의학석사 학위논문

Bone marrow analysis of immune cells and apoptosis in patients with systemic lupus erythematosus

전신성 홍반성 낭창 환자의 골수 내 면역세포 및 세포 자살에 대한 분석

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지도 교수 이 은 봉

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박 준 원

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위 원 장 정 경 천 (인)

부위원장 이 은 봉 (인)

위 원 이 은 영 (인)

Bone marrow analysis of immune cells and apoptosis in patients with systemic lupus erythematosus

by

Jun Won Park M.D

(Directed by Eun Bong Lee M.D,, Ph.D)

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Professor Kyeong Cheon Jung **Chairman**

Professor Eun Bong Lee **Vice chairman**

Professor Eun Young Lee

Abstract

Objectives: To examine the immune cell profile in the bone marrow (BM) of systemic lupus erythematosus (SLE) patients and to assess its clinical relevance.

Methods: Sixteen BM samples from 14 SLE patients were compared with seven healthy control samples. The numbers of CD4+ T cells, CD8+ T cells, B cells, plasmacytoid dendritic cells (pDC), macrophages and plasma cells in the BM, and the levels of interleukin-6 (IL-6) expression, were examined by immunohistochemistry. The number of apoptotic cells (active caspase-3+) in the BM was also measured. The association between immune cell subsets and clinical features was also investigated.

Results: CD4+ T cells, macrophages and plasma cells were more common in the BM of SLE patients than in healthy controls (1.82±1.45% *vs.* 0.26±0.12%, p=0.001; 16.35±7.17% *vs.* 8.04±1.38%, p=0.004; and 9.72±5.64% *vs.* 3.44±0.64%, p<0.001, respectively). Greater numbers of CD4+ T cells and macrophages were associated with high-grade BM damage. The percentage of apoptotic cells in BM specimens from SLE patients was significantly higher than that in controls (2.47±1.35% *vs.* 0.19±0.22%, p<0.001) and was positively correlated with the number of pDCs (r=0.606, p=0.013). Increased numbers of plasma cells and high IL-6 expression were correlated with anti-double stranded DNA (dsDNA) antibody levels and the SLE disease activity index (r=0.538, p=0.031 and r=0.581, p=0.013, respectively).

Conclusion: BM samples from SLE patients showed a distinct immune cell profile and increased numbers of apoptotic cells. This, coupled with a correlation with disease activity, suggest that the BM may play a critical role in the pathogenesis of SLE.

Key word: Systemic Lupus Erythematosus, Bone marrow, Apoptosis

Student number 2011-23772

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LIST OF ABBREVIATIONS

BAFF	B-cell activating factor
BM	Bone marrow
CXCL12	C-X-C motif chemokine 12
dsDNA	Double-stranded DNA
IFN	Interferon
IL-6	Interleukin-6
pDC	Plasmacytoid dendritic cell
SLE	Systmic lupus erythematosus
SLEDAI	Systemic lupus erythematosus disease activity index

Introduction

Systemic lupus erythematosus (SLE), a chronic autoimmune disease of unknown etiology, is characterised by the production of autoantibodies against nuclear autoantigens. Haematological abnormalities such as anaemia and thrombocytopenia, are common clinical manifestations.(1, 2) Exact mechanism of haematological abnormalities in SLE is still uncertain. Peripheral cellular destruction (mediated by autoantibodies), dysfunction of haematopoietic stem cells, and autoimmune-mediated bone marrow (BM) damage are thought to be immunological contributors.(3, 4) Few studies have examined the BM characteristics associated with SLE. Voulgarelis et al., examined the histological features of BM specimens from 40 cytopenic SLE patients and reported prominent disruption of the normal architecture and the presence of atypical cells belonging to the erythroid and megakaryocytic lineages.(5) Fibrosis, necrosis and gelatinous transformation were also relatively common histologic findings in other reports.(6, 7) By contrast, BM findings of patients with SLE appeared almost normal in another study.(8, 9) However, no study has undertaken a detailed analysis of the immune cell profile in the BM of SLE patients and its relationship with disease activity.

Several studies suggest that dysregulated apoptosis and prolonged exposure to nuclear autoantigens play a role in the pathogenesis of SLE,(10, 11) and others report the accumulation of apoptotic cells in various tissues in these patients.(12, 13) Hepburn et al. were the first to report multiple apoptotic bodies in the BM of SLE patients.(14) However, the clinical relevance of these apoptotic cells and their relationship with other immune cells are unknown. Therefore, the aims of the present study were to investigate the role of the BM in the pathogenesis of SLE by examining the immune cell profile and level of apoptosis in the BM of SLE patients and to assess their clinical relevance.

Patients and methods

Patients and clinical information

Between 2000 and 2013, 16 BM biopsy specimens were obtained from 14 SLE patients and seven healthy controls (BM donors) at Seoul National University Hospital. All SLE patients fulfilled the revised American College of Rheumatology criteria for classification of SLE at diagnosis.(15) Information regarding specific organ involvement, disease activity (measured using the SLE disease activity index; SLEDAI) and laboratory findings such as serum complement levels and titer of autoantibodies (Anti-dsDNA, Anti-Sm, Anti-Ro, Anti-La and Anti-cardiolipin) was obtained from medical records. SLE patients with a culture-proven infection in any organ or concurrent haematological disorders at the time of BM examination were excluded. The study was approved by the institutional review board (IRB) of Seoul National University Hospital [IRB No. H-1210-040-432].

Bone marrow examination

Haematoxylin and eosin (H&E)-stained paraffin-embedded BM sections and Wright-Giemsa-stained BM aspirate smears were evaluated by two independent investigators blinded to the patient's clinical information. BM cellularity was analysed according to previous report and graded into three groups: hypercellular, normocellular and hypocellular.(16) Morphological dysplasia of tri-lineage haematopoietic cells was assessed. Other histological manifestations, including oedema, fibrosis, gelatinous changes, necrosis, lymphoid follicular formation and haemophagocytosis were also evaluated. BM necrosis was graded semiquantitatively as described by Maisel et al.(17) High-grade BM damage was defined as extensive BM necrosis (≥50% of the biopsy) and/or severely decreased

cellularity (<30%), and low-grade BM damage was defined as mild-to-moderate BM necrosis (<30%).

Immunohistochemistry

For immunohistochemistry, formalin-fixed, paraffin-embedded BM sections were deparaffinised in xylene and hydrated in a graded series of ethanol solutions followed by distilled water. After heat-induced antigen retrieval in sodium citrate buffer, endogenous peroxidase activity was blocked by incubation in a peroxide-blocking solution (Dako A/S, Glostrup, Denmark). After incubation in 5% normal goat serum for 30 minutes, the specimens were incubated with specific primary antibodies (see below) for 60 minutes at room temperature or overnight at 4°C, followed by horseradish peroxidase-conjugated anti-mouse or anti-rabbit Envision+ (Dako A/S) for 30 minutes. Staining was visualised using diaminobenzidine. Cell nuclei were counterstained with haematoxylin. The results were expressed as the percentage of positively stained cells in five or more randomly selected fields (magnification: ×400). Interleukin (IL)-6 expression was assessed semiquantitatively as follows: 0, no staining; 1, <25% of cells stained; 2, 26–50% of cells stained; 3, 51–75% of cells stained; and 4, >75% of cells stained. All specimens were examined in a blinded manner by two independent investigators using a digital quantification system (Leica Application Suite version 3.8) connected to a light microscope (Leica DFC240, Wetzlar, Germany).

Primary antibodies

The following primary antibodies were used in the present study: mouse monoclonal anti-CD3 (1:50, Dako A/S), mouse monoclonal anti-CD4 (1:250, Abcam, Cambridge, UK), rabbit monoclonal anti-CD8 (1:100, Abcam), rabbit monoclonal anti-CD20 (1:100, Abcam), mouse monoclonal anti-CD68

(1:50, Dako A/S), mouse monoclonal anti-CD138 (1:50, Abcam), rabbit monoclonal anti-active caspase 3 (1:50, Abcam), mouse monoclonal anti-CD303 (BDCA-2) (1:50, Merck Millipore, Darmstadt, Germany) and rabbit polyclonal anti-IL-6 (1:400, Abcam).

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation and compared using the Mann-Whitney U test. Fisher's exact test was used to compare categorical variables. Spearman's correlation coefficient was used to examine the correlation between two continuous variables. All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and p values < 0.05 were considered significant.

Results

Clinical features

Most of the SLE patients were female (14/16, 87.5%) and the mean age (± SD) at the time of BM examination was 32.4 ± 12.5 years. For 10 patients, BM examination was performed to investigate peripheral cytopenia or fever of unknown origin before a diagnosis of SLE was made. For the remainder, BM examination was performed after a diagnosis of SLE due to a suspicion of concurrent haematological malignancy. The patients' mean (± SD) SLEDAI score was 13.8 ± 8.3, which indicates that most were experiencing an active flare-up at the time of BM examination. In most cases, patient had anaemia with decreased reticulocyte (15/16, 93.8%). Thrombocytopenia and pancytopenia occurred in 11 and 4 cases, respectively. Only one patient had received immunosuppressive drugs; this patient was treated with oral cyclosporine due to refractory thrombocytopenia. Five patients had active nephritis at the time of BM biopsy and there was one case of death due to full-blown SLE two months after the test. The patients' clinical information is summerised in the Table 1.

Histologic features of the BM

Histological examination revealed six patients had normocellular BM (37.5%), eight had hypocellular BM (50.0%) and two had hypercellular BM (12.5%). Further examination of the specimens revealed reduced erythropoiesis in 10 (62.5%), and reduced myelopoiesis and megakaryopoiesis in 8 (50.0%) and 7 (43.8%), respectively. Dysmegakaryopoiesis, characterised by micromegakarocyte, hypolobulated nuclei and denuded cytoplasm with pyknotic nuclei was the most common feature in

Table 1. Clinical characteristics of the patients

Female	14 (87.5%)
Age at diagnosis	28.9 ± 11.7.
Age at bone marrow examination	32.4 ± 12.5
Reason for bone marrow examination	
Evaluation for cytopenia before the diagnosis of SLE	10 (62.5%)
Clinical suspicion about haematologic malignancy	6 (27.5%)
Clinical features	
Fever	7 (43.8%)
CNS [a)] symptom	1 (6.3%)
Nephritis	5 (31.3%)
Vasculitis	1 (6.3%)
Anaemia (Hb [b)] <12.0g/dl)	15 (93.8%)
Leukopenia (WBC [c)] < 4,000/mm^3)	9 (56.3%)
Thrombocytopenia (Platelet < 150,000/ mm^3)	11 (68.8%)
Pancytopenia	5 (31.3%)
Serositis	8 (50.0%)
SLEDAI score at bone marrow examination	13.8 ± 8.3
Laboratory and serological feature	
WBC (/mm^3)	4526 ± 3956
Reticulocyte (%)	1.52 ± 1.94
Hemoglobin (mg/dl)	9.26 ± 1.58
Platelet (x1,000/mm^3)	116.31 ± 81.90
Anti-dsDNA titer (IU/ml)	927.28 ± 2149.69 (max=7820.00)
C3 (mg/dl)	58.94 ± 35.09
C4 (mg/dl)	10.19 ± 7.11

Continuous value = mean ± standard deviations

a, central nervous system; b, hemoglobin; c, white blood cell

Table 2. Histological features of bone marrow specimens

Bone marrow cellularity	
Hypocellular	8 (50.0%)
Normocellular	6 (37.5%)
Hypercellular	2 (12.5%)
Decreased erythropoiesis	10 (62.5%)
Decreased myelopoiesis	8 (50.0%)
Decreased megakayopoiesis	7 (43.8%)
Dyserythropoiesis	2 (12.5%)
Dysmyelopoiesis	6 (37.5%)
Dysmegakaryopoiesis	8 (50.0%)
Bone marrow oedema	5 (31.3%)
Gelatinous transformation	4 (25.0%)
Necrosis	6 (37.5%)
Fibrous change	11 (68.8%)
Lymphocyte aggregate	3 (18.8%)
Haemophagocytosis	2 (12.5%)
High-grade BM damage [a)]	5 (31.3%)

a, defined as extensive BM necrosis (>50% of the biopsy) and/or severely decreased cellularity (<30%).

BM aspiration (8/16, 50.0%). Dysplastic feature in erythropoiesis and myelopoiesis was observed in 2 (12.5%) and 6 (37.5%) cases, respectively. Blast was not found in any cases.

In BM biopsy, prominent stromal change such as oedema, fibrosis and necrosis were observed in most of the specimens (12/16, 75.0%). Gelatinous transformation, which appears in the condition of severe malnutrition and weight loss, was relatively common features (4/16, 25.0%). Five specimens showed high-grade BM damage. Benign lymphoid cell hyperplasia was observed in four cases, but none showed lymphoid follicular formation. The histological features of the BM and the patients' clinical information are summarised in the Table 2.

Immune cell profiles in the BM

There were no significant differences in the percentages of CD3+ and CD8+ cells in the BM of SLE patients and controls. By contrast, CD4+ T cells were more common in the BM of SLE patients than in healthy controls (1.82 ± 1.45% *vs*. 0.26 ± 0.12%, p = 0.001) (Fig 1), as were CD68+ cells (16.35 ± 7.17% *vs.* 8.04 ± 1.38%, p=0.004) (Fig 2). The cells were distributed throughout the parenchyma and showed a macrophage-like morphology, characterised by a stellate-appearance and numerous cytoplasmic processes. The percentage of CD68+ cells correlated with that of CD4+ cells (r = 0.826, p < 0.001).

The percentage of CD20+ cells in the BM was lower in SLE patients than in controls (2.04 ± 1.30% *vs.* 4.48 ± 0.72%, p < 0.001); however, the percentage of CD138+ cells was higher (9.72 ± 5.64% *vs.* 3.44 ± 0.64%, p < 0.001) (Fig 3). CD138+ cells were more prominent in the perisinusoid area in SLE patients and were morphologically similar to plasma cells.

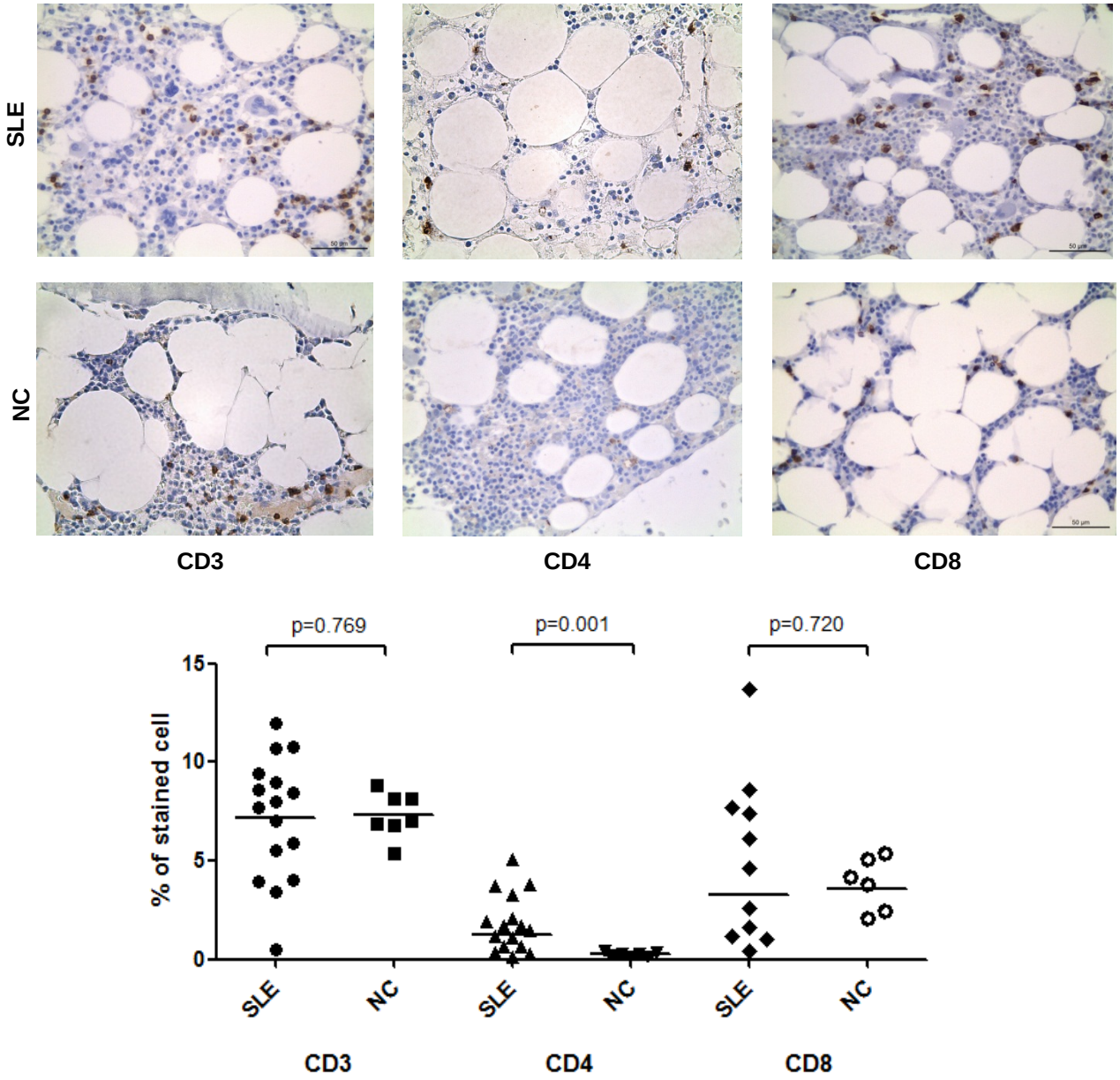

Figure 1. Cellular proportion of T cell lineage in BM of SLE. There is no difference in percentage of CD3+ cell and CD8+ cell. However, proportion of CD4+ cell was significantly increased in SLE group. All positively stained cells had morphology of lymphocyte.

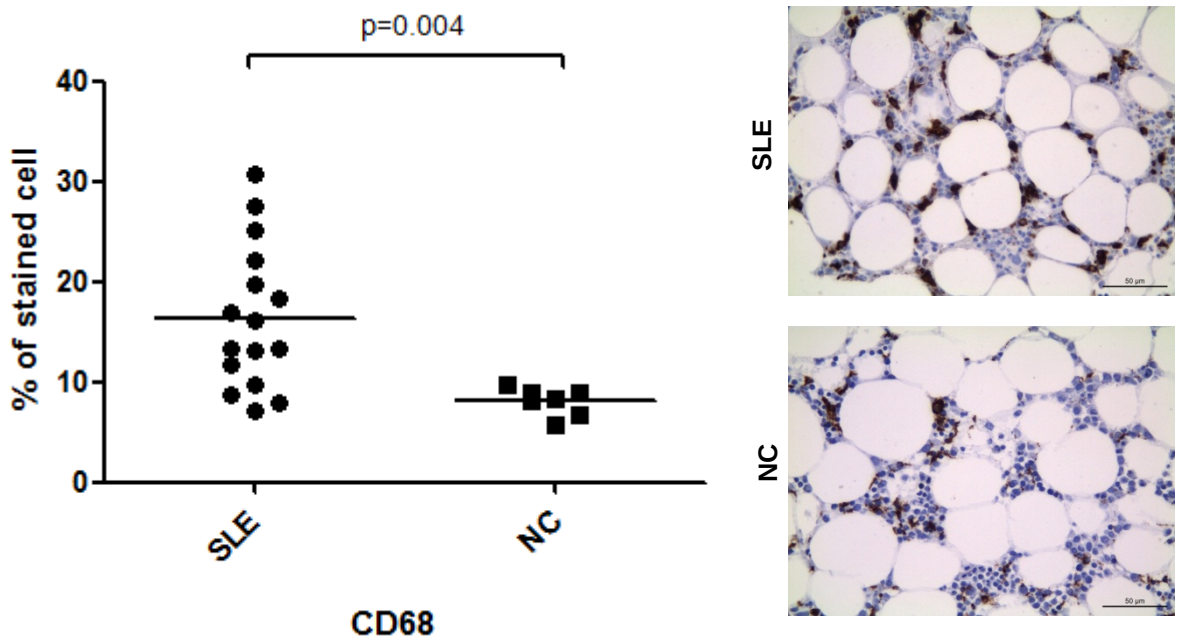

Figure 2. Cellular proportion of monocyte/macrophage in BM of SLE. CD68 positive cells revealed increased frequency in the bone marrow of SLE patient compared to that of normal control. In SLE group, Most CD68+ cells had morphology of macrophage, which is defined as strong cytoplasmic staining pattern with cytoplasmic process.

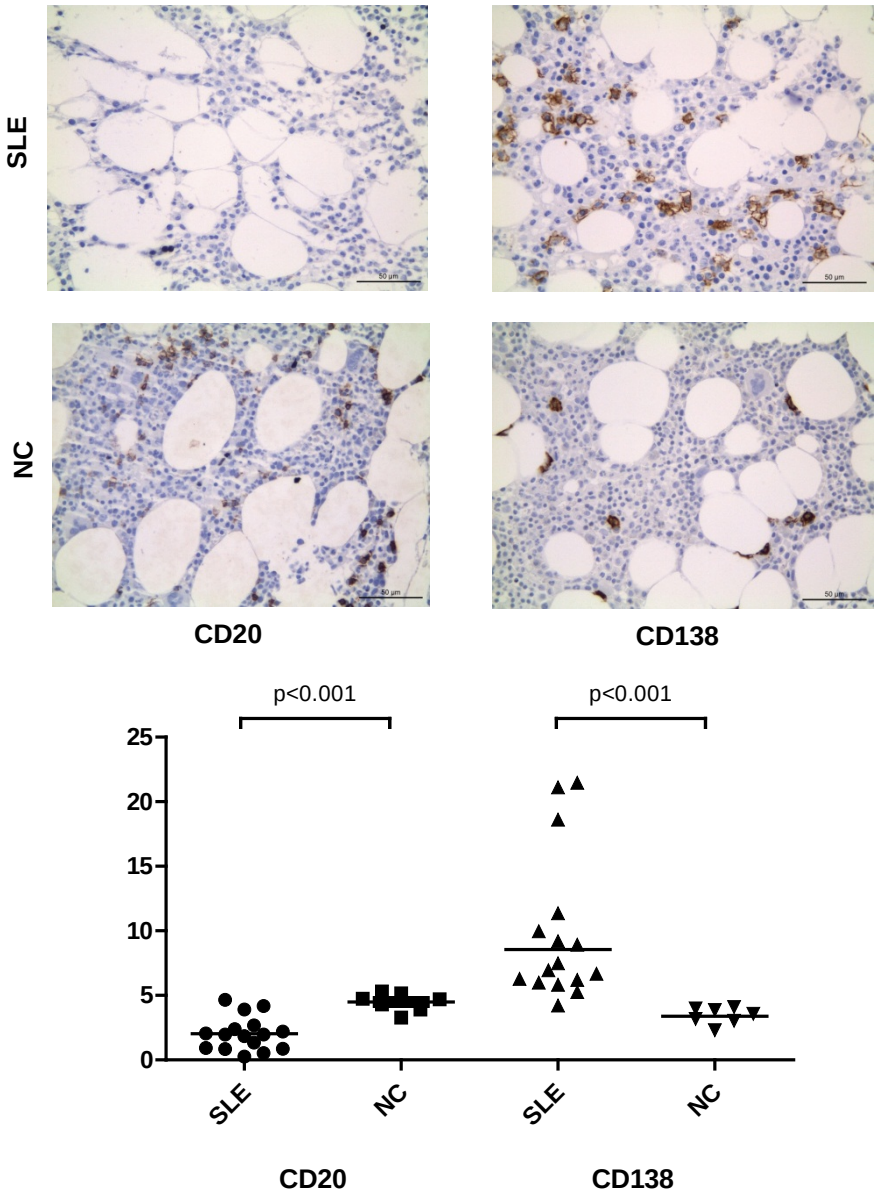

Figure 3. Cellular proportion of B cell lineage in BM of SLE. CD20+ cells showed lower frequency in the BM of SLE patient. In contrast, CD138+ cells were significantly increased in the SLE group. Their distribution showed interstitial and perisinusoidal pattern.

Relationship between immunohistochemical and clinicopathologic features

The percentages of CD4+ T cells and CD68+ macrophages in the BM were negatively correlated with BM cellularity (r=-0.518, p=0.040 and r=-0.549, p=0.028, respectively), which is an indirect representation of haematopoietic function. The five BM specimens showing high-grade damage also showed higher percentages of CD4+ T cells and CD68+ macrophages than the other 11 BM specimens of SLE patients (3.06 ± 1.50% *vs.* 1.26 ± 1.06% p = 0.038; 22.10 ± 7.19% *vs.* 13.09 ± 4.67%, p = 0.027, respectively). Also, patients with high-grade BM damage showed evidence of more severe thrombocytopenia as compared with other 11 SLE patient (62.60 ± 68.90×10^3/mm^3 *vs.* 140.73 ± 77.88×10^3/mm^3, p=0.027) (Fig 4). These results suggest that CD4+ T cells and macrophages may play a pivotal role in BM damage and subsequent thrombocytopenia.

Percentage of CD138+ plasma cell was not associated with BM destruction or abnormal peripheral blood cell count. However, it was significantly correlated with markers of disease activity, including anti-dsDNA antibodies and serum C4 levels (r=0.538, p=0.031 and r=-0.652, p=0.006, respectively). The SLEDAI scores also correlated with the percentage of plasma cells in the BM (r = 0.581, p = 0.031) (Fig 5). Interestingly, BM samples from five patients with active lupus nephritis contained more plasma cells than those from other patients without nephritis (18.28 ± 7.16% *vs.* 7.19 ± 2.16%, p=0.038). The levels of other autoantibodies, including anti-Ro/La, anti-Sm and anti-cardiolipin antibodies, were not correlated with the percentage of plasma cells in the BM.

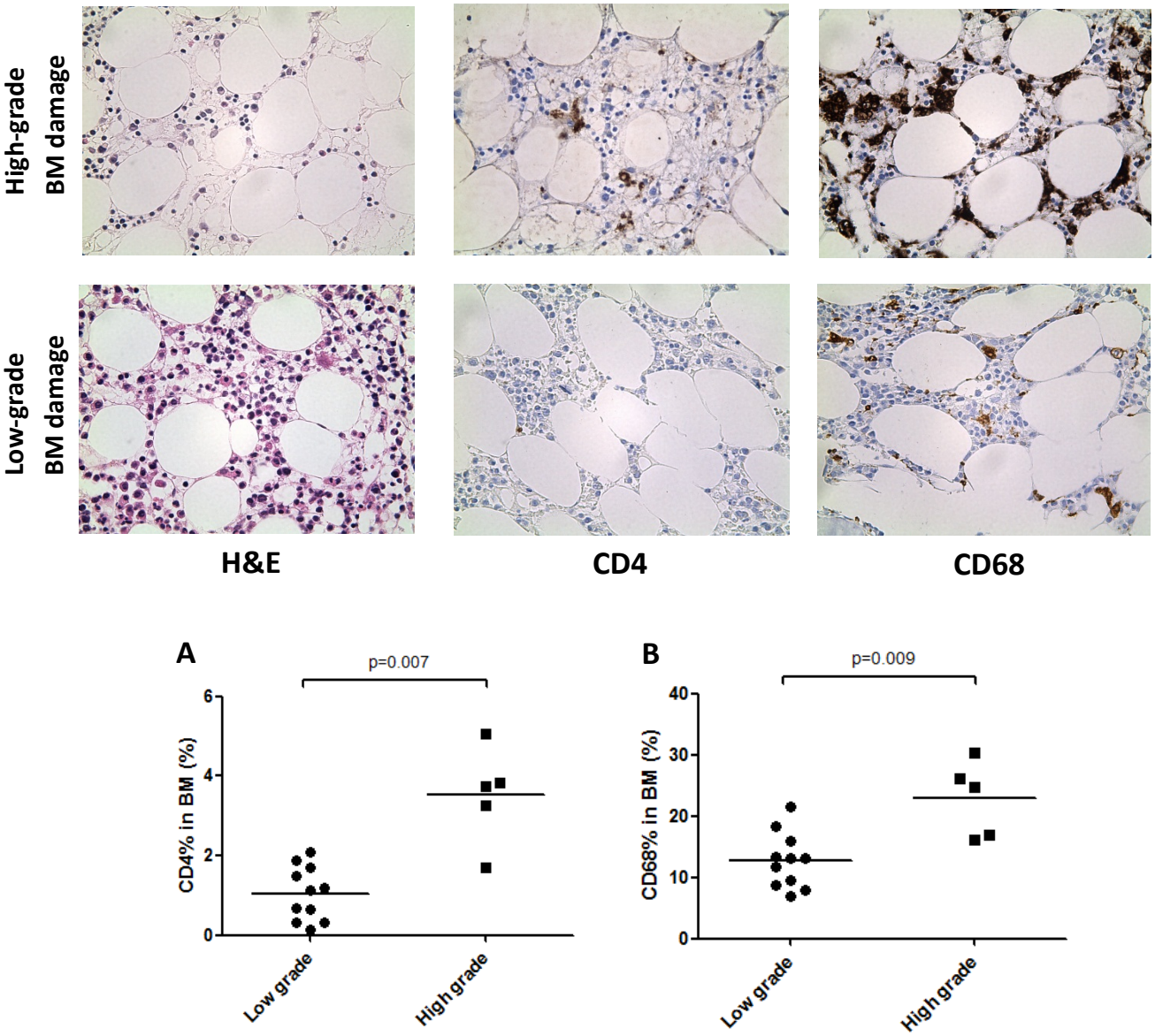

Figure 4. Relationship between organ damage and cellular component of immune cells in bone marrow of SLE. BM specimens with high-grade damage are significantly associated with more cellular infiltration of CD4+ T cells (A) and CD68+ macrophages (B).

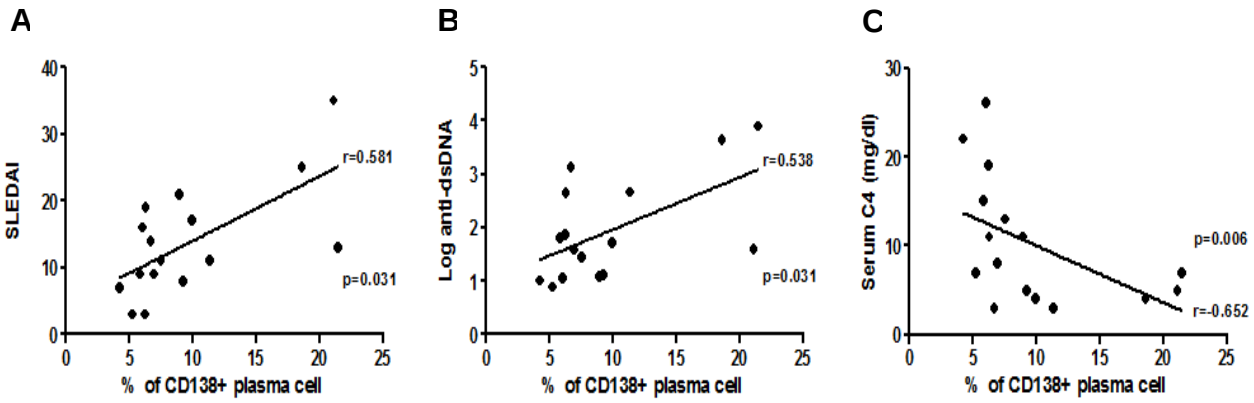

Figure 5. Correlation between percentage of bone marrow CD138+ plasma cell and disease activity in SLE; SLEDAI score (A), Log anti-dsDNA level (B) and serum C4 level (C).

Up-regulation of IL-6 expression in the BM of SLE patients

To investigate factors underlying the higher percentage of plasma cells in the BM of SLE patients, we stained the BM sections for IL-6. IL-6 expression was significantly higher in the SLE group; 12 (75%) SLE specimens showed grade 3 or 4 staining, whereas all the healthy control samples showed grade 0 or 1 ($p = 0.001$) (Fig 6). SLE patients with high IL-6 expression in the BM (≥grade 3) also had higher percentages of macrophages and plasma cells than those with low expression (18.12 ± 6.44% *vs.* 9.26 ± 1.99%, $p = 0.008$ and 11.09 ± 5.89% *vs.* 5.60 ± 1.16%, $p = 0.020$, respectively). This subgroup also showed higher anti-dsDNA levels (2.21 ± 0.96 *vs.* 1.13 ± 0.31, $p = 0.008$, expressed on a log scale) (Fig 7).

The BM of SLE patients contains higher numbers of plasmacytoid dendritic cells and shows higher levels of apoptosis

pDCs are a major source of type I interferon (IFN) and are activated by immune complexes or apoptotic cells in SLE patients. Therefore, I next examined the expression of active caspase-3 and BDCA-2 (both of which are specific markers for apoptotic cell and pDCs, respectively) in the BM.(18) Apoptotic cells were present in all BM specimens from SLE patients. They also appeared in specimens of healthy control, although their occurrence was very scarce. The percentage of apoptotic cells in the BM of SLE patients was significantly higher than that in healthy controls (2.47 ± 1.35% *vs.* 0.19 ± 0.22%, $p < 0.001$), as was the percentage of BDCA-2+ pDCs (4.60 ± 2.50% *vs.* 2.00 ± 1.32%, $p = 0.020$). There was a positive correlation between the percentage of BDCA-2+ cells in the BM and that of apoptotic cells ($r = 0.606$, $p = 0.013$) (Fig 8). The percentage of apoptotic cells in the BM was not correlated with that of other cell types, such as T lymphocytes, B lymphocytes and plasma cells, in BM but it was negatively correlated with serum C3 levels ($r = -0.618$, $p = 0.011$).

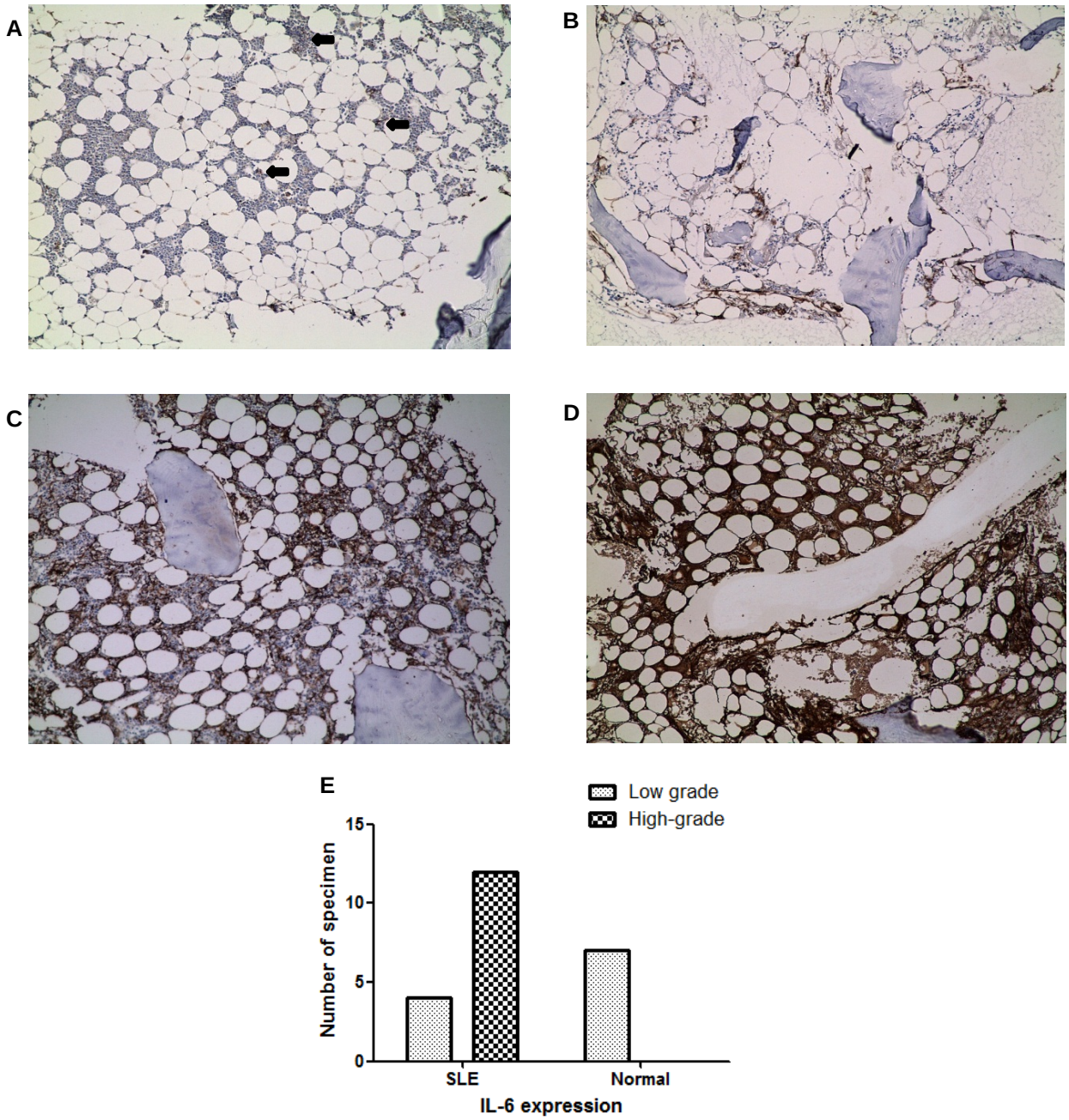

Figure 6. IL-6 expression in the BM is assessed with semiquantitative method. (A) Grade 1 (≤ 25% positively stained cellular area). (B) Grade 2 (26~50% positively stained cellular area). (C) Grade 3 (50~75% positively stained cellular area). (D) Grade 4 (>75% positively stained cellular area). All BM specimens of control group show low-grade IL-6 expression, whereas high-grade IL-6 expression exclusively appeared in SLE group (12/16).

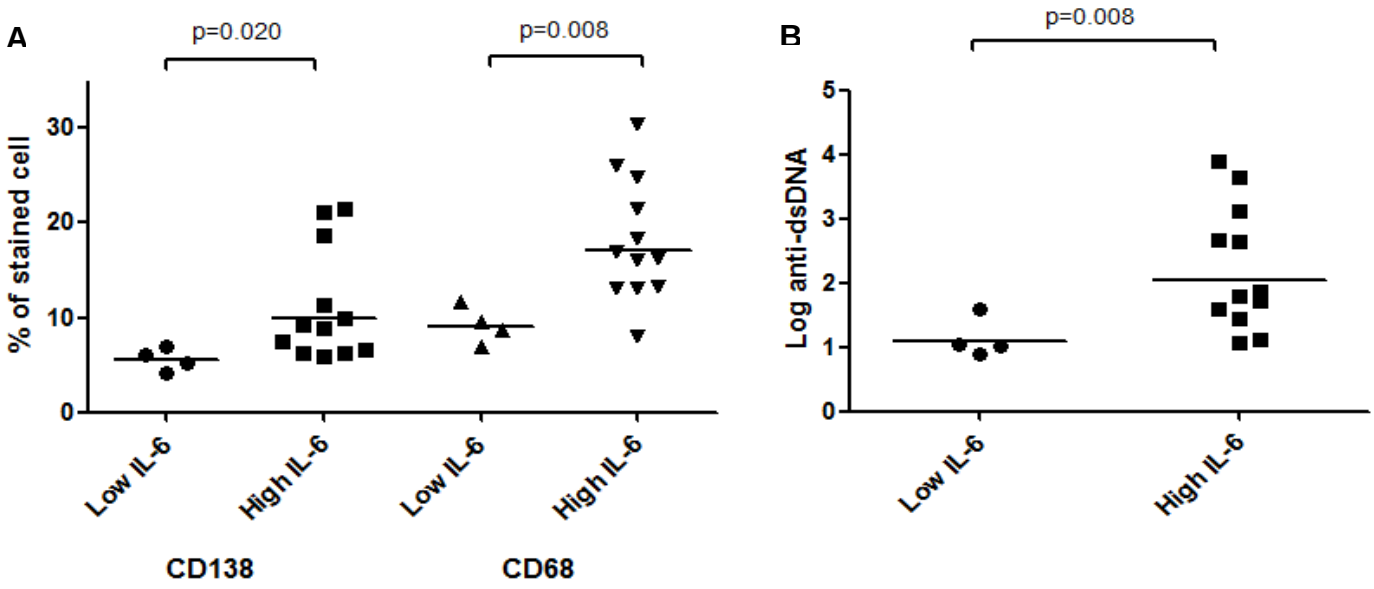

Figure 7. Correlation between IL-6 expression in BM and clinicopathologic features of SLE. High-grade IL-6 expression in BM is significantly associated with percentage of CD138+ plasma cells and CD68+ macrophages (A). And, SLE patients with high-grade IL-6 expression in BM have higher titer of serum anti-dsDNA antibody (B).

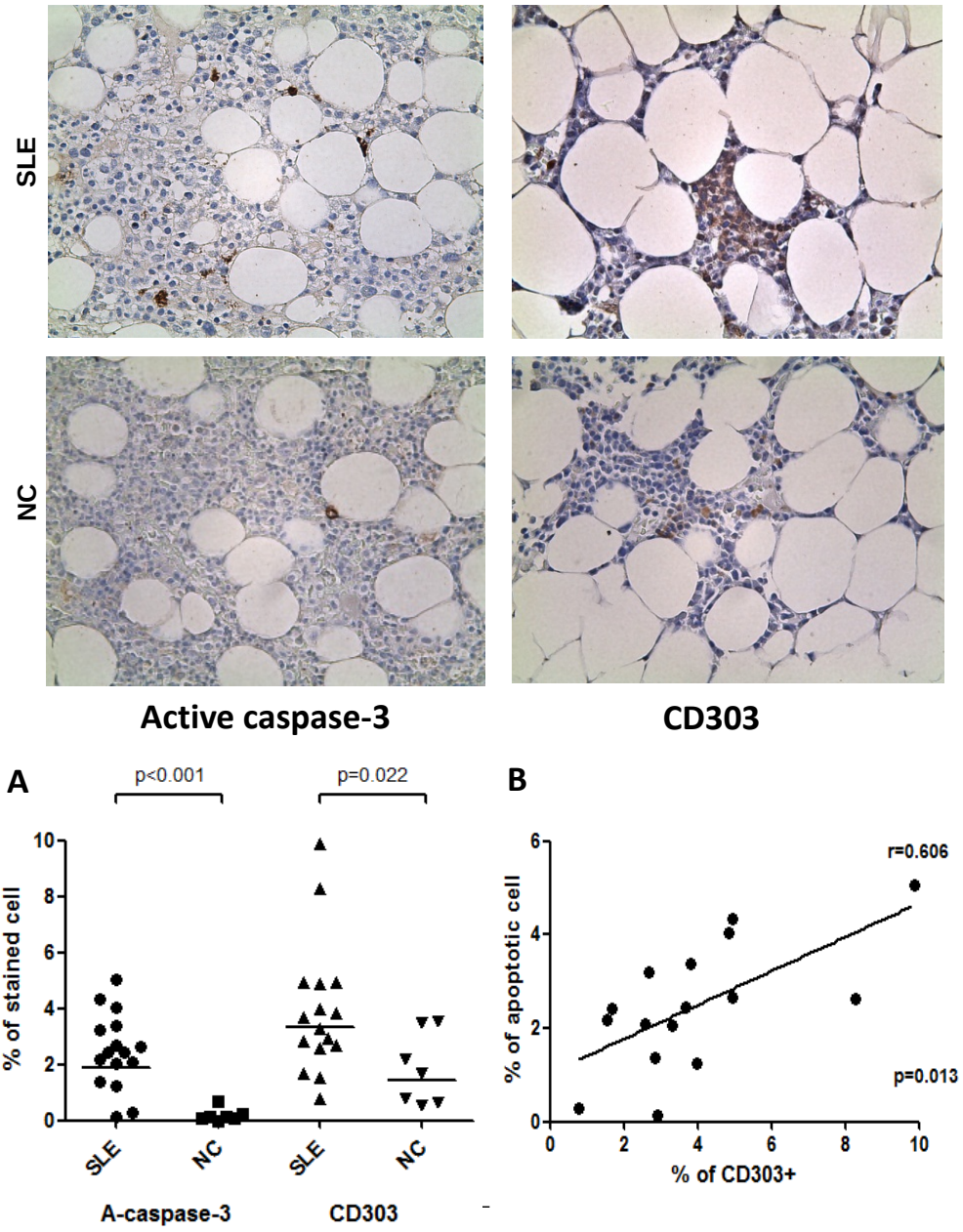

Figure 8. Expression of active-caspase3+ apoptotic cells and CD303 (BDCA-2)+ plasmacytoid dendritic cells (pDCs) in the bone marrow. Percentage of apoptotic cells and pDCs in bone marrow of SLE patient is higher than that of normal control (A). Furthermore, cellular proportions of these two cells have a significant correlation (B).

Discussion

The results of the present study showed that the immune cell profile in the BM of SLE patients was significantly different from that in healthy controls, and that there was an association between the immune cell profile and the clinical features of SLE. Increased numbers of CD4+ T cells and macrophages in the BM were related to more extensive BM damage, and increased BM plasma cell infiltration was significantly correlated with disease activity and the levels of anti-dsDNA antibodies. In addition, we found an association between an increase in the number of apoptotic cells and the presence of pDCs.

The immunohistochemical and clinicopathologic features of the target organ were mainly investigated in patients with lupus nephritis. Renal biopsy specimens from patients with lupus nephritis show infiltration of the renal interstitium by CD4+ lymphocytes, which is associated with a worsening of renal function.(19) In addition, samples from patients with more aggressive form of nephritis show evidence of glomerular and interstitial macrophage accumulation, which is the best clinical marker of renal activity.(20, 21) Likewise, I found that the percentages of CD4+ lymphocytes and CD68+ macrophages in the BM of SLE patients were significantly higher than those in the controls, and were closely associated with hypocellularity and high-grade organ destruction. Taken together, these results suggest that CD4+ lymphocytes and CD68+ macrophages may be central players in BM damage as they are in damage caused to other target organs in SLE patients.

The observation that the increased percentage of plasma cells in the BM of SLE patients was significantly correlated with anti-dsDNA levels is rather interesting. This result is consistent with previous pathologic studies of lupus nephritis; increased tubulointerstitial plasma cell number was associated with ds-DNA antibody titer and increased severity of the disease but it was not related to

activity of lupus nephritis.(22) The majority of long-lived plasma cells reside in the BM, where they produce antibodies for a long time in the absence of antigenic stimulation or T cell help.(23, 24) Long-lived plasma cells originate from proliferating plasmablasts in the spleen or lymph nodes, from where they migrate to the BM along a chemokine gradient.(25) After arriving in the BM, they must occupy a specific survival niche to mature into long-lived plasma cells. Some cytokines and adhesion molecules function as survival niches for BM plasma cells. IL-6, in conjunction with B-cell-activating factor (BAFF) and C-X-C motif chemokine 12 (CXCL12) expressed by stromal cells, provides a survival signal.(26, 27) In the present study, there is no case of lymphoid follicular formation and CD20+ lymphocytes were decreased in the BM of SLE patients. It suggests an increased migration of plasma cells from peripheral lymphoid organ. Because long-lived plasma cells in the BM are resistant to immunosuppressive agents and B cell depletion therapy, it is assumed that they are the main cause of refractory autoimmune disease.(28) Recently, Cheng Q et al. demonstrated that BM plasma cells continuously secreted anti-dsDNA antibodies and caused immune complex nephritis after they were transferred from NZB/W mice to Rag1-/- mice.(29) It is consistent with the results of the present study, which showed that the increased percentage of plasma cells in the BM of SLE patients correlated with increased anti-dsDNA antibody and serum complement levels, which are significantly associated with lupus nephritis. This indicates that increased numbers of plasma cells in the BM may trigger lupus by secreting anti-dsDNA antibodies; this increases the formation of anti-dsDNA-containing immune complexes, which then activate the complement system. Although I did not directly show that the plasma cells in the BM secreted autoantibodies, I did identify an association between BM plasma cell numbers and immune complex nephritis in SLE patients. In addition, the results indicate that increased expression of IL-6 in the BM of SLE patients may be an important factor for plasma cell survival and subsequent autoantibody production. IL-6 is the most efficient survival factor for BM plasma cells *in vitro*, and monocytes-macrophages are the main producers of the IL-6 required for plasma cell maturation in murine lymph nodes.(27, 30) Also, several studies have suggested the role of IL-6 in the pathogenesis of SLE. For example, IL-6-deficient MRL-*Fas*^{lpr}

mice showed delayed onset of proteinuria, decreased deposition of immune complex in glomerulus and reduced infiltration of lymphocyte and macrophage infiltration.(31) In NZB/NZW lupus mice, exogenous IL-6 increased autoantibody production from B cells and blocking of IL-6 receptor by monoclonal antibody reduced such autoantibodies.(32, 33) As in murine SLE model, lupus patients had elevated serum IL-6 level, which was correlated with disease activity and anti-dsDNA levels in some studies.(34) In addition, tocilizumab, humanised monoclonal antibody IL-6R, treatment in SLE patients decreased peripheral plasma cell frequency and leaded to improvement of the disease.(35) Although little is known about the cellular source of cytokines that promote plasma cell survival in humans, I found that IL-6 expression was significantly associated with the number of macrophages in the BM of SLE patients.

Dysregulated apoptosis can generate nuclear autoantigens, which act as important triggers for autoimmunity. I identified an increase in the number of apoptotic cells in the BM of SLE patients, which is consistent with previous report.(14) However, I also identified an important association between the degree of apoptosis and an increase in the number of pDCs in the BM of SLE patients. pDCs are a major producer of type I IFN, which plays an important role in the pathogenesis of SLE.(36, 37) In normal condition, production of type I IFN is triggered by exogenous stimuli, such as viral pathogen or to endogenous self-nucleic acid and nucleic-acid containing immune complex via TLR-dependent or independent pathway. This cytokine have broad effects on overall immune system. Exposure of cells to type I IFN induces antiviral state for the prevention of additional viral infection. This cytokine also activates classical dendritic cells, and promotes activation of lymphocytes. Thus, type I IFN has been proposed to have a pivotal role that serves as a link between innate and adoptive immunity. In SLE, pDCs recognise immune complexes containing self-nucleic acids and then produce type I IFN.(38, 39) Lovgren et al. demonstrated that late apoptotic cells release nuclear material, which induces pDCs to secrete IFN-alpha.(40) SLE patients show elevated levels of circulating apoptotic cells, which are cleared very slowly. As a result, pDCs continuously produce type I IFN.

This activates conventional DCs, which in turn stimulate autoimmune T cells.(41) Patients with lupus nephritis show a marked increase in glomerular infiltration by pDCs, which is not observed in normal kidneys. Such infiltration is prominent in the kidneys of patients with active class III/IV lupus nephritis. Likewise, the results reported herein suggest that increased apoptosis and increased numbers of pDCs in the BM of SLE patients may promote T cell-medicated BM damage via the unabated production of type I IFN.

There have been a few reports about histologic features of BM of SLE patients and their results have been inconsistent. Decreased cellularity, reticulin fibrosis and necrosis were common findings.(6, 7, 9) In contrast, Chen JL et al evaluated morphologic characteristics of 72 SLE BM and observed normal BM feature in about half of specimens.(8) Among these studies, only few investigated the relationship between BM histology and clinical features of SLE. Oka et al examined BM aspirates from 17 SLE patients and reported that reversible BM dysplasia appeared to be related with disease activity.(42) Voulgarelis et al, in their study about histologic features of 40 SLE BM, found that hemoglobin level inversely correlated with the presence of abnormal localization of immature precursors.(5) However, previous studies have not focused on the association between cellular composition of BM and clinical feature of SLE patients. Recently, Hunt et al identified morphologic and immunohistochemical abnormalities in 25 BM specimens of SLE patients.(43) They showed normal appearance and mean number of lymphocytes and histiocytes in BM were also same. However, this study did not show any information about disease activity of enrolled patients and mean blood cell count of SLE patients was normal. Therefore, it is possible that ‘mild’ SLE patients constituted the majority of study group and this result may not be able to represent BM characteristics of SLE. In the present study, immune cell profile in the BM of SLE patients, which was clearly different from that of control group, was consistent in most specimens and closely correlated with clinical features. Moreover, multiple apoptotic cells and concomitant increase of pDCs correspond with known pathogenesis of SLE. To sum it up, these results demonstrated that BM may be an important target organ in SLE and same

pathologic process in other target organs are also applied in BM.

This study has several limitations. First, the number of SLE BM specimens was small, and most patients showed haematological manifestations at the time of BM examination. Therefore, it is uncertain whether the pathological mechanism suggested by this study is relevant to other SLE patients, particularly those without haematological abnormalities. Same immunohistochemical analyses of BM from more SLE patients with various clinical manifestations may be required for consolidation of my results. Second, the results are based on *in situ* analyses rather than on functional studies. However, previous studies (both *in vitro* studies and animal studies) support my conclusion that the BM is not only a major target organ in SLE, but also plays an important role in disease pathogenesis by acting as a source of anti-dsDNA antibodies and allowing prolonged exposure to apoptosis-induced autoantigens.

In conclusion, the results reported herein suggest that the BM may play two critical roles in the pathogenesis of SLE. First, it is a target organ that is damaged by CD4+ T cells and macrophages, resulting in an increase in the number of apoptotic cells (a process promoted by pDCs); second, BM plasma cells, supported by IL-6, generate anti-dsDNA antibodies, which increase systemic manifestations such as nephritis.

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국문 초록

서론: 전신홍반 루프스에서 골수 내 면역세포 및 세포 자살이 질병의 병태생리에 어떠한 영향을 끼치는 지에 대해서는 명확히 알려지지 않았다. 본 연구에서는 전신홍반 루프스 환자의 골수 내 면역세포 및 사멸세포의 비율이 정상 골수 소견과 어떠한 차이를 보이는 지 보고, 이러한 소견과 질병의 임상 양상 간의 관계를 알아보았다.

방법: 14 명의 전신홍반 루프스 환자에서 얻은 16 개의 골수 조직에 대해서 면역 조직화학 염색을 시행하여 T 림프구, B 림프구, 대식세포, 형질세포, 형질세포양 수지상 세포, 사멸 세포의 비율을 파악하여 이를 7 개의 정상 골수 조직 소견과 비교하였다. 골수 검사를 시행할 당시의 환자의 임상 양상은 후향적 의무기록 분석을 통해 파악하였다.

결과: CD4+ T 림프구와 대식세포, 형질세포는 전신홍반 루프스 환자의 골수에서 빈도가 증가한 양상을 보였다 (1.82±1.45% vs. 0.26±0.12%, p=0.001; 16.35±7.17% vs. 8.04±1.38%, p=0.004; and 9.72±5.64% vs. 3.44±0.64%, p<0.001, respectively). 많은 수의 골수 내 CD4+ T 림프구와 대식세포의 분포는 심한 골수의 괴사 소견과 유의한 연관 관계를 보였다. 전신홍반 루프스 환자의 골수 내 사멸세포의 비율은 정상인의 골수에 비해서 현저히 증가한 소견을 보였고 (2.47±1.35% vs. 0.19±0.22%, p<0.001), 이는 골수 내 형질세포양 수지상세포의 비율과 유의한 양의 상관 관계를 보였다 (r=0.606, p=0.013). 전신홍반 루프스 환자에서 증가한 골수 내 형질세포의 비율과 높은 IL-6 의 발현은 항 이중가닥 DNA 항체의 수치 및 SLEDAI 점수와 유의한 양의 상관 관계를 보였다 (r=0.538, p=0.031 and r=0.581, p=0.013, respectively).

결론: 전신홍반 루프스 환자의 골수 내 면역 세포 및 사멸 세포는 정상 소견과 다르며, 질병의 임상 양상과 밀접한 관련을 보인다. 이는 전신홍반 루프스에서 골수가 질병의 병태생리에 중요한 역할을 한다는 것을 시사한다.

주요어: 전신홍반 루프스, 골수, 세포자살

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