In Vitro Regulation of Immunoglobulin Synthesis After Marrow Transplantation. I. T-Cell and B-Cell Deficiencies in Patients With and Without Chronic Graft-Versus-Host Disease

By Lawrence G. Lum, Margaret C. Seigneuret, Rainer F. Storb, Robert P. Witherspoon, and E. Donnall Thomas

Twenty-four patients with aplastic anemia or acute leukemia were treated by marrow grafts from HLA-identical donors after conditioning with high doses of cyclophosphamide and/or total body irradiation. They were studied between 4 and 63 mo (median 14.2) after transplantation. Seventeen patients had chronic graft-versus-host disease (C-GVHD) and 7 were healthy. They were studied for defects in their T- and B-cell function using an indirect hemolytic plaque assay for Ig production after 6 days of culture in the presence of pokeweed mitogen. T or B cells from the patients with or without C-GVHD were cocultured with T or B cells from their HLA-identical marrow donors or unrelated normal controls. Intrinsic B-cell defects, lack of helper T-cell activity, and suppressor T-cell activity were more frequently found in patients with C-GVHD than in healthy patients. Fifteen of the 17 patients with C-GVHD showed one or more defects in their T- and B-cell function compared to only 3 of the 7 patients without C-GVHD. None of the healthy controls, including the marrow donors, showed defects in their T- and B-cell functions. These in vitro findings may be helpful in assessing the process of immune reconstitution and the immunologic aberrations found after human marrow transplantation.

Bone marrow transplantation from HLA-identical siblings is effective treatment for aplastic anemia and hematologic malignancies. The lymphoid systems after marrow transplantation are derived from cells of donor origin. Most long-term survivors are healthy without evidence of graft-versus-host disease (GVHD). Within 1 yr of grafting, they recover normal or near-normal antibody responses to pneumococcal polysaccharide antigens and the neoa antigen bacteriophage \( \Phi X174 \). However, even in these seemingly normal patients, some immune deficiencies persist, as illustrated by lower-than-normal antibody responses to primary and secondary immunizations with keyhole limpet hemocyanin. One-third of the long-term survivors develop chronic GVHD (C-GVHD), which resembles some autoimmune disorders and is characterized by severe combined immunodeficiencies, impaired granulocyte chemotaxis, and recurrent bacterial and fungal infections. Patients with C-GVHD have significantly lower primary and secondary antibody responses to all antigens tested at all times after transplantation than those without C-GVHD. They display immunoglobulin and complement deposits at the dermoepidermal junction of the skin (86% of patients), circulating antineutrophil and/or antimitochondrial antibodies (65%), lymphocytes capable of suppressing the response of marrow donor cells in mixed leukocyte culture (50%), and more recently, the presence of suppressor cells and the inability to synthesize immunoglobulin in vitro. Chronic GVHD, therefore, provides a unique immunologic model for investigating the roles that T cells, T-cell subpopulations, and B cells play in the immune dysfunction that likely exists in C-GVHD.

Previous studies of in vitro immunoglobulin secretion post-marrow grafting were done on unfractinated peripheral blood mononuclear cells using killed Cowan bacteria to stimulate Ig production. A major disadvantage of those studies was that if no immunoglobulin-secreting cells were detected after culture, the identity of the cell or cells responsible for the failure of the B cells to secrete Ig could not be identified.

This investigation seeks to analyze the nature of the immunoregulatory defects found in patients with C-GVHD. These studies were directed at answering the following questions: (1) Are there deficiencies in B-cell function? (2) Are there deficiencies in the ability of T cells to provide helper activity to B cells? and (3) What role do suppressor T cells play in C-GVHD? These questions were addressed using pokeweed mitogen-stimulated polyclonal Ig secretion that was assessed after culture with an indirect hemolytic plaque assay.

MATERIALS AND METHODS

Patients

Twenty-four patients, 7 without and 17 with C-GVHD, were studied between 128 and 1896 days after transplantation of marrow donors.

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from HLA-identical donors. They were conditioned for transplantation either with cyclophosphamide, total body irradiation, or a combination thereof. Tables 1 and 2 summarize the data on the underlying diseases, the days on which each patient was tested after transplantation, and the serum Ig levels on these patients. Lymphocyte subpopulations, prepared as outlined below from the HLA-identical marrow donors, if available, or from random unrelated individuals, served as “controls” for the in vitro cultures. Note that 3 patients with C-GVHD (unique patient numbers UPNJ 568, 863, and 938) were studied twice.

### Purification of T and Non-T Lymphocyte Subpopulations

Peripheral blood mononuclear cells were prepared from 50–75 ml of heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. The washed peripheral blood mononuclear cells from patient or control were mixed with 2-amino-ethylisothiouronium bromide (AET) modified sheep red blood cells (SRBC-AET) at a ratio of 1:50 SRBC to 1 lymphocyte in balanced salt solution (BSS) containing 20% fetal calf serum (FCS). The E-rosette-positive pellet (T cells) and the E-rosette-depleted interface cells (non-T) were washed, counted, adjusted, and placed in RPMI 1640 (Grand Island Biological Company, Grand Island, N.Y.) supplemented with 10% FCS, penicillin-streptomycin, and 4 mM glutamine unless otherwise mentioned. The E-rosette-positive pellets routinely contained 95%–99% E-rosette-positive cells before lysis of the SRBCs in the pellet, with no more than 2%–3% surface immunoglobulin-positive (SmIg+) cells using a rabbit-fluoresceinated anti-human (Fab) reagent, and no more than 2% nonspecific esterase-positive cells. The non-T (B-cell-enriched) interface consistently contained less than 2% E-rosette-positive cells when they were restested using SRBC-AET rosetting. The number of monocytes found in the non-T-cell subpopulations ranged up to 35% (mean 20%) by nonspecific esterase staining in both the control and patient non-T-cell subpopulations. Unless otherwise specified, the non-T populations will be referred to as B cells.

### PWM-Stimulated Immunoglobulin Secretion

The various T and B subpopulations from patients or controls were cocultured in the presence or absence of pokeweed mitogen (PWM) (1:200–1:400 dilution of stock, obtained from Grand Island Biological Company). Duplicate cultures containing 1.0–1.5 x 10^6 lymphocytes were done at a cell concentration of 10^6 cells/ml in RPMI 1640 for 6 days in 15-ml conical tubes (Corning Glass Works, Corning, N.Y.). When T- and B-cell combinations were done, 5.0 x 10^5 T cells and 5.0 x 10^5 B cells were added to each duplicate macroculture. In the case of (IN+BN+T), 5.0 x 10^5 normal T cells (IN) 5.0 x 10^5 normal B cells (BN), and 5.0 x 10^5 patient T cells (Tv) were cocultured. Unstimulated cocultures of patient T and B cells or normal T and B cells consistently made fewer than 50 plaque-forming cells (PFC) per culture. Unless otherwise mentioned, irradiated T cells (Ta) received 1200 rad from a cesium source.

Adherent accessory cells were not removed from the non-T subpopulations of the patient or control since, in many instances, the...
yields of patient non-T subpopulations were limiting in number. Monocytes composed no more than a maximum of 40% of the non-T subpopulation; therefore, the total monocyte composition would approximate 16%-20% of the entire culture (a concentration of monocytes unlikely to cause suppression).

**Indirect Hemolytic Plaque Assay**

The method for assessing plaque-forming cells (PFC) has been described. In brief, after 6 days of culture in an humidified atmosphere containing 5% CO2 at 37°C, the cultures for Ig secretion were harvested, washed 3 times in BSS, counted, and adjusted to a cell concentration of 10⁶ cells/ml. Each culture was plated separately and results are reported as a mean of duplicate cultures. Viability in the cells recovered was usually >90%. Total immunoglobulin production was determined using a rabbit anti-human polyvalent (specific for gamma-, mu-, and alpha-heavy chains) developing antiserum obtained from Dako Corporation (Santa Barbara, Calif.). Staphylococcus protein A (SPA) coated SRBC were made as described previously. One-hundred microliters of the above lymphocyte suspensions (10⁶ cells/ml), 25 μl of developing antiserum (titrated to be optimally effective at 1:20 dilution of stock) and 25 μl of SRBC were added to tubes containing 0.25 ml of agarose (2%). The entire mixture was pipetted rapidly onto subcoated Petri dishes and swirled to obtain a uniform lawn. After 4 min of cooling, 1 ml of isogevers (gelatin veronal buffer) was added to each dish and incubated 1 hr at 37°C in a 5% CO₂-humidified atmosphere. Guinea pig complement diluted 1:12 in isogevers solution was added to each plate following aspiration of the isogevers for an additional 1.5 hr of incubation at 37°C in a 5% CO₂-humidified atmosphere. Guinea pig complement diluted 1:12 in isogevers solution was added to each plate following aspiration of the isogevers for an additional 1.5 hr of incubation at 37°C in a 5% CO₂-humidified atmosphere. At the end of the incubation, the guinea pig complement was aspirated and the plates were stored at 4°C overnight. The number of PFC/plate were harvested, washed 3 times in BSS, counted, and adjusted to a cell concentration of 10⁶ cells/ml. Each culture was plated separately and results are reported as a mean of duplicate cultures.

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**Analysis of Data**

The cocultures for B-cell function, T-cell helper activity, and T-cell suppressor activity for each patient have been expressed as a percentage of the control culture (marrow donor or normal individual). The mean PFC response of duplicate cultures was used for these computations. The following equations were used to express the data presented in the Results section unless otherwise mentioned:

% normal B-cell function =

\[
\frac{(T_N + B_n)}{(T_N + B_n + T_p)} \times 100\% \quad (1)
\]

% normal T-cell helper function =

\[
\frac{(T_N + B_n)}{(T_N + B_n + T_p)} \times 100\% \quad (2)
\]

% suppressor T-cell activity =

\[
\frac{((T_N + B_n) - (T_N + B_n + T_p))}{(T_N + B_n)} \times 100\% \quad (3)
\]

(Note: Negative suppression denotes enhancement.)

Irradiated patient T cells (Tf) were set up in a parallel coculture experiment to establish radiosensitivity of the suppressor cells in the event that suppression was detected in the appropriate coculture. The suppressor T-cell activity was considered radiosensitive if the suppression observed in equation 3 was decreased. B-cell and T-cell helper function was defined as “moderately” impaired when the number of mean PFC/culture in the experimental coculture was less than 20% of that of the control culture. “Severe” impairment was defined as experimental mean PFC/culture numbers fewer than 5% of those of control cultures.Suppressor activity of patient T cells was considered “moderate” when it decreased PFC numbers by more than 75% of those of control cultures, and “severe” when it decreased PFC numbers by more than 90%.

**Statistics**

Unless otherwise mentioned, statistical analyses are done with a two-sided Wilcoxon rank sum test.

**RESULTS**

**The Response of Normal T and B Cells to Allogeneic Cocultures**

Table 3 shows the comparison of autologous T- and B-cell mixtures with allogeneic T- and B-cell mixtures. The effect of culturing allogeneic T and B cells for T-cell helper function, irradiated T-cell helper function, B-cell function, and suppressor T-cell activity are shown. The allogeneic cocultures for nonirradiated or

<p>| Table 3. Normal Ig Production in Allogeneic Cocultures of T and B Cells |
|------------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>Autologous Control (T₁ + B₁)</th>
<th>T-Cell Helper Activity</th>
<th>T-Cell Suppressor Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (PFC/culture)</td>
<td>1,600</td>
<td>4,250</td>
</tr>
<tr>
<td>Range (PFC/culture)</td>
<td>175-15,600</td>
<td>80-25,500</td>
</tr>
<tr>
<td>Data expressed as percent of individual controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Interval</td>
<td>112%†</td>
<td>145%†</td>
</tr>
<tr>
<td>n</td>
<td>23</td>
<td>19</td>
</tr>
</tbody>
</table>

†Includes the 5th to 95th percentile of all determinations.

‡Expressed as percent of the control for each run using (T₁ + B₁/T₁ + B₁) × 100% or (Tᵢ + Bᵢ/T₁ + B₁) × 100%.

§The median for 23 such allogeneic mixtures in the suppressor assay as defined by equation (3) was −88% suppression (88% enhancement).

This interval includes the 5th to 95th percentile of the normalized values.
irradiated T-cell helper function (or B-cell function) yielded medians of 4250 and 4800 PFC/culture, respectively. Such allogeneic cocultures tended to enhance helper T-cell activity compared to the autologous cultures; irradiated T cells were not significantly better or worse in providing T-cell helper activity at the T/non-T ratio of 1:1, as indicated in Materials and Methods. Similar experiments using a T/non-T ratio of 2:1 were not different.

Experiments were also done to assess the effects of adding normal T cells (0.5 x 10^6) from one individual to a second normal individual's T (0.5 x 10^6) and non-T (0.5 x 10^6) in the suppressor assay (equation 3) using the cell numbers and conditions described previously. After the normalization of the data in 23 such cocultures, the 5th to 95th percentile interval ranged from 40% suppression of the control to greater than 500% enhancement above the control, with a median of 88% enhancement.

**A Coculture Experiment From a C-GVHD Patient**

A representative coculture experiment with lymphocytes from a patient with C-GVHD 224 days after marrow transplantation (UPN 995) is shown in Fig. 1, where the results in the various coculture combinations are compared to results obtained with the control lymphocytes from the healthy HLA-identical donor. In this experiment, coculture of donor’s T and B cells resulted in 8800 PFC/culture; patient T and B cells failed to secrete Ig; irradiated patient T cells failed to provide helper activity to donor’s B cells; patient B cells secreted minute amounts of Ig in the presence of donor helper T cells; patient T cells suppressed the ability of donor T and B cells to make Ig by 90%; and the patient’s suppressor T cells were not radiosensitive. In this example where there is a radiosensitive suppressor T cell, the “absence of helper activity” seen in the T^P_+ + B_N coculture cannot be interpreted as “lack of help.” This patient with C-GVHD was not able to produce an in vivo antibody response to primary and secondary immunizations with pneumococcal polysaccharide antigens, keyhole limpet hemocyanin, and bacteriophage ÖX174, but had near-normal or normal levels of circulating polyclonal immunoglobulins at the time of study (Table 2).

**Ig Synthesis in Autologous T-Cell and B-Cell Cocultures**

Figure 2 shows the number of PFC obtained in T- and B-cell cultures from marrow graft patients compared to T- and B-cell cultures from healthy individuals. The range of PFC numbers with control T and B cells was 175–20,500 PFC/culture with a geometric mean of 1622 PFC/culture (n = 33). The patients with C-GVHD showed severely depressed PFC responses (geometric mean 27 PFC/culture) when compared to the controls (p = 0.0004). The patients without C-GVHD also had less severe but significantly depressed responses (geometric mean 420 PFC/culture) when compared to the controls (p = 0.057). Eight of the 16 patients with C-GVHD tested (1 autologous culture was not interpretable) failed to produce any PFC compared to only 1 of the 9 patients without GVHD (p = 0.03).

Subsequent experiments were aimed at determining whether the impairment in Ig synthesis seen with cocultures of patient cells represented impaired B-cell function, lack of T-cell helper function, active T-cell suppression or combinations thereof.

**B-Cell Function**

To access patient B-cell function, control T cells were added to patient B cells, and results were normalized to those seen with coculture of control T and B
cells as outlined in equation 1. Figure 3 and Tables 4 and 5 show the results. Of the 7 healthy long-term patients without GVHD, one had moderately impaired B-cell function, one had severe impairment, and 5 had no impairment of B-cell function. In contrast, 5 of 17 patients with C-GVHD had moderate impairment of B-cell function, 5 had severe impairment, and 7 had no impairment.

**T-Cell Helper Activity**

In order to eradicate radiosensitive suppressor activity and optimize helper activity within the T-cell populations from the patients, patient T cells were irradiated with 1200 rad prior to coculture with control B-cell subpopulations. The results of such cultures were compared to those obtained in the control T and B cocultures as detailed in equation 2 (Fig. 4 and Tables 4 and 5). Irradiated T cells from only 2 of 7 patients without GVHD had impaired T-cell helper activity, and in these 2 patients, 1 had moderately impaired T-cell helper activity and another had severely impaired T-cell helper activity. The T-cell helper defect was more pronounced and more frequent in patients with C-GVHD than in patients who were long-term healthy individuals. Three of 17 patients with C-GVHD had moderately impaired T-cell helper activity and 5 had severe impairment of their T-cell helper activity. In only 1 patient (UPN 995) with C-GVHD, lack of helper activity could not be confirmed due to the presence of a radioresistant suppressor T cells.

**Suppressor T-Cell Activity**

Suppressor T-cell activity in the patients was assessed by coculturing patient T cells with control T

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**Table 4. Immune Defects in Healthy Long-Term Patients**

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>B-Cell Defect</th>
<th>Helper Defect</th>
<th>T-Cell Defects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 20% of Normal</td>
<td>&lt; 20% of Normal</td>
<td>&gt; 75%</td>
</tr>
<tr>
<td>953</td>
<td>+</td>
<td>+</td>
<td>*</td>
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<tr>
<td>732</td>
<td>-</td>
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<tr>
<td>700</td>
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<td>762</td>
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<td></td>
<td>2/7</td>
<td>2/7</td>
<td>1/7</td>
</tr>
</tbody>
</table>

+ Finding present; - , finding absent.

* Suppression partially reversed with 1200 rad.
In the three C-GVHD patients studied twice, there were changes between the earlier study and their later study. These results were compared to cultures of control T and B cells, as outlined in equation 3. The normalized results of the suppressor T-cell assays are shown in Fig. 5 and Tables 4 and 5. T cells from 1 of the 7 healthy patients without GVHD showed moderate suppressor activity (>75% suppression), which was partially reversed when the patient's T cells were irradiated. T cells from the remaining 6 healthy patients did not suppress control T- and B-cell Ig synthesis. In contrast, T cells from 7 of 17 patients with C-GVHD exhibited suppression >75% and 5 of these 7 exhibited >90% suppression. In 4 of these 5, the "severe" suppression was totally or partially abrogated by irradiation of the patient's T cells prior to culture.

Although statistical comparisons of the results in the 2 groups of patients failed to reach significance (Fig. 5), more patients with C-GVHD had suppressor T cells (7 of 17 compared to only 1 of 7 patients without GVHD). If severe (>90%) suppression were considered, 5 of 17 patients with C-GVHD had suppressor T cells and none of the long-term healthy patients had T cells capable of mediating >90% suppression.

Serial Studies

In the three C-GVHD patients studied twice, there were changes between the earlier study and their later study.

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Table 5. Immune Defects in Patients With Chronic GVHD

<table>
<thead>
<tr>
<th>Unique Patient Number</th>
<th>B-Cell Defect</th>
<th>T-Cell Defects</th>
<th>Suppression</th>
</tr>
</thead>
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<tr>
<td></td>
<td>&lt;20% of Normal</td>
<td>&lt;20% of Normal</td>
<td>&gt;75%</td>
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<td>568</td>
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<td>863</td>
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<td>-/+</td>
<td>-/+</td>
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</tr>
<tr>
<td>759</td>
<td>-/+</td>
<td>-/+</td>
<td>-/-</td>
</tr>
<tr>
<td>892</td>
<td>-/+</td>
<td>-/+</td>
<td>-/-</td>
</tr>
</tbody>
</table>

-/+ Finding present; -/- finding absent.
*Second study.
†Suppression either completely or partially reversed with 1200 rad of irradiation.

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Fig. 4. T-cell helper activity in the healthy patients (I) and chronic GVHD (II) patients: [(T+ + B+)/T+ + B+] × 100%. I versus II. p = 0.2 (Wilcoxon rank sum comparison).

Fig. 5. Suppressor T-cell activity in long-term healthy patients (I) and chronic GVHD (II). I versus II. p = 0.25 (Wilcoxon rank sum comparison).
The normalized data from the second study on these three patients is presented in Table 5. Patients UPN 863 and 938 (Table 5) showed improvement in their helper T-cell function (+ → −, going from defective to the normal range). These patients showed clinical improvement during the interval between the two test dates. One patient (UPN 568) had intact B-cell function and intact helper T-cell activity, but had suppressor T-cell activity when he was studied at 1346 days posttransplant. This T-cell suppressor activity no longer was present on restudy (day 1651); however, a B-cell defect was found on the restudy. During the interval between the two studies, the activity of his C-GVHD decreased on treatment.

Types and Distribution of Abnormalities

Tables 4 and 5 summarize the types and the distribution of the various T- and B-cell abnormalities found in long-term patients without and with C-GVHD. These findings in patients are contrasted with cultures done between random normal individuals in Table 6. Both groups of patients showed some T- and/or B-cell abnormalities, but the overall probability of finding a particular defect was higher among patients with C-GVHD.

DISCUSSION

Past studies were directed at analyzing the mechanisms that determine the rate of immunologic recovery or the persistence of immunodeficiency in the lymphoid cells of donor origin after marrow grafting. The proportion of lymphocyte subpopulations (T and B cells), lymphocyte reactivity to antigens, mitogens, and alloantigens, and analysis of cytotoxic effector cells mediating natural killing, antibody-dependent, and lectin-dependent cellular cytotoxicity lacked clear associations with in vivo immune function. These findings demonstrated the need for developing new in vitro methodology.

In recent years, in vitro assays of antibody production have become available and have allowed the investigation of Ig secretion in marrow transplantation patients. Ringden et al. studied class-specific Ig secretion in 50 bone marrow transplant recipients and 42 healthy controls using peripheral blood mononuclear cells stimulated in vitro with killed S. aureus bacteria. Although most of the patients were studied within the first 100 days posttransplant, there were 7 patients with C-GVHD within the study population who had deficiencies in their in vitro Ig secretion when compared to the 4 long-term healthy patients (>1 yr after transplant). This investigation, in contrast to the previous studies, was directed at analyzing C-GVHD as a model of immunologic dysfunction by the detailed evaluation of B-cell function, T-cell helper activity, and suppressor T-cell activity in patients with and without C-GVHD. PWM, a well known T-dependent activator, was used as the polyclonal activator instead of Staphylococcal bacteria, and peripheral blood mononuclear cells were separated into T and non-T subpopulations prior to culture.

B-cell function was indeed defective in that B cells failed to respond to PWM polyclonal activation. There are multiple plausible explanations for B-cell failure. First, this failure to respond to a PWM signal may be related to a differentiation block or failure of B-cell maturation. Second, it is possible that an in vivo immunization to host alloantigens rendered the B cells unresponsive to further stimulation by PWM. The possibility of such in vivo immunization and the production of antibodies directed at host antigens is supported by the findings of increased levels of circulating serum immunoglobulins, the presence of "autoantibodies" and immunoglobulin deposition at the dermo-epidermal junction. Finally, there is a real possibility that the failure of B cells to secrete Ig may be a result of active suppression exerted by monocytes directly on B cells and not totally an intrinsic failure of B cells. This mechanism is being explored.

The evaluation of T-cell helper activity revealed lack of T-cell helper activity in these patients with C-GVHD. The quantitative lack of T-cell helper function could have been related to the inability of T-cell precursors to differentiate into mature helper T cells or due to the in vivo inactivation of the helper T cells by suppressor T cells in that patient in whom radioreistant suppression was detected.

The hypergammaglobulinemia found in many of these C-GVHD patients remains an enigma, especially in the presence of lymphocytic abnormalities detected. An earlier report suggested that defects in lymphocytic elements contributed to the hypogammaglobulinemia seen during the early period post-

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**Table 6. Summary of T- and B-Cell Defects**

<table>
<thead>
<tr>
<th>T-Cell Defects</th>
<th>B-Cell Failure</th>
<th>Lack of Help</th>
<th>Suppression ≥ 75%</th>
<th>Suppression ≥ 90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic GVHD patients</td>
<td>10/17 (58%)</td>
<td>8/17 (47%)</td>
<td>7/17 (41%)</td>
<td>5/17 (29%)</td>
</tr>
<tr>
<td>Healthy long-term patients</td>
<td>2/7 (28%)</td>
<td>2/7 (28%)</td>
<td>1/7 (14%)</td>
<td>0/7 (0%)</td>
</tr>
<tr>
<td>Controls</td>
<td>0/33 (0%)</td>
<td>0/17 (0%)</td>
<td>0/23 (0%)</td>
<td>0/23 (0%)</td>
</tr>
</tbody>
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*Data from Table 3.
†Where normal T cells from one individual were irradiated with 1200 rad before coculture with a second individual's B cells.
GVHD may represent a compensatory attempt by the immune system to regulate overactive helper T cells or overstimulated B cells. Evidence that lymphocytes from patients with C-GVHD actively suppress those of the donor in mixed lymphocyte culture has been previously reported. The mixed lymphocyte culture suppressor cells and the PWM suppressor cells found in these patients may be different. They may also play different roles from those described during the earlier period post-marrow grafting.

Although this study was not a kinetic study and the majority of these long-term patients were examined once, the results revealed a spectrum of immunologic defects in these marrow graft recipients consisting of: failure of B cells to secrete Ig, impaired or absent T-cell helper function, active suppressor T-cell activity, or combinations thereof. These defects were more common and more severe in the patients with C-GVHD, and severe T-cell suppressor activity was not seen in the healthy long-term patients.

The serial studies in three patients were useful in identifying changes in the functional characteristics of particular subpopulations; however, a larger number of patients studied in this fashion will clarify or establish in vivo/in vitro correlations. Though limited, these studies suggest that changes in the activity of C-GVHD may indeed affect biosynthesis. Immunosuppressive therapy per se could not be responsible for the defects encountered in patients with C-GVHD, since only 6 of the 17 patients received drugs (4 received prednisone only, 1 received both azathioprine and prednisone, and 1 received azathioprine) when the in vitro studies were done. Eleven of the 17 had received no immunsuppressive therapy. A number of our patients with C-GVHD have been entered into serial studies to evaluate the effects of immunosuppressive therapy on their T- and B-cell functions. The immunologic mechanisms involved in the pathogenesis of C-GVHD are the subject of ongoing investigations. Whether the immunologic aberrations found in the present study are the cause or the result of C-GVHD remain to be elucidated.

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REFERENCES

4. Storb R for the Seattle Marrow Transplant Team: Decrease in the graft rejection rate and improvement in survival after marrow transplantation for severe aplastic anemia. Transplant Proc 11:196, 1979
16. Reinherz EL, Parkman R, Rappeport J, Rosen FS, Schloss-
In vitro regulation of immunoglobulin synthesis after marrow transplantation. I. T-cell and B-cell deficiencies in patients with and without chronic graft-versus-host disease

LG Lum, MC Seigneurat, RF Storb, RP Witherspoon and ED Thomas