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References


Stem Cell Transplantation

Long-term follow-up of lymphocyte populations and cellular cytokine production in patients with chronic graft-versus-host disease treated with extracorporeal photopheresis

We studied lymphocyte populations and cytokine-expression profiles of ten patients with chronic graft-versus-host disease who at least transiently responded to photoimmunotherapy. The numbers of lymphocytes, monocytes and dendritic cells rose in most cases. Th1 cells always increased during therapy, supporting the hypothesis that a more favorable immune balance contributes to clinical responses.

Letters to the Editor

Extracorporeal photoimmunotherapy (ECP) is partially effective in controlling cutaneous and visceral chronic graft-versus-host disease (GVHD). The mechanisms of action include T-cell apoptosis and modulation of dendritic and regulatory cells and cytokine production. A Th1-polarization induced by ECP has been suggested, this, and a Th2-polarized cytokine profile found in some patients with chronic GVHD, prompted us to evaluate Th1/Th2 cytokine-expression profiles and circulating cell compartments in 10 patients (8 men, 2 women, median age 35 years, 16-61) with steroid-refractory, extensive chronic GVHD under ECP. Four patients had acute lymphoblastic leukemia, six had chronic myeloid leukemia and all had received bone-marrow and/or donor-lymphocyte infusions from identical-sibling donors. ECP was started a median of 31 (5-124) months after the onset of chronic GVHD, when ocular and/or extensive cutaneous disease, refractory to at least two immunosuppressants was present. The UVAR XTS System (Therakus, Johnson & Johnson) was used as described previously. Treatment was given on two consecutive days every 2-4 weeks for 3-6 months and then reduced according to the clinical response. Peripheral blood samples were collected before the first and after the second ECP in each therapeutic session. Phenotypic analysis for surface markers and intracellular cytokines employed phycoerythrin (PE)-conjugated interleukin (2), IL4 and IL10 and fluorescein isothiocyanate (FITC)-conjugated interferon-γ (Pharmigen, S. Diego, CA, USA); FITC-conjugated CD3, CD8, CD14, CD19 and CD56; PE-conjugated CD4, CD56 and CD80; HLA-DR and peridin chlorophyl protein (PerCP)-conjugated HLA-DR and CD3 (BDIS, San José, CA, USA). Cell populations were compared before and at different time-points during photoimmunotherapy in each patient, and between patients exhibiting complete or partial responses (good-responders) and patients without response in at least one organ or dependent on frequent ECP for control of their GVHD (bad-responders). The Wilcoxon and Student’s t tests were used to compare cell populations in each patient and between groups; the null hypothesis was rejected for p<0.05.

Most patients had oral and cutaneous chronic GVHD. Intestinal, hepatic, ocular and lung involvement was present in five, three, two and two patients, respectively. With a median follow-up of 36 (26-38) months after starting ECP, all patients had at least a partial response. Major improvements occurred in cutaneous and ocular disease; pulmonary disease did not respond to ECP. Seventy-percent of patients were good-responders (binomial exact confidence interval 34.8%-93.3%); three patients were bad responders. All patients were profoundly immunosuppressed before ECP, with T, B and NK-cell counts well below normal (Figure 1). The numbers of CD4+ and CD8+ B and NK-cells, respectively, did not change significantly after each session; the small increments detected did not persist until the next treatment (data not shown). However, after at least 9 months of therapy, the T-cell count had increased a median of 1.65-fold; CD4+cells increased in eight patients, although only five reached the normal range and the number of CD8+ cells did not change significantly in the majority of patients. B and NK cells were normal in seven and eight patients, respectively, at last follow-up (Figure 1). Monocytes and dendritic cells increased in most cases (median increases 1.9 and 11.4-fold, respectively). The changes in lymphocyte populations were similar in good and bad-responders (p>0.05). T cells (particularly CD4+) increased significantly with photopheresis (p=0.01) and were higher after ECP in good-responders (p=0.02); no differences were found in other cell populations before or after treatment. Th1 and Th2 cells were almost undetectable before ECP. An immediate, transient increase in circulating Th1 cells after ECP occurred in ≤20% sessions. However, in the long-term photoimmunotherapy consistently increased the number of Th1-producing cells in all patients (Figure 2). IFN-γ and IL2-producing T-cell numbers were normal in all but one case at the last follow-up (median fold-increase of 92.6, 171.5 and 70.7 for IFN-γ producing CD3+, CD4+ and CD8+ cells, respectively, and 87.4, 122.3 and 25.4 for IL2-producing CD3+, CD4+ and CD8+lymphocytes, respectively). Bad and good-responders had similar increases (p>0.05), possibly because all had at least a partial response to therapy. ECP did not modify Th2-cell percentages. In conclusion, only transient modifications in immune cells and cytokine-expression profiles occurred shortly after ECP, but prolonged therapy consistently increased the numbers of CD4+ T cells (without significant changes in CD8+ counts), NK cells and B cells in ≥50% cases and Th1-producing cells in
all patients. These changes, expected during post-transplant immune recovery, may depend on ECP, in addition to the resolution of chronic GVHD and reduction of immunosuppressants. If Th2 cells play a role in chronic GVHD, the effects of ECP may be partially explained by restoration of the normal Th1/Th2 balance. An ECP-induced increase in Th1 cells was not found by others; however, it may improve the immune response against the autoreactive lymphocytes responsible for tissue damage. ECP could also reduce the number of donor-derived CD4+CD25hi, IL10-producing regulatory T cells found in chronic GVHD, allowing the proliferation of Th1 cells. The stable clinical improvements observed may be related to the achievement of a more balanced immune system.

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References
was employed to quantify the extent and severity of oral mucosal involvement by chronic GVHD. The aim of this study was to investigate the clinical relevance of oral involvement by chronic graft-versus-host disease. The presence of oral changes in association with skin and other target organs including eye, lung or joint may adversely influence the probability of discontinuing systemic immunosuppressive treatment.

The importance of oral manifestations of chronic graft-versus-host disease (GVHD) remains ill-defined and it might be surmised that selected patients with oral involvement may not require the aggressive immunosuppressive treatment (IST) that would naturally be used for extensive chronic GVHD. We evaluated the incidence and the clinical characteristics of oral chronic GVHD and investigated the impact of oral chronic GVHD on IST requirements, overall survival and non-relapse mortality (NRM). Between November 1990 and April 2001, 147 adult patients (median age 38 years, range 18-71 years) with hematologic malignancies who received a hematopoietic stem cell transplantation (HSCT), survived at least 100 days after HSCT and were at risk of chronic GVHD. Patients underwent HSCT from HLA-matched sibling donors (n=120), partially matched related donors (n=8) or matched unrelated donors (n=19). The anatomic areas of the oral cavity evaluated for assessment of involvement by chronic GVHD were the buccal mucosa (cheeks), tongue, lips and palate.

A modified model of the Oral Mucosal Rating Scale (OMRS) was employed to quantify the extent and severity of oral mucosal involvement by chronic GVHD. Objective changes, including erythema, lichenoid and atrophy, were rated on scales ranging from 0 to 3 (0, normal; 1, mild; 2, moderate; 3, severe changes). Mild changes were defined as <25% involvement in one or more anatomic areas, moderate changes were defined as 25-50% involvement and severe changes as >50% involvement. Ulceration and pseudomembrane formation were rated on scores based on estimated surface area involved (0, none; 1, >0 1 cm²; 2, >1 cm² 2 cm²; 3, 2-4 cm²; 4, >4 cm²).

### Table 1. Characteristics of patients with oral chronic GVHD.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total (n=56)</th>
<th>Mild/moderate oral involvement (n=40)</th>
<th>Severe oral involvement (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day of onset of oral chronic GVHD (range)</td>
<td>(76-925)</td>
<td>(76-925)</td>
<td>(78-290)</td>
</tr>
<tr>
<td>Clinical Manifestations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>25 (44%)</td>
<td>20 (50%)</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>Lichenoid Mucosal Changes</td>
<td>38 (68%)</td>
<td>29 (72%)</td>
<td>9 (56%)</td>
</tr>
<tr>
<td>Oral Atrophy</td>
<td>11 (19%)</td>
<td>1 (2%)</td>
<td>10 (62%)</td>
</tr>
<tr>
<td>Ulcerations &amp; Pseudomembrane</td>
<td>15 (26%)</td>
<td>2 (5%)</td>
<td>13 (81%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sites</th>
<th>Total (n=56)</th>
<th>Mild/moderate oral involvement (n=40)</th>
<th>Severe oral involvement (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheeks</td>
<td>55 (98%)</td>
<td>39 (97%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>17 (30%)</td>
<td>8 (20%)</td>
<td>9 (56%)</td>
</tr>
<tr>
<td>Lips</td>
<td>10 (18%)</td>
<td>7 (17%)</td>
<td>3 (18%)</td>
</tr>
<tr>
<td>Palate</td>
<td>9 (16%)</td>
<td>4 (10%)</td>
<td>5 (31%)</td>
</tr>
</tbody>
</table>

| No. of sites involved  |            |                                     |                             |
|------------------------|-------------|                                     |                             |
| 1                      | 28 (50%)    | 23 (57.5%)                          | 5 (31%)                     |
| 2                      | 21 (37.5%)  | 16 (40%)                            | 5 (31%)                     |
| 3 - 4                  | 7 (12.5%)   | 1 (2.5%)                            | 6 (38%)                     |

<table>
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<th>Symptoms</th>
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<th>Severe oral involvement (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>30 (54%)</td>
<td>27 (68%)</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Dryness</td>
<td>14 (25%)</td>
<td>12 (30%)</td>
<td>2 (12.5%)</td>
</tr>
<tr>
<td>Difficulty in swallowing</td>
<td>9 (16%)</td>
<td>0</td>
<td>9 (56%)</td>
</tr>
<tr>
<td>Pain</td>
<td>3 (5%)</td>
<td>1 (2%)</td>
<td>2 (12.5%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other organs involved</th>
<th>Total (n=56)</th>
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<th>Severe oral involvement (n=16)</th>
</tr>
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<tbody>
<tr>
<td>Local</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Single systemic</td>
<td>17 (30%)</td>
<td>16 (40%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Double systemic</td>
<td>24 (43%)</td>
<td>17 (43%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>Triple systemic</td>
<td>14 (225%)</td>
<td>6 (15%)</td>
<td>8 (50%)</td>
</tr>
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<table>
<thead>
<tr>
<th>Immunosuppressive therapy</th>
<th>Total (n=56)</th>
<th>Mild/moderate oral involvement (n=40)</th>
<th>Severe oral involvement (n=16)</th>
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<tr>
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<thead>
<tr>
<th>Median duration of immunosuppressive therapy (range)</th>
<th>Total (n=56)</th>
<th>Mild/moderate oral involvement (n=40)</th>
<th>Severe oral involvement (n=16)</th>
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<tr>
<th>Follow-up from transplantation, median days (range)</th>
<th>Total (n=56)</th>
<th>Mild/moderate oral involvement (n=40)</th>
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25-50% involvement and severe changes as > 50% involvement. Ulceration and pseudomembrane formation were rated on scores based on estimated surface area involved (0, none; 1, >0 1 cm²; 2, >1 cm² 2 cm²; 3, 2-4 cm²; 4, >4 cm²).

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