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 GVHD (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 (≤100/4 fields [few T cells; FT] vs >100/4 fields [many T cells; MT]), numbers of infiltrating CD163+ macrophages (≤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 (≤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
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 that of 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 χ² 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 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).

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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Total no. of patients</td>
<td>104</td>
</tr>
<tr>
<td>Median age at allo-SCT, y (range)</td>
<td>40.5 (19-61)</td>
</tr>
<tr>
<td>Male/female</td>
<td>65/39</td>
</tr>
<tr>
<td>Disease risk, low/high</td>
<td>51/53</td>
</tr>
<tr>
<td>HLA, match/mismatch</td>
<td>72/32</td>
</tr>
<tr>
<td>Donor, unrelated/related</td>
<td>67/37</td>
</tr>
<tr>
<td>Graft, BM/PB/CB</td>
<td>89/11/4</td>
</tr>
<tr>
<td>Conditioning, conventional/RIST</td>
<td>78/26</td>
</tr>
<tr>
<td>Median date of highest stage of skin biopsy (range)</td>
<td>34 (9-90)</td>
</tr>
<tr>
<td>Median observation period, mo (range)</td>
<td>31.5 (6-82)</td>
</tr>
</tbody>
</table>

Infiltrating CD1a

The median number of 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 χ² 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.

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

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

Acknowledgments
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References

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