Brief report

Impact of macrophage infiltration of skin lesions on survival after allogeneic stem cell transplantation: a clue to refractory graft-versus-host disease

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We retrospectively reviewed 104 biopsy specimens of previously untreated skin acute graft-versus-host disease (GVHD) within 100 days after allogeneic stem cell transplantation, and analyzed the relationship between types of infiltrating cells and clinical outcomes. Counting the total number of CD8$^+$ T cells, CD163$^+$ macrophages, and CD1a$^+$ dendritic cells in 4 fields under original magnification ×200, the infiltration of more than 200 cells of CD163$^+$ macrophages (many macrophages [MM]) was the only significant predictor for refractory GHVD (odds ratio, 3.79; 95% confidence interval, 1.22-11.8; $P = .02$). In 46 patients given steroid treatments, MM was the only significant predictor for refractory acute GVHD (odds ratio, 5.05; 95% confidence interval, 1.19-21.3; $P = .03$). Overall survival of patients with MM was significantly lower than that of those with an infiltration of less than 200 cells of CD163$^+$ macrophages. Macrophage infiltration of skin lesions could be a significant predictive factor for refractory GVHD and a poor prognosis. (Blood. 2009;114: 3113-3116)

Introduction

Macrophages are phagocytic cells with various abilities, such as phagocytosis, antigen-presenting, and secretion of cytokines.[1,2] Recently, it was revealed in human sequential biopsy data that recipient macrophages contributed to acute graft-versus-host disease (GVHD) by antigen-presenting and secreting cytokines, causing the activation and proliferation of CD8$^+$ T cells.[3] We focused on macrophage involvement in acute GVHD, especially on the relationship between the macrophage infiltration of skin lesions and refractory GVHD.

Methods

Between January 1997 and October 2007 at the Japanese Red Cross Nagoya First Hospital, we used skin biopsy specimens within 100 days after allogeneic stem cell transplantation (allo-SCT) of skin lesions clinically considered acute GVHD without any GVHD treatment from 104 patients who underwent allo-SCTs. We analyzed the relationship between types of infiltrating cells and clinical outcomes by counting the total number of CD8$^+$ T cells, CD163$^+$ macrophages, and CD1a$^+$ dendritic cells in 4 fields of a skin biopsy specimen under original magnification ×200. Immunohistochemical analysis using paraffin sections was performed using monoclonal antibodies against CD8, CD163, and CD1a (Novocastra). CD163 is a member of the scavenger receptor cystein-rich superfamily and is an exclusive marker for macrophages, playing a major role in the scavenging components of damaged cells.[4-7]

The endpoints of this study were the outcomes of acute GVHD and overall survival (OS). Acute GVHD was diagnosed and graded according to the consensus criteria.[8] We defined refractory GVHD as that exhibited by patients who had persistent lesions after primary steroid treatments. To establish parameters, we analyzed the numbers of infiltrating CD8$^+$ T cells ($\leq$ 100/4 fields [few T cells; FT] vs $>$ 100/4 fields [many T cells; MT]), numbers of infiltrating CD163$^+$ macrophages ($\leq$ 200/4 fields [few macrophages; FM] vs $>$ 200/4 fields [many macrophages; MM]), disease risk (low vs high), human leukocyte antigen (HLA) disparity (match vs mismatch), donor source (related vs unrelated), graft source (bone marrow vs peripheral blood), age at allo-SCT ($\leq$ 50 years vs $>$ 50 years), conditioning regimen (conventional regimens vs reduced intensity regimens), and skin GVHD stage at biopsy (stages 1-2 vs stages 3-4). A significance level of $P < .05$ was used for all analyses, which were based on all data available as of August 31, 2008. Protocols were approved by the Japanese Red Cross Nagoya First Hospital's Institutional Review Board, and all patients provided informed consent in accordance with the Declaration of Helsinki.

Results and discussion

Table 1 summarizes the characteristics of patients and information gathered about GVHD. We divided patients into 4 groups according to the amount of infiltrating cells (FM and FT, 60.6%; MT and FM, 18.2%; MT and MM, 10.6%; and FT and MM, 10.6%). We noted a striking difference among patients in the types of infiltrating cells in skin GVHD lesions (Figure 1A). The distributions of numbers of infiltrating cells also exhibited a

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Table 1. Information on patient characteristics, acute GVHD, and skin biopsy

Characteristic	Value
Patient characteristics	
Total no. of patients	104
Median age at allo-SCT, y (range)	40.5 (19-61)
Male/female	65/39
Disease risk, low/high	51/53
HLA, match/mismatch	72/32
Donor, unrelated/related	67/37
Graft, BM/PB/CB	89/11/4
Conditioning, conventional/RIST	78/26
Median observation period, mo (range)	13.7 (0.7-120.7)
Acute GVHD	
Stage skin (at the time of biopsy), 1/2/3/4	22/57/25/0
Skin (the maximal severity), 1/2/3/4	16/25/52/11
Gut, 0/1/2/3/4	69/9/8/15/3
Liver, 0/1/2/3/4	82/5/3/9/5
Grade (at the time of the biopsy), I/II/III/IV	58/41/4/1
Grade (the maximal severity), I/II/III/IV	28/44/19/13
Primary steroid treatment, yes/no	46/58
Second treatment, yes/no	18/30
Outcome of GVHD, improved/refractory	84/20
Skin lesion	
Median date of appearance, days (range)	24 (5-81)
Median date of skin biopsy, days (range)	31.5 (6-82)
Median date of highest stage of skin GVHD, days (range)	34 (9-90)
No. of infiltrating $CD8^+$ cells	65 (2-305)
No. of infiltrating $CD163^+$ cells	132.5 (38-372)
No. of infiltrating $CD1a^+$ cells	7 (0-122)

Disease risk low indicates acute leukemia in first remission; CML, in first chronic phase; MDS, refractory anemia or nonmalignant hematologic disease; disease risk high, all other diagnoses; HLA match, identical HLA-A, -B, and -DRB1 loci; HLA mismatch, at least one disparity at one of these loci; BM, bone marrow; PB, peripheral blood; CB, cord blood; and RIST, reduced intensity conditioning regimens.

considerably wide variety (Figure 1B). The median number of infiltrating $CD8^+$ T cells was 65 (range, 2-305), that of infiltrating $CD163^+$ macrophages was 132.5 (range, 38-372), and thatof infiltrating $CD1a^+$ dendritic cells was 7 (range, 0-122). We used 3 skin biopsy specimens of drug rash from autologous transplantation patients as non-GVHD controls; the median numbers of $CD8^+$, $CD163^+$, and $CD1a^+$ infiltrating cells were 11 (range, 6-15), 26 (range, 19-30), and 68 (range, 65-83), respectively. MT was correlated with an HLA mismatch ($P = .047$), grade III-IV acute GVHD ($P = .03$), and MM ($P = .01$), whereas MM was correlated with unrelated donor ($P = .04$), an HLA mismatch ($P = .049$), refractory GVHD ($P = .004$), and MT ($P = .01$) using χ^2 analyses. The sensitivity and specificity of MT for refractory GVHD were 25.0% and 70.5% in all 104 patients, and 25.0% and 73.3% in 46 receiving steroids, whereas those of MM were 43.8% and 82.9%, and 43.8% and 86.7%, respectively.

In 46 patients undergoing steroid treatments, the median date of the appearance of skin lesions was 17.0 days (range, 5-54 days), whereas that of skin biopsy was 27.5 days (range, 6-63 days) and that of the highest skin stage was 32.0 days (range, 9-68 days). Treatments for GVHD were considered for GVHD patients without spontaneous regression and with progression to a higher grade, except for those in which enhanced immunosuppression would not be preferable, such as encephalopathy resulting from calcineurin inhibitor or a pathologic diagnosis of intestinal transplantation-associated microangiopathy.[9,10] The median interval from the initial clinical manifestation of GVHD to the primary treatments was 4.5 days (range, 0-97 days). The dose of prednisolone was 0.5 mg/kg in 4 patients, 1 mg/kg in 20 patients, 2 mg/kg in 19 patients, 500 mg/body in 1 patient, and 1000 mg/body in 2 patients. Only MM was identified as a negative predictive factor for refractory GVHD (Table 2). In 46 patients undergoing steroid treatments, only MM was identified as a negative predictive factor for refractory GVHD (odds ratio, 5.05; 95% confidence interval [CI], 1.19-21.3; $P = .03$).

In the Cox proportional hazard model, age more than 50 years, high risk, and MM were identified as significant risk factors by univariate analyses, with MM and high risk remaining a significant risk in a multivariate analysis (Table 3). OS rates were significantly higher in FM patients compared with those in MM (Figure 1C). Uncontrolled GVHD was the cause of 6 MM patients of 11 (54.5%) who died because of transplantation-related mortality (TRM), whereas in FM patients, 3 of 24 (12.5%) died because of uncontrolled GVHD. The causes of death for the 5 MM patients who did not die of uncontrolled GVHD were infection in 3 patients, intestinal transplantation-associated microangiopathy in 1 patient, and liver failure in 1 patient. In 46 patients who underwent steroid treatments, only MM was identified as a significant risk factor in the Cox proportional hazards model (Hazard ratio 3.25; 95% CI, 1.46-7.26; $P = .004$). OS rates were significantly higher in FM patients compared with those in MM (Figure 1D). Uncontrolled GVHD was the cause of 6 MM patients of 9 (66.7%) who died because of TRM, whereas in FM patients, 3 of 15 (20.0%) died because of uncontrolled GVHD.

Our study suggested that macrophages are involved in a specific type of acute GVHD that tended to be systemic and refractory to conventional therapies, such as corticosteroids or calcineurin inhibitors.[11-13] Differences in treatment efficacy could be explained by the difference in infiltrating cell types. Although efforts have been made at predicting refractory GVHD,[14-17] no confirmed factor has been established to date. Our findings could prove to be a relatively simple and useful method directly related to the prognosis of patients.

Macrophages could not be completely suppressed by current therapies for acute GVHD mainly targeting T cells. Considered together with

Table 2. Analyses of predictive factors for refractory GVHD in all 104 patients

Parameter	Odds ratio (95% CI)	*P*
More than 50 y old	1.21 (0.35-4.19)	.76
High disease risk	1.29 (0.44-3.76)	.65
Graft PB (vs BM)	1.19 (0.23-6.11)	.83
Unrelated donor	0.91 (0.30-2.73)	.86
HLA mismatch	1.96 (0.66-5.84)	.23
Conventional regimens	0.94 (0.27-3.23)	.92
Skin stage 3 or 4 at biopsy	1.97 (0.69-5.68)	.21
MT (> 100 $CD8^+$ cells)	1.26 (0.37-4.27)	.71
MM (> 200 $CD163^+$ cells)	3.79 (1.22-11.8)	.02

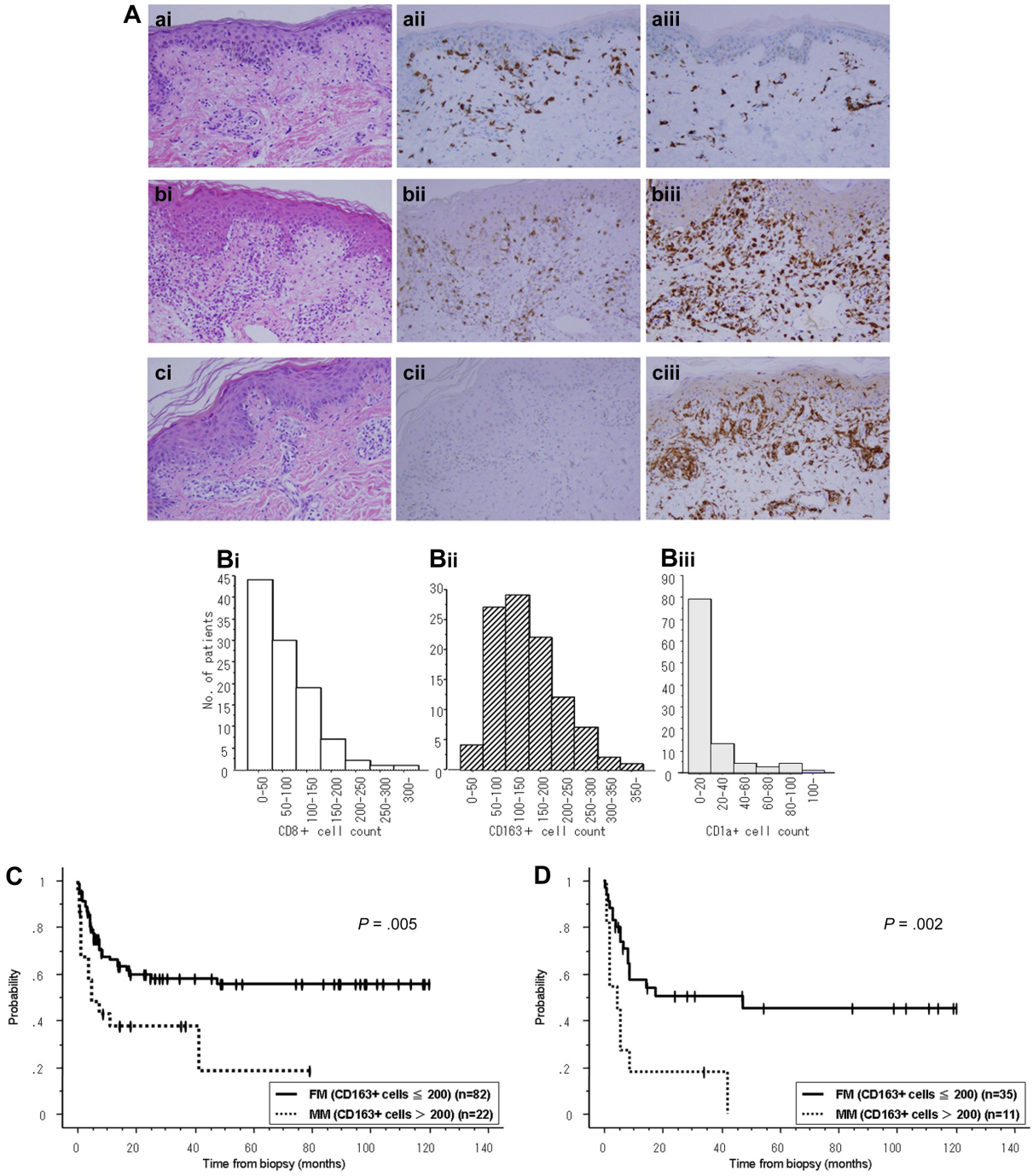

Figure 1. Skin biopsy specimens, infiltrating cells, and overall survival. Immunohistochemical analysis of representative skin biopsy specimen (A), the cell count distribution of $CD8^+$, $CD163^+$, and $CD1a^+$ cells (B), and the impact of MM on OS (C-D). (A) Tissue sections of skin biopsy were stained with hematoxylin and eosin (ai,bi,ci), or antibodies to $CD8^+$ (aii, bii, cii), or $CD163^+$ (aiii, biii, ciii) as detailed in "Methods." Shown are representative specimens of an MT/FM patient (ai-iii), a MT/MM patient (bi-iii), and an FT/MM patient (ci-iii). Original magnifications ×200. (B) Distribution of infiltrating cell counts (Bi, $CD8^+$; Bii, CD163; and Biii, CD1a). (C) OS according to $CD163^+$ cell counts (≤ 200 [FM] vs > 200 [MM]) in all patients. OS of patients with MM was significantly lower than that of those with FM (FM: 66.2% ± 10.6% at 1 year and 58.3% ± 11.4% at 3 years, MM: 37.8% ± 21.0% at 1 year and 37.8% ± 21.0% at 3 years, respectively; $P = .005$). (D) OS according to $CD163^+$ cell counts (≤ 200 vs > 200) in 46 patients undergoing steroid treatments. OS of patients with MM was significantly lower than that of those with FM (FM: 57.7% ± 17.1% at 1 year and 50.7% ± 17.4% at 3 years; MM: 18.2% ± 22.7% at 1 year and 18.2% ± 22.7% at 3 years, respectively; $P = .002$).

the fact that skin biopsies can be performed safely without any critical complications, our results support the importance of conducting skin biopsies of posttransplantation skin lesions. In cases where macrophage-dominant infiltration is observed in a skin biopsy specimen, macrophage-targeted therapies[18-23] could provide a clue to refractory GVHD.

In conclusion, macrophage infiltration of skin lesions after allo-SCT was shown to be a significant predictive factor for

Table 3. Analyses of risk factors for OS in all 104 patients

	Univariate		Multivariate	
Parameter	**Hazard ratio (95% CI)**	***P***	**Hazard ratio (95% CI)**	***P***
Older than 50 y	2.27 (1.23-4.20)	.009	—	—
High disease risk	1.87 (1.03-3.40)	.04	1.92 (1.06-3.50)	.03
Graft PB (vs BM)	2.04 (0.91-4.61)	.08	—	—
Unrelated donor	0.71 (0.40-1.29)	.26	—	—
HLA mismatch	1.13 (0.61-2.10)	.70	—	—
Conventional regimens	0.74 (0.39-1.39)	.35	—	—
Skin stage 3 or 4 at biopsy	1.11 (0.57-2.15)	.76	—	—
MT (> 100 $CD8^+$ cells)	1.10 (0.59-2.08)	.76	—	—
MM (> 200 $CD163^+$ cells)	2.38 (1.27-4.49)	.006	2.45 (1.30-4.61)	.006

— indicates not applicable.

refractory GVHD, as well as being a negative prognostic factor for OS. Our results indicate the importance of skin biopsies after allo-SCT and suggest the possibility of developing infiltrating cell-based strategies.

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Authorship

Contribution: S.N., S.T., M.I., and K.M. designed the research; S.N., S.T., and M.I. analyzed and interpreted the data; S.N. and S.T. performed statistical analysis; and S.N., T.G., A.S., K.W., M.Y., N.I., S.T., M.S., Y.O., M.I., and K.M. collected clinical data and wrote the paper.

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