High Levels of Regulatory T Cells in Blood Are a Poor Prognostic Factor in Patients With Diffuse Large B-Cell Lymphoma

Chen Chang, MD,[1,2] Shang-Yin Wu, MD,[2,3] Yu-Wei Kang,[1] Kun-Piao Lin,[1] Tsai-Yun Chen, MD,[3] L. Jeffrey Medeiros, MD,[4] and Kung-Chao Chang, MD, PhD[1,2]

From the [1]Department of Pathology, College of Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; [2]Institute of Clinical Medicine, College of Medicine and [3]Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan; and [4]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston.

Key Words: Diffuse large B-cell lymphoma; Antitumor immunity; Peripheral blood; Flow cytometry; Regulatory T cells

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ABSTRACT

Objectives: *Host immunity likely plays a role in preventing progression of diffuse large B-cell lymphoma (DLBCL). Analysis of host immune cells may provide useful information for assessing prognosis or possibly clinical management.*

Methods: *Peripheral blood samples from 77 patients with DLBCL and 30 healthy volunteers were analyzed using flow cytometry immunophenotyping. CBC counts, T-cell subsets, and dendritic cells (DCs) were detected, and the results were correlated with clinicopathologic characteristics.*

Results: *Compared with healthy volunteers, patients with DLBCL had significantly higher leukocyte and monocyte counts (*P < *.001); higher percentages of neutrophils (*P < *.001), "natural" regulatory T cells (Tregs; CD3+Foxp3+,* P < *.001), and immature DCs (CD83–CD1a+,* P = *.005); and lower percentages of lymphocytes (*P < *.001) and helper T cells (*P = *.038). In univariate analysis, high neutrophil counts (≥6,000/μL,* P = *.014) and "induced" Tregs (CD4+CD25+,* P = *.026) were poor survival factors along with high International Prognostic Index scores (*P < *.001) and other high-risk clinical parameters. In multivariate analysis, high Tregs retained significance. Suppression of lymphocytes correlated with poor clinical factors; higher natural Tregs correlated with a lower CD4+/CD8+ ratio (*P = *.035) and more immature DCs (*P = *.055).*

Conclusions: *Changes in blood immune cells occur in patients with DLBCL. The results also support a suppressive role of Tregs in adaptive immunity and correlate with poor-risk prognostic factors.*

CME/SAM

Upon completion of this activity you will be able to:
- define markers used for regulatory T cells.
- describe the differences of immune compositions between diffuse large B-cell lymphoma (DLBCL) patients and a healthy population.
- list the prognostic factors in DLBCL patients.

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The host immune system has anticancer effects, but tumor cells can evade the immune system by interfering with the presentation of self-antigens to dendritic cells, suppressing effector T-cell responses, and enhancing regulatory T-cell (Treg) responses.[1] Immunotherapies that target the host immune system include various cytokines, antibodies, and dendritic cell–based vaccines that aim to reverse immune evasion by solid tumors.[2] We and others have shown that the composition of the immune infiltrate in patients with diffuse large B-cell lymphoma (DLBCL) correlates with survival.[3,4] Dendritic cells (DCs)[3] and CD4+ helper T (Th) cells[5] are generally considered beneficial to the host, whereas the prognostic value of cytotoxic T cells is controversial.[6-8] The role of Tregs in patients with DLBCL is multifaceted because both host immune cells and lymphoma cells can be targeted.[9] Published studies have reported positive, negative, or no influence of Tregs on survival in tumor tissues.[8,10,11]

Tregs act to suppress other immune cells and are important for preventing autoimmunity. Immune tolerance generated by

Tregs was initially evidenced by experiments in thymectomized mice.[12,13] These T cells, belonging to a specific CD4+ T-cell subpopulation, were then reported to express CD25 (interleukin [IL]–2 receptor α-chain).[14] Subsequently, Tregs expressing CD4+CD25 high were found in humans.[15] Tregs function by a direct cell contact manner and produce transforming growth factor–β (TGF-β) and IL-10 and can be separated into two forms: "natural" Tregs (Foxp3+CD4+), which originate in the thymus, and "induced" Tregs (CD25+CD4+), which are derived from naive T cells in the periphery.[16] They are similar, both phenotypically and functionally, but are reported to be different in "Helio" expression, which is a zinc finger transcription factor.[17] Inducible Tregs are derived from naive T cells by costimulation with T-cell receptors and TGF-β.[18] Several subsets of Tregs have been reported, including CD4+, CD8+,[19] and T follicular regulatory cells.[20] Tregs can further specialize for suppression of Th1, Th2, and Th17 reactions in response to different environmental stimuli.[21] However, Treg status is plastic and may acquire an effector phenotype and function.[22,23]

The prognostic role of Tregs in patients with lymphoma is controversial. Tregs can be categorized into at least two groups: suppressor Tregs and direct tumor-killing Tregs.[24] The former suppress antitumor immune response mediated by CD8+ cytotoxic T cells; the latter, known as activated Tregs, preferentially kill antigen-presenting B cells.[25] Further complicating the issue are differences in methods between studies, such as calculation methods, flow markers, and population characters. In addition, the role of Tregs might be influenced by lymphoma characteristics, such as the germinal center vs activated B-cell phenotype.[10] DLBCL is heterogeneous, and various subgroups may elicit different immune responses.[10]

In the present study, we analyzed peripheral blood (PB) samples from patients with newly diagnosed DLBCL and healthy volunteers by using flow cytometry immunophenotypic methods to examine the possible roles of DCs and T cells, including CD4+ Th, CD8+ cytotoxic T cells, and Tregs. Understanding the roles of blood immune cells might be helpful for predicting the clinical outcome of patients with DLBCL and possibly may be targets suitable for therapy.

Table 1
Summary of Clinical Features of Diffuse Large B-Cell Lymphoma Cases

Characteristic	No. (%)
Sex	
Male	44 (57.1)
Female	33 (42.9)
Age, y	
<60	37 (48.1)
>60	40 (51.9)
Stage[a]	
I	21 (27.6)
II	13 (17.1)
III	15 (19.7)
IV	27 (35.5)
LDH,[a] IU/L	
<200	25 (32.9)
>200	51 (67.1)
ECOG score	
0-1	48 (62.3)
2-4	29 (37.7)
Location	
Nodal	19 (24.7)
Extranodal	58 (75.3)
IPI score	
0-2	40 (51.9)
3-5	37 (48.1)
Bulky disease (>10 cm)	
Yes	16 (20.8)
No	61 (79.2)
Bone marrow involvement[a]	
No	60 (84.5)
Yes	11 (15.5)
Tumor EBV associated[a]	
Yes	3 (5)
No	57 (95)
B symptoms	
Yes	57 (74.0)
No	20 (26.0)
Effusions[a]	
Yes	24 (31.6)
No	52 (68.4)
Radiotherapy	
Yes	25 (32.5)
No	52 (67.5)
Chemotherapy	
Yes	66 (85.7)
No	11 (14.3)
Stem cell transplant	
Yes	18 (23.4)
No	59 (76.6)
Relapse[a]	
Yes	26 (41.9)
No	36 (58.1)

EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase.
[a] Some data were not available.

Materials and Methods

Patients

Blood samples were collected from 77 patients with newly diagnosed DLBCL, including 44 males and 33 females with a mean age of 59.1 years (range, 15.1-93.1 years). All samples were obtained from patients at the National Cheng Kung University Hospital from April 2006 through December 2010. Patient information was collected through chart review, including sex, age, tumor stage, serum lactate dehydrogenase (LDH) level, Eastern Cooperative Oncology Group (ECOG) performance status, International Prognostic Index (IPI) score, bone marrow involvement,

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Table 2
Differences Between Cases and Healthy Control Groups[a]

Parameter	Cases (n = 77)	Control (n = 30)	*P* Value
Age, y	59.1 ± 17.4	56.3 ± 4.8	.132
Male/female sex, No.	44/33	16/14	.830
WBC, /μL	8,930.0 ± 4,320.0	5,350.0 ± 2,120.0	<.001[b]
Neutrophils, %	74.2 ± 15.0	58.1 ± 8.1	<.001[b]
Absolute neutrophil count, /μL	6,981.3 ± 4,345.1	3,155.3 ± 1,632.8	<.001[b]
Monocytes, %	7.9 ± 5.4	7.0 ± 1.8	.750
Absolute monocyte count, /μL	654.3 ± 496.7	374.9 ± 159.8	<.001[b]
Lymphocytes, %	15.3 ± 12.3	31.3 ± 7.1	<.001[b]
Absolute lymphocyte count, /μL	1,126.5 ± 719.9	1,628.3 ± 531.3	<.001[b]
CD3+, %	68.3 ± 14.9	67.6 ± 6.5	.398
Absolute CD3+ count, /μL	775.2 ± 504.4	1,097.0 ± 361.1	.001[b]
CD3+CD4+, %	37.7 ± 13.6	42.0 ± 7.0	.038[b]
Absolute CD3+CD4+ count, /μL	422.2 ± 287.2	693.0 ± 290.5	<.001[b]
CD3+CD8+, %	28.5 ± 11.5	24.9 ± 7.1	.152
Absolute CD3+CD8+ count, /μL	333.1 ± 278.6	396.3 ± 134.1	.006[b]
CD4+/CD8+, ratio	1.6 ± 1.1	1.9 ± 0.9	.038[b]
CD25+CD4+, %	8.3 ± 6.5	7.0 ± 2.6	.816
Absolute CD25+CD4+ count, /μL	87.3 ± 84.6	114.7 ± 68.7	.008[b]
CD25+CD8+, %	1.4 ± 1.6	2.0 ± 2.8	.561
Absolute CD25+CD8+ count, /μL	14.9 ± 21.6	33.2 ± 45.0	.003[b]
CD25+, %	11.1 ± 8.5	11.2 ± 3.3	.303
Absolute CD25+ count, /μL	114.7 ± 108.6	184.5 ± 91.9	<.001[b]
CD3+Foxp3+, %	1.05 ± 1.58	0.05 ± 0.06	<.001[b]
Absolute CD3+Foxp3+ count, /μL	10.8 ± 18.2	0.8 ± 1.0	<.001[b]
CD83+CD1a–, %	0.27 ± 0.28 (n = 36)	0.13 ± 0.11	.055
Absolute CD83+CD1a– count, /μL	2.4 ± 2.9	2.1 ± 2.0	.872
CD83–CD1a+, %	0.18 ± 0.27 (n = 36)	0.04 ± 0.05	.005[b]
Absolute CD83–CD1a+ count, /μL	1.9 ± 3.3	0.8 ± 1.00	.020[b]

[a] Values are presented as mean ± SD unless otherwise indicated.
[b] Statistically significant.

tumor location, B symptoms, effusions, treatment, relapse, and survival **Table 1**. Epstein-Barr virus (EBV) association was determined by using in situ hybridization for EBV-encoded RNA on the tissue sections, as described previously.[26] The IPI score was calculated according to age, stage, serum LDH levels, ECOG performance status, and number of extranodal sites. The presence of effusions was evaluated based on image findings or cytology. The mean follow-up duration was 28.0 months (range, 0.3-80.4 months).

Blood samples were also collected from 30 healthy volunteers, including 16 men and 14 women, with a mean age of 56.3 years (range, 50.36-68.4 years). No obvious differences in age and sex existed between the patients with DLBCL and the healthy volunteers **Table 2**. All studies were conducted following a laboratory protocol approved by the institutional review board (NCKUH-ER-100-351) and were in accordance with the 1975 Declaration of Helsinki, as revised in 1983.

Flow Cytometry

Peripheral venous blood was drawn into EDTA tubes. The total leukocyte count and differential counts, including neutrophils, monocytes, and lymphocytes, were determined using an automated blood counter (LH 750 analyzer; Beckman Coulter, Brea, CA). Cell surface markers were detected using a four-color flow cytometer (Cytomics FC500; Beckman Coulter) equipped with the CXP software program, as has been described.[27] Experimental procedures and analytic methods were performed according to the worksheet of the original equipment manufacturer (Beckman Coulter). The samples were diluted with phosphate-buffered saline (PBS) solution if the concentration was higher than 12,000 WBCs/μL. Fluorochrome-conjugated antibodies (20 μL) were added to the prepared samples (100 μL), and samples were processed with OptiLyse C solution (500 μL; Beckman Coulter), which lyses human RBCs. Subsequently, repeated centrifugation (800-1,000*g*, 5-10 minutes), disposure of the supernatant liquid, and washing with PBS solution (500 μL) were performed. All antibodies were ready to use (Beckman Coulter): fluorescein isothiocyanate (FITC)–conjugated CD3, FITC-conjugated CD25, FITC-conjugated CD83, phycoerythrin (PE)–conjugated CD4, PE-conjugated CD8, PE-conjugated CD1a, and PE-conjugated Foxp3. CD83 and CD1a were mature and immature DC markers, respectively, as reported in our previous study.[3] The lymphocytes were selected by gating CD45 (Beckman Coulter) and using adequate forward and sidelight scatters. Subsequently, specific immunolabeling was performed.

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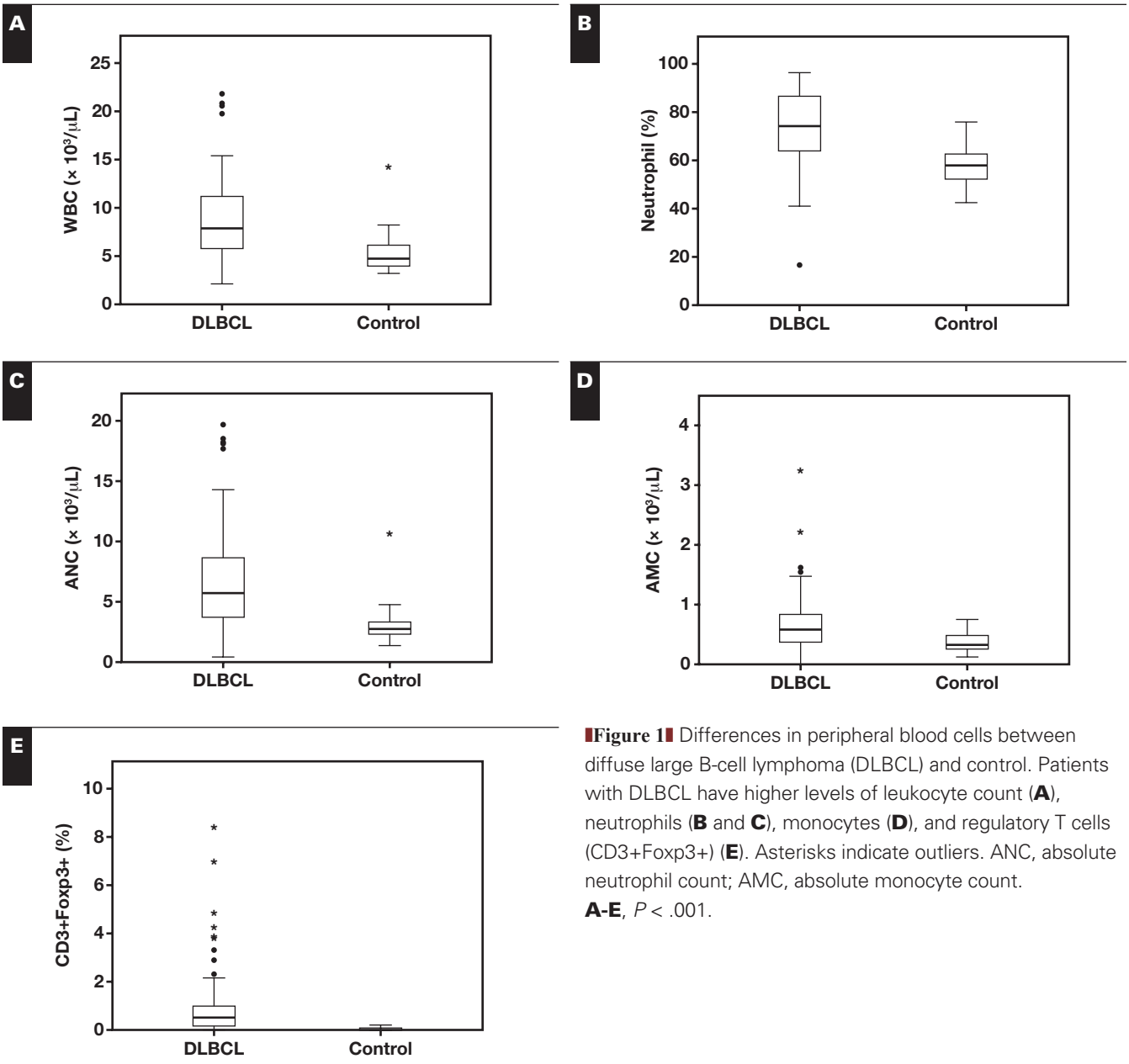

Figure 1 Differences in peripheral blood cells between diffuse large B-cell lymphoma (DLBCL) and control. Patients with DLBCL have higher levels of leukocyte count (**A**), neutrophils (**B** and **C**), monocytes (**D**), and regulatory T cells (CD3+Foxp3+) (**E**). Asterisks indicate outliers. ANC, absolute neutrophil count; AMC, absolute monocyte count. **A-E**, $P < .001$.

Statistical Analysis

The differences between the patients with DLBCL and the healthy volunteers were analyzed using the Mann-Whitney U test and χ^2 tests for continuous and categorical variables, respectively. Bonferroni correlation was used to avoid spurious positive results in multiple comparisons. Overall survival was measured from initial diagnosis to death from any cause, with follow-up data of the surviving patients assessed on the last contact date. Estimates of overall survival distribution were calculated using the Kaplan-Meier method. Time-to-event distributions were compared using the log-rank test. The simultaneous influence on overall survival of selected covariates demonstrated to be significant in the univariate analysis was tested using the Cox regression model. Relationships and correlations between the variables were analyzed using Kendall's τ test. Two-sided P values were used. All analyses were performed using SPSS 17.0 statistical software (SPSS, Chicago, IL).

Results

PB Immune Cells Differed Between Patients With DLBCL and Healthy Volunteers

The patients with DLBCL had a significantly higher leukocyte count ($P < .001$), a higher percentage of neutrophils ($P < .001$), a higher absolute monocyte count ($P < .001$), and a lower percentage and absolute count of lymphocytes

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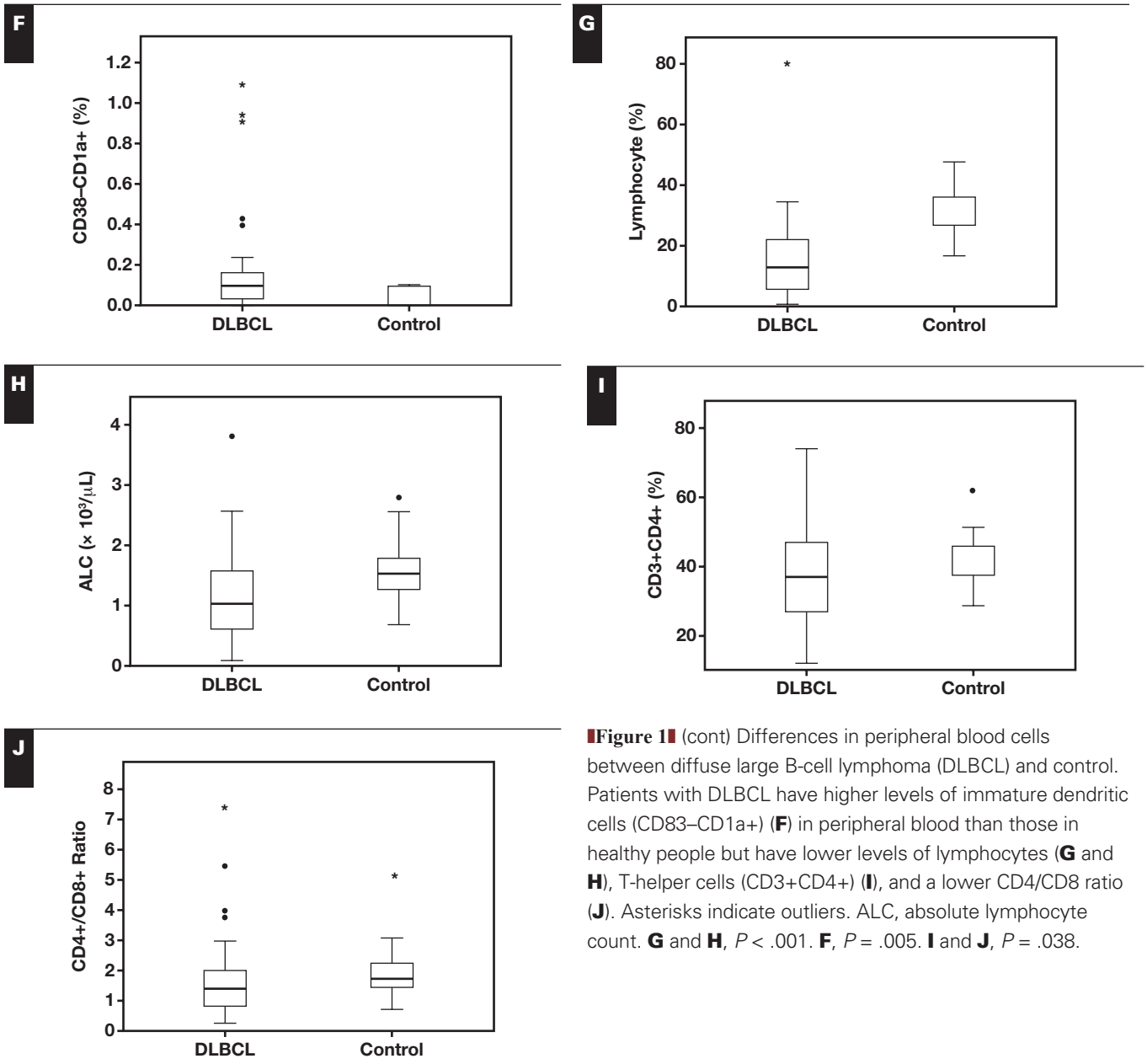

Figure 1 (cont) Differences in peripheral blood cells between diffuse large B-cell lymphoma (DLBCL) and control. Patients with DLBCL have higher levels of immature dendritic cells (CD83–CD1a+) (**F**) in peripheral blood than those in healthy people but have lower levels of lymphocytes (**G** and **H**), T-helper cells (CD3+CD4+) (**I**), and a lower CD4/CD8 ratio (**J**). Asterisks indicate outliers. ALC, absolute lymphocyte count. **G** and **H**, $P < .001$. **F**, $P = .005$. **I** and **J**, $P = .038$.

($P < .001$) than the healthy volunteers (Table 2) **Figure 1**. The patients with DLBCL had a lower percentage of Th cells (CD3+CD4+, $P = .038$), lower CD4/CD8 ratios ($P = .038$), and a higher percentage of Tregs (CD3+ Foxp3+, $P < .001$), and immature DCs (CD83–CD1a+, $P = .005$) (Table 2, Figure 1). However, the percentage of T cells detected by CD25 ($P = .303$), including those of CD25+CD4+ ($P = .816$) and CD25+CD8+ ($P = .561$), showed no significant difference. The absolute numbers of each of the various lymphocyte and DC subsets were also significantly different between patients with DLBCL and healthy controls (Table 2). No significant differences were observed in the percentage of monocytes ($P = .750$), T cells (CD3+, $P = .398$), and cytotoxic T cells (CD3+CD8+, $P = .152$).

Clinical Factors and Immune Cells Correlated With Patient Survival

Unfavorable clinical factors included age 60 years or older ($P = .001$), high stage (III-IV, $P = .001$), high serum LDH levels (≥200 IU/L, $P = .019$), high ECOG scores (2-4, $P < .001$), bone marrow involvement ($P < .001$), high IPI scores (3-5, $P < .001$), presence of effusions ($P = .002$), no treatment with chemotherapy ($P < .001$), stem cell transplantation ($P < .001$), and relapse ($P < .001$) **Table 3**. For immune cells, a higher leukocyte count (≥9,000/μL, $P = .037$), higher absolute neutrophil count (≥6,000/μL, $P = .014$), and higher percentage of Tregs (CD25+ cells ≥11%, $P = .038$; CD4+CD25+ cells ≥8%, $P = .026$) in PB correlated with worse survival (Table 3) **Figure 2**. However,

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Table 3
Unfavorable Clinical and Immunocellular Factors for Patients With Diffuse Large B-Cell Lymphoma

			Multivariate	
Parameter	**Unfavorable Factor, %**	**Univariate *P* Value**	***P* Value**	**HR (95% CI)**
Male sex	57.1	.960	—	
Age ≥60 y	51.9	.001[a]	.319	0.42 (0.08-2.29)
High stage (III-IV)	55.3	.001[a]	.571	0.55 (0.07-4.34)
LDH ≥200 IU/L	67.1	.019	.015[a]	29.3 (1.91-451.0)
High ECOG score (2-4)	37.7	<.001[a]	.181	3.38 (0.57-20.13)
Extranodal location	75.3	.060	—	
Bone marrow involvement (yes)	15.5	<.001[a]	.357	0.23 (0.01-5.32)
High IPI score (3-5)	48.1	<.001[a]	.001[a]	60.7 (1.14-71.18)
Bulky disease >10 cm (yes)	20.8	.773	—	
EBV-associated (yes)	5.0	.728	—	
B symptoms (yes)	74.0	.153	—	
Effusions (yes)	31.6	.002[a]	.701	0.70 (0.11-4.47)
Radiotherapy (no)	67.5	.085	—	
Chemotherapy (no)	14.3	<.001[a]	.590	2.62 (0.08-86.9)
Stem cell transplantation (yes)	23.4	<.001[a]	.908	1.10 (0.20-5.99)
Relapse (yes)	41.9	<.001[a]	.001[a]	61.2 (4.88-767.8)
WBC ≥9,000/μL[b]	43.4	.037[a]	.707	1.83 (0.08-43.3)
Neutrophils ≥74%	50.6	.325	—	
Absolute neutrophil count ≥6,000/μL[c]	42.9	.014[a]	.532	0.42 (0.03-6.28)
Monocytes ≥8%	44.0	.613	—	
Absolute monocyte count ≥654/μL	41.3	.886	—	
Lymphocytes <15%	48.0	.346	—	
Absolute lymphocyte count <1,127/μL	56.0	.436	—	
CD3+ <68%	39.0	.100	—	
CD3+CD4+ ≥38%	49.4	.087	—	
CD3+CD8+ <29%	54.5	.820	—	
CD4+/CD8+ ≥1.6 (ratio)	40.3	.260	—	
CD25+CD4+ ≥8%[b]	41.6	.026[a]	0.035[a]	51.4 (1.33-1,980.1)
CD25+CD8+ ≥1.4%	30.1	.108	—	
CD25+ ≥11%[b]	44.2	.038[a]	.127	0.070 (0.002-2.124)
CD3+Foxp3+ ≥1%	28.6	.523	—	
CD3–Foxp3+ ≥0.5%	20.8	.203	—	
CD83+CD1a– <0.27%	63.9	.208	—	
CD83–CD1a+ <0.18%	76.3	.582	—	

CI, confidence interval; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; IPI, International Prognostic Index; LDH, lactate dehydrogenase.
[a] Statistically significant.
[b] The approximate mean values shown in Table 2 as cutoffs.
[c] The approximate value (6,000/μL) is more powerful than the mean value (6,981/μL).

the absolute numbers of each of the various lymphocyte and DC subsets were insignificant for survival (Supplementary Table 1; supplemental material can be found at http://bit.ly/ChangDec15). In the multivariate model, high serum LDH level (P = .015), high IPI score (P = .001), relapse (P = .001), and a high percentage of Tregs (CD4+CD25+, P = .035) were significant factors (Table 3).

PB Immune Cells Correlated With Clinical Factors

Higher leukocyte counts correlated with higher stage (r = 0.245, P = .035) and IPI scores (r = 0.235, P = 0.041). The higher percentage of neutrophils correlated with higher ECOG scores (r = 0.236, P = .042), and a higher absolute neutrophil count correlated with higher ECOG (r = 0.289, P = .013) and IPI scores (r = 0.254, P = .029). A lower percentage of lymphocytes correlated with a higher serum LDH level (r = −0.277, P = .018), higher ECOG (r = −0.270, P = .020) and IPI scores (r = −0.307, P = .008), and the presence of effusions (r = −0.420, P < .001). Disease relapse correlated with a lower percentage of T cells (CD3+, r = 0.295, P = .021) at diagnosis. Bulky disease correlated inversely with the percentage of Th cells (CD3+CD4+, r = −0.249, P = .030). A higher percentage of Tregs (CD3+Foxp3+) correlated with a lower CD4+/CD8+ ratio (r = –0.242, P = .035) but a higher percentage of immature DCs (CD83–CD1a+, r = 0.325, P = .055) at time of diagnosis.

Discussion

The immune system is composed of innate and adaptive immune responses. The innate system is nonspecific,

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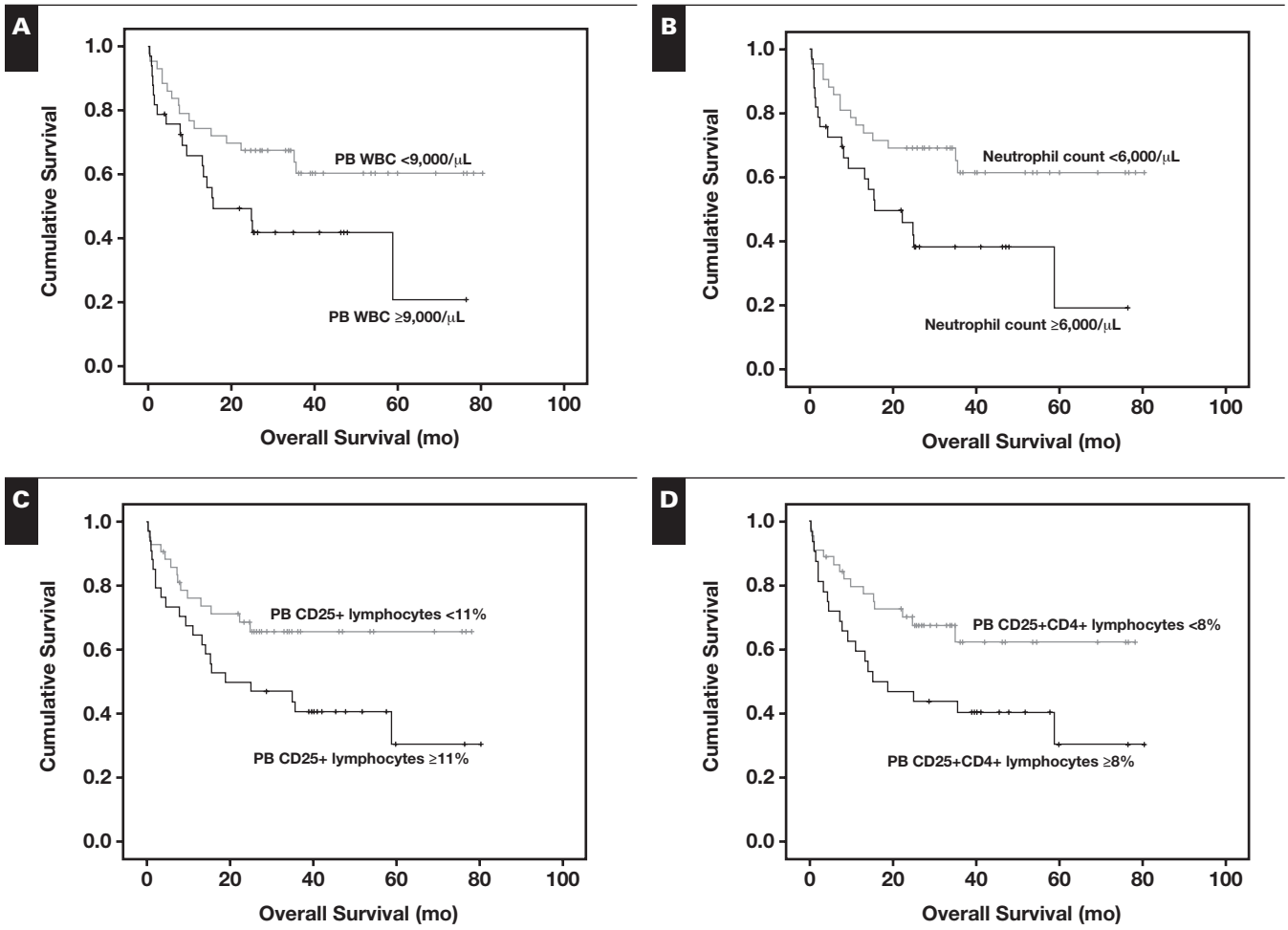

Figure 2 Differences in blood immune cells correlate with overall survival in patients with diffuse large B-cell lymphoma. Patients with higher levels of leukocytes (**A**), neutrophils (**B**), CD25+ lymphocytes (**C**), and regulatory T cells (CD4+CD25+) (**D**) have worse overall survival. PB, peripheral blood. **A**, $P = .037$. **B**, $P = .014$. **C**, $P = .038$. **D**, $P = .026$.

is often triggered by microbes, and reacts mainly through activation of phagocytes, including neutrophils, monocytes (macrophages), and DCs. The adaptive immune system is antigen specific, triggered by antigen presentation, and reacts through lymphocytes.[2] In this study we compared PB immune cells in patients with DLBCL with those of healthy persons and found that patients with DLBCL had more neutrophils, monocytes, Tregs, and immature DCs, but lymphocytes, including Th cells, were suppressed. These data suggest that patients with DLBCL have active innate immunity but suppressed adaptive immunity. In addition, increased neutrophils and monocytes may result from tumor-derived cytokine effects or reflect the underlying inflammation or morbidity status of the host. Others have shown that tumor-derived chemokines induce proliferation of neutrophils and monocytes, thus benefiting tumor growth.[28-30] Furthermore, a high neutrophil count might correlate with infection, one of the reasons that patients with DLBCL seek medical attention from a physician, particularly in this vulnerable population.

Many reports have shown that absolute lymphocyte and monocyte counts and their ratio are crucial prognostic factors in patients with DLBCL.[31-41] These values did not show statistical significance on prognosis in this study, although a lower lymphocyte count correlated with high-risk factors (eg, higher serum LDH levels, higher ECOG and IPI scores, and presence of effusions). Instead, we found that a higher percentage of baseline Tregs (CD25+CD4+) was a statistically significant poor survival factor. It is noteworthy that CD3+Foxp3+ Tregs correlated with more immature DCs but a lower CD4+/CD8+ ratio. The latter is possibly attributable to lowered CD4+ Th cells, because patients with DLBCL had fewer absolute Th cells. The former relationship is of interest because DCs are the most potent antigen-presenting cells. By expressing high levels of major histocompatibility complex and costimulatory molecules, DCs can present tumor-associated antigens to elicit T-cell–mediated tumor destruction.[42] In humans, DCs are a heterogeneous group, as reflected by different precursor populations, anatomical

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localization, and functions.[43] Immature DCs have more phagocytic ability than mature DCs, whereas mature DCs have more potent antigen-presenting ability.[43] On the other hand, tolerogenic DCs are required for both differentiation and activation of Tregs.[44] Although DLBCL in patients triggers recruitment of immature DCs, one possibility is that elicited Tregs may suppress the DCs and effector T cells in blood. Another possibility is that the increased Tregs mediated by tolerogenic DCs suppress the antitumor response generated by effector lymphocytes.[16,45] Recently, others have reported that inhibition of p38 mitogen-activated protein kinase in DCs overcomes Treg-mediated immunosuppression and prompts antitumor immune responses.[46] These findings may help improve DC-based immunotherapy.

In this study, adaptive immunity in patients with DLBCL was composed of a lower percentage of Th cells (CD4+), lower CD4+/CD8+ ratio, and higher percentage of Tregs (CD3+Foxp3+). Interestingly, the higher percentage of Tregs (CD4+CD25+) correlated with poorer survival in both univariate and multivariate analyses. Likewise, Mittal et al[47] reported that the percentage of Tregs (CD25+/Foxp3+/CD127 low/CD4+) was increased in patients with non-Hodgkin lymphoma (n = 30) compared with healthy people and that the higher percentage of Tregs correlated with higher stage disease and serum LDH levels. Furthermore, tumor cells induced expansion of Tregs, which subsequently contributed to systemic T-cell hyporesponsiveness in the host.[47] However, Glowala-Kosinska et al[48] showed that the circulating Treg numbers (CD4+/CD25 high/Foxp3+) were lower in patients with DLBCL than in healthy controls and were associated with poorer prognosis. The discrepancy might be attributable to methodologic differences, such as the percentage vs numbers of Tregs, cutoff values for gating (population characters), and Treg marker selection. The markers commonly used for Treg selection include CD4+CD25+,[14] Foxp3,[49] and their combinations. However, these markers are not unique for Tregs and can be upregulated in other activated T cells, although at a lower level and in a transient manner.[15,50-52] In addition, the role of Tregs might be influenced by location (within tumor vs blood),[8] tumor characteristics (germinal center–like vs activated B-cell phenotype),[10] and disease course (at diagnosis vs after therapy). More important, the diverse functions of Tregs also may be influenced by interaction with different subtypes of DCs, such as conventional vs tolerogenic.[53,54] Collectively, the data suggest that CD4+CD25+ Tregs in the blood of patients with DLBCL govern the escape of tumor from immunosurveillance through the systemic effects of immunosuppression.

In conclusion, compared with healthy volunteers, patients with DLBCL exhibit lymphocyte suppression, particularly Th cells, but elevated percentages of Tregs and activated innate immunity in the blood. The higher percentages of neutrophils and CD4+CD25+ Tregs correlate with worse patient survival. A lower lymphocyte count and T-cell (CD3+) percentage correlate with high-risk prognostic factors and disease relapse. These results show changes in the immune system of patients with DLBCL and support the suppressive role of Tregs in adaptive immunity that is detrimental to patient survival.

Corresponding author: Kung-Chao Chang, MD, PhD, Dept of Pathology, College of Medicine, National Cheng Kung University Hospital, 138 Sheng-Li Rd, Tainan, 704 Taiwan; changkc@mail.ncku.edu.tw.

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