Interferon Gamma/IL10 Ratio Production in Response to Host Antigens may Predict Acute Graft Versus Host Disease after Allogeneic Stem Cell Transplantation from a Sibling

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Abstract

Background: Graft versus host disease (GVHD) continues to be the main concern of transplanter. Cytokines’ contribution to its pathogenesis has been widely addressed in the literature. Previous studies have mainly evaluated the cytokine’s level after the actual occurrence of GVHD. In this work, we investigated the possibility of predicting the occurrence of acute GVHD through an experimental setup to mimic the response of the immune cells of the graft to host antigens expressed by interferon gamma (IFNγ) and interleukin 10 (IL10) production.

Methods: The study included 45 patients receiving allogeneic HSCT from an identical sibling. An aliquot from the graft prior to infusion was used for in vitro culture for 3 days with patients’ mitomycin treated mononuclear cells. IFNγ and IL10 were measured in culture supernatant using microbead array technology.

Results: Acute GVHD was encountered in 14 cases. IFNγ was detectable in the culture supernatant of 9/14 (64.3%) cases with GVHD at a level of 6.2 - 19.000 with a median of 159.3 pg/ml versus 3/31 (9.6%) cases without GVHD at a level of 1.1, 8.1 and 80.01 pg/ml (p<0.001). IL10 was detectable in the culture supernatant of 7/14 (50%) cases with GVHD at a level of 9.5 – 85.5 with a median of 128 pg/ml versus 6/31 (19.3%) cases without GVHD at a level of 14.0 – 359.0 with a median of 45.39 pg/ml (p<0.05). At a cutoff of 1.13, IFNγ/IL10 ratio could predict GVHD with a sensitivity of 85.7%, specificity of 83.3% and a total accuracy of 84.6%.

Conclusion: In vitro cytokine production by graft immune cells in response to host antigens is extremely variable. IFNγ production apparently reflects potential development of acute GVHD while IL10 production is apparently protective. When both are produced the IFNγ/IL10 ratio is more informative than either alone.

Keywords: GVHD; HSCT; IFNγ; IL10; Cytokines

Abbreviations: HSCT: Hematopoietic Stem Cell Transplantation; GVHD: Graft Versus Host Disease; IFN γ: Interferon Gamma; IL10: Interleukin 10; BMT: Bone Marrow Transplantation; PBSC: Peripheral Blood Stem Cell Transplantation; MHC: Major Histocompatibility. IL2: Interleukin 2; TGF β: Transforming Growth Factor β; Th: T Helper

Introduction

Stem cell transplantation is a widely used therapeutic modality [1]. Graft versus host disease (GVHD) continues to be the main concern of transplanter [1-3]. If not, so far, preventable, prediction of its occurrence will help proper management and reducing its severity.

The conditioning regimen with radiation and chemotherapy produces host tissue injury leading to release of chemokines and cytokines and the host dendritic cells in spleen and peripheral target tissues (skin, liver, gut) are activated. This cytokine storm initiates a cascade of immune reactions leading to Th1-polarized immune response [4]. These events are followed by the generation of cytotoxic and inflammatory cytokines, cytotoxic effector cells, large granular lymphocytes, and nitric oxide. Interactions of innate and adaptive immune responses lead to organ damage.

On the other hand, these effector mechanisms are accompanied by endogenous release of IL10 and other Th2 cytokines [5,6]. There is substantial evidence to implicate that different cytokines play a major role in GVHD induction and grade. GVHD was reported to be associated with increased production of IFN γ [7], IL2 [8] and IL10 [9]. IL 10 has been used as a predictor of GVHD [10]. Other reports demonstrated a protective role for IL10 [11]; the increased level may be a physiological response to counteract the effect of Th1 cytokines. A very good positive correlation between the frequency of Th1 and Th2 (r=0.951, p=0.001) in acute GVHD has been reported [12]. In general cytokines associated with Th1 response are claimed to be contributing to the occurrence and severity of acute GVHD while those associated with Th2 response are claimed to be protective [13]. All reports addressed the roles of cytokine(s) individually and, in most, the evaluation was performed after the actual occurrence of GVHD. We hypothesized that it is the balance between Th1 and Th2 rather than either alone would determine the potential occurrence and severity of GVHD. In the present study, we attempted to develop an experimental setup to mimic the response of the immune cells of the graft to the host antigens expressed by Th1 (IFNγ) and Th2 (IL10) cytokine production.

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and study the potential impact of the pattern of production of each cytokine separately and the balance between both on the development of acute GVHD. We also aimed to verify if this pattern could possibly predict the occurrence of acute GVHD.

Patients and Methods

Forty five patients who received fully matched allogeneic peripheral blood stem cell transplantation from a sibling donor at Nasser Institute, Cairo, Egypt in the period from November 2008 until May 2010 were enrolled in the study. They included 26 males and 19 females with an age range from 6 to 41 with a mean of 22.5 ± 9.3 and a median of 22 years. Eighteen patients had Acute Myeloid Leukemia, 15 had Chronic Myeloid Leukemia, 4 had Acute Lymphoblastic Leukemia, 4 had aplastic anemia, 3 had Myelodysplastic Syndrome and one patient had Fanconi’s anemia. All CML patients were transplanted in chronic phase 1 except one case (case 21) that was transplanted in accelerated phase. Of the 18 AML patients, 9 were transplanted in first and 9 in second remission. Two of the four ALL cases were transplanted in first, one was transplanted in second and one in third remission. All patients and donors were ABO compatible. Conditioning regimen and GVHD prophylaxis were applied as previously reported [14]. The different conditioning regimens according to age and diagnosis are displayed in table 1. GVHD prophylaxis consisted of Cyclosporin-A (CSA) 3 mg/kg IV from day -1 to be changed to oral form (3 mg/kg) once the patient can swallow for 9-12 months post-transplant; as well as a short course of IV leucovorin rescue.

The work was performed according to Helsinki declaration and the protocol was approved by the IRB of the NCI, Cairo University. Under informed consent, a heparinized venous blood sample was obtained from the patient before conditioning, mononuclear cells separated by Ficoll-Hypaque density gradient centrifugation and cryopreserved. On the day of transplant, the cryopreserved cells were thawed, viability test was done and cells were incubated with 200 μl of mitomycin C (MUTAMYCIN (5 mg) Bristol Mayers Squibb) at a concentration of 50 μg/ml at room temperature for one hour then washed in RPMI and readjusted to 5 × 10⁶/ml to serve as stimulators while the mononuclear cells of the graft at a concentration of 5 × 10⁶/cells/ml served as responders in a mixed lymphocyte culture setup. Culture plates were incubated for 3 days at 37°C in a CO2 incubator; the culture supernatant, cytokines were below the detection limit in 25/31 (80.6%) cases who did not develop acute GVHD after allogeneic PBSCT from a sibling.

Results

Fourteen/45 patients developed acute GVHD, 4 grade I, six grade II and 4 grade III. Seven patients developed chronic GVHD; 3 of them on top of acute GVHD. Patients who developed chronic GVHD showed no statistically significant differences in any of the tested parameters and will not be mentioned any more. IFN γ and IL10 were measured in the culture supernatant. Both cytokines were significantly higher in the 14 cases that developed as compared to the 31 cases that did not develop GVHD (Table 2). Both cytokines were more frequently detected in the culture supernatant of the cases that developed GVHD and at a significantly higher level as compared to those who did not develop GVHD (Table 3).

In the culture supernatant, cytokines were below the detection limit in 25/31 (80.6%) cases who did not and in 3/14 (16.13%) cases who did develop acute GVHD. The level of cytokines in the other cases varied widely. Of the 11 cases that showed cytokine production, 4 produced IFNγ only and two produced IL10 only. The other 5 cases produced both; IL10 was higher in one case; in the other 4 cases, IFNγ was much higher (Table 4). In the 6/31 cases without GVHD, IL10 only was detected in 3 cases. The other 3 cases produced both; IFNγ was higher in one, in the other two cases IL10 was much higher (Table 5).

All patients were positive for anti CMV IgG except two cases; both had undetectable cytokines in the culture supernatant and both did not predict the occurrence of acute GVHD.
develop GVHD. Seven AML patients had detectable cytokines in their culture supernatant, four developed GVHD and 3 did not. Among the 4 who developed GVHD, 3 were transplanted in second (cases 5, 8 and 25) and one in first remission (case 42). The fifth AML case that developed grade 1 GVHD (case 38) had no detectable cytokines in the culture supernatant. Of the three cases that did not develop GVHD, one (case 20) was transplanted in second and two (cases 9 and 11) were transplanted in first remission. There was no relation between the pattern of cytokines production and age or remission status of patients at the time of transplantation. However, the single CML case that was transplanted in accelerated phase showed high level of cytokines in the culture supernatant (case 21) (Table 4).

At a cutoff level of 15.9 pg/ml, IFNγ was predictive of acute GVHD with a sensitivity of 64.3%, specificity of 96.8% and a total accuracy of 80.4%. At a cutoff of 2.27 pg/ml, IL10 showed a sensitivity of 50%, specificity of 80.6% and a total accuracy of 71.1%. At a cutoff of 1.13, IFNy/IL10 ratio showed a sensitivity of 85.7%, specificity of 83.3% and a total accuracy of 84.6 (Figure 1).

Discussion

GVHD is an exaggerated, undesirable manifestation of a normal inflammatory response [15,16]. The donor CD4+ T-cell interaction with host APCs leads to the activation of the donor T cells and their differentiation into Th1, Th2, and Th17 cells, depending on the cytokine milieu [16,17]; the T helper cells then secrete a variety of cytokines to mediate GVHD inflammation [9,16,18].

The role of Th1, Th2, and Th17 cells in acute GVHD pathogenesis is still controversial. However, in general, Th1 cells play a critical role in mediating acute GVHD pathogenesis; Th2 cells can mediate acute GVHD under special circumstances but they are generally protective [16].

Accordingly the balance between Th1 and Th2 rather than either alone might reflect the outcome in any particular immune response.

In this work, we measured IFNγ and IL10 in the supernatant of a MLC as a surrogate of the in vivo donor immune response to patient’s antigens. Our data suggest that IFNγ production apparently reflects potential development of acute GVHD while IL10 production is apparently protective. When both are produced the IFNγ/IL10 ratio is more informative than either alone. To the best of our knowledge, this is the first study to address the impact of the Th1/Th2 balance, in a MLC setup, on the development of acute GVHD. Only one previous study used the MLC setup to evaluate cytokine production in the sibling transplant situation [19]. This study, however, relied on calculating the number of IFNγ, IL10 and IL4 secreting cells by the Ellispot technique and concluded that only IL10 is informative; they did not measure the actual level of the secreted cytokines [19]. Donor T-cells are the main effectors in the induction of GVHD [20]. Conversely, host B cells may attenuate GVHD by secreting IL10 [11]. The differentiation of CD4+ T cell populations into two distinct groups, Th1 and Th2 cells, is reflected on the pattern of cytokines secreted by the T cells. Th1 and Th2 mediated immune responses cross inhibit each other through the secreted cytokines [21]. In our study, the level of IFNγ was found to be significantly higher in culture supernatant of patients who developed acute GVHD; it was encountered at a higher frequency and at significantly higher levels.

In concordance with our results, serum IFNγ was reported to be increased in patients with acute GVHD [22]. Furthermore, Remmerber et al. [23] had reported a significantly higher levels of IFNγ among patients who had moderate to severe acute GVHD compared to patients with little , if any, GVHD when IFNγ was measured after engraftment.

In the current study, the level of IL10 was significantly higher in

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**Table 4:** IFNγ and IL10 levels in MLC culture supernatant of 11/14 cases with acute GVHD after allogeneic PBSCT from a sibling.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>IFNγ pg/ml</th>
<th>IL10 pg/ml</th>
<th>GVHD Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>22</td>
<td>CML</td>
<td>&lt;DL</td>
<td>9.5</td>
<td>G2</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>AML</td>
<td>6.2</td>
<td>128</td>
<td>G2</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>CML</td>
<td>&lt;DL</td>
<td>131</td>
<td>G2</td>
</tr>
<tr>
<td>8</td>
<td>31</td>
<td>AML</td>
<td>41.66</td>
<td>&lt;DL</td>
<td>G1</td>
</tr>
<tr>
<td>15</td>
<td>29</td>
<td>CML</td>
<td>651.2</td>
<td>22.3</td>
<td>G3</td>
</tr>
<tr>
<td>21</td>
<td>34</td>
<td>CML</td>
<td>2914</td>
<td>520.8</td>
<td>G1</td>
</tr>
<tr>
<td>24</td>
<td>45</td>
<td>CML</td>
<td>19000</td>
<td>858.6</td>
<td>G3</td>
</tr>
<tr>
<td>25</td>
<td>23</td>
<td>AML</td>
<td>23.7</td>
<td>7.9</td>
<td>G3</td>
</tr>
<tr>
<td>36</td>
<td>29</td>
<td>MDS</td>
<td>118.6</td>
<td>&lt;DL</td>
<td>G2</td>
</tr>
<tr>
<td>37</td>
<td>41</td>
<td>CML</td>
<td>159.3</td>
<td>&lt;DL</td>
<td>G3</td>
</tr>
<tr>
<td>42</td>
<td>10</td>
<td>AML</td>
<td>7.12</td>
<td>&lt;DL</td>
<td>G1</td>
</tr>
</tbody>
</table>

Only cases with detectable IFNγ and/or IL10 are included

**Table 5:** IFNγ and IL10 levels in MLC culture supernatant of 6/31 cases without acute GVHD after allogeneic PBSCT from a sibling.

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (years)</th>
<th>Diagnosis</th>
<th>IFNγ pg/ml</th>
<th>IL10 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>23</td>
<td>ALL</td>
<td>1.1</td>
<td>121</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>CML</td>
<td>&lt;DL</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>CML</td>
<td>&lt;DL</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>AML</td>
<td>&lt;DL</td>
<td>46</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>AML</td>
<td>8.1</td>
<td>359</td>
</tr>
<tr>
<td>20</td>
<td>44</td>
<td>AML</td>
<td>80.01</td>
<td>44.78</td>
</tr>
</tbody>
</table>
patients who developed acute GVHD as compared to those who did not when it was measured in culture supernatant. Increased IL-10 level may represent a compensatory mechanism to counteract the effect of Th1 cytokines. The same explanation was stated by Miura et al. [10] reporting high serum IL-10 levels in patients with severe GVHD.

IL-10 and TGF-β have similar and overlapping roles in regulation by the adaptive immune system; a study suggested that GVHD that develops after allogeneic SCT is attenuated by the additive effects of donor derived TGF-β and IL-10 [24]. Yet we did not encounter any impact of TGF-β in the current study (data not included). Macrophages, the major source of IL-10, are stimulated to produce IL-10 by several endogenous and exogenous factors [25]. Considering IL-10 as just immunosuppressive and anti-inflammatory might be oversimplifying [12]. Considering IL-10 as immune-regulatory instead of immunosuppressive might be more appropriate [26]. IL-10 inhibits allo-antigen specific T cell responses through its strong endogenous anti-inflammatory effects [5]. IL-10 controls inflammatory processes and inhibits IFN induced gene transcription; IL-10 inhibition can be overcome by increasing IFN concentrations [27].

A strong Th1 response tends to be hostile during transplantation, playing a major role in exacerbating graft versus host reactions [9]. On the other hand, Th2 response could inhibit allo-antigen specific T cell responses through secretion of cytokines as IL-10. IL-4 and IL-10 are more often perceived as being anti-Inflammatory protective with respect to transplantation [28]. In the current study IL-4 was detectable in two cases only; both developed GVHD and both expressed IFNγ and IL-10 as well (data not presented).

Cytokine polarization is a relative definition that reflects the relative ratio of type 1 cytokines over type 2 cytokines [29]. Th1 cytokines are the main mediators in GVHD production. Generation of CD4+ IL4 and IL10 producing Th2 cells inhibited GVHD and the balance between these two responses determine the outcome of an inflammatory response; hence was the idea to investigate if IFNγ/IL10 ratio could potentially predict the occurrence of acute GVHD rather than either alone. ROC curve for IFNy, IL10 and IFNγ/IL10 ratio in MLC supernatant proved that the IFNy/IL10 is superior to IFNy with IL10 coming last with regards to sensitivity, specificity and total accuracy in predicting the occurrence of acute GVHD.

In the current study, there was no association between the pattern of cytokine production and age or remission status at the time of transplantation. However, it is worth mentioning that the single CML case transplanted in accelerated phase showed a high level of cytokine production; more cases are needed to verify this observation.

Conclusions

In vitro cytokine production by graft immune cells in response to host antigens is extremely variable; it may serve as a surrogate system of the immune reaction following allogeneic stem cell transplantation. IFNγ production apparently reflects potential development of acute GVHD while IL10 production is apparently protective. When both are produced the IFNγ/IL10 ratio is more informative than either alone. These conclusions, however, are drawn from a relatively small number of cases. A validation cohort is essential to verify these results.

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References


