T-cell levels were lower in MCL compared with reactive lymph nodes irrespective of subtype ($P < 0.001$, all comparisons) and there was a marked further reduction of $CD4^+$ cells in more aggressive subtypes; mantle zone to diffuse, $P < 0.001$; mantle zone to nodular, $P = 0.003$; nodular to diffuse, $P = 0.015$. C, the $CD8^+$ T-cell levels were lower in MCL compared with reactive lymph nodes; reactive to diffuse, $P < 0.001$; reactive to nodular, $P = 0.007$. Within MCL, there were significantly lower $CD8^+$ T cells in mantle zone compared with diffuse histology, $P < 0.001$. D, B-cell levels were higher in reactive lymph nodes than in MCL of all subtypes ($P < 0.001$, all comparisons). Within MCL, there were lower levels of malignant B cells in mantle zone compared with nodular subtype, $P = 0.008$ and in mantle zone compared with diffuse, $P < 0.001$. E, the CD4:CD8 ratios were lower in MCL cases compared with reactive lymph nodes and decreased according to growth pattern mainly due to lower CD4 levels (shown in Fig. 3B). Statistically significant differences were: reactive to nodular and reactive to diffuse, $P < 0.001$; reactive to mantle zone, $P = 0.015$; mantle zone to diffuse, $P = 0.002$.

pathologic characteristics and our main findings are: (i) T-cell levels were lower in MCL compared with reactive lymph nodes. (ii) T-cell levels were highest in MCL cases with predominant or partial mantle zone growth pattern and lowest in the diffuse subtype, suggesting a change in the immune microenvironment during development to more aggressive histologic subtypes. (iii) Importantly, the reduction in T-cell levels was mostly due to a decrease of $CD4^+$ T cells and to a lesser extent due to reduction of $CD8^+$ T cells. (iv) This decline of $CD4^+$ T cells was reflected in a decreasing

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Table 3. Multivariate (Cox) analysis with respect to OS ($n = 110$)

Factor	HR (95% CI)	*P*
MIPI		<0.0001
Low risk	1	
Intermediate risk	11.82 (2.68–52.07)	
High risk	38.24 (8.77–166.72)	
p53		<0.0001
>20% positive cells	8.70 (4.21–18.01)	
CD4:CD8 ratio		0.023
Lowest tertile	1	
Middle tertile	0.65 (0.35–1.21)	
Highest tertile	0.40 (0.21–0.77)	

CD4:CD8 ratio in MCL compared with reactive lymph nodes and with a further decrement in nodular and diffuse MCL subtypes as compared with those with mantle zone growth pattern. (v) The CD4:CD8 ratio was inversely correlated to tumor cell proliferation in MCL but not to blastoid morphology or IHC overexpression of p53 protein. (vi) Higher levels of total T cells, $CD4^+$ T cells, and higher CD4:CD8 ratios were associated with indolent disease. (vii) A high CD4:CD8 ratio in diagnostic lymph node samples, but not the histologic subtype, was associated with longer OS in MCL in both univariate and multivariate analysis.

The lower T-cell levels ($CD3^+$ cells) in lymph nodes involved by MCL than in nonmalignant, reactive lymph nodes is best explained by the effacement of normal lymphoid architecture by overgrowth of the malignant cells. Higher levels of $CD3^+$ T cells in cases with partial lymphoma involvement, as reflected by a mantle zone growth pattern, also support this notion. However, further analysis of the $CD4^+$ and $CD8^+$ T-cell subsets demonstrated that the decrease in total T-cell levels was mainly an effect of a gradual decline in the proportion of the $CD4^+$ T-cell subset when the lymphoma cells expanded to nodular or diffuse histologic subtypes. This selective reduction of $CD4^+$ T cells could imply that MCL tumor cells become less dependent on supporting signals from the surrounding T cells with histologic progression or that lymphoma cells actively suppress certain local T-cell responses or regulatory subsets. There is some evidence supporting the latter alternative. Wang and colleagues (21) recently investigated T-cell responses to primary MCL cells and to MCL cell lines. Interestingly, the lymphoma cells efficiently inhibited $CD4^+$ and $CD8^+$ T-cell proliferation and T cell–mediated cytolysis (21). Even though the clinical relevance of this *in vitro* study is somewhat limited because isolated tumor cells from blood or cell lines were used, the results are interesting and may support our conclusion that perturbations of T-cell populations in MCL could be clinically relevant in as much as that we found T-cell levels and subset distributions highly correlating to certain prognostic features in MCL.

Although there was no correlation with age, total T cells, $CD4^+$ T cells, and CD4:CD8 ratio were lower in males compared with females. We do not have any clear explanation for this finding but correlations between gender and T-cell numbers has earlier been shown in healthy adults with lower absolute number of $CD4^+$ T cells in men compared with women (51). In MCL, low tumor cell proliferation, partial involvement as in the mantle zone subtype, and no immediate requirement for treatment are all favorable prognostic features (4, 14, 29–34). In our study, we found that a high CD4:CD8 ratio was positively correlated with all these features and also with longer OS. Thus, it seems that preservation of lymph node T-cell levels and especially the $CD4^+$ subset is a favorable predictor in MCL. Similarly, in CLL, an inverted CD4:CD8 ratio is a negative prognostic feature and has been associated with shorter doubling time of lymphocytes, shorter time to first treatment, and shortened progression-free survival (52). This is in marked contrast to findings in FL in which $CD4^+$ T cells and a high CD4:CD8 ratio is instead a poor prognostic sign (53). The reason for this difference is not known but could be related to CD4 subset differences or functional activity. Among $CD4^+$ T cells, there are several subpopulations including $CD4^+$ $FOXP3^+$ regulatory T cells and $CD4^+PD1^+$ T follicular helper cells. Myklebust and colleagues (54) recently showed that in FL and in reactive tonsils, $PD1^+$ T cells were unresponsive to cytokines. This unresponsive subset was $CD4^+$, $CD45RO^+$, and $CD62L^-$ and had high expression of PD1. That study and a previous study by the same group (55) suggested that a high frequency of nonresponding T cells was a clinically adverse feature. Comparison with other lymphoma subtypes showed that the nonresponding $CD4^+$ T-cell population was significantly more frequent in FL compared with MCL and CLL (54). Such phenotypic and functional difference of $CD4^+$ T cells in different lymphoma entities might be an explanation for the association between high CD4 levels, high CD4:CD8 ratio, and poor survival in FL but not in MCL and CLL but there could also be other, yet unknown, underlying reasons. Thus, T cells seem to have an impact on disease outcome not only in FL and CLL but also in MCL even if the underlying mechanisms for perturbation of T cells and T-cell subsets may be different in the lymphoma subtypes. Further studies are clearly needed to better characterize the T-cell response in MCL including refined studies on regulatory subpopulations of $CD4^+$ cells.

In summary, this study reveals marked alterations of T-cell levels and T-cell subpopulations in MCL that correlates to indolent and aggressive disease features. Our data indicate that the local immune microenvironment in MCL lymph nodes may be of importance during disease progression toward more aggressive features, including high tumor cell proliferation. Because better preservation of $CD4^+$ T cells and a high CD4:CD8 ratio correlate to longer OS,

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novel targeted therapy aiming to restore T-cell function could be considered in MCL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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