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Association of CD57+ Natural Killer Cells with Better Overall Survival in DLBCL Patients

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Background: Malignant tumor cells may evoke the innate and adaptive immune systems. Various immune cells are involved in this immune reaction, and tumor infiltrating lymphocytes, macrophages, natural killer (NK) cells are associated with patient prognosis for solid tumors. **Methods:** Seventy-eight patients who were diagnosed with diffuse large B cell lymphoma (DLBCL) between 2001 and 2009 were selected. CD57+ NK cells, CD68+ tumor associated macrophages (TAMs), and CD4+ and CD8+ T cells were evaluated in tissue sections using immunohistochemical staining and compared with clinical parameters including age, gender, performance status, clinical stage, serum lactic dehydrogenase level, number of extranodal sites, international prognostic index score, chemotherapy response, and survival. **Results:** Patients with high numbers of CD57+ NK cells had a significantly higher overall survival rate than patients with low numbers of CD57+ NK cells. However, no significant difference was observed between the number of CD57+ NK cells and other prognostic parameters. The number of CD68+ TAMs and CD4+ or CD8+ T cells was not significantly correlated with prognostic factors in patients with DLBCL. **Conclusions:** An evaluation of tumor infiltrating CD57+ NK cells is recommended as a prognostic indicator in patients with DLBCL.

Key Words: Lymphoma, large B-cell, diffuse; Killer cells, natural; CD57; Prognosis

Diffuse large B-cell lymphoma (DLBCL) is defined as a diffuse proliferation of large neoplastic lymphoid B cells and can be divided into prognostically important subgroups of a germinal center (GC) type and non-GC (NGC) type according to gene expression profile.[1] Currently, the prognosis of patients with DLBCL is estimated using the clinical parameters of the International Prognostic Index (IPI). IPI incorporates patient age, performance status, serum levels of lactate dehydrogenase (LDH), Ann Arbor clinical stage, and number of involved extranodal lesions.[2]

As with a variety of solid tumors, it has been suggested that lymphoproliferative disorders can also evoke both innate and the adaptive immune reactions, and that tumor infiltrating lymphocytes, tumor associated macrophages (TAMs), and natural killer (NK) cells are associated with patient prognosis.[3-9]

This study was designed to investigate the prognostic implications of CD57+ NK cells, CD68+ TAMs, CD4+ helper-, and CD8+ cytotoxic T lymphocyte infiltration in patients with DLBCL.

MATERIALS AND METHODS

Patients

Among DLBCLs diagnosed in our hospital between 2001 and 2009, 78 cases, in which the paraffin blocks were available, were retrieved. The tissues were obtained by excisional biopsy, processed in 10% buffered formalin, and embedded in paraffin. The histological features and immunohistochemical findings fulfilled the World Health Organiztion classification of lymphoid neoplasms criteria for DLBCL.[10] B-cell lymphoma with features intermediate between DLBCL and Burkitt's lymphoma was not included in this study.

Clinical and follow-up data were obtained from the medical records. Clinical parameters included age, gender, performance status, Ann Arbor clinical stage, serum LDH levels, number of extranodal sites, IPI score, response to chemotherapy, and survival.

Construction of the tissue microarray (TMA)

An appropriate area containing the tumor lesion of each case was selected on hematoxylin and eosin stained slides, and the representative area was marked on the paraffin block. One core with a 3 mm diameter was punched out from each case and incorporated into a recipient paraffin block using a precision instrument (UNITMA Co. Ltd., Seoul, Korea).

Immunohistochemical staining

The TMA blocks and three paraffin blocks that were difficult to construct a TMA block because of the small size of the tissue sample were cut to a thickness of 4 μm. The sections were deparaffined in xylene and rehydrated through a graded ethanol series. The sections were placed in 10 mM EDTA (pH 8.0), heated in a microwave for 5 minutes, and then immersed in 0.3% hydrogen peroxide for 20 minutes to block endogenous peroxidase activity. The sections were incubated with their respective primary antibodies at room temperature for 1 hour and with secondary antibody at room temperature for 30 minutes. Then, the sections were developed with 3, 3´-diaminobenzidine and counterstained with Mayer's hematoxylin. Based on the immunohistochemical staining pattern for CD20, CD3, bcl-2, CD10, bcl-6, and MUM1, the histological diagnosis was confirmed, and the cases were grouped into GC and NGC types (Fig. 1).[11] Antibodies against CD57, CD68, CD4, and CD8 were used to identify NK cells, TAMs, helper-, and cytotoxic T lymphocytes. The characteristics of the primary antibodies against CD3, CD20, CD10, bcl-2, bcl-6, MUM1, CD4, CD8, CD57, and CD68 are summarized in Table 1. For a positive control, we applied the same method to reactive lymph nodes, the spleen and tonsils, which have positive cells for the aforementioned antibodies.

To enumerate tumor infiltrating cells, one image from a representative area of each case was created with an Olympus DP70 camera connected to Olympus microscope equipped with a UPlanApo 40× objective (Olympus, Tokyo, Japan). An area of

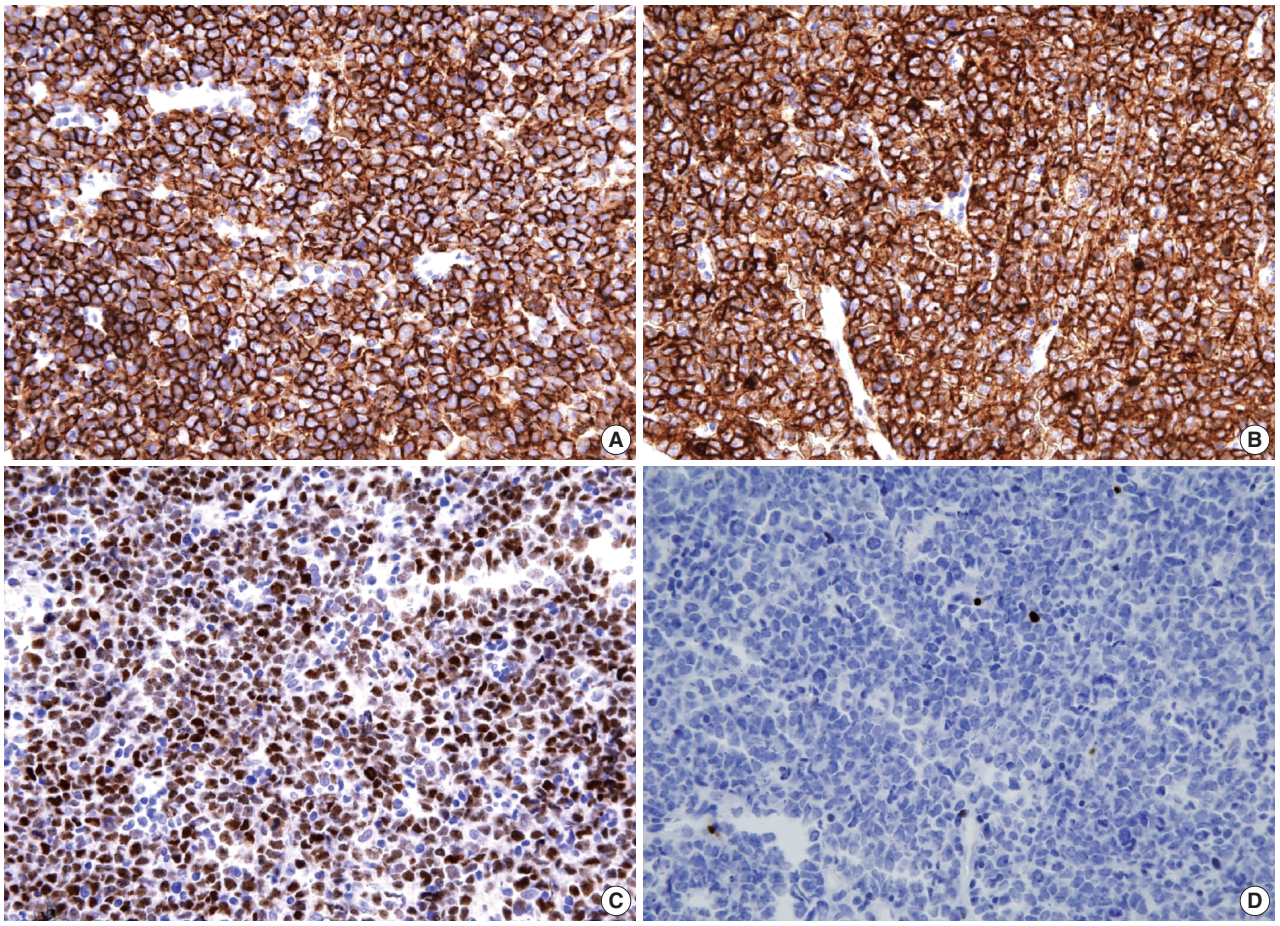

Fig. 1. Germinal center type shows positive stain for CD20 (A), CD10 (B), bcl-6 (C), but negative stain for MUM1 (D).

Table 1. Characteristics of the primary antibodies

Antibody	Type	Clone	Source	Dilution
CD3	Monoclonal mouse	F7.2.38	DAKO, Glostrup, Denmark	1:100
CD20	Monoclonal mouse	L26	Invitrogen, Carlsbad, CA, USA	1:400
CD10	Monoclonal mouse	56C6	DAKO, Glostrup, Denmark	1:100
Bcl-2	Monoclonal mouse	124	DAKO, Glostrup, Denmark	1:200
Bcl-6	Monoclonal mouse	PG-B6p	DAKO, Glostrup, Denmark	1:20
MUM1	Monoclonal mouse	MUM10	DAKO, Glostrup, Denmark	1:100
CD4	Monoclonal mouse	NCL-L-CD4-368	Novocastra, Newcastle, UK	1:50
CD8	Monoclonal mouse	C8/144B	DAKO, Glostrup, Denmark	1:200
CD57	Monoclonal mouse	NK-1	Zymed, San Francisco, CA, USA	1:100
CD68	Monoclonal mouse	KP1	DAKO, Glostrup, Denmark	1:4,000

0.88 mm^2 was investigated for each case. All interpretations of immunohistochemical stains were made without knowledge of clinical outcome. Tumor infiltrating cells were divided into low (same or lower than median number/0.88 mm^2) and high (higher than median number/0.88 mm^2) groups.

Clinical staging, response criteria, and survival

Each patient was evaluated for clinical staging by a physical examination; computed tomography scans of the thorax, abdomen, and pelvis; a bilateral bone marrow biopsy; complete blood cell count with a differential count; liver and kidney function tests; and serum LDH and was staged according to the Ann Arbor system at the time of diagnosis. Response to chemotherapy was assessed according to Cheson's criteria and categorized as complete remission, partial remission, stable disease, or progressive disease.[12] Survival was determined by whether the patient was alive or not at the latest follow-up date.

Statistical analyses

Statistical analyses were performed using Pearson's χ^2 test for dichotomized variables. Comparison tests were regarded as significant if the two-sided p-value was <0.05. All statistical analyses were performed using SPSS ver. 14.0.1 (SPSS Inc., Chicago, IL, USA).

RESULTS

Characteristics of the patients and tumors

The clinical characteristics of the 78 patients diagnosed with DLBCL are summarized in Table 2. The average age of the study group at the time of diagnosis was 59 years (median, 62 years; range, 14 to 89 years). The patients included 48 males and 30 females. Primary sites were categorized into nodal sites (32 patients, 41.0%) and extranodal sites (46 patients, 59.0%). Extranodal sites included the gastrointestinal tract (19), nasal cavity (6), soft tissue (4), testis (4), retroperitoneum (3), adrenal glands (2), brain (2), eyelids (1), liver (1), ovaries (1), pleura (1), skin (1), and spinal cord (1). Treatment consisted of chemo-radiotherapy (more than three cycles of standard chemotherapy followed by radiotherapy), chemotherapy (including rituximab after 2007) or a debulking operation. The median follow-up time was 3.8 years (range, 6 months to 11.0 years).

Nine patients were not fully evaluated at the time of initial diagnosis; eight patients refused further evaluation and treatment or wanted to transfer to other hospital and one patient died two days after biopsy. Therefore, several missing values occurred: seven in the clinical stage, four in performance status, seven for the number of extranodal lesions, nine for IPI score, nine for the response to therapy, and eight for survival.

Based on the immunohistochemical staining pattern for CD-10, bcl-6, and MUM1, 22 cases were classified as GC type (Fig. 1) and 55 cases as NGC type (Fig. 2). One case was unclassifiable. The patients with the NGC type (18/51, 35.3%) tended to have a higher IPI (3-5) than the patients with the GC type (3/18, 16.7%), but no statistical significance was found ($p=0.140$). No statistical significance was observed between the GC and NGC types for survival rate or response to therapy ($p=0.823$ and $p=0.650$, respectively).

Relationship between CD57+ NK cells and prognostic factors

The average and median number of CD57+ NK cells in all cases was 27.05/0.88 mm^2 and 25.00/0.88 mm^2 (range, 1 to 123/0.88 mm^2), respectively (Table 3). The patients were divided into 32 high (>25/0.88 mm^2) and 46 low (≤ 25/0.88

Table 2. Patient characteristics

Variables	No. of patients (%)	Variables	No. of patients (%)
Gender		Immunophenotype	
Male	48 (61.5)	Germinal center	22 (28.2)
Female	30 (38.5)	Non-germinal center	55 (70.5)
Age (yr)		Unclassifiable	1 (1.3)
11-20	3 (3.8)	Ann Arbor clinical stage	
21-30	3 (3.8)	I	22 (28.2)
31-40	2 (2.6)	II	27 (34.6)
41-50	9 (11.5)	III	4 (5.1)
51-60	19 (24.4)	IV	18 (23.1)
61-70	30 (38.5)	Unknown	7 (9.0)
71-80	10 (12.8)	International prognostic index	
81-90	2 (2.6)	0	17 (21.8)
Serum LDH level		1	17 (21.8)
Normal	38 (48.7)	2	14 (17.9)
Above normal	33 (42.3)	3	12 (15.4)
Unknown	7 (9.0)	4	7 (9.0)
No. of extranodal sites		5	2 (2.6)
0 or 1	55 (70.5)	Unknown	9 (11.5)
≥2	16 (20.5)	Response to therapy	
Unknown	7 (9.0)	Complete remission	35 (44.9)
Performance status		Partial remission	11 (14.1)
0	29 (37.2)	Stable disease	0 (0.0)
1	25 (32.1)	Progressive disease	23 (29.5)
2	10 (12.8)	Unknown	9 (11.5)
3	9 (11.5)	Survival	
4	1 (1.3)	Survival	39 (50.0)
Unknown	4 (5.1)	Death	31 (39.7)
		Unknown	8 (10.3)

LDH, lactate dehydrogenase.

Table 3. The relationship of CD57+ NK cells with clinical parameters

Variables		Low NK cell group (≤25 cells/0.88 mm^2)	High NK cell group (>25 cells/0.88 mm^2)	p-value
Performance status (n=74)	0-1	32 (74.4)	22 (71.0)	0.746
	2-5	11 (25.6)	9 (29.0)	
No. of extranodal sites (n=71)	0-1	31 (77.5)	24 (77.4)	0.994
	2 or more	9 (22.5)	7 (22.6)	
Clinical stage (n=71)	I and II	29 (72.5)	20 (64.5)	0.478
	III and IV	11 (27.5)	11 (35.5)	
Immunophenotype (n=77)	GC type	10 (22.2)	12 (37.5)	0.242
	NGC type	35 (77.8)	20 (62.5)	
IPI (n=69)	0-2	29 (76.3)	19 (61.3)	0.182
	3-5	9 (23.7)	12 (38.7)	
Response to therapy (n=69)	CR	17 (43.6)	20 (66.7)	0.093
	PR, SD or PD	22 (56.4)	10 (33.3)	
End point survival (n=70)	Survival	18 (45.0)	23 (76.7)	0.037
	Death	22 (55.0)	7 (23.3)	

Values are presented as number (%).
NK, natural killer; GC, germinal center; NGC, non-germinal center; IPI, International Prognostic Index; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

mm^2) CD57+ NK cell groups based on the cell number/0.88 mm^2 (Fig. 3). During follow-up, 22 of 40 patients (55.0%) in the low CD57+ NK cell group died, whereas seven of 30 patients (23.3%) in the high CD57+ NK cell group died. The low CD57+ NK cell group had a significantly lower survival rate than that of the high CD57+ NK cell group (p=0.037).

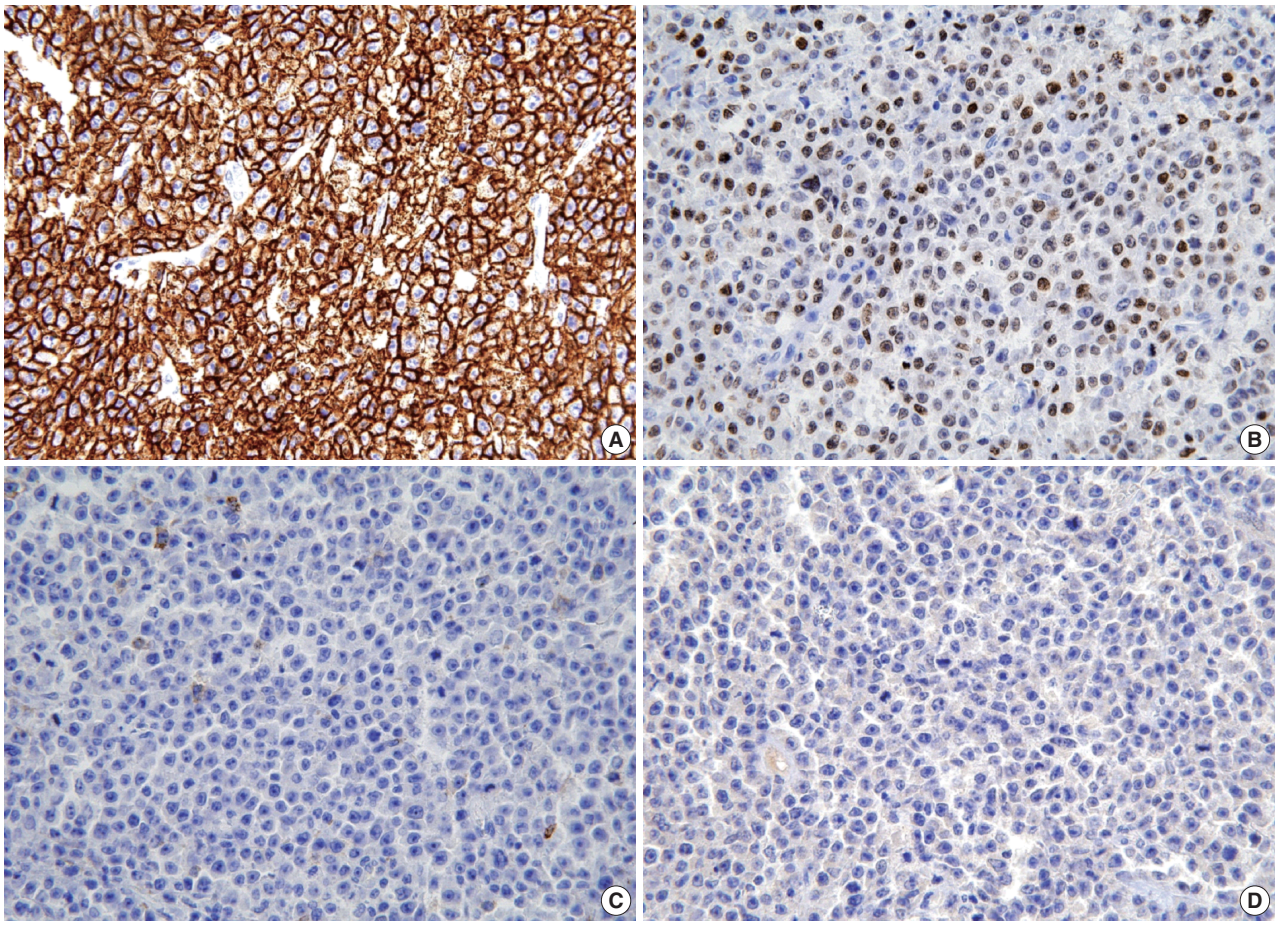

Fig. 2. Non-germinal center type shows positive stain for CD20 (A) and MUM1 (B), but negative stain for CD10 (C) and bcl-6 (D).

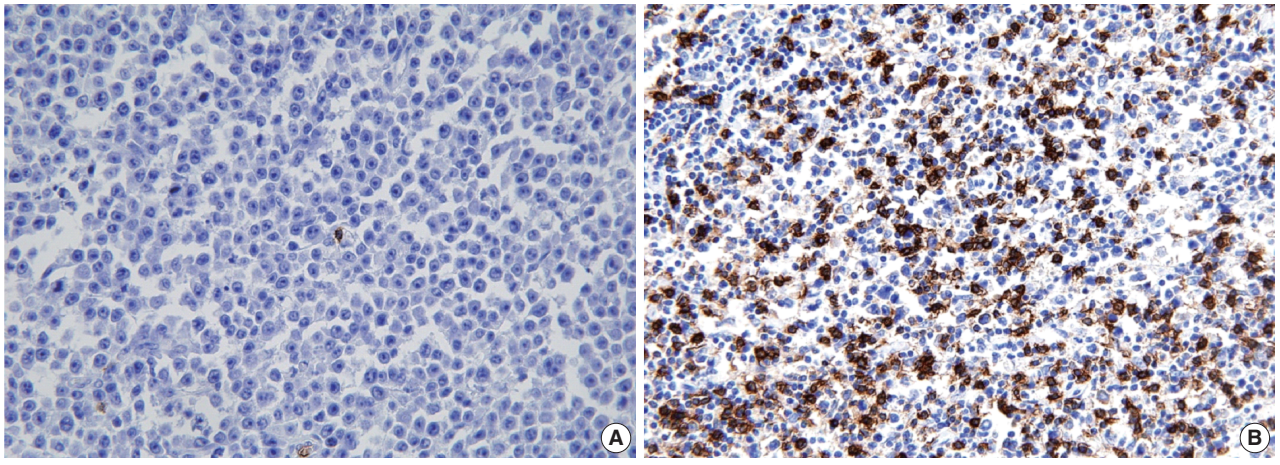

Fig. 3. CD57+ natural killer (NK) cells. Low NK cell group (A) and high NK cell group (B).

Twenty of 30 patients (66.7%) in the high CD57+ NK cell group had higher complete remission rates than those in the low CD57+ NK cell group (17/39, 43.6%); however, no statistical difference in response to therapy was observed between the two groups (p=0.093). The proportion of patients with a high IPI in the low CD57+ NK cell group (9/38, 23.7%) tended to be lower than that of patients in the high CD57+ NK cell group (12/30, 38.7%), but no statistical significance was found (p=

Table 4. The relationship of TAMs, CD4+ T cells, CD8+ T cells, and CD8+/CD4+ T cell ratio with clinical parameters

	CD68+ TAMs		CD4+ T cells		CD8+ T cells		CD8+/CD4+ ratio	
	Low	High	Low	High	Low	High	Low	High
Performance status (n=74)								
0-1	26	28	27	27	28	26	29	25
2-5	9	11	12	8	9	11	8	12
p-value	0.813		0.451		0.606		0.302	
No. of extranodal sites (n=71)								
0-1	26	29	28	27	27	28	28	27
≥2	7	9	10	6	8	8	7	9
p-value	0.807		0.421		0.950		0.620	
Clinical stage (n=71)								
I and II	22	27	25	24	22	27	23	26
III and IV	11	11	13	9	13	9	12	10
p-value	0.695		0.535		0.275		0.560	
Immunophenotype (n=77)								
GC type	11	11	8	14	10	16	12	10
NGC type	26	29	32	23	28	23	28	27
p-value	0.872		0.146		0.562		0.793	
IPI score (n=69)								
0-2	21	27	24	24	22	26	24	24
3-5	10	11	12	9	11	10	11	10
p-value	0.770		0.591		0.623		0.858	
Response to therapy (n=69)								
CR	13	22	17	18	16	19	16	19
PR, SD or PD	18	16	19	15	17	17	18	16
p-value	0.431		0.611		0.824		0.703	
End point survival (n=70)								
Survival	16	23	17	22	17	22	22	17
Death	17	14	20	11	17	14	13	18
p-value	0.257		0.084		0.357		0.235	

TAM, tumor associated macrophages; GC, germinal center; NGC, non-germinal center; IPI, International Prognostic Index; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

0.182). The degree of CD57+ NK cell infiltration was not different by immunophenotype (p=0.242), performance status (p=0.746), number of extranodal sites (p=0.994), or Ann Arbor clinical stage (p=0.478).

Relationship between CD68+ TAMs and prognostic factors

The average and median number of CD68+ TAMs was 66.91/0.88 mm^2 and 56.00/0.88 mm^2 (range, 0 to 207/0.88 mm^2), respectively (Table 4). The patients were divided into 40 high (>56/0.88 mm^2) and 38 low (≤56/0.88 mm^2) TAM groups (Fig. 4). During follow-up, 17 of 33 patients (51.5%) in the low TAM group died, whereas 14 of 37 patients (37.8%) in the high TAM group died (p=0.257). No significant differences were observed between the low and high TAM groups for other prognostic factors, including performance status, number of extranodal sites, clinical stage, immunophenotype, IPI score, or response to therapy.

Relationship between tumor infiltrating T lymphocytes and prognostic factors

The patients were divided into high and low groups based on the count per 0.88 mm^2 of CD8+ cytotoxic T lymphocytes, CD4+ helper T lymphocytes, and the ratio of CD8+/CD4+ T lymphocytes (Table 4).

Tumor infiltrating CD8+ T lymphocytes

The average and median number of CD8+ cells was 89.21/0.88 mm^2 and 74.00/0.88 mm^2 (range, 4 to 309/0.88 mm^2), respectively. High and low CD8+ T cell groups were defined as >74/0.88 mm^2 and ≤74/0.88 mm^2, respectively (Fig. 5). At the time of initial diagnosis, 13 of 35 patients (37.1%) in the low CD8+ T cell group showed an advanced clinical stage (III and IV), whereas nine of 36 patients (25.0%) in the high CD8+ T cell group showed clinical stage III or IV (p=0.275). Seventeen of 34 patients (50.0%) in the low CD8+ T cell group died, whereas 14 of 36 patients (38.8%) in the high CD8+ T cell

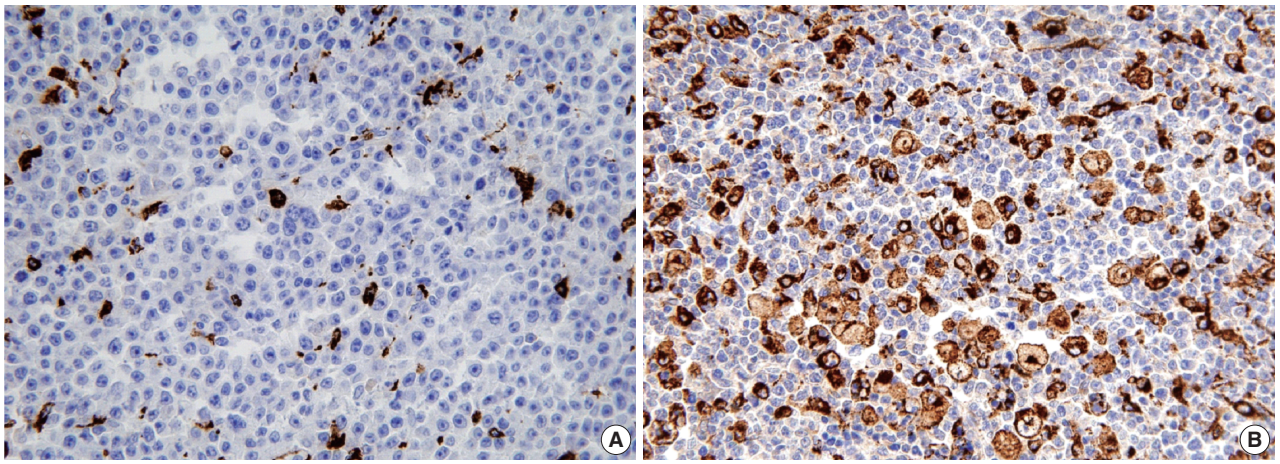

Fig. 4. CD68+ tumor associated macrophages (TAMs). Low TAM cell group (A) and high TAM cell group (B).

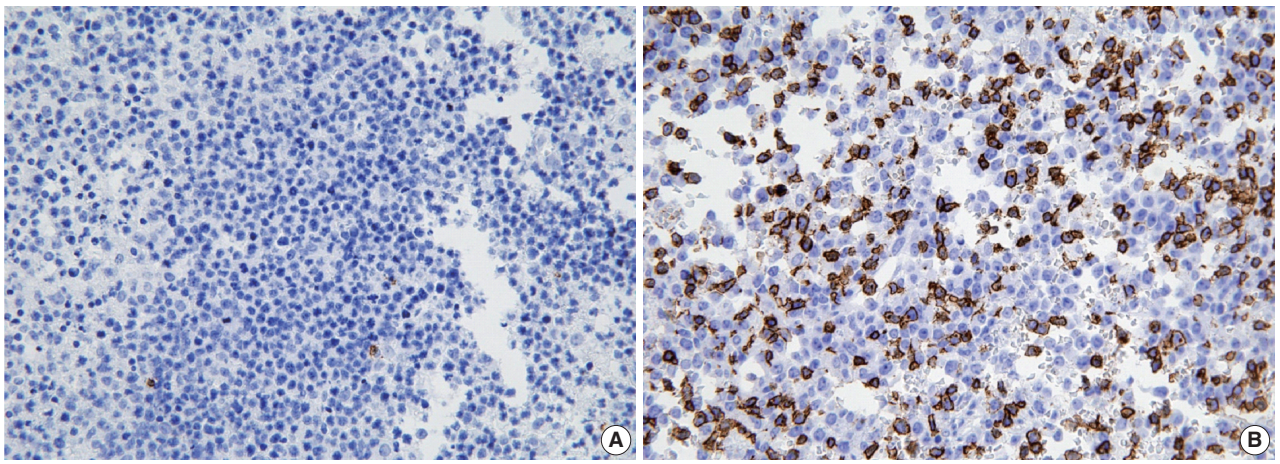

Fig. 5. CD8+ T cytotoxic cells. Low cytotoxic cell group (A) and high cytotoxic cell group (B).

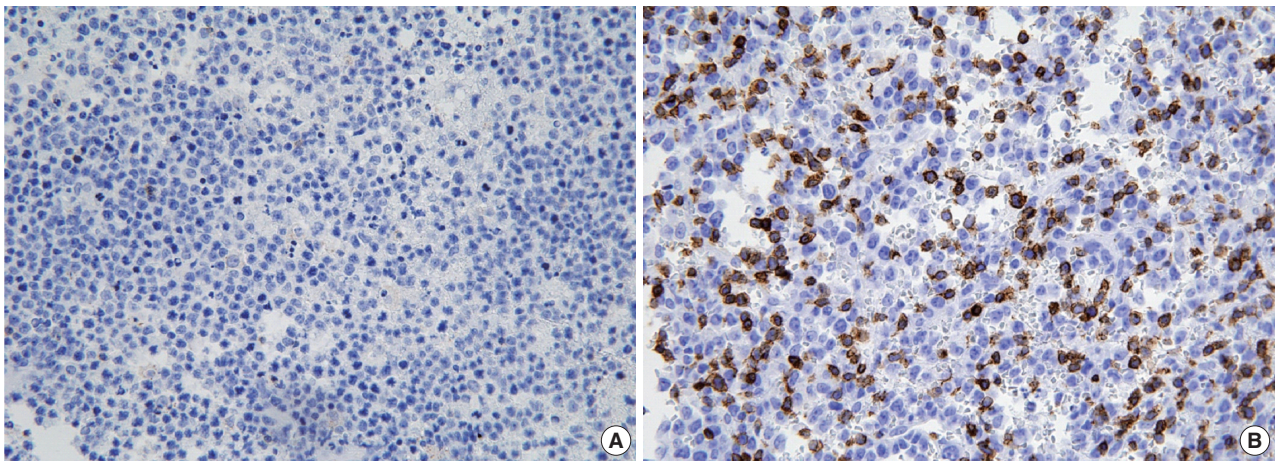

Fig. 6. CD4+ T helper cells. Low helper cell group (A) and high helper cell group (B).

group died during follow-up ($p=0.357$). No statistical differences were found between the number of CD8+ T cells and performance status, number of extranodal sites, immunophenotype, IPI score, or response to therapy.

Tumor infiltrating CD4+ T lymphocytes

The average and median number of CD4+ cells was 31.68/0.88 mm^2 and 15.00/0.88 mm^2 (range, 0 to 218/0.88 mm^2), respectively. High and low CD4+ T cell groups were defined as >15 cells/0.88 mm^2 and ≤15/0.88 mm^2, respectively (Fig. 6). Eight of 40 patients (20.0%) in the low CD4+ T cell group were classified as GC type, whereas 14 of 37 patients (37.8%) in the high CD8+ T cell group were GC type (p=0.146). During follow-up, more patients in the low CD4+ T cell group (20/37, 54.1%) tended to die than patients in the high CD4+ T cell group (11/33, 33.3%), but the difference was not significant (p=0.084). No significant differences were found between the number of CD4+ T cells and performance status, number of extranodal sites, clinical stage, IPI score, or response to therapy.

The ratio of CD8+/CD4+ T lymphocytes

The range of CD8+/CD4+ T lymphocyte ratio was from 0 to ∞. The high and low ratios of the CD8+/CD4+ T lymphocyte groups were defined as >4 and ≤4, respectively. Overall survival rates in low the CD8+/CD4+ T lymphocyte ratio group tended to be better than those in the high CD8+/CD4+ T lymphocyte ratio group (62.9% vs 48.6%), but the result was not significant (p=0.235). No statistical difference was observed between the CD8+/CD4+ T lymphocyte ratio and prognostic factors, including performance status, number of extranodal sites, clinical stage, immunophenotype, IPI score, or response to therapy.

DISCUSSION

The response of the body to a cancer is not a unique mechanism but has many parallels with inflammation and wound healing.[13] The tumor microenvironment is characterized by a reactive stroma with an abundance of inflammatory mediators and leukocytes, dysregulated vessels, and proteolytic enzymes. Inflammatory cells in the tumor microenvironment consist of macrophages, dendritic cells, and lymphocytes, whereas NK cells are rare.[13] The role of intratumoral infiltration of NK cells is controversial for various solid tumors. Most studies have suggested that NK cells play a role in tumor rejection in carcinomas, control of cancer spread, and the interaction between innate and adaptive immunity.[7,8,14,15] Compared to solid tumors, few large-scale studies have considered the role of NK cells in lymphoproliferative diseases.

In our study, the high CD57+ NK cell group showed a higher survival rate (76.7%) than that in the low CD57+ NK cell group (45.0%), and the average number of CD57+ NK cells in surviving patients was higher than the average number in dead patients (34.7/0.88 mm^2 vs 59.5/0.88 mm^2). This finding agrees with other studies in which high number of intratumoral NK cells was associated with a better prognosis. Dewan *et al.*[16] suggested that NK cells play an important role in growth and infiltration of tumor cells in primary effusion lymphoma, and that activated NK cells could be a promising immunotherapeutic tool against tumors either alone or in combination with conventional therapy. Alvaro-Naranjo *et al.*[17] also reported a significantly longer survival in patients with Hodgkin's lymphoma who had a high level of infiltrating CD57+ NK cells. Our results support the notion that NK cell therapeutic measures favor their potential clinical value in patients with malignant lymphoma.

NK cells are the primary effector cells of the innate immune system, and the primary role of NK cells is limiting or eliminating dangerous challenges to the host. In addition to inducing tumor cell apoptosis by producing a variety of cytokines and chemokines, the anti-tumoral function of NK cells includes promoting immunity as regulators of innate and adaptive immunity by interacting with dendritic cells.[14] Enhancing NK cell function is more efficient for the therapeutic response in patients with various tumors.[8,14,16] This NK cell function has been also described for lymphoproliferative diseases. Perez *et al.*[18] ascribed a role for glucocorticoids in cellular adoptive immunotherapy of erythroleukemia and Burkitt's lymphoma, as they act on mature NK cell expansion and function. Screpanti *et al.*[19] showed that NK cells play a central role in death receptor-mediated apoptosis, and result in tumor rejection in patients with T cell lymphoma. Our study showed that the high NK cell group appeared to be related to a better response to therapy (66.7% vs 43.6%), although the result was not statistically significant. We speculate that this might be due to the limited case numbers and different treatment modalities. None of the patients diagnosed before 2007 underwent rituximab therapy, which is the gold standard for DLBCL. The result that high NK cell infiltration in patients with DLBCL was associated with better survival suggests that evaluating tumor-infiltrating NK cells can be routinely performed.

TAM is a major component of the non-neoplastic infiltrate in most tumors.[20] TAMs are derived from circulating monocytic precursors and are directed into the tumor by chemokines. Many tumor cells also produce cytokines called colony-stimulating factors, which prolong TAM survival. After activation, TAMs

kill tumor cells or elicit tissue destructive reactions centered on the vascular endothelium. However, TAMs also produce growth and angiogenic factors as well as protease enzymes that degrade the extracellular matrix, favoring tumor progression. Some studies have suggested that TAMs are more likely to contribute to tumor growth and progression than they are to mount an effective host anti-tumor response in many solid tumors.[9] Studies on lymphoproliferative diseases have come to various conclusions. Steidl *et al.*[5] reported that an increased number of TAMs is strongly associated with shortened survival in patients with classic Hodgkin's lymphoma, whereas Hasselblom *et al.*[4] and Wahlin *et al.*[6] reported that patients with follicular lymphoma and DLBCL with high TAMs had a better prognosis. However, our study did not show the prognostic significance of CD68+ TAMs in patients with DLBCL.

The clinical significance of tumor-infiltrating lymphocytes has been suggested for malignant melanoma. Clark *et al.*[21] suggested that tumor-infiltrating lymphocytes in malignant melanoma can result in partial tumor destruction, and Mihm *et al.*[22] further showed that brisk T-cell reactions in malignant melanomas are predictors of improved survival. The significance of T lymphocytes as well as helper- and cytotoxic T cells in lymphoproliferative disorders has been described.[3,6-8,23,24] Ansell *et al.*[23] suggested that an increase in the percentage of activated memory CD4+ T cells rather than cytotoxic T lymphocytes is correlated with good outcomes in patients with large B cell lymphoma and suggested that CD4+ T cells may be responsible for costimulating CD8+ T cells and antigen-presenting cells. Recently, it has been suggested that T-helper cells may also have cytolytic activity.[24] However, Wahlin *et al.*[6] reported that CD4+ cells are associated with poor outcomes, whereas CD8+ cells are associated with good outcome in patients with follicular lymphoma. Our result did not show the prognostic significance of CD4+ or CD8+ tumor-infiltrating lymphocytes or the ratio of CD8+/CD4+ T lymphocytes.

In conclusion, our results show the prognostic implication of intratumoral CD57+ NK cells in patients with DLBCL. The high-CD57+ NK cell group had a better survival rate regardless of clinical prognostic parameters. However, no clinical implications were found for CD68+ TAMs, CD4+, or CD8+ T lymphocytes.

REFERENCES

1. Rosenwald A, Wright G, Chan WC, *et al.* The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002; 346: 1937-47.
2. Ziepert M, Hasenclever D, Kuhnt E, *et al.* Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. J Clin Oncol 2010; 28: 2373-80.
3. Xu Y, Kroft SH, McKenna RW, Aquino DB. Prognostic significance of tumour-infiltrating T lymphocytes and T-cell subsets in de novo diffuse large B-cell lymphoma: a multiparameter flow cytometry study. Br J Haematol 2001; 112: 945-9.
4. Hasselblom S, Hansson U, Sigurdardottir M, Nilsson-Ehle H, Ridell B, Andersson PO. Expression of CD68+ tumor-associated macrophages in patients with diffuse large B-cell lymphoma and its relation to prognosis. Pathol Int 2008; 58: 529-32.
5. Steidl C, Lee T, Shah SP, *et al.* Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med 2010; 362: 875-85.
6. Wahlin BE, Aggarwal M, Montes-Moreno S, *et al.* A unifying microenvironment model in follicular lymphoma. Outcome is predicted by programmed death-1: positive, regulatory, cytotoxic, and helper T cells and macrophages. Clin Cancer Res 2010; 16: 637-50.
7. Qiu H, Xiao-Jun W, Zhi-Wei Z, *et al.* The prognostic significance of peripheral T-lymphocyte subsets and natural killer cells in patients with colorectal cancer. Hepatogastroenterology 2009; 56: 1310-5.
8. Hyakudomi M, Matsubara T, Hyakudomi R, *et al.* Increased expression of fractalkine is correlated with a better prognosis and an increased number of both CD8+ T cells and natural killer cells in gastric adenocarcinoma. Ann Surg Oncol 2008; 15: 1775-82.
9. Dirkx AE, Oude Egbrink MG, Wagstaff J, Griffioen AW. Monocyte/macrophage infiltration in tumors: modulators of angiogenesis. J Leukoc Biol 2006; 80: 1183-96.
10. Swerdlow SH, Campo E, Harris NL, *et al.* WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: International Agency for Research on Cancer, 2008; 439.
11. Hans CP, Weisenburger DD, Greiner TC, *et al.* Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004; 103: 275-82.
12. Cheson BD, Pfistner B, Juweid ME, *et al.* Revised response criteria for malignant lymphoma. J Clin Oncol 2007; 25: 579-86.
13. Balkwill F, Mantovani A. Cancer and inflammation: implications for pharmacology and therapeutics. Clin Pharmacol Ther 2010; 87: 401-6.
14. Wallace ME, Smyth MJ. The role of natural killer cells in tumor control: effectors and regulators of adaptive immunity. Springer Semin Immunopathol 2005; 27: 49-64.
15. Woan K, Reddy V. Potential therapeutic role of natural killer cells in

cancer. Expert Opin Biol Ther 2007; 7: 17-29.
16. Dewan MZ, Terunuma H, Toi M, *et al.* Potential role of natural killer cells in controlling growth and infiltration of AIDS-associated primary effusion lymphoma cells. Cancer Sci 2006; 97: 1381-7.
17. Alvaro-Naranjo T, Lejeune M, Salvadó-Usach MT, *et al.* Tumor-infiltrating cells as a prognostic factor in Hodgkin's lymphoma: a quantitative tissue microarray study in a large retrospective cohort of 267 patients. Leuk Lymphoma 2005; 46: 1581-91.
18. Perez SA, Mahaira LG, Demirtzoglou FJ, *et al.* A potential role for hydrocortisone in the positive regulation of IL-15-activated NK-cell proliferation and survival. Blood 2005; 106: 158-66.
19. Screpanti V, Wallin RP, Ljunggren HG, Grandien A. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. J Immunol 2001; 167: 2068-73.
20. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. Immunol Today 1992; 13: 265-70.
21. Clark WH Jr, From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. Cancer Res 1969; 29: 705-27.
22. Mihm MC Jr, Clemente CG, Cascinelli N. Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response. Lab Invest 1996; 74: 43-7.
23. Ansell SM, Stenson M, Habermann TM, Jelinek DF, Witzig TE. Cd4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. J Clin Oncol 2001; 19: 720-6.
24. Hishii M, Kurnick JT, Ramirez-Montagut T, Pandolfi F. Studies of the mechanism of cytolysis by tumour-infiltrating lymphocytes. Clin Exp Immunol 1999; 116: 388-94.