Low Serum Thymic Hormone Levels in Patients With Chronic Graft-Versus-Host Disease


We tested the hypothesis that chronic graft-versus-host disease (GVHD) is due to inadequate thymic function by examining pretransplant serum levels of facteur thymique sérique (FTS). Four of five patients with no detectable FTS activity developed chronic GVHD, while one of four with some FTS activity did. Further patient numbers are needed to confirm or reject this hypothesis. We further postulated that chronic GVHD, whatever its cause, involves thymic epithelium as a target organ. When tested 11 mo or more posttransplant, patients with chronic GVHD had lower absolute FTS levels \( p < 0.02 \) and lower age-corrected levels \( p = 0.05 \) than patients without chronic GVHD. Low values in chronic GVHD were associated with the disease itself and not its therapy. These findings show that thymic epithelial secretory function is impaired in chronic GVHD, and this may in part be responsible for the immunodeficiency characteristic of these patients.

CHRONIC graft-versus-host disease (GVHD) is emerging as a major complication of allogeneic bone marrow transplantation for aplastic anemia (AA) and acute leukemia as increasing numbers of patients become long-term survivors. The mechanisms underlying chronic GVHD are not well defined. Both severe acute GVHD and increasing patient age predispose to the development of chronic GVHD, and the latter finding suggested the possibility that inadequate thymic function may play an important etiologic role in chronic GVHD. In this study we assessed thymic epithelial secretory function by measuring serum levels of facteur thymique sérique (FTS) in the serum of marrow transplant patients with and without chronic GVHD. FTS is a nonapeptide secreted by thymic epithelial cells. In mice, FTS induces the conversion of normal murine spleen cells to theta-positivity and induces concanavalin-A responsiveness in spleen cells of nude mice. In man, serum levels are low in DiGeorge’s syndrome and most cases of severe combined immunodeficiencies as well as in systemic lupus erythematosus; low levels have also been found in some patients with acute leukemia and aplastic anemia treated by conventional means. The significance of this finding is unclear, but it may be related to immunosuppressive therapy.

MATERIALS AND METHODS

Patients

Details on the selection of patients and donors for transplantation, on the conditioning regimens for transplantation, and on the transplantation procedure have been previously described, as have details of the diagnosis, classification, and treatment of acute and chronic GVHD. All patients and their donors were HLA-identical siblings as determined by serologic histocompatibility typing and mutual nonreactivity in mixed leukocyte culture. All recipients were treated with the immunosuppressive agent methotrexate within the first 100 days posttransplantation to prevent or ameliorate GVHD. Evidence for allogeneic marrow engraftment was obtained by monitoring the peripheral blood count, determination of marrow cellularity, and frequent monitoring of blood genetic markers.

Serum Samples

Sera were stored at \(-80^\circ\text{C}\) until used. No serum utilized in this study had been heat inactivated or previously thawed, and only one was older than 4 yr (4.9 yr): this showed a normal FTS value. (Normal sera stored for 3 yr maintain their activity.) If sufficient serum in a given sample was available, a repeat FTS estimation was made. In such cases both values are given in the tables. When one estimation gave a normal level while the other gave a low level, the patient was said to have a borderline level. Borderline levels were treated as low levels for the purposes of statistical analysis. All serum samples were coded prior to analysis, which was performed without any knowledge as to the origin of the samples.

FTS Assay

Levels of FTS activity in the sera of patients were quantitated by the rosette inhibition assay of Dardenne and Bach with minor modifications. Rosette-forming cells in the spleens of thymectomized mice are less sensitive to azathioprine than are those from normal mice. After a short incubation at 37°C, human serum with FTS activity restores to normal the sensitivity to azathioprine of rosette-forming cells from adult thymectomized mice, resulting in inhibition of rosette formation. This change has been used to establish a reproducible and quantitative bioassay for determination of circulating FTS in serum.
### Table 1. Pretransplant FTS Levels

<table>
<thead>
<tr>
<th>Unique Patient Number (UPN)</th>
<th>Age (yr)/Sex</th>
<th>Diagnosis</th>
<th>Disease Status at Admission (Leukemias)</th>
<th>Immunosuppressive Treatment in Preceding 4 mo</th>
<th>FTS Titer</th>
<th>Long-term Status After Grafting</th>
</tr>
</thead>
<tbody>
<tr>
<td>705 16/F ANL</td>
<td>Remission</td>
<td>Combination cytotoxic chemotherapy</td>
<td>&lt; 1:4</td>
<td>Chronic GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>735 28/F ANL</td>
<td>Relapse</td>
<td>Combination cytotoxic chemotherapy</td>
<td>1:64</td>
<td>Chronic GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>613 16/M AA</td>
<td>—</td>
<td>Prednisone</td>
<td>&lt; 1:4</td>
<td>Chronic GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>733 28/M AA</td>
<td>—</td>
<td>Nil</td>
<td>&lt; 1:4</td>
<td>Chronic GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>686 15/F AA</td>
<td>—</td>
<td>Prednisone</td>
<td>&lt; 1:4</td>
<td>Chronic GVHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>593 15/M AA</td>
<td>Nil in preceding 7 mo</td>
<td>—</td>
<td>&lt; 1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>370 29/M AA</td>
<td>—</td>
<td>Nil</td>
<td>1:8</td>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>619 13/F AA</td>
<td>—</td>
<td>Nil</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>538 18/F ALL</td>
<td>Remission</td>
<td>Combination cytotoxic chemotherapy</td>
<td>1:8</td>
<td>Healthy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANL, acute nonlymphoblastic leukemia; ALL, acute lymphoblastic leukemia; AA, aplastic anemia; GVHD, graft-versus-host disease.

### Table 2. FTS Levels 11 mo or Later Posttransplant

<table>
<thead>
<tr>
<th>Unique Patient Number (UPN)</th>
<th>Age (yr)/Sex</th>
<th>Diagnosis</th>
<th>Time Posttransplant (mo)</th>
<th>FTS Titer</th>
<th>Age-Corrected FTS Level</th>
<th>Allogeneic Factor</th>
<th>Long-Term Status at Time of FTS Estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Healthy long-term survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>593 15/M AA</td>
<td>13</td>
<td>1:32, 1:64 Normal</td>
<td>NE</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>370 29/M AA</td>
<td>11</td>
<td>1:8, 1:16 Normal</td>
<td>NE</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>619 13/F AA</td>
<td>11</td>
<td>1:32 Normal</td>
<td>NE</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>538 18/F ALL</td>
<td>12</td>
<td>1:64 Normal</td>
<td>NE</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>776 11/M AML</td>
<td>13</td>
<td>1:128 Normal</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>879 23/F AA</td>
<td>12</td>
<td>1:16 Normal</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>700 16/M AA</td>
<td>12</td>
<td>1:32, 1:64 Normal</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>574 3/M CML-BT</td>
<td>12</td>
<td>1:16 Low normal</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>385 12/M AA</td>
<td>12</td>
<td>1:16 Low</td>
<td>1:4</td>
<td>Healthy</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(B) Patients with untreated chronic GVHD</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>387 30/F AA</td>
<td>13</td>
<td>&lt; 1:4 Low</td>
<td>NE</td>
<td>Limited chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>872 11/F AA</td>
<td>12</td>
<td>1:4 Low</td>
<td>NE</td>
<td>Limited chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>660 19/M AA</td>
<td>15</td>
<td>1:32 Normal</td>
<td>NE</td>
<td>Limited chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>683 6/M ALL</td>
<td>12</td>
<td>&lt; 1:4 Low</td>
<td>NE</td>
<td>Extensive chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>748 4/M CML-BT</td>
<td>12</td>
<td>1:8, 1:16 Low</td>
<td>&lt; 1:4</td>
<td>Extensive chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>803 25/F ANL</td>
<td>13</td>
<td>1:4 Low</td>
<td>NE</td>
<td>Extensive chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>366 34/F AA</td>
<td>18</td>
<td>1:128 High</td>
<td>1:4</td>
<td>Extensive chronic GVHD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(C) Patients given immunosuppressive therapy for chronic GVHD</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>705 16/F ANL</td>
<td>11</td>
<td>1:4 Low</td>
<td>NE</td>
<td>Extensive chronic GVHD; on prednisone and azathioprine at time of FTS estimation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>735 28/F ANL</td>
<td>16</td>
<td>1:4 Low normal</td>
<td>NE</td>
<td>Extensive chronic GVHD; prednisone and azathioprine discontinued 4 mo prior to FTS estimation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>613 16/M AA</td>
<td>12</td>
<td>1:8 Low</td>
<td>NE</td>
<td>Extensive chronic GVHD; prednisone and procarbazine discontinued 3 mo prior to FTS estimation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>733 28/M AA</td>
<td>13</td>
<td>1:8 Normal</td>
<td>NE</td>
<td>Extensive chronic GVHD; on prednisone and procarbazine at time of FTS estimation</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>686 15/F AA</td>
<td>12</td>
<td>&lt; 1:4 Low</td>
<td>NE</td>
<td>Extensive chronic GVHD; on prednisone and azathioprine at time of FTS estimation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AA, aplastic anemia; ANL, acute nonlymphoblastic leukemia; CML-BT, chronic myelogenous leukemia in blastic transformation; NE, not estimated; GVHD, graft-versus-host disease.
Since FTS activity is dependent on age, the range of normal levels in human serum is as follows: birth to 20 yr, 1:128-1:32; 21-30 yr, 1:64-1:8; 31-40 yr, 1:32-1:4; 41-50 yr, 1:8-1:2; 51 yr and over, 1:4-1:2.

**Allogeneic Factor Assay**

An allogeneic factor (AF) has been detected in mice undergoing a graft-versus-host reaction. Since AF has activity in the rosette inhibition assay, the possibility of its presence in the serum of recipients of allogeneic bone marrow transplants was evaluated. We performed a separate assay for this factor in patients showing elevated FTS levels whenever a sufficient quantity of serum permitted. In brief, sera were incubated overnight at 4°C with either specific anti-FTS immunosorbent prepared by coating anti-FTS antibodies to Sepharose using cyanogen bromide or with the control immunosorbent, anti-human alpha-2 macroglobulin. The Sepharose beads were then spun down and the activity of the supernatant evaluated by the rosette inhibition assay.

**RESULTS**

**FTS Levels Pretransplant (Nine Patients)**

Nine patients had FTS levels measured immediately prior to the initiation of the conditioning regimen for marrow transplantation. Five of the nine had undetectable (<1:4) levels of FTS (Table 1). Of these five patients, four subsequently developed chronic GVHD, while three of the four patients in whom some FTS was evident pretransplant became healthy long-term survivors without chronic GVHD. FTS levels did not correlate with immunosuppressive therapy given in the 4 mo before admission for marrow transplantation.

**FTS Levels 11 Mo or Later Posttransplant (21 Patients)**

Long-term healthy survivors (9 patients). Nine patients became healthy survivors and had no recurrence of their original disease, chronic GVHD, or additional immunosuppressive therapy (Table 2A). All patients had detectable levels of FTS. When corrected for age, FTS levels were normal in seven of the nine, borderline in one, and low in one (Fig. 1). Allogeneic factor measured in five patients was detectable only at a 1:4 dilution and thus did not account for the elevation of the corresponding FTS titer.

Patients with untreated chronic GVHD (7 patients). Seven patients had chronic GVHD but received no treatment for it (Table 2B). Two patients had undetectable levels of FTS, while five showed some activity. When corrected for age, FTS levels were low in five of the seven, normal in one, and high in one (Fig. 1). Allogeneic factor levels measured in two patients were undetectable or detectable only at a 1:4 dilution, and thus again, did not account for the elevation of the corresponding FTS titer.

Patients given immunosuppression for chronic GVHD (5 patients). Five patients received immunosuppressive therapy for chronic GVHD (Table 2C). In one patient, FTS activity was undetectable while four showed some activity. When corrected for age, three patients had low levels, one had a borderline level, and one had a normal level (Fig. 1).

If all 12 patients with chronic GVHD (7 untreated, 5 treated) are combined, eight had low levels of FTS at 11-18 mo (median 12.5) posttransplant, 1 had a borderline level, 2 had normal levels, and 1 had a high level. In contrast, at 11-13 mo (median 12) posttransplant, 7 of 9 healthy long-term survivors without chronic GVHD had normal levels, 1 had a borderline level, and 1 had a low level. The normal levels of FTS could not be explained by elevations of allogeneic factor. At approximately 1 yr posttransplant, patients with chronic GVHD had lower absolute FTS levels ($p < 0.02$, Mann-Whitney U test [two-tailed]) and lower age-corrected levels ($p = 0.05$, Fisher's exact test [two-tailed]) than patients without chronic GVHD. An additional 3 patients (UPN 644, 614, 300) whose chronic GVHD had become quiescent had levels measured 33, 36, and 54 mo posttransplant, respectively (Table 3). UPN 644 (no previous estimations) had a low level at 33 mo posttransplant, 3 mo after his chronic GVHD was determined to have become inactive. UPN 613 and UPN 300, however, had normal levels at 36 and 54 mo posttransplant, respectively, and 12 and 4 mo after cessation of chronic GVHD activity. An elevation of allogeneic factor was excluded as the mechanism of the normal FTS titers.

**DISCUSSION**

Chronic GVHD is a debilitating and sometimes fatal complication that occurs in 30% of patients surviving at least 6 mo after allogeneic bone marrow transplantation.
transplantation for aplastic anemia or acute leukemia.1
Since 75% of patients with aplastic anemia,2 55% of
patients with acute nonlymphoblastic leukemia trans-
planted in first remission,17 and 35% of patients with
acute lymphoblastic leukemia transplanted in second
or subsequent remission18 become long-term survivors,
chronic GVHD is becoming an increasingly common
problem. In addition to skin, eye, mouth, liver, esopha-
geal, gut, and joint involvement, patients with chronic
GVHD have a severe combined immunodeficiency19-20
and are prone to recurrent bacterial infections.21
While the mechanism of acute GVHD is relatively
well defined and is thought to be due primarily to
immunocompetent cytotoxic T cells present in the
donor marrow inoculum,22 the mechanism of chronic
GVHD is less clear. One component may be alloreact-
ivity mediated by cells of donor origin present in the
chimera and directed against host cells bearing trans-
plantation antigens present in the recipient and not in
the donor. It has been detected in both canine23 and
human24 chimeras. Additionally, there appears to be an
inadequate attempt to control this alloreactivity by the
evolution in the chimera of a population of cells
nonspecifically suppressive of the donor’s response to
unrelated alloantigens.25 In contrast, chimera cells
specifically suppressive of the donor’s response to
trinitrophenyl-modified host but not trinitrophenyl-
modified donor, trinitrophenyl-modified unrelated or
unmodified unrelated cells are noticeable by their
absence in patients with chronic GVHD but are char-
acteristically present in long-term surviving chimeras
without chronic GVHD.26
Since chronic GVHD is more common in older
recipients of allogeneic bone marrow transplants,1 we
first postulated that an age-related defect of thymic
function pretransplant predisposed to chronic GVHD.
With the data presented here we were unable to
confirm or negate this hypothesis. It is of interest,
however, that most patients with undetectable pre-
transplant FTS activity developed chronic GVHD,
while most of those with at least some FTS activity
became healthy long-term survivors without chronic
GVHD. More patients, and in particular more very
young patients, will have to be studied to determine
whether or not pretransplant FTS activity is predictive
of the development of chronic GVHD.
We next examined the hypothesis that chronic
GVHD, regardless of its cause, involved thymic epithe-
lium as a target organ. Seddik et al. have shown a
thymic epithelial injury to occur in murine GVHD.27
The present study documents that a defect of thymic
epithelial secretory activity is an integral component
of chronic GVHD and provides another parameter of the
severe immunodeficiency from which patients with
active chronic GVHD suffer and by which they differ
from their long-term surviving counterparts without
GVHD. Furthermore, the fact that 5 of 7 patients with
untreated chronic GVHD had low FTS levels 12–18
mo posttransplant provides evidence that the disease
itself, and not its therapy, is associated with this state
of impaired thymic epithelial secretory function.

ACKNOWLEDGMENT
We thank Lisa Eldred and Dottie Thomas for their skillful
preparation of the manuscript.

REFERENCES
1. Sullivan KM, Shulman HM, Storb R, Weiden PL, With-
erness R, MacDonald GB, Schubert MM, Atkinson K, Thomas
ED: Chronic graft-versus-host disease in 52 patients: Adverse

Table 3. FTS Levels in Patients With Inactive Chronic Graft-Versus-Host Disease

<table>
<thead>
<tr>
<th>Unique Patient Number (UPN)</th>
<th>Age (yr)/Sex</th>
<th>Diagnosis</th>
<th>Prior FTS Levels</th>
<th>FTS Activity When Chronic GVHD Inactive</th>
<th>Age-Corrected Levels</th>
<th>Clinical Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>644</td>
<td>8/M</td>
<td>ALL</td>
<td>NEO</td>
<td>&lt;1:4 (33 mo posttransplant)</td>
<td>Low</td>
<td>Limited chronic GVHD diagnosed at 14 mo; not treated. Considered inactive at 30 mo</td>
</tr>
<tr>
<td>613</td>
<td>16/M</td>
<td>AA</td>
<td>Low pretransplant, and 14 wk, 12 and 24 mo posttransplant</td>
<td>1:32, 1:64 (36 mo posttransplant, allogeneic factor &lt;1:4)</td>
<td>Normal</td>
<td>Extensive chronic GVHD treated by prednisone and procarbazine till 9 mo posttransplant. Chronic GVHD considered inactive at 24 mo</td>
</tr>
<tr>
<td>300</td>
<td>10/M</td>
<td>AA</td>
<td>NEO</td>
<td>1:32, 1:64 (54 mo posttransplant, allogeneic factor 1:8)</td>
<td>Normal</td>
<td>Limited chronic GVHD diagnosed at 5 mo posttransplant. Not treated. Considered inactive at 50 mo</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AA, aplastic anemia; NE, not examined.
THYMIC HORMONE LEVELS IN CHRONIC GVHD


9. Incefy GS, O'Reilly R: (Manuscript in preparation)


Low serum thymic hormone levels in patients with chronic graft-versus-host disease

K Atkinson, GS Incefy, R Storb, KM Sullivan, T Iwata, M Dardenne, HD Ochs, RA Good and ED Thomas